Welcome from the Director

Ravi Bhatia, M.D.
Interim Director, O'Neal Comprehensive Cancer Center at UAB
Professor of Medicine, Director, Division of Hematology/Oncology
Martha Ann and David L. May Chair in Cancer Research

I welcome each of you to the O'Neal Comprehensive Cancer Center's 21st Annual Research Retreat. We are honored to have three keynote speakers who will focus on major paradigm shifts in our understanding of tumor progression.

Our first keynote speaker, Dr. Dimitry Gabrivilovich, will describe recent advances in understanding how the immune response is attenuated by Myeloid Derived-Suppressor cells, which migrate into the tumor microenvironment and promote cancer progression. Our second keynote address by Dr. David Solit will focus on how the power of genomics can be harnessed by identifying those genes and pathways that can be therapeutically targeted in each cancer patient. Finally, our third keynote speaker, Dr. Stephen Schoenberger, will describe how novel proteins, or neoantigens, expressed on cancer cells, can be used to create personalized cancer vaccines and cellular immunotherapies.

We are also happy to welcome the many new cancer researchers recently recruited to UAB and have invited six of them to speak at this Retreat.

This event truly represents the stellar quality of cancer research being conducted here on a variety of levels. This year, we hit a record number in attendance and in abstract submissions. We also have the largest turnout of guests from neighboring institutions and organizations. We hope that our Retreat will continue to grow and bring together scientists from across the Southeast to exchange ideas and forge collaborations.

Finally, I want to extend my thanks to Drs. Troy Randall and Soory Varambally and our wonderful staff for putting together this excellent program.

Acknowledgements

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Fan Yang
Yang Yang
George Yang
Eddy Yang
Allan Zajac
Jianhua Zhang
Xinyang Zhao

Featured Exhibitor

Program Agenda - Friday, October 11, 2019

7:30 – 8:00 am	**Continental Breakfast**
8:00 – 8:05 am	**Ravi Bhatia, M.D.** *Opening Remarks*
8:10 – 8:55 am	**Dmitry Gabrilovich, M.D., Ph.D., Keynote Speaker** *Regulation of Immune Response and Tumor Progression by Myeloid-derived Suppressor Cells.*
9:00 – 10:15 am	**Poster Session 1**
10:20 – 10:40 am	**Benjamin Larimer, Ph.D.** *Predicting Response to Immunotherapy Using Granzyme B PET Imaging*
10:45 – 11:30 am	**David Solit, M.D., Keynote Speaker** *Defining the Actionable Genome*
11:35 – 11:55 am	**Narendra Wajapeyee, Ph.D.** *Unbiased Proteomics Approaches for Deciphering Dysregulated Oncogenic Signaling Pathways*
12:00 – 1:25 pm	**Poster Session 2 and Lunch**
1:25 – 1:30 pm	***Presentation of Albert F. LoBuglio Distinguished Faculty Award***
1:30 – 1:50 pm	**Lewis Shi, M.D., Ph.D.** *Drivers in Cancer Immunotherapy*
2:00 – 2:20 pm	**Ryan Miller, M.D., Ph.D.** *Probing the Glioblastoma Kinome*
2:25 – 3:10 pm	**Stephen Schoenberger, Ph.D., Keynote Speaker** *Going Natural with Neoantigens*
3:15 – 3:35 pm	**Elizabeth Worthey, Ph.D.** *Application of Omic-Based Data Science to Extract Clinically Relevant Information from Relatively Big Data*
3:40 – 4:00 pm	**Brittany Lasseigne, Ph.D.** *Genome-Guided Cancer Signatures*
4:05 – 4:20 pm	**Presentation of Awards and Closing Remarks**
4:20 – 4:40 pm	**Award Winner photographs**

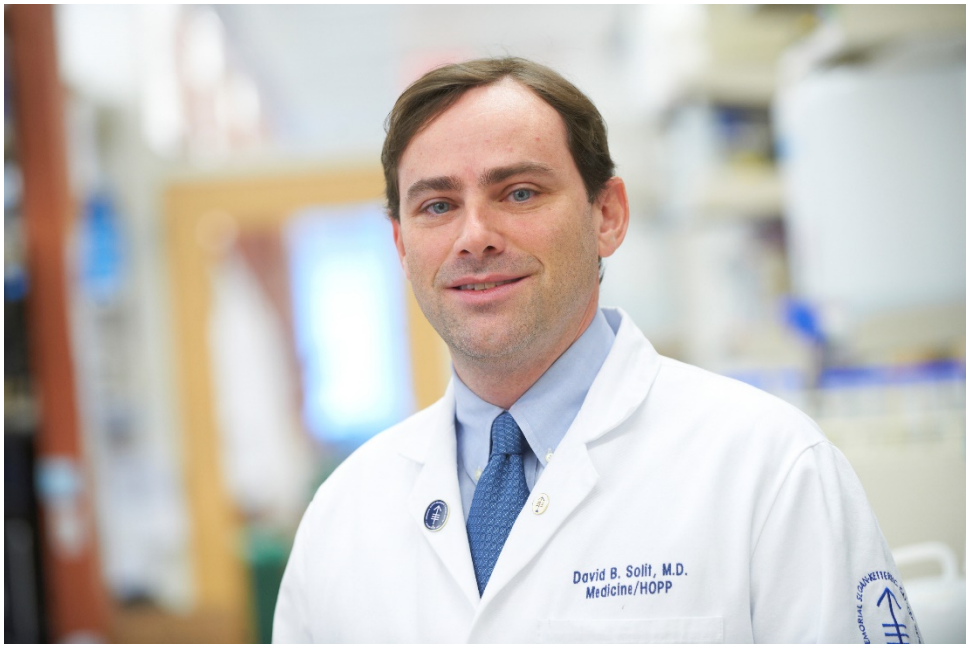
Keynote Speaker

Dmitry Gabrilovich, M.D., Ph.D.

Dmitry Gabrilovich, M.D., Ph.D., is currently a Christopher M. Davis Professor in Cancer Research and Program Leader, Immunology, Microenvironment, and Metastasis at the Wistar Institute in Philadelphia. He is the Wistar Professor at the Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania. Dr. Gabrilovich studied dendritic cell (DC) biology under Dr. Stella C. Knight at the Imperial College of London and then trained in cancer research at U.T. Southwestern Medical School and Vanderbilt University in the laboratory of Dr. D. Carbone. In the mid-1990s, his group demonstrated that DCs in cancer were functionally impaired. They described the first tumor-derived factor directly implicated in DC defects in cancer and suggested that myeloid progenitor cells were the main targets for this negative effect. His group implicated lipid accumulation as one of the mechanisms negatively regulating function of DCs in cancer. Dr. Gabrilovich was one of the discoverers of cells that are now called myeloid-derived suppressor cells (MDSCs). His group characterized a number of molecular mechanisms regulating expansion and function of these cells and that MDSC can be therapeutically targeted in patients. Dr. Gabrilovich was involved in a number of clinical trials testing the effect of cancer vaccines, small molecules and antibodies that target myeloid cells.

Keynote Speaker

David Solit, M.D.,



David Solit, MD is a graduate of the University of Pennsylvania School of Medicine and completed a residency in Internal Medicine at Barnes-Jewish Hospital/Washington University in St. Louis. He is a practicing Medical Oncologist and a Laboratory Scientist and the Geoffrey Beene Chair for Cancer Research at Memorial Sloan-Kettering Cancer Center. As a member of the Genitourinary Oncology Service, he specializes in the treatment of prostate and bladder cancers. Dr. Solit is very involved in clinical trials, particularly trials of targeted drugs known as kinase inhibitors. These drugs block pathways inside cancer cells that cause the cells to grow or spread. Dr. Solit has had a long-standing interest in the development of selective inhibitors of the MAP kinase pathway, and his laboratory was a leader in defining mechanisms of resistance to RAF and MEK inhibitors. One long-standing problem in oncology is the variability among patients with the same cancer type to standard and investigational treatments. Dr. Solit has pioneered the use of whole genome sequencing methods to identify occult predictors of drug response, work that serves as the basis for the NCI Extraordinary Responder Initiative. As the Director of the Marie-Josée and Henry R. Kravis Center for Molecular Oncology, Dr. Solit leads a multidisciplinary team of clinicians, geneticists, bioinformaticians and laboratory scientists whose mission is to integrate molecular and clinical information to develop therapies that are individualized to each patient's cancer. The Kravis Center for Molecular Oncology has led efforts to profile all patients with recurrent or metastatic cancer treated at MSK. Using the MSK-IMPACT targeted assay, MSK has prospectively sequenced over 23,000 patient tumors over the past 3 years.

Keynote Speaker

Stephen Schoenberger, Ph.D.

Dr. Schoenberger joined La Jolla Institute for Immunology in 1998 as an Assistant Professor in the Division of Immune Regulation. In 2002, Dr. Schoenberger became an Associate Professor in the Division of Cellular Immunology, and in 2005 gained Tenure. In 2008, he was promoted to Professor. Dr. Schoenberger's research focuses on the regulation of cellular immune responses.

Dr. Schoenberger received his B.S. from the University of California, Los Angeles in 1987 and his Ph.D. from the same university in 1992. In 1993, Dr. Schoenberger was a Postdoctoral Fellow in Immunohematology at the University of Leiden Hospital, the Netherlands, from 1993-1998.

Dr. Schoenberger is a member of numerous grant review panels and a reviewer for many scientific publications. He is also a member of the editorial advisory board for the *Journal of Experimental Medicine*.

UAB Speakers

Benjamin Larimer, Ph.D., is an Assistant Professor in the Department of Radiology. He obtained his Ph.D. in Biochemistry from the University of Missouri, where he utilized phage display to develop novel cancer-targeted peptides for molecular imaging. He then performed his post-doctoral studies at Massachusetts General Hospital and the Harvard Medical School, developing targeted PET imaging agents for characterizing response to cancer immunotherapy. While there, he developed a peptide-based imaging agent for granzyme B, which has been shown to be predictive of immunotherapy response in mouse models and is currently being translated for first-in-human studies at UAB.

Narendra Wajapeyee, Ph.D., is Professor and Vice Chair of Research in the Department of Biochemistry and Molecular Genetics and Co-leader of the O'Neal Comprehensive Cancer Center's Experimental Therapeutics Program. Dr. Wajapeyee's research is focused on oncogenic cell signaling pathways and gene regulation mechanisms in cancer.

Lewis Shi, M.D., Ph.D., received his M.D. in preventive medicine and M.S. in toxicology from China. He then obtained his Ph.D. from Purdue University in 2005. While at St. Jude Children's Research Hospital, he was among the first to show that the HIF-1a-glycolysis pathway functions as a metabolic checkpoint in reciprocal regulation of T_H17 and iT_{reg}, contributing to the establishment of the immuno-metabolism field. Since 2013, he has been working on cancer immunotherapy, particularly immune checkpoint blockade. His work revealed tumor cell-intrinsic (e.g., loss of IFN-γ signaling genes) and tumor cell-extrinsic resistance mechanisms (e.g., upregulation of inhibitory molecules VISTA in the tumor microenvironment) to anti-CTLA-4 therapy. Currently, his lab focuses on understanding the underlying mechanisms of cancer immunotherapies.

Ryan Miller, M.D., Ph.D., is a Professor and Director of the Division of Neuropathology in the Department of Pathology. His research focuses on primary brain tumors, particularly gliomas. He is a graduate of the UAB Medical Scientist Training Program and completed a residency in Anatomic Pathology and a fellowship in Neuropathology at Washington University in St. Louis and Barnes-Jewish Hospital. He was a member of the Lineberger Comprehensive Cancer Center at UNC Chapel Hill for 12 years, rising through the ranks to Professor. He currently serves as Co-Chair of Neuropathology and is a member of the Neuro-oncology Committee for NCI Alliance for Clinical Trials in Oncology. He was a member of the TCGA Disease and Analysis Working Groups for glioblastoma and lower-grade gliomas for over a decade. Since establishing his independent research laboratory in 2010, he has focused on developing genetically-engineered mouse models of gliomas and their genomic and proteomic characterization as preclinical models for experimental therapeutics.

Elizabeth Worthey, Ph.D., received her Ph.D. at the Imperial College London, England, and completed her postdoctoral training at the Center for Global Infectious Disease Research in Seattle. Her research interests include the development and application of omic, informatic, and data science based methods and technologies in order to identify and understand causal molecular variation in rare, undiagnosed or misdiagnosed disease. Her lab also focuses on the identification and analysis of variation that alters an individual's response to therapeutics or modifies clinical presentation, progression, and/or outcome.

Brittany Lasseigne, Ph.D., is an Assistant Professor in the Department of Cell, Developmental, and Integrative Biology. Her research focuses on integrating omic data, functional annotations, and patient information through data science approaches to discover novel mechanisms in disease etiology and progression, therapeutic targets, and circulating biomarkers. An Assistant Professor in the Department of Cell, Developmental and Integrative Biology, Dr. Lasseigne has a Ph.D. in Biotechnology Science and Engineering from The University of Alabama in Huntsville, and a postdoctoral fellowship in genetics and genomics from the HudsonAlpha Institute for Biotechnology. She has received several honors including the National Merit and Hosmer Engineering Excellence Awards, William J. Maier III Fellowship in Cancer Prevention, and the NIH K99/R00 Pathway to Independence Award.

Albert F. LoBuglio Distinguished Faculty Award

The Albert F. LoBuglio Distinguished Faculty Award is presented to a faculty member of the O'Neal Comprehensive Cancer Center who has made distinguished contributions to the research activities of the Center. The award is presented by the Director at the Annual Research Retreat.

1999	Seng-jaw Soong, Ph.D.
2000	Edward E. Partridge, M.D.
2001	Clinton Grubbs, Ph.D.
2002	Donald Buchsbaum, Ph.D.
2003	Stephen Barnes, Ph.D.
2005	William E. Grizzle, M.D., Ph.D.
2006	Ronald Alvarez, M.D.
2007	J. Michael Ruppert, M.D., Ph.D.
2008	Tong Zhou, M.D.
2009	Andres Forero, M.D.
2011	Louis Burt Nabors, M.D.
2012	Mona N. Fouad, M.D., MPH
2013	Francisco Robert, M.D.
2014	Mary-Ann Bjornsti, Ph.D.
2016	Wendy Demark-Wahnefried, Ph.D.
2017	Ralph Sanderson, Ph.D.
2018	Upender Manne, Ph.D.

RESIDENTS & FELLOWS

Abstract Number 100

STRAP Promotes the Malignant Phenotype in Neuroblastoma

Laura Bownes, MD[1]; Adele Williams, MD[1]; Raoud Marayati, MD[1]; Colin Quinn, BS[1]; Laura Stafman, MD[1]; Jerry Stewart, BS[1]; Pran Datta, PhD[2]; Juliet Easlick, BS[3]; Elizabeth Beierle, MD[1]

[1]Division of Pediatric Surgery, Department of Surgery, UAB; [2]Division of Hematology/Oncology, Department of Medicine, UAB; [3]Division of Transplantation, Department of Surgery, UAB

Background: Serine-threonine kinase receptor associated protein (STRAP) is upregulated in several malignancies and plays an important role in tumor growth and metastasis. The role of STRAP in pediatric malignancies and specifically in neuroblastoma (NB) has not been explored. We sought to determine whether STRAP functions to promote the malignant phenotype in NB.
Methods: CRISPR-Cas9 gene editing was utilized to establish stable genetic knockout (KO) of STRAP in the human NB cell line SK-N-AS (AS). Immunoblotting confirmed STRAP KO. Growth curves for STRAP wild type (WT) and KO cells were documented at 24, 48, and 72 hours and cell survival and proliferation were compared using alamarBlue and CellTiter96 assays, respectively. Cell motility was assessed using modified Boyden chamber assays. Anchorage independent growth was evaluated using soft agar assay. To investigate the role of STRAP in NB tumorigenesis in vivo, 1.8x106 WT or KO cells were injected into flanks of 6-week old athymic nude mice. Tumor volumes were measured with calipers three times per week. An in vivo model of NB metastasis was also utilized to assess the metastatic potential of the WT compared to the KO cells.
Results: Cell proliferation and survival were significantly decreased in the KO cells by 23% (p=0.002) and 32% (p=0.0001), respectively. Cell growth was also significantly decreased in the KO cells at 48 (p=0.004) and 72 (p=0.02) hours. STRAP KO cells showed significantly decreased migration (p=0.0001) and invasion (p=0.0001) compared to WT cells, and formed fewer colonies in anchorage independent growth conditions (p=0.09). In vivo, there was a decrease in average tumor volumes as well as relative tumor growth between mice injected with WT and KO cells. The metastatic model demonstrated a trend toward decreased number (5 vs. 8) and average volume (26.7 vs. 65.4 cm3, p=0.1) of liver metastases in mice injected with KO compared to WT cells.
Conclusion: STRAP KO in NB cells led to decreased cell growth, survival, proliferation, and motility in vitro, as well as a trend toward decreased tumor volume and metastatic burden in vivo. These novel findings demonstrated that STRAP plays a role in promoting the malignant phenotype in NB and warrants further investigation as a potential therapeutic target in NB.

Abstract Number 101

PRMT5 as a Target for Inhibition of FLT3-ITD AML Cell Growth in Combination with TKI

Aditi Dhir, MD[1]; Andrew Paterson, PhD[2]; Ravi Bhatia, MD[2]

[1]Fellow, Pediatric Hematology/Oncology, UAB; [2]Division of Hematology/Oncology, UAB

FLT3-ITD mutations are seen in 15% of pediatric acute myeloid leukemia(AML) patients and are associated with poor prognosis and higher rates of relapse. FLT3 tyrosine kinase inhibitors(TKIs) have shown transient success in treatment, and multiple potential mechanisms of drug resistance have been postulated including altered epigenetic regulation. Mutations involving epigenetic modifiers such as DNMT3A, IDH1/2, TET2, ASXL1 and MLL1 are often co-expressed with FLT3-ITD. The objective of our study was to identify epigenetic targets in FLT3-ITD AML and study their role in regulating growth of FLT3-ITD AML cells and to modulate ability of TKIs to target FLT3-ITD AML cells.

We used a well-validated collection of epigenetic inhibitors(Structural Genomics consortium, Epigenetics Probes Collection, SGC Toronto; www.thesgc.org) that target a spectrum of proteins

involved in epigenetic regulation. MOLM-13 and MV4-11 human FLT3-ITD positive AML cells were exposed to each inhibitor by itself and in combination with the TKI Quizartinib/AC220(500pM, IC20). Cell growth was measured using the CellTiter-Glo®Luminescent Cell Viability Assay. The FLT3-ITD negative AML cell line OCI-AML3(FLT3-WT) was studied as a control. Apoptosis was measured by flow cytometry using Annexin-V/FITC labeling and data analyzed by FlowJoV10. Statistical analysis performed using GraphPadPrism 8.1.1 software.

Of a total of 38 epigenetic inhibitors, less than a third resulted in reduction in cell proliferation after 72 hours of treatment. When used in combination with TKI, several epigenetic inhibitors resulted in significantly increased inhibition of proliferation of FLT3-ITD positive AML cells compared to either agent alone (21 for MOLM13, 10 for MV4-11 cell line, $p<0.05$). These included several Bromodomain inhibitors (L-moses, SGC-CBP30, OF1), EZH2/H1 inhibitors (GSK343, UNC1999) and protein arginine methyl-transferase (PRMT) inhibitors (PRMT1 MSO23; PRMT5 GSK591, LLY283). Given the selective effect of the combination of PRMT5 and TKI on FLT3-ITD positive AML cells in our screen, and reports of over-expression of PRMT5 in hematologic malignancies and its described direct influence on FLT3 gene transcription, we further studied this effect. The effectiveness of the combination of PRMT5 inhibitor and TKI on FLT3-ITD positive AML cells was confirmed by fixed-ratio dose-response analysis. Use of PRMT5 inhibitors(5μM) with AC220(500pM) resulted in increased inhibition of cell proliferation and increased induction of apoptosis compared to TKI alone, $p<0.05$.

In conclusion, our studies have identified PRMT5 inhibition as a novel and selective approach to enhance targeting of FLT3-ITD AML cells in combination with FLT3 TKI. These results support our ongoing studies to evaluate the cellular and molecular mechanisms underlying these combinatorial effects.

Abstract Number 103

GLI1 Occupies Sites of Ribosomal DNA Damage and Facilitates their Repair

Victor Lin, MD, PhD[1]; Tshering Lama-Sherpa[2], Brandon Metge[2], Shannon Weeks[2], Dongquan Chen, PhD[3]; Rajeev Samant, PhD[2]; Lalita Shevde, PhD[2]

[1]Division of Hematology and Oncology, Department of Medicine, UAB; [2]Department of Pathology, UAB; [3]Division of Preventive Medicine, Department of Medicine, UAB

Ribosomal DNA (rDNA) is comprised of highly repeated sequences encoding the 5S and 45S ribosomal RNAs. Double-strand breaks (DSBs) within 45S rDNA repeats are particularly deleterious, as they are spread across five different chromosomes and misrepair can result in translocations and genomic deletions. Here, we show that GLI1, the terminal effector of the Hedgehog (Hh) pathway, participates in the repair of rDNA damage. Previous work in our lab has implicated Hh signaling in DNA repair. To extend on these findings, we undertook an unbiased screening approach in triple-negative breast cancer (TNBC) cells using ChIP-Seq to investigate cistromic changes in GLI1 following ionizing radiation (IR). Unexpectedly, we found that GLI1 is highly enriched at rDNA loci in cells treated with IR. GLI1 also occupies site-specific DSBs within rDNA induced by the restriction enzyme I-PpoI. Inhibiting GLI1 with pharmacologic agents and shRNA interferes with the repair of rDNA DSBs. Combining Hh inhibition with IR compromises the recovery of RNA polymerase I in response to rDNA damage and diminishes the viability of TNBC cells. Our findings support the investigation of rational combinations of Hh inhibitors with genotoxic agents in the treatment cancer.

Abstract Number 104

PIM3 Kinase Promotes Tumor Growth and Metastasis in Hepatoblastoma

Raoud Marayati, MD[1]; Laura L. Stafman, MD, PhD[1]; Adele P. Williams, MD[1]; Colin H. Quinn, BS[1]; Hooper R. Markert[1], Juliet L. Easlick, BS[2]; Jamie M. Aye, MD[3]; Jerry E. Stewart, BS[1]; Anita B. Hjelmeland, PhD[4]; Elizabeth Mroczek-Musulman, MD[5]; Elizabeth A.Beierle, MD[1]

[1]Division of Pediatric Surgery, Department of Surgery, UAB; [2]Division of Transplantation, Department of Surgery, UAB; [3]Division of Pediatric Hematology Oncology, Department of Pediatrics UAB; [4]Department of Cell Developmental and Integrative Biology, UAB; [5]Department of Pathology, Children's of Alabama

Introduction: Hepatoblastoma (HB) is the most common primary liver tumor in children. Despite increasing incidence, therapy for HB has remained unchanged for the last 20 years. Over half of patients with HB initially present with metastatic or advanced disease and their prognosis remains dismal. We previously demonstrated that PIM3 kinase is overexpressed in human HB cells and functions to promote tumorigenesis. Using CRISPR-Cas9 mediated PIM3 knockout (KO), we have confirmed the role of PIM3 in promoting cell proliferation and survival in vitro, as well as in promoting migration and invasion of HB cells as distinct early steps of the metastatic cascade. We aimed to assess the role of PIM3 in promoting HB tumor growth and metastasis in vivo.
Methods: CRISPR-Cas9 gene editing was used to achieve stable PIM3 KO in the human HB cell line, HuH6. PIM3 KO or HuH6 wild-type (WT) cells were injected subcutaneously into flanks of athymic nude mice and tumor volumes were measured 3 times weekly with calipers. Formalin-fixed, paraffin-embedded tumors were examined for cell proliferation using immunohistochemical staining for Ki-67. Tail vein injections of PIM3 KO and WT cells with stable expression of luciferase reporter gene were performed, and bioluminescence imaging was used to monitor mice for the development of lung metastasis and to quantify metastatic burden. Student's t-test and Chi-square were used with mean ± standard error of the mean reported and p=0.05 significant.
Results: Immunoblotting confirmed stable KO of PIM3. Mice bearing PIM3 KO flank tumors exhibited significantly decreased tumor volumes compared to those with WT tumors (p=0.016). Mice bearing PIM3 KO tumors survived a median of 12 days longer (p=0.002), and their tumors had significantly less Ki-67 staining than WT tumors (p=0.039) indicating decreased proliferation. PIM3 KO impaired the formation of lung metastasis: 5 of 6 mice injected with WT compared to 0 of 7 mice injected with PIM3 KO cells developed lung metastasis (p=0.002). Average bioluminescence in lungs of WT injected mice was 2000-fold higher than PIM3 KO lungs (p=0.038). Hematoxylin and eosin staining confirmed metastasis formation while PIM3 KO lungs showed no histological evidence of metastases.
Conclusions: PIM3 KO led to decreased tumor growth, increased survival, and decreased lung metastasis of HB cells in vivo. These findings suggest that PIM3 plays an important role in promoting the metastatic phenotype in HB and that targeting PIM3 may provide a novel therapeutic strategy for the treatment of children with metastatic disease.

Abstract Number 105

Fluorescence-guided Surgery of Head & Neck Cancer: da Vinci Robot and Targeted Agents

Lindsay Moore, MD[1]; Bailey Luke, BS[2]; Melanie Hicks, MD[1]; Daniel Morrison, MD[1]; Eben Rosenthal, MD[3]; William Carroll, MD[1]; Jason Warram, PhD[1]

[1]Department of Otolaryngology, UAB; [2]School of Medicine, UAB; [3]Department of Otolaryngology, Stanford University

Purpose: In this novel clinical trial subanalysis, we demonstrate, for the first time, the ability of tumor-targeted near-infrared (NIR) fluorophores to detect the presence of tumor in head and neck

cancer patients using the da Vinci Xi surgical system and Firefly/Advanced Firefly integrated NIR fluorescence imaging systems.
Study Design: The Firefly and Advanced Firefly fluorescence imaging systems integrated within the da Vinci Xi (Intuitive Surgical) robotic surgical system were repurposed to fluorescently localize cetuximab-IRDye800CW and panitumumab-IRDye800CW (fluorescent dye conjugated to anti-EGFR antibodies) in 8 patients scheduled for curative surgical resection of squamous cell carcinoma of the head and neck within the context of two Phase I clinical trials performed/ongoing at UAB and Stanford University. The Firefly and Advanced Firefly of the da Vinci robot Xi system were used for real-time imaging of the primary specimen and postresection wound bed was performed intraoperatively in the surgical field, and the resected primary specimens and resected margins were imaged ex vivo immediately after resection in the operating room. Fluorescence was quantified using integrated instrument software and standardized quantitative imaging analysis software. Relative fluorescence units (RFU) were measured for tumor, background, and post-resection wound bed, and averaged among 6 individual frames per imaging time point. TBR was calculated by dividing tumor RFU by respective background RFU, and fluorescence tissue assessment for tumor positivity was performed using a previously validated ratiometric threshold analysis.
Results: Intraoperative fluorescence imaging with the robot showed significant cancer-specific tumor-fluorescence in vivo and with immediate back table ex vivo imaging of the resected tumor, with tumor-to-background ratios (TBR >2.0 in all cases; maximum mean TBR 3.67, p0.05). This technology also was able to detect residual tumor in a post-resection wound bed in two cases that lead to the resection of pathology confirmed cancerous tissue.
Conclusion: Optical imaging in these patients showed potential for this technology to be utilized to guide real-time tumor resection, margin assessment, and immediate post-resection pathological processing, and the ongoing advancements in the current imaging technology have correlated with improved optical imaging. This pilot study clinical trial subanalysis is the first to explore the use of targeted fluorescent optical imaging agents to guide surgical resection of head and neck cancer using the da Vinci Xi robotic surgical system and integrated NIR fluorescence imaging technology. These promising results have served as the foundational evidence to propel this research to a Phase II clinical trial currently under review.

Abstract Number 106
Predicting Schwannoma Growth in a Mouse Flank Tumor Model using Targeted Imaging
Alyssa Ovaitt, MD

Otolaryngology, UAB

Introduction: Vestibular schwannoma (VS) is a common pathology encountered in neurotology clinics. Many patients are observed with a “wait and scan” approach. Prior efforts to determine radiographic indicators of future growth have been unsuccessful. Using a mouse flank tumor model, we seek to determine if fluorescent imaging with directed immunotargets could be used to predict schwannoma growth rate.
Methods: Anti-VEGFR2 and anti-Her2/Neu were covalently linked to a near-infrared probe (IRDye800). Immunodeficient mice underwent flank injections with a rat-derived schwann (R3) cell line. When tumor growth was evident, either Anti-VEGFR2-IRDye800, anti-Her2/Neu-IRDye800, or IgG Isotype-IRDye800 (control) were injected via tail vein. The mice were serially imaged in a closed field device (LUNA, Novadaq, Toronto, Ontario, Canada). Fluorescent data were analyzed for mean tumor signal and correlated with tumor growth. Tumor heterogeneity of fluorescence signal was assessed through quantification of histogram analysis through automated MATLAB code. Wilcoxon rank sum test (or t-test) and person correlation was used to evaluate statistical significance (p=0.05).

Results: All mice grew clinically evident tumors with variable growth rate. In both study groups, there were strong correlations between day 1 mean tumor fluorescence and eventual maximum tumor volume (p=0.002, 0.001; r2=0.92, 0.86). There was also strong correlation with maximum tumor signal and maximum tumor volume (p=0.003, 0.008; r2 = 0.90, 0.91) There was no such correlation in the control group. Differences in heterogeneity were quantified in between group histograms.
Conclusion: VS is a challenging problem for neurotologists and patients alike, with many patients opting for observation. We seek to identify immunotargets in a murine model that show promise in predicting schwannoma growth with advanced imaging techniques. Both Her2/Neu and VEGFR2 are promising targets that merit further investigation. Assessing tumor heterogeneity may identify spatial regions of increase growth patterns.

Abstract
Heparanase from Multiple Myeloma Causes Kidney Injury
Sunil Rangarajan, MD[1]; Rada Ali, PhD[2]; Kaushlendra Tripathi, PhD[2]; Shyam Bandari, PhD[2]; Elizabeth Brown[2], PhD; Ralph Sanderson, PhD[2]

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Multiple myeloma is a malignant plasma cell disorder that constitutes 12-13% of hematologic malignancies in the United States. Kidney injury in myeloma patients can occur due to free light chain mediated proximal tubulopathy, cast nephropathy, light chain deposition disease, amyloidosis or hypercalcemia to enumerate a few. Nearly half of patients who have myeloma have concomitant kidney injury and serum creatinine >2.0 mg/dL portends a worse prognosis and limits the use of some front line chemotherapeutic agents. In a recent publication, we showed that light chains can cause proximal tubulopathy and activate pro-inflammatory and pro-fibrotic pathways much before it is clinically reflected by means of serum creatinine elevation. Our lab has previously shown that heparanase plays a key role in pathogenesis of multiple myeloma related bone disease and patients with high heparanase activity have increased disease burden. Heparanase has been previously implicated in diabetes mellitus related nephropathy. Using an in vitro model, we now show that recombinant, enzymatically active heparanase causes injury to Human Kidney-2 cells (HK-2). Increasing doses of recombinant heparanase decreased the clonogenic potential of HK-2 cells. We established for the first time a co-culture system where multiple myeloma spheroids were co-cultured with HK-2 spheroids. It was noted that there were many fewer HK-2 spheroids that survived over prolonged co-culture periods when the myeloma cells over expressed heparanase. Together these data indicate that heparanase secreted by myeloma tumor cells may promote kidney injury. We are currently in the process of developing a mouse model to evaluate the effects of heparanase secreted by multiple myeloma on the kidneys. In this model, we will also attempt to identify biomarkers that will potentially identify novel pathways involved in multiple myeloma related nephropathy.

Abstract Number 108
PP2A Inhibition Does Not Impede the Cytotoxic Effects of Oncolytic HSV In Vitro
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Background: Brain tumors are the most common solid tumor in children and have now surpassed leukemias as the leading cause of cancer-related morbidity and mortality. More effective and less-toxic therapies for pediatric brain tumors are greatly needed. Immunovirotherapy with engineered oncolytic herpes simplex virus (oHSV) is a promising approach. HSV G207 has been proven safe with evidence of efficacy in recurrent adult glioblastoma and is currently in clinical trial in recurrent

pediatric brain tumors. Our ongoing laboratory studies are focused on modulating the immune microenvironment to enhance tumor cytotoxicity and the anti-tumor immune response generated by oHSV with the goal of providing durable anti-tumor immunity. Protein phosphatase 2A (PP2A) is a protein that regulates multiple cellular processes: metabolism, apoptosis, DNA repair, and mitosis. Inhibition of PP2A by LB-100 has been shown to modulate the tumor microenvironment by decreasing T regulatory cells and increasing cytotoxic T cells. We hypothesize that low dose LB-100 used in conjunction with IL-12 producing oHSV M002 will not negatively impact M002 cytotoxicity and will act synergistically with the virus in vivo against pediatric brain tumor models.
Methods: To determine if LB-100 impedes M002 cytotoxicity, we first assessed the effects of LB-100 alone and combined with M002 in vitro. 1x104 cells per well of human Group 3 D341 Medulloblastoma (MB) cells or murine 15-405-7 DIPG cells were added to 96-well plates and incubated 24 hrs in Neurobasal medium. LB100 was added at increasing concentrations (0 - 30μM) and incubated for 48 hrs followed by 25μL of alamarBlue. Color changes were quantified with a BioTek microplate spectrophotometer and OD595-562 nm values were used to calculate the IC50. Subsequent experiments measured the effect of LB100 on M002 cytotoxicity by adding several molar concentrations of LB-100 1 hr prior to viral inoculation with M002 at various doses (10 plaque-forming units/cell). 72 hrs after virus inoculation, AlamarBlue assays were performed, and IC50 was measured.
Findings: LB-100 alone had an IC50 of 3.6 and 4.8 with D341 and 15-405-7 respectively. LB100 at several concentrations had no negative effects on M002 cytotoxicity in either cell line.
Conclusions: LB-100 does not interfere with the cytotoxic effects of oHSV against pediatric brain tumor cell lines in vitro. Our next step is to evaluate the safety and efficacy of LB-100 with oHSV in vivo murine models.

Abstract Number 109

PORCN Inhibition Improves Survival, Alters the Immune Microenvironment in Ovarian Cancer

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Wnt/β-catenin pathway upregulation has been correlated with immune evasion in multiple cancers, including epithelial ovarian cancer (EOC). Porcupine (PORCN) is an enzyme necessary for cells to produce WNT ligand, which is necessary for the activation of the pathway. We hypothesized that administration of PORCN inhibitor (CGX1321) would decrease Wnt signaling and thereby lead to a decrease in immune evasion, creating a “hot” tumor microenvironment (TME), resulting in increased survival and a decrease tumor burden. RNA sequencing was performed in a cohort of primary ovarian cancer samples. CGX1321 was administered to C57Bl6 mice injected intraperitoneally with ID8 or ID8p53-/- cells. Tumor growth was measured by omental weight. Amount of tumor infiltrating lymphocytes (TILs), Tregs, dendritic cells (DCs), and macrophages in the TME were quantified via flow cytometry. CD11c-cre x β-catenin-flox C57Bl6 mice, a mouse model with no DC-intrinsic β-catenin and the administration of anti-CD8β antibody was used to evaluate tumor burden with and without CGX1321. Increased Wnt activity was correlated with a decreased T-cell signature in our human ovarian cancer samples. Treatment with CGX1321 prolonged survival (P=0.0001), and decreased omentum weight (P=0.0056) in mice injected with ID8 cells. There was an increase of DCs (P=0.0159), and macrophages (P=0.0079) with treatment, which was only significant for mice injected with ID8 cells, not ID8p53-

/- mice. Tumor burden was decreased in CD11c-cre x β-catenin-flox mice (P=0.1014) without treatment, and with CGX1321 treatment (P=0.0450). Tumor burden was not decreased with CGX1321 treatment in the absence of CD8+ T-cells (P=0.3175). Consistent with EOC TCGA data, we identified a correlation between increased Wnt signaling and decreased T-cell signature (i.e. "cold" tumor). ID8 cells have a higher level of total β-catenin and expression of WNT related genes compared with ID8p53-/-, which may explain the prolonged survival, decreased tumor burden, and more profound increase of DCs, macrophages, and monocytes in the TME. This may suggest a reliance on these cells for tumor recognition with PORCN inhibition. Furthermore, tumor burden was decreased in a DC-intrinsic β-catenin absent model, suggesting the presence of β-catenin in this antigen-presenting cell contributes to immune-evasion in the TME. The lack of effect of CGX1321 on tumor burden in the absence of CD8+ T-cells indicates a partial reliance on CD8+ T-cells for tumor recognition. This data warrants further investigation of Wnt inhibition in both pre-clinical models and clinical trials in EOC.

Abstract Number 110

The Impact of Depression in Cancer Patients on Healthcare Utilization

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Background: The Oncology Care Model (OCM) has set several initiatives to improve payment and care delivery in the Medicare patient population, including screening for depression in cancer patients. We evaluated the prevalence of depression in OCM patients and the relationship between depression and healthcare utilization.

Methods: This cross-sectional study used patient-reported outcome (PRO) surveys administered in the outpatient setting as part of OCM at the University of Alabama at Birmingham (UAB). Depression scores and Eastern Cooperative Oncology Group performance status were obtained from PRO surveys. Moderate to severe depression was defined as a score 10 on the Patient Health Questionnaire 2/9 (PHQ-2/9). Sex, marital status, phase of care, race, disease aggressiveness (stage, progression, cancer type), number of emergency department (ED) visits and inpatient admissions within a 3-month period from survey completion were abstracted from the electronic health record. The relationship between depression and hospital visits was assessed using rate ratios and 95% confidence limits from Poisson regression models adjusting for clinical and demographic characteristics.

Results: Of 1038 patients surveyed, 68% of patients were female, and 27% of patients were non-Caucasian. Notably, 13% of patients had moderate to severe depression (PHQ-2/10). The cancer-specific prevalence of at least moderate depression was 2% in breast, 1% in gastrointestinal, 2% in genitourinary, 5% in gynecologic, and 2% in hematologic cancers. In adjusted models, the inpatient admission and ED visit rate in the 3 months following PRO survey completion did not differ by depression category (RR: 1.25; CI: 0.97-1.62).

Conclusions: Approximately 13% of cancer patients report clinically significant depression during routine screening, which highlights the continued need for outpatient counseling and behavioral services. Although rates of inpatient admissions and ED visits were not impacted by the presence of depression, further analysis is needed to evaluate the impact of treating depression on healthcare utilization over time.

Abstract Number 111

Effect of DNA Methyltransferase Inhibitors on Gastroenteropancreatic Neuroendocrine Cells

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Introduction: Pancreatic neuroendocrine tumors (pNETs) are a heterogeneous group of tumors in which up to 60% of patients present with metastatic disease with limited treatment options. Epigenetic modulation of pNETs has shown some early clinical promise with histone deacetylase (HDAC) inhibitors upregulating pNET tumor suppressor, Notch3. Prior studies have shown Notch3 is likely under the transcriptional regulation of insulator CTCF and activator BORIS, which have not been studied in pNETs. We aim to assess whether epigenetic modulation of Notch3 with DNA methyltransferase (DNMT) and/or HDAC inhibitors could be an effective anti-proliferative treatment in pNETs.

Methods: Cytotoxicity was determined by Cell proliferation (MTT) assay. CompuSyn software was used for in silico drug combination analysis. Quantitative RT-PCR was used to measure mRNA expression. Protein expression was determined by Western Blot analysis. HDAC inhibitor, valproic acid (VPA), and DNMT inhibitor, 5-azacytidine (AZA), were utilized for drug testing.

Results: Cytotoxicity threshold to HDAC and DNMT inhibitors were compared between BON-1 (pNET), PANC-1 (pancreatic adenocarcinoma), and HEK293 (fibroblast) cell lines by cell proliferation (MTT) assay. IC50 for BON-1, PANC-1, and HEK293 were 10.6 μM, 368.7 μM, and 26.4 μM respectively. When combined, DNMT and HDAC inhibitors act synergistically with combination indices (CI) of 0.89, 0.67, and 0.80 for Fa 0.25, 0.5, and 0.75 respectively of treated BON-1 cells. Drug-reduction indices (DRI) were favorable (DRI>1) for both drugs. NOTCH3 mRNA expression in BON-1 increased 2-fold with valproic acid and 7-fold with 5-azacytidine over DMSO control. This corresponds to the easily visualized Notch3 band after treatment with AZA, but limited visualization of the Notch3 band with VPA. Protein expression differed after AZA treatment compared to VPA. BORIS displayed dose-dependent increase in intensity while CTCF remained unchanged with AZA, but BORIS was unchanged while CTCF fluctuated in a dose-dependent manner with VPA. AZA caused Chromogranin A to change similarly to NOTCH3, but VPA found Chromogranin A to change in tandem with fluctuations of CTCF.

Conclusion: DNMT inhibitors are more selectively cytotoxic for GEP-NETs than other cell lines and act synergistically with HDAC inhibitors with favorable DRI's, suggesting that favorable therapeutic responses can be obtained at lower drug toxicities. AZA and VPA affect pNET protein expression differently, suggesting differing pathway of effect, but VPA failed to show the same dose-related upregulation of NOTCH3 previously seen with TDP-A, which may suggest that the specific HDAC being inhibited may play a role in NOTCH3 expression.

POSTDOCTORAL FELLOWS

Abstract Number 200

TRIM29 is Involved in Metastasis of Microsatellite Stable Types of Colorectal Cancer

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Colorectal cancer (CRC) is the third leading cause of cancer deaths in USA. CRC has two major molecular subtypes: Microsatellite instability (MSI) having dysfunction of the mismatch repair (MMR) genes are less aggressive, and microsatellite stable (MSS) with mismatch repair active genes harboring 85% of the CRCs are more aggressive. Various studies suggested that MSI status have favorable prognosis and treatment for CRC while MSS is associated with poor prognosis and resistance to various therapies. Microarray gene expression profiling studies from the previous study showed association of Tripartite motif-containing 29 (TRIM29) with MSS. In this study, we have investigated TRIM29 expression in CRC tissues and cells having different MSI and p53 status. We found overexpression of TRIM29 in MSS as well as p53 mutated types of colon cancer cells as compared to MSI and p53 wild type. TRIM29 knockdown studies showed the role of TRIM29 in malignancy of MSS types of colon cancer. Our study demonstrated the role of TRIM29 in MSS types of colon tumor metastasis and found fastest increase in colon cancer cells colonization in liver and dissemination in bone in control shRNA group as compared to TRIM29 knockdown. Thus our study suggested that targeting TRIM29 could be potentially an effective therapeutic strategy with small molecule inhibitors in future for treatment of CRC.

Abstract Number 201

ST6Gal-I Overexpression in Gastric Adenocarcinoma Blocks Epithelial Cell Apoptosis

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Overexpression of the glycosyltransferase ST6Gal-I in the epithelium is associated with several epithelioid cancers, including colonic, ovarian, and pancreatic adenocarcinoma. ST6Gal-I promotes the addition of 2,6-linked sialic acids to N-glycans on epithelial cell death receptors TNFR1 and Fas, blocking receptor internalization and disrupting homeostatic apoptosis. To explore the role of ST6Gal-I in gastric epithelioid tumor biology, we generated human gastric epithelial stem cell organoids and investigated the role of ST6Gal-I in gastric epithelial cell apoptosis. Epithelial stem cell organoids generated from normal gastric antral mucosa expressed the canonical Wnt target gene Lgr5, as well as ST6Gal-I mRNA and protein. Organoids cultured in reduced concentrations of Wnt-conditioned media exhibited diminished Lgr5 and ST6Gal-I mRNA levels (p=0.004), indicating that ST6Gal-I expression is, at least in part, regulated by the Wnt pathway. The differentiation of gastric epithelial stem cell organoids into epithelial monolayers resulted in sharp decreases in Lgr5 mRNA expression (p0.0001), along with ST6Gal-I protein and mRNA levels (p=0.0008), suggesting ST6Gal-I expression reflects epithelial cell stemness and is significantly reduced in differentiated gastric epithelium. Importantly, ST6Gal-I was highly expressed in gastric adenocarcinoma tissue together with differentiated epithelial cell monolayers generated from tumor-derived epithelial stem cell organoids. To mimic gastric adenocarcinoma expression of ST6Gal-I, we transduced epithelial stem cell organoids generated from normal

gastric tissue to overexpress ST6Gal-I. ST6Gal-I expression remained highly elevated in epithelial stem cell monolayers generated from the transduced organoids, resulting in markedly increased resistance to TNF-mediated apoptosis, as evidenced by decreased caspase signaling. In conclusion, ST6Gal-I is a marker of gastric epithelial stem cells and is strongly upregulated in gastric adenocarcinoma, implicating a potential role for ST6Gal-I in the dysregulation of homeostatic apoptosis and tumor longevity in mucosal epithelioid cancers.

Abstract Number 202
Chemoexosomes secreted by Myeloma cells enhance Osteoclast Formation
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Extreme bone destruction is a major complication of myeloma; 85% of myeloma patients suffer from destructive bone lesions. Bone lesions are formed when the rapidly proliferating myeloma cells produce soluble factors responsible for the formation of bone resorbing cells called osteoclasts. Recent studies have revealed that exosomes, 50 to 150 nm vesicles secreted in large numbers by cancer cells, are involved in osteoclast formation. However, nothing is known about how osteoclast formation is impacted by exosomes that are secreted by myeloma cells following their exposure to chemotherapy. I recently published work showing that exposure of myeloma cells to chemotherapeutic drugs ignites a burst of exosome secretion by those cells. We refer to these chemotherapy-induced exosomes as chemoexosomes. In the current study, I explored the role of chemoexosomes in promoting osteoclast formation. Murine macrophages were incubated with control exosomes from cells not exposed to chemotherapy or with chemoexosomes for 6 days and TRAP staining was performed to assess osteoclast formation. Results demonstrated that chemoexosomes increased osteoclast formation compared to that of control exosomes. Further, when murine macrophages were incubated with chemoexosomes isolated from myeloma patient plasma, enhanced osteoclast formation was observed. Previously we have shown that Roneparstat, a modified heparin that inhibits heparanase enzyme activity, also can block exosome docking to recipient cells. When chemoexosomes were added to the murine macrophages in the presence of Roneparstat, results demonstrated a decrease in osteoclast formation compared to murine macrophages incubated with chemoexosomes alone. This suggests the direct role of chemoexosomes in osteoclast formation and the ability of Roneparstat to interfere with the docking of chemoexosomes with murine macrophages thereby decreasing osteoclast formation. This study has revealed for the first time that a negative side effect of chemotherapy in myeloma is the release of chemoexosomes that can contribute to bone destruction in myeloma patients. Moreover these studies imply that therapeutic disruption of chemoexosomes could dramatically reduce tumor related osteolysis thereby enhance myeloma patient quality of life and survival.

Abstract Number 203
Age-dependent Heterogeneity of Lymph Node Metastases in a National Breast Cancer Registry
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Department of Pathology, UAB

Background: For several cancers, including breast, young age of diagnosis is related to an adverse prognosis. While this effect is often attributed to heritable mutations such and BRCA1/2, the relationship between clinical/pathologic features, young age of onset, and prognosis in breast cancer remains unclear. In this study, we highlight links between age of onset and lymph node

metastasis (NM) in U.S women with breast cancer. NM in breast cancer was consistently associated with young age of onset across a 27 year period of SEER data.
Methods: Case listings from registry data for women with breast cancer were used in this study. NM and NM-associated outcomes were evaluated in a test set of women with receptor subtype information and then compared against a larger, pre-subtype validation set of data from the same registries. Age of diagnosis was a 5-category variable; under 40 years, 40-49 years, 50-59 years, 60-69 years and 70+ years.
Results: In adjusted logistic regression models, women under 40 years old at diagnosis had 1.67 times the odds of NM as women 60-69 years of age. Odds of NM in Luminal B and Her2 subtypes did not differ significantly from those in Luminal A. Triple negative (TNBC) tumors were found to have significantly decreased odds of NM when compared to the same referent (OR 0.63). In subtype-stratified adjusted models, age of diagnosis had a consistent trend of decreasing odds of NM by age category, most noticeable in hormone receptor positive (HR+) subtypes of Luminal A and B.
Conclusions: Lymph node metastasis is age dependent, yet not all molecular subtypes are clearly affected by this relationship. NM may also be a large reason for decreased survival in 40 yr. olds, with over 50% of the adjusted hazard of death in this age group attributable to NM .When stratified by subtype, the strongest associations were in HR+ groups (Luminal A and B), suggesting a possible hormonal connection between young age of onset and NM. Similarly, the lack of importance of age to NM in TNBC tumors may point to alternate, non-NM means of disease severity. This study is supported by a T32 training award to Dr. Behring and by the institutional support to Dr. Manne

Abstract Number 204
89Zr-panitumumab, a Radiolabeled EGFR Antibody, for Imaging Ameloblastomas In Vivo
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Background: Ameloblastomas demonstrate locally aggressive and destructive behavior primarily in the posterior mandible. The ability to accurately assess tumor margins with specific, non-invasive imaging could result in the preservation of healthy tissue and improve long-term local tumor control, thereby reducing the risk of recurrence and providing appropriate reconstructive therapies with minimal morbidity.
Hypothesis: Epidermal growth factor receptor (EGFR) expression in ameloblastomas may be used to specifically visualize tumors intraosseously, which may be used to assess tumor margins preoperatively. The objective is to measure the specificity of radiolabeled 89Zr-panitumumab (an EGFR antibody) in vivo using patient-derived tumor models of ameloblastoma and positron emission tomography/computed tomography (PET/CT) scans.
Methods: Patient-derived xenografts (PDX) of ameloblastoma were implanted subcutaneously into the flanks of immunocompromised mice. Following tumor establishment, mice receive 89Zr-panitumumab and are imaged 120 hours post-injection by PET/CT.
Results: In PDX of ameloblastomas from three patients (AB-36, AB-37, and AB-39), the biodistribution of 89Zr-panitumumab was measured 120 hours post-injection and was reported as the injected dose per gram of tissue (%ID/g). The average tumor uptake was ~40 %ID/g for AB-36, ~65 %ID/g for AB-37, and ~20 %ID/g for AB-39. The radiolabeled %ID/g was significantly greater in AB-36 and AB-37 tumors of 89Zr-panitumumab-treated mice that did not receive

unlabeled panitumumab as a blocking control. The standardized uptake values (SUV) in tumors receiving 89Zr-panitumumab were significantly higher in tumors from AB-37 and AB-39. MicroPET/CT imaging showed high uptake of 89Zr-panitumumab in the ameloblastoma tumors compared to other areas of the mouse, including low uptake in the bone. Radiolabeled anti-EGFR demonstrates specificity for ameloblastoma PDX tumor xenografts.
Conclusion: We believe 89Zr-panitumumab is an attractive target for imaging EGFR-expressing tumors. With this technology, we believe we can more accurately assess neoplastic margins for the surgical removal of ameloblastomas, thus improving patient outcomes.

Abstract Number 205
BOP1 Loss Confers Resistance to BRAF Kinase Inhibitors in Melanoma by Activating MAPK
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Acquired resistance to BRAF kinase inhibitors (BRAFi) is the primary cause for their limited clinical benefit. Although several mechanisms of acquired BRAFi resistance have been identified, the basis for acquired resistance remains unknown in over 40% of melanomas. We performed a large-scale short-hairpin RNA screen, targeting 363 epigenetic regulators and identified Block of Proliferation 1 (BOP1) as a factor, the loss of which results in resistance to BRAFi both in cell culture and in mice. BOP1 knockdown promoted down-regulation of the MAPK phosphatases DUSP4 and DUSP6 via a transcription-based mechanism, leading to increased MAPK signaling and BRAFi resistance. Finally, analysis of matched patient-derived BRAFi or BRAFi+MEKi pre- and progressed melanoma samples revealed reduced BOP1 protein expression in progressed samples. Collectively, our results demonstrate that loss of BOP1 and the resulting activation of the MAPK pathway is a clinically relevant mechanism for acquired resistance to BRAFi in melanoma.

Abstract Number 206
Three-Dimensional Lung Tumor Mimics to Investigate Tumor-Stromal Interactions
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Introduction: Lung cancer is the leading cause of cancer-related death in the United States, with the five-year survival rate remaining below 20%. The tumor microenvironment (TME) is a key regulator of tumor biology and response to therapeutic intervention, with intercellular communication between tumor and stromal cells known to regulate tumor growth and progression. Extracellular vesicles (EVs) facilitate cell-cell communication, yet the impact of this form of tumor-stromal cross-talk is not fully understood. Recently, in vitro engineered tissues, in which an extracellular matrix scaffold containing cellular components generates a three dimensional (3D) volume, have been implemented as surrogates of human pathophysiology in biomedical and pharmaceutical research. Herein we utilize tissue engineering strategies to develop in vitro non-small cell lung tumor mimics, which include multiple components of the TME, to characterize the role of EVs in tumor-stromal crosstalk.
Methods: Engineered tumor mimics were generated utilizing a bioreactor platform and maintained via a closed perfusion loop to provide nutrient circulation. This platform allows for isolation of circulating EVs without disrupting the 3D volume. Using this model system, the EV profile was characterized in tumor mimics containing non-small cell lung carcinoma cells (H358

or A549) pre-stained with PKH26 and IMR90 lung fibroblasts pre-stained with PKH67 (seeded at a 2 to 1 ratio) following 6 days culture. A monocyte cell line, THP1, was also added to the tumor mimics to evaluate the impact EV- mediated tumor-stromal cross-talk within the engineered tumor mimics.
Results: Expression of two tetraspanins, CD63 and CD81, that are common markers of EVs were observed in vesicle populations derived from both tumor and fibroblast populations. Furthermore, a 5.5 fold increase in PDL-1 expression was observed in tumor cell derived EVs (PKH26+) when compared to fibroblast derived EV (PKH67+). Tumor-immune cell cross talk was monitored in tumor mimics that contained human monocytes in addition to NSCLC cells and lung fibroblasts. When monocyte phenotype was evaluated following 7 days co-culture, increases in the macrophage population (CD11b+) and M2 markers, CD206 and CD163 were seen when compared to engineered tissues containing THP1 cells alone. Interestingly, macrophage populations contained both tumor- and fibroblast-derived material (indicated by PKH signal).
Conclusions & Future Directions: These results suggest differential EV profiles within the TME that may regulate tumor-stromal interactions, particularly those involving infiltrating immune populations. Moving forward, this tumor mimic platform will allow for extensive characterization of tumor-stromal interactions, potentially in a patient-specific manner.

Abstract Number 207

Phosphoproteomic Unravels Dynamic Regulation of Energy Sensor by CDK5 in NE-Tumors

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Background: Pheochromocytoma (PC) is a rare Neuroendocrine (NE) tumor that usually originates from adrenal gland's chromaffin cells. These tumors are often benign but can become malignant, causing excessive secretion of catecholamines leading to life threatening situation. Pro-tumorigenic role of CDK5, a proline-directed serine/threonine neuronal kinase has been implicated in tumor development. However, the broader downstream signalling events contributing to the function of CDK5 are largely unknown in NE tumors, particularly Pheochromocytoma.
Results: Here we showed for the first time that aberrant CDK5/p25 signaling attributes human PC lesions both sporadic and in tumours, incorporating mutations in key susceptibility genes such as SDHx and VHL. Unique doxycycline regulated bi-transgenic (TG) mouse model system was designed to drive CDK5/p25 overexpression (OE) in a NE cell specific manner localized within adrenal medulla. PC lesions developed in the TG mouse model accurately recapitulate human PC pathobiology and thus rendered a platform for discovering precision use of novel CDK5 inhibitors. Picking CDK5 pocket with a novel CDK5 inhibitor "MRT3-007" induced significant tumor regression in TG/ SDHB knockdown xenograft/ & metastatic PC models. Next, to identify putative CDK5 substrates, we analysed phosphoproteomic profile of ["turned On"/ Proliferating] versus ["turned Off"/ Arrested] PC lesions. The analyses identified dynamic downregulation of 122 phosphosites in proliferating tumors. Further interrogation of biological relevance of top 5 downregulated phosphorylation sites unleashed anti-proliferative function of Phospho-Ser-65 (pS65) on PRKAG2 which is a non-catalytic regulatory gamma subunit of metabolic regulator-AMP-activated protein kinase (AMPK). Biochemical characterization uncovered mystery of CDK5-GSK3β-AMPK crosstalk in regulation of human PC cellular (hPheo1) proliferation. We show that our hit compound inhibits CDK5 with simultaneous elevation of novel phosphorylation on pS65-PRKAG2 embarking catalytic activation of AMPK kinase. Moreover, in vitro kinase data suggests that CDK5 influences phosphorylation on AMPK enzymatic components via regulation

of pS9/21-GSK3β activatory dephosphorylation. Once activated, AMPK invokes anti-proliferative response by promoting excessive mitochondrial fission in PC cells.
Conclusion: This study illuminates comprehensive view of phosphorylation events downstream of complex CDK5 signaling in human PC. Identification of novel CDK5/p25-GSK3-PRKAG2-AMPK signalling cascade may provide new insights into target-directed Neuroendocrine Cancer therapeutics.

Abstract Number 208
Podocalyxin Regulates Proliferation and Migration of the Pancreatic Cancer Cells
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Podocalyxin (PODXL), a glycosylated transmembrane protein, belongs to CD34 family. PODXL has been implicated in more than 10 human malignancies, yet its roles in the cancer cells are poorly understood. In the present study, we aimed to investigate the role of PODXL in the SUIT-2 cells, an aggressive pancreatic cancer cell line. Our RNA-seq data revealed that PODXL is highly expressed in the pancreatic cancer cell lines. We used doxycycline (DOX)-inducible shRNA vector to downregulate the expression of PODXL in SUIT-2 cells. The downregulation of PODXL expression decreased the proliferation of SUIT-2 cells. Moreover, wound healing assay indicated that PODXL downregulation reduced the migration of SUIT-2 cells. Furthermore, the downregulation of PODXL expression declined the SUIT-2 cell migration through the trans-well membrane. Taken together, PODXL may play an important role in the growth and metastasis of pancreatic cancer. A thorough investigation of the function of PODXL may facilitate to develop new strategies to treat pancreatic cancer.

Abstract Number 209
MMP-14 as a Noninvasive Marker for PET and NIRF Imaging of Glioblastoma Multiforme
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Background: Glioblastoma multiforme (GBM) is a rapidly proliferating and invasive cancer originating from the glial cells of the brain. GBMs are diffuse, with indistinct tumor margins leading to incomplete surgical resection and subsequent recurrence. Matrix metalloproteinase 14 (MMP-14) is an enzyme that degrades the extracellular matrix. MMP-14 is highly expressed in GBM, and is involved in the invasion of the cancerous cells into the surrounding tissue. The primary treatment for GBM is surgery, with an increasing percentage of the tumor removed correlating to improved survival of the patient. However, current imaging of GBM pre-surgery is difficult to translate during surgery due to shift in the tissue. Combinatorial imaging of MMP-14 pre-surgery with PET and intra-operatively with near infrared fluorescence (NIRF) would allow for improved surgical resection of the tumor. We have developed peptides to bind MMP-14, which are suitable for both PET and NIRF imaging.
Methods: Several peptide constructs were developed and used for imaging in mice bearing intracranial implants of patient derived xenograft GBMs. Construct 1 exhibited binding affinity to MMP-14, construct 2 was a substrate for MMP-14, and construct 3 was a combination of 1 and 2. Constructs 1 and 3 were labeled with 68Ga or 64Cu and used for PET imaging. Constructs 2 and 3 were fluorescent and were used for NIRF imaging.
Results: Immunohistochemistry showed the presence of MMP-14 in the tumor areas, which was co-localized with fluorescence signal from probes 2 and 3. Radiolabeled probes 1 and 3 showed

accumulation in the tumor, which could be significantly reduced with the addition of non-labeled blocking peptides (p0.01 and p0.05 respectively). Additionally, in vivo PET and ex vivo NIRF was well correlated as shown using construct 3 (R2 = 0.80).
Conclusions: All 3 constructs showed accumulation in the tumor area. Results warrant further investigation of probes in additional preclinical GBM models. Development of probes to image MMP-14 could improve noninvasive detection of the tumor area before surgery, guided tumor resection, and surveillance for recurrence.

Abstract Number 210
Repurposing Clofazimine as a Novel Drug Against Cancer Stemness in PI-resistant Myeloma
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Multiple myeloma/MM, the second-most common hematopoietic malignancy in USA, remains a difficult-to-cure disease due to differential drug response/resistance. Cancer stem cell-like sub-populations/MM-CSCs, including CD19-CD138- quiescent stem cells, dormant cells, ALDH+ cells and side populations/SP may significantly contribute towards resistance to proteasome inhibitors/PIs, the standard-of-care drug for MM. However, no study so far has attempted to develop drugs specifically targeting drug-resistant MM-CSCs. Further, the gene signature underlying these sub-cellular populations are yet to be discovered. We have previously demonstrated that Clofazimine/CFZ, an anti-leprosy drug, could be successfully repurposed for the treatment of Chronic myeloid leukemia/CML by specifically targeting quiescent stem-cell population (CD34+CD38-, CFSE-bright) in drug-resistant patients. Here, we hypothesized that CFZ can be used to treat PI-resistant MM by targeting the sub-cellular MM-CSCs. A panel of >60 human myeloma cell lines/HMCLs and clonally-derived PI-resistant HMCLs (PI-R: generated using dose escalation over a period of time) representing innate and acquired PI-response/resistance, respectively, were treated with CFZ as single agent or in combination with PIs (CFZ+PI) and in vitro cytotoxicity was determined using CellTiter-Glo assay. Synergy between CFZ and PIs was analyzed by Calcusyn software based on Chou-Talalay's combination index (CI) theorem. Tumorigenic potential of purified MM-CSCs was determined using colony forming assay, carboxyfluorescein succinimidyl ester (CFSE) assay and analysis of side population/SP. We found that CFZ alone showed potent inhibition of cell viability, while CFZ+PIs significantly improved the therapeutic index of PI administration to HMCLs, including PI-R lines. Next, using flow cytometry, we found that CFZ alone or CFZ+PI significantly reduced the number of quiescent CD138+ cells in parental/P vs PI-R lines and increased CFSE-dim (dividing cell) population. Evaluation of apoptosis in these cells revealed that CFZ alone caused apoptosis in both CFSE-bright and CFSE-dim cells while combining CFZ+PI caused a more robust effect amounting to their near obliteration. Further, %SP was found higher in PI-R cells as compared to P lines. CFZ alone or CFZ+PI significantly reduced the %SP in PI-R cell lines. Based on these interesting preliminary results, we propose that CFZ may have strong potential to increase the therapeutic efficacy of PIs when used in combination by specifically targeting cancer stemness (MM-CSCs sub-cellular population) in MM-which we are investigating further. Currently, we are using bulk mRNA sequencing and single-cell transcriptomic analysis of MM-CSCs to evaluate top genes and molecular pathways involved in stem-cell based PI-resistance and to characterize PI-resistant sub-cellular stem cell populations based on gene expression signatures.

Abstract Number 211
14-3-3 Protects AMPK Phosphorylated TET2 from Dephosphorylation by PP2A
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Ten-eleven translocation-2 (TET2) is a member of the methylcytosine dioxygenase family of enzymes implicated in cancer and in aging due to its role as a global epigenetic modifier. TET2 has a large N-terminal domain followed by a catalytic C-terminal. Previous reports have demonstrated that the catalytic domain remains active independent of the N-terminal domain. As such, the function of the N-terminus of this large protein remains poorly characterized. Here, we identify that several isoforms of the 14-3-3 family of proteins binds TET2. 14-3-3s bind TET2 when phosphorylated at serine 99 (S99). AMPK-mediated phosphorylation at S99 promotes TET2 stability and increases global DNA 5-hydroxymethylcytosine. 14-3-3s interaction with TET2 serves to protect S99 phosphorylation. Disruption of this interaction leads to both reduced TET2 phosphorylation and decreased protein stability. Furthermore, we identify that the protein phosphatase 2A (PP2A) can interact with TET2 and dephosphorylates S99. Collectively, our study provides novel insights into the role of the N-terminal domain in TET2 regulation. Moreover, they demonstrate the dynamic nature of TET2 protein regulation that could have therapeutic implications for disease states resulting from reduced TET2 levels and/or activity.

Abstract Number 212
Prediction and Validation of Novel Secondary Drugs against Drug-resistant Prostate Cancer
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Response to anti-cancer drugs is heterogeneous. All patients do not respond equally well to treatment (innate resistance) and those who do often acquire resistance over the course of treatment (emerging resistance). Therefore, identifying and validating secondary drug regimens to circumvent drug resistance is essential for more effective alternative/combination therapy strategies. We have used a novel greedy algorithm-based set-covering computational optimization method followed by a regularization technique (secDrug) on sex-hormone related human cancer cell line subtypes (including breast, cervical, endometrial, ovarian, prostate, testicular, vulval cancers) from the Genomics of Drug Sensitivity in Cancer (GDSC) database, the largest public database of drug sensitivity in a vast array of human cancer cell lines, to identify potential secondary drug combinations in sex-hormone related cancers resistant to standard-of-care drugs. In this study, our aim is to validate these in silico predictions using in vitro chemo-sensitivity assays using our panel of solid tumor cell lines representing patient diversity in treatment response/resistance. To evaluate the potency of predicted secondary drugs in circumventing drug resistance in Prostate Cancer (PCa) cell lines, we treated the PCa cancer cell lines with the top predicted secondary drugs, alone or in combination with the FDA-approved prostate cancer drugs, Docetaxel, Cabazitaxel and Enzalutamide. In vitro cytotoxicity assays (MTT and SRB) were then performed to calculate the half maximal inhibitory concentration (IC50) of single-agent treatment and to evaluate drug-drug synergy of combination treatments using Chou-Talalay's combination index (CI) method and the isobologram algorithm. Our results showed that the in silico predicted top secondary drugs, YM155 and FK866, exhibited potency in a panel of PCa cell lines (n=12), alone and in combination with CI values exhibiting high levels of

synergistic effect. Currently, we are performing single-cell and bulk gene expression profiling (GEP) analysis using next generation sequencing methods to identify differentially expressed (DE) genes and pathways associated with successful drug combinations. Combining in silico and in vitro approaches, we have thus identified novel potent secondary drugs in drug-resistant prostate cancers. Ultimately, we aim to create a universally applicable software application for predicting and functionally validating unique secondary therapies in drug-resistant cancers for any cancer type and any test drug.

Abstract Number 213

Multi-omics Analysis of Conventional vs. Metronomic Dosing in Prostate Cancer Risk Groups

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Repetitive, low-dose drug administration (metronomic; METRO) has the potential to overcome drug resistance and increase efficacy in many cancers; however, the mechanisms mediating the increase in efficacy are not understood fully. We previously showed that METRO dosing of Topotecan/TOPO was more potent than conventional (CONV) dosing in prostate cancer cell lines and xenograft mouse models.

We performed targeted mRNA and microRNA expression studies to explore possible mechanisms by which METRO dosing alters tumor growth, and identified potential candidate cancer pathway genes, miRNAs and miRNA-mRNA pairs as potential treatment-related biomarkers for TOPO-METRO therapy. To determine drug response, androgen-independent (PC3, PC3-M, DU-145) and androgen-dependent (LNCaP, 22Rv1) human prostate cancer cell lines were treated with TOPO following CONV and METRO dosing schedules. Cancer pathway genes and miRNA expression profiles were assessed at the calculated IC50 of TOPO. Expression signatures were identified using differential expression analysis and a Spearman's rank-based correlation was used to assess associations between drug response, mRNA and miRNA expression. Gene signatures associated with TOPO-METRO therapy were queried against patient profiles in The Cancer Genome Atlas/TCGA database. Protein expression of most significant genes was confirmed by immunoblotting.

We identified disparate treatment-related mRNA and miRNA expression signatures for TOPO-METRO vs CONV treatment. We also identified a gene signature (top five genes: SERPINB5, CDKN1A, TNF, FOS, ANGPT1) for both PC-3 and LNCaP following TOPO-METRO dosing. Ingenuity Pathway Analysis identified that upregulation of tumor suppressor, anti-proliferation (eg, SERPINB5), activation of the immune system (RPL13A) and down-regulation of genes involved in apoptosis, invasion, metastasis, and inflammation (TNF, FOS, and MMP1) are associated with the observed treatment response. MicroRNA expression changes may influenced differential gene expression (miRNA-mRNA pairs) for TOPO-METRO dosing. Twenty (20) miRNAs genes (including miR-30c, miR-19a, mir-20a, mir-17, let7i, let7b) were associated with TOPO cytotoxicity ($p<0.05$); seven of these bind to 28 mRNAs ($p<0.05$) as mRNA-miRNA pairs (TargetScan); three of these are conserved. Furthermore, expression of our top genes (SERPINB5, CDKN2A, MMP9) correlated with patient survival. Interestingly, MMP1, B2M, CXCL8, PDGFA, ERBB2, ITGA1, ITGA3 and JUN was associated with survival in African American patients ($p<0.05$). Immunoblotting results (SERPINEB5, FOS, MMP1, MMP9, ANGPT2, VEGF, HIF-α) corroborated our mRNA expression results.

Overall, these studies begin to address a fundamental gap in knowledge related to the effects of METRO dosing on key miRNAs, genomic and transcriptomic factors and overall treatment efficacy. Using a multi-omics approach, we determined gene signatures may be used to individualize patient therapy.

Abstract Number 214

EGFR Activation Attenuates Mechanical Threshold for Integrin Tension & Adhesion Formation

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Mechanical forces, growth factors, and the extracellular matrix, all play critical roles in cell adhesion. To understand how epidermal growth factor receptor (EGFR) impacts the mechanics of adhesion, we used tension gauge tether (TGT) probes displaying integrin ligand cRGDfK and quantified integrin tension during cell adhesion. EGF exposure increased spread area, cell circularity, integrated integrin tension, tension occupancy, radial organization and size of focal adhesions (FAs) significantly in Cos-7 cells on TGT surfaces. These findings suggest EGFR acts as a mechano-organizer, regulating integrin tension and FA spatial organization. Additionally, the mechanical force threshold for outside-in integrin activation is tunable by EGFR. Parallel genetic and pharmacologic strategies demonstrated that these phenotypes are driven by ligand-dependent EGFR signaling. Our results establish a novel mechanism where EGFR allosterically regulates integrin activation and cell adhesion, providing control over cellular responses to the environment.

Abstract Number 215

Investigating the Synthetic Lethality of EZH2 Inhibition in ARID1A Mutant Bladder Cancer

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Next-gen sequencing of bladder cancer (BCa) has revolutionized our understanding of the disease and promises to move the field towards better risk stratification, therapeutic target identification, and more personalized therapies for patients. Specifically, genes involved in epigenetic modifications such as ARID1A have been shown to be frequently mutated in BCa. Previously, we and others have shown that the histone methyltransferase EZH2, which is a transcriptional repressor, is over-expressed and required for cancer growth, including BCa. EZH2 functions as the catalytic subunit of the polycomb repressive complex 2 (PRC2) which methylates lysine 27 on histone 3 (H3K27me), resulting in transcriptional silencing. Herein, we show that ARID1A mutations sensitize BCa cells in vitro and in vivo to EZH2 inhibition with the small molecule GSK-126.

Results: In silico analysis using the TCGA dataset revealed that ARID1Amut BCa shows worse disease-free survival compared to ARID1Awt tumors (p-value: 0.007). Western blot using lysates from matched bladder tumors and normal urothelium from cystectomy samples revealed that EZH2 and resultant H3K27me3 levels are dramatically increased in bladder tumors, with a concomitant decrease in ARID1A protein levels. Using 3 ARID1Awt BCa cell lines (T-24, 5637, RT-112) and 3 ARID1Amut cell lines (HT-1197, HT-1376, VM-CUB1) we showed that the

proliferation of only ARID1Amut cell lines is inhibited by EZH2 inhibitor GSK-126. ARID1A knockdown in 5637 cells resulted in de novo GSK-126 sensitivity in proliferation assays. These findings were also recapitulated in vivo using murine xenograft models with intraperitoneal GSK-126 treatment. To determine the molecular mechanisms behind the synthetic lethality of EZH2 inhibition in ARID1mut cell lines, we performed transcriptomic analysis comparing ARID1Awt and mut cell lines with and without GSK-126. Several genes were differentially expressed that could explain the differential sensitivity to GSK-126 inhibition: MTSS1 (or “missing in metastasis”), optineurin (OPTN), an autophagy regulator, and the tumor suppressor Protein Tyrosine Phosphatase, Receptor Type, R (PTPRR). The GSK-126-mediated induction of these proteins only in ARID1Amut cell lines was confirmed by western blot. ChIP analysis using antibodies to EZH2 and H3K27me3 in combination with PCR amplicons in the promoters of MTSS1, OPTN, and PTPRR showed that GSK-126 inhibition abrogated EZH2 binding to and methylation of those foci only in ARID1Amut cell lines. In conclusion, ARID1A mutation is a biomarker for EZH2 inhibitor sensitivity in BCa cells and may represent a new epigenetic therapeutic target for patients.

Abstract Number 216

Integrative "Omics" Analysis Identifies PAK4 as a Target in Muscle Invasive Bladder Cancer

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Muscle invasive bladder cancer (MIBC) is an aggressive form of bladder cancer. The biology and drivers of muscle-invasive urothelial bladder carcinoma (MIBC) are poorly understood. Kinases appear to be major molecular drivers of muscle-invasive bladder cancer (MIBC). Given that kinases are therapeutically actionable, we performed comprehensive multiplatform analysis of kinases in matched MIBC tumor and normal tissue to prioritize their relevance. We collected fresh frozen tumor and adjacent normal tissue from 24 MIBC patients. After macrodissection to demarcate tumor from normal tissue, the samples underwent analysis of kinases using platforms to assess DNA, RNA activity. Next Generation Sequencing (NGS) of 517 kinase genes was performed using the Agilent Kinome capture and run on the Illumina HiSeq at PE150bp. The Nanostring nCounter™ platform analyzed the expression of 519 kinase genes. We found amplification and/or over-expression of PAK4 (P21 (RAC1) Activated Kinase 4) in subset of MIBC patients. Multiplex kinase assay indicated enzymatic activity of PAK4 and in vitro experiments confirmed the role of PAK4 in bladder cancer cell proliferation and invasion. Transcriptome sequencing PAK4 inhibited or PAK4 knock down bladder cancer cells revealed increased expression of another kinase PTK6 (Protein Tyrosine Kinase 6), thereby suggesting combinatorial treatment of PAK4 and PTK6 inhibitors could form a better treatment option. In summary, our study shows that PAK4 can be therapeutic target in subset of MIBC patients.

Abstract Number 217

ERO1L, a Therapeutic Target, is a Predictor of Poor Survival in Lung Adenocarcinoma

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Lung Adenocarcinoma (LUAD) is one of the most common cancers across the world and a leading cause of cancer related death in both men and women. To improve patient outcomes it is essential to find genes to target for treatment. This is especially important for aggressive cancer like Lung Adenocarcinoma. Using pan-cancer analysis of The Cancer Genome Atlas (TCGA) data using the "UALCAN" portal, we identified that endoplasmic reticulum oxidoreductase 1 alpha (ERO1L) is upregulated in Lung adenocarcinoma. ERO1L (or ERO1A) is a gene that codes for Endoplasmic Reticulum Oxidoreductase 1 Alpha (ERO1A), an enzyme that catalyzes disulfide bond formation of proteins in the endoplasmic reticulum (ER) and involved in proper protein folding and stability. ERO1L overexpressed in lung adenocarcinoma. ERO1L gene expression is increased in lung adenocarcinoma tissues compared to normal lung. Furthermore, immunohistochemical analysis using ERO1L antibody indicated overexpression of the protein as well in lung adenocarcinoma. Critically, analysis found that high expression of ERO1L RNA predicts poor patient survival suggesting that it can be a potential biomarker for aggressiveness and could be an oncogene target to treat lung adenocarcinoma. siRNA knockdown experiments suggests a critical role of ERO1L in cancer progression. Future studies will focus on investigating the ERO1L expression in a large cohort of lung adenocarcinoma and understanding its role in lung cancer progression.

Abstract Number 218

Antitumor Activity of Vitamin D3 (Calcitriol) in Melanoma Cells

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In the present study, we have evaluated the influence of 1,25(OH)D3 (the most active form of vitamin D3- calcitriol) in concentration of 10-7 M on tumorogenesis gene expression pattern in human melanoma cells (WM164 cell line) after 24 hour incubation in in vitro conditions. Performing Gene Set Enrichment Analysis of the results from RNA sequencing we have found several enriched pathways connected with tumorogenesis (Reactome FI Viz). One of the most important is signaling by NOTCH1 PEST Domain Mutants in Cancer. In this pathway, the NOTCH1 Coactivator Complex:HES1 gene is overexpressed. It has been established that activation of NOTCH1 signaling determine proliferation of melanoma cell line. NOTCH target-gene HES1 can cause inhibition of NOTCH1 signaling and induce growth arrest of melanoma cells. Comparing 1,25(OH)D3 treated melanoma cells with EtOH treated cells by DISeq2 differential expression of RNAseq. data we have found also upregulation of HES2 gene (FPKM fold change= 2.2 vs. control). The HES factors are known as repressors of gene transcription at two different mechanisms: active and passive repression.

Abstract Number 219

Enhancement of Oncolytic Virotherapy for Diffuse Intrinsic Pontine Glioma

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Diffuse intrinsic pontine glioma (DIPG) is the leading cause of brain tumor-related death in children and is uniformly fatal on average 8-14 months from diagnosis. Our overarching goal is to develop an innovative immunovirotherapy by combining a novel vaccination platform with our next-generation, engineered oncolytic HSV (oHSV) M002 that produces interleukin-12 (IL-12) to maximize an anti-tumor immune response against DIPG. We hypothesize that vaccination against DIPG tumor antigens using vaccines comprising peptide-TLR-7/8a conjugates will synergize with M002 to create and sustain a robust anti-tumor immune response against DIPG. To determine the efficacy of M002 in vitro, cytotoxicity assays were conducted against two murine DIPG cell lines. Sensitivity of DIPG tumors growing orthotopically in C57BL/6 mice were tested by intratumoral injection with either M002 or saline. To determine DIPG antigens to vaccinate against, expression of several proteins was assessed by flow cytometry and Western blot. To assess the vaccine against DIPG, mice injected orthotopically with syngeneic transgenic DIPG tumor cells will be administered the vaccination or scrambled control by intravenous injection. To determine the combined effects of the vaccination and M002, mice bearing DIPG will be vaccinated prior to M002 administration and survival will be compared to either therapy alone or saline. The anti-tumor immune response will be established via peptide-specific assays and flow cytometry analyses. Both murine DIPG cell lines were sensitive to M002 in vitro with a lethal dose required to kill 50% of the cells (LD50) of 0.2-0.4 plaque-forming units/mL. M002 significantly prolonged survival in mice bearing DIPG; however, surviving mice rechallenged with tumor ultimately succumbed to disease. Western blot and flow cytometry analyses confirmed expression of survivin. Experiments to determine the safety and immunogenicity of the survivin vaccine are ongoing. Thus far, we have identified survivin as a target antigen for the innovative peptide vaccination. Murine DIPG cell lines are sensitive to oncolysis by M002 in vitro and in vivo. Mice rechallenged with tumor after initial treatment with M002 did not survive, indicating anti-tumor immunity was not durable. Thus, our next step is to test whether the combination of the vaccination and M002 produces a durable response. This will be the first time this innovative vaccination approach alone and combined with a novel cytokine-expressing oHSV has been developed to target DIPG, and if successful, the approach can be applied to treat other brain and solid tumors in both children and adults.

Abstract Number 220

Elevated Adiposity Reduces Response to a Combinatorial CTLA-4 Therapy in Diet-matched Mice

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Background: Associations between modifiable factors (e.g., adiposity) and immunotherapeutic efficacy remain uncertain. Preclinical studies suggest increased adiposity improves response rates to immune checkpoint blockade; however, few studies control for diet effects between chow-fed lean and high-fat diet-induced obese (DIO) mice. We found previously that DIO reduces the efficacy of an immunotherapy consisting of replication-deficient adenovirus (Ad) encoding tumor-necrosis factor-related apoptosis-inducing ligand (TRAIL) (AdT) plus class B oligonucleotides (CpG1826) in renal tumor-bearing mice. To eliminate the compounding effects of diet and explore

if therapy response can be improved in DIO mice, the current study evaluated response rates to AdT/CpG combined with anti-cytotoxic T-lymphocyte-associated protein-4 (CTLA-4) in diet-matched obese-resistant (OBR) versus DIO mice.
Methods: BALB/c mice were administered a high-fat diet for 20-weeks. Following diet administration, mice were characterized as either OBR or DIO. DIO mice possess hallmarks of obesity, including increases in systemic glucose, insulin, and leptin; whereas, OBR mice display phenotypic characteristics similar to chow-fed, lean controls. Mice were injected orthotopically with Renca-Luc renal cancer cells into the left kidney. At day seven post-tumor challenge, mice were randomized to receive saline or AdT/CpG injected intra-tumorally, followed by isotype control (n=7-9/group) or anti-CTLA-4 therapy (n=12/group). At day 21 post-tumor challenge, immunogenetic profiling of whole-tumors was performed via nanoString and tumor-infiltrating lymphocytes were characterized by flow cytometry.
Results: Both OBR and DIO therapy-treated mice had significant reductions in tumor weight compared to therapy-free controls (66% reduction for OBR; p=0.001 versus 55% reduction for DIO; p=0.007). However, therapy was effective in 75% of OBR mice compared to 50% of DIO mice. Over 30 differentially expressed genes were shared between OBR and DIO therapy-treated responders compared to therapy-free controls. A third of these “responder” genes were related to T cell function or migration (e.g., Cd8a, CD274, Icos, Icosl, Zap70, Cxcr3, Xcr1, Il16). Cellular analyses demonstrated that OBR therapy-treated responder mice had a greater percentage of tumor-infiltrating activated (CD44+) CD8+ T cells compared to DIO therapy-treated responder mice (p=0.004); however, CD8+ T cell IFNγ production and PD-1 expression were comparable between these groups.
Conclusions: When controlling for diet, increased adiposity reduced the response rate to combinatorial anti-CTLA-4 based therapy. However, both OBR and DIO therapy-treated responder mice shared immunogenetic and T cell profiles independent of weight status. To improve treatment strategies, future studies are needed to understand the mechanistic drivers of the differential response rates to immunotherapy observed with obesity.

Abstract Number 221
Proteomic Analysis of MYB-regulated Secretome Identifies Functional Pathways and Biomarkers: Potential Pathobiological and Clinical Applications
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Our earlier studies establish the oncogenic property of MYB in pancreatic tumor pathobiology. To better understand the role of MYB in the tumor microenvironment and identify MYB-associated secreted biomarker proteins, we conducted mass spectrometry analysis of the secretome from MYB-modulated and control pancreatic cancer cell lines. We also performed in silico analyses to determine MYB-associated biofunctions, gene networks and altered biological pathways. Our data demonstrated significant modulation (p=0.05) of 337 secreted proteins in MYB-silenced MiaPaCa cells whereas 282 proteins were differentially present in MYB-overexpressing BxPC3 cells, compared to their respective control cells. Alteration of several phenotypes such as cellular movement, cell death and survival, inflammatory response, protein synthesis etc. was associated with MYB-induced differentially expressed proteins (DEPs) in secretomes. DEPs from MYB-silenced MiaPaCa PC cells were suggestive of the downregulation of genes primarily associated with glucose metabolism, PI3K/AKT signaling and oxidative stress response, among others.

DEPs from MYB-overexpressing BxPC3 cells suggested enhanced release of proteins associated with glucose metabolism and cellular motility. We also observed that MYB positively regulated the expression of four proteins with potential biomarker properties, i.e. FLNB, ENO1, ITGB1 and INHBA. Mining of publicly available databases using Oncomine and UALCAN demonstrated that these genes are overexpressed in pancreatic tumors and associated with reduced patient's survival. Altogether, these data provide novel avenues for future investigations on diverse biological functions of MYB, specifically in the tumor microenvironment, and could also be exploited for biomarker development.

GRADUATE **STUDENTS**

Abstract Number 300
Understanding the Role of STAT4 in CD4 T cell Mediated Neuroinflammation
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Department of Cell, Developmental and Integrative Biology, UAB

Multiple Sclerosis (MS) is an autoimmune disease that affects over two million people worldwide. MS is characterized by the demyelination of axons in the central nervous system (CNS), leading to vision problems, muscle weakness and poor coordination. Among the various immune cells that contribute to the disease, a subset of CD4 T cells, Th17 cells has been associated with MS pathogenesis. Recently, there has been a distinction between homeostatic Th17 cells, which do not directly contribute to disease and pathogenic Th17 cells that directly influence inflammation. Importantly, IL-23 signaling in Th17 cells is essential for the pathogenicity. The transcription factor STAT4 has been historically correlated with Th1 cells and is downstream of IL-23 signaling. As STAT4 deficiency does not prevent Th17 differentiation, but still limits inflammation, we hypothesize that STAT4 is necessary for the pathogenic properties of Th17 cells in MS. To study the influence of STAT4 in the context of neuroinflammation, our laboratory uses the adoptive transfer model of experimental autoimmune encephalomyelitis EAE. Our laboratory also utilizes in vitro CD4 T cell differentiate to identify how STAT4 influences the expression of genes in CD4 T cells cultured in “homeostatic” Th17 culture conditions compared to “pathogenic” Th17 conditions. We find that while STAT4 expression does not impact the development of Th17 cells but is necessary to induce CNS inflammation. Furthermore, global gene expression analysis indicates that STAT4 regulates the recently described pathogenic and nonpathogenic Th17 gene signatures. In the absence of STAT4, the levels of pathogenic Th17 genes including Tbx21, Il22 and Cxcl3 are significantly reduced, while the expression of nonpathogenic genes including Il10 and Ahr is increased. Furthermore, we find a significant increase of genes that rely on both IL-23 and STAT4 when compared to genes that rely solely on STAT4 in Th17 differentiated CD4 T cells. This leads us to further hypothesize that the IL-23/STAT4 signaling axis is vital to neuroinflammation. Together, these data reveal a novel role of STAT4 in controlling Th17 pathogenicity, which may provide a promising therapeutic target for MS patients.

Abstract Number 301
Investigating the Role of Neuropeptide Y in the Vitiligo Disease
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Vitiligo is an autoimmune disease that is characterized by destruction of the pigment-producing cells called melanocytes, resulting in de-pigmentation of the skin and hair. This disease is known to be associated with mutations in a number of genes and is thought to be caused by a combination of intrinsic melanocyte stress, autoimmunity, and immune cell-mediated melanocyte destruction. There is no cure for vitiligo, and the current treatments that are available address different aspects of the disease in untargeted and often inefficient manners. Because vitiligo is such a complex disease composed of multi-genic, multi-cellular, and autoimmune factors, it is important to understand the molecules and signaling pathways that are involved in the disease’s pathogenesis, progression, and maintenance in order to design more efficient treatments for the disease. It has been shown that neuropeptide Y (NPY), a neuropeptide with implications in stress-resiliency, has known mutations that are associated with both increased NPY expression and increased susceptibility for vitiligo. NPY has also been shown to be upregulated in the circulation

of vitiligo patients when compared to control patients and is also upregulated in vitiliginous skin compared to unaffected skin of the same patient. In a mouse model with a targeted mutation that results in the overexpression of endogenous NPY, our preliminary data has shown that these mice have upregulated NPY expression in the skin, among other tissues, and exhibit a premature and progressive loss of pigmentation in the hair, resulting in a vitiligo-like hair-graying phenotype. The purpose of this project is to investigate how the upregulation of NPY, as is seen in the circulation and skin of vitiligo patients, plays a role in the vitiligo disease. Preliminary findings from RNA sequencing of skin from NPY-overexpressing mice indicate that overexpression of NPY changes the expression of genes involved in both keratinocyte and adipocyte biology, which implicates these two cell types as potential mediators of hair graying in these animals. Future studies for this project aim to identify whether and how these skin constituents are responsible for effecting NPY-mediated signaling and to determine the pathogenic effects of upregulated NPY on the regulation of follicular pigmentation.

Abstract Number 302

Identifying the LSC Niche Mediating Drug Resistance in Flt3-ITD Acute Myeloid Leukemia

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Flt3-ITD, the most common mutation in AML, defines a distinct disease subtype with unique features and biology and poor prognosis related to high recurrence rates. Although several FLT3 TKIs have been developed for clinical use, responses are limited and transient. The objective of our study was to determine the contribution of bone marrow stromal populations to LSC drug resistance to Flt3-targeted TKI in Flt3-ITD AML. We utilized a new Flt3-ITD mouse model of AML to identify phenotypic populations with leukemia initiating capacity (LIC). Transplantation of selected ST-HSC, MPP and GMP populations revealed that LIC were almost exclusively found within the phenotypic ST-HSC population. We similarly found that LIC capacity in human Flt3-ITD AML patient samples was restricted to primitive HSPC populations (Lin-CD34+CD38-) and was not seen in committed GMP (Lin-CD34+CD38+CD123+CD45RA+). We transplanted murine AML cells into CXCL12-GFP mice to assess alterations in CXCL12-expressing stromal populations in AML bone marrow. We found several stromal populations expanded in AML vs. WT mice, including mesenchymal stem cells and osteoprogenitors. Expression of CXCL12, a key factor mediating niche localization of HSC and LSC, was significantly increased in osteoprogenitors but 2-fold lower in mesenchymal stem cells in AML vs. WT mice. We also showed that Flt3-ITD AML HSPCs have significantly higher CXCR4 expression than WT HSPCs. These data taken together supported further exploration of the role of CXCL12-expressing osteoprogenitors in Flt3-ITD AML. We transplanted murine AML cells into global or osteoprogenitor-specific CXCL12-KO mice to assess the effect on AML progression and drug response. We found that this AML model was resistant to single-agent Flt3 TKI. Global CXCL12-KO modestly improved response to TKI. A combination regimen including standard-of-care chemotherapy (cytarabine + doxorubicin) and Flt3 TKI results in more effective targeting of leukemia cells in this model. We are currently treating osteoprogenitor-specific CXCL12-KO AML mice with the combination regimen to investigate the contribution of osteoprogenitors to disease progression and drug resistance in Flt3-ITD AML LSC. In conclusion, our results suggest that LSC in Flt3-ITD AML are present within a primitive phenotypic ST-HSC population, unique from some other types of AML. Our studies support a potential role for a CXCL12-expressing osteoprogenitor niche in supporting Flt3-ITD AML LSC growth and drug resistance, targeting of which could improve responses and outcomes in Flt3-ITD AML.

Abstract Number 303

The Glycosyltransferase ST6Gal-I Confers Resistance against NK Cell Mediated Cytotoxicity

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ST6Gal-I, a glycosyltransferase that adds Î±2-6-linked sialic acids to N-glycosylated proteins, is upregulated in multiple cancers and correlates with decreased patient survival. Previous studies by our group and others have demonstrated that sialylation of select cell surface receptors by ST6Gal-I modulates receptor function, leading to alterations in downstream signaling. Additionally, our lab has shown that high ST6Gal-I activity confers a cancer stem cell phenotype and promotes several of the hallmarks of cancer, including increased cell invasiveness, dysregulated energetics, resistance to apoptosis, and acquisition of metastatic potential. However, the role of ST6Gal-I in tumor escape from the immune system has received limited attention. In the present study, we investigated ST6Gal-I's contribution to immune evasion by modulating ST6Gal-I expression in ovarian or pancreatic cancer cells, and then co-culturing cells with the NK-92 natural killer (NK) cell line. We find that ST6Gal-I overexpression protects cancer cells against NK-mediated cell death, as measured by cell morphology, caspase 3/7 activity, and annexin/PI staining. Contrarily, ST6Gal-I knockdown sensitizes cancer cells to the action of NK cells. Furthermore, using SNA precipitation, we have identified that the two death receptors, Fas and TNFR1, are targets of ST6Gal-I mediated sialylation. By immunoblotting and examining caspase 3/7 activity, we find that sialylation of these two receptors leads to a decrease in apoptotic signaling. Additionally, we have shown that ST6Gal-I-mediated sialylation of the Fas and TNFR1 prevents receptor internalization as measured by flow cytometric analysis and immunofluorescent imaging. In turn, receptor sialylation promotes the survival of tumor cells exposed to FasL and TNF-α, both of which are secreted by NK cells in order to kill their targets. Based upon these data, we hypothesize that tumor cells with high levels of ST6Gal-I evade NK-mediated cytotoxicity through the sialylation of Fas and TNFR1 and the activation of pro-survival pathways.

Abstract Number 304

Catheter Placement Analysis of Drug Delivery to Brain Tumors

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In 2019, the National Brain Tumor Society expects physicians to diagnose approximately 86,000 new brain tumor cases, adding to almost 700,000 existing cases [1]. To deliver drugs to treat these tumors, some clinicians perform injections using catheters. However, the effectiveness of this approach may be a function of primarily catheter placement and time. In this study, investigators considered diffusion, momentum, porosity, and permeability to conduct simulations in the brain to compare drug volume distribution in the tumor and peri-tumoral cells based on catheter placement and time. From T1-weighted and diffusion-weighted images the investigators derived the brain and tumor geometry and the corresponding diffusion tensors to simulate diffusion using various catheter placements. The volume distribution of the drug in the tumor and peri-tumoral cells varied based on the catheter placement. These distributions suggest that the catheter placement influences the potential therapeutic value of the drug. [1] "Quick Brain Tumor

Facts." National Brain Tumor Society, National Brain Tumor Society, 2019, braintumor.org/brain-tumor-information/brain-tumor-facts/.

Abstract Number 305

Endocan Bridging the Gap: Tumor Endothelial Cell Secretome and the Tumor Edge

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Background: Glioblastoma (GBM) is a highly invasive, angiogenic and therapeutically resistant primary brain tumor. It is impossible to completely resect the tumor, which subsequently leaves behind tumor "edge" that can act as seeds for recurrence. We recently reported the leading "edge" and tumor core have the presence of two distinct types of glioma stem-like cells (GSCs) that resemble proneural (PN) and mesenchymal (MES) subtypes, respectively. GSCs are enriched in perivascular niches, and vascular endothelial (VE) cells are known to provide paracrine factors, radioresistance properties to GSCs. Therapeutically, targeting GBM using an anti-angiogenic anti-VEGF inhibitor, Bevacizumab, did not aid in overall survival and instead resulted in aggressive MES phenotype. Hence, it is imperative to study this nexus in greater detail to develop novel targeted therapies for VE and GBM cells. The purpose of this study was to understand the molecular mechanisms emanating from VE cells that cause the invading "edge" to recur and determine if targeting these molecular cascades can provide therapeutic benefit.
Results: Using an unbiased approach to identify paracrine factors from GBM patient-derived VE cells, we identified endocan, a vascular endothelial cell-secreted molecule, as a pro-tumorigenic factor essential for neovascularization, radioresistance, and stemness of GBM edge tumor cells. Utilizing patient-derived and mouse glioblastoma sphere models and the corresponding intracranial tumor models, we demonstrate that endocan maintains the "edge"-located glioma stem cell signature and protects glioblastoma cells from radiation-induced differentiation and apoptosis. Mechanistically, we show that endocan is a novel ligand that interacts with PDGFRa and induces its phosphorylation, leading to phosphorylation of the downstream targets PI3K and MAPK in GBM.
Conclusions: Collectively, these findings demonstrate a novel signaling axis of endocan-PDGFRa in GBM that is activated by tumor endothelial cell and plays an essential role in glioma stem cells maintenance and radioresistance in tumor "edge."

Abstract Number 306

Maintenance Therapy for Platinum-sensitive (PS) Recurrent Ovarian Cancer (rOC)

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Background: Treatment for platinum-sensitive (PS) recurrent ovarian cancer (rOC) patients generally consists of retreatment with platinum-based chemotherapy. Maintenance therapy with a targeted agent can extend the interval before progression. Patients with a somatic or germline BRCA mutation (BRCAm) have a greater clinical benefit with maintenance PARPi compared to BRCA wildtype (BRCAwt). Data has shown that ~50% of patients eligible for maintenance therapy do not receive it, and variation in type of maintenance therapy prescribed (BEV vs. PARPi) exists both nationally and within institutions. The objective of this study was to analyze the utilization of maintenance therapy in PS rOC.
Methods: This retrospective cohort study evaluated PS rOC patients seen at the University of Alabama at Birmingham who completed 2nd line or greater platinum-based chemotherapy for 3-

8 cycles since March 27, 2017 (FDA approval date for PARPi maintenance). Patient medical records were reviewed from 9/2018 - 2/2019 to capture a recent representative sample. Percent of patients and what type of maintenance therapy they received after platinum-based treatment was assessed. The difference in BRCA status between these groups was evaluated. Progression free survival (PFS) was compared between those who received maintenance therapy and those that did not. PFS was defined as the date of initiation of platinum-based chemotherapy until progression of disease or death regardless of whether or not they were on maintenance therapy.
Results: There were 61 PS rOC patients seen during this time period that were eligible for maintenance therapy. 72% received maintenance therapy: 86% (n=38) received PARPi and 14% (n=6) received BEV. PFS following platinum-based chemotherapy with at least one dose of maintenance therapy was 13.4m (6.5-23.6) vs. 11.4m (7.5-27.0) with no maintenance therapy. Germline and somatic BRCA status were known in ~75% of the cases. 60% (9/15) of the patients with a BRCAm (somatic or germline) received maintenance therapy; all patients received a PARPi. PFS in these patients was 17.5m (15.6-19.0) vs. 10.7m (8.0-27.0) with no maintenance therapy.
Conclusions: Consistent with randomized control trials, maintenance therapy prolonged PFS in PS rOC patients which was more pronounced in BRCAm patients who received a PARPi; although, not statistically significant in our small cohort of patients. At our institution, >50% of patients that were eligible for maintenance therapy received it, which is higher than data obtained from national databases. Only 60% of the patients with known BRCAm received maintenance PARPi, which warrants further investigation to the potential barriers that exist.

Abstract Number 307
ST6Gal-I Potentially Dampens Immune Response during PDAC Progression
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Sialyltransferase ST6Gal-I adds α±2-6 sialic acids to select N-glycosylated cell surface receptors leading to alterations in receptor function and downstream intracellular signaling. Prior studies from our group have shown that: (1) ST6Gal-I is upregulated in multiple tumor types including Pancreatic Ductal Adenocarcinoma (PDAC); (2) oncogenic Ras signaling leads to upregulated ST6Gal-I; and (3) ST6Gal-I promotes a cancer stem cell phenotype. We generated a genetically engineered mouse model (GEMM) of PDAC with either pancreas specific knock-in of oncogenic K-Ras alone (KC mouse) or K-ras in combination with ST6Gal-I (KSC mouse). Single cell RNA sequencing studies revealed that genes involved in B cell response are repressed, whereas genes involved in B cell anergy are upregulated, in KSC mice in comparison to KC mice. KSC mice also exhibited dramatically accelerated PDAC progression and mortality compared to KC mice. This led us to hypothesize the role of ST6Gal-I activity in dampened immune response and accelerated PDAC progression. To test this hypothesis, we used another GEMM generated in the lab, wherein ST6Gal-I alone was knocked into the pancreas of the mouse (SC mouse). The sialic acids added by ST6Gal-I serve as ligands for the Sialic acid binding Immunoglobulin-like lectins (Siglec). Siglecs are lectin receptors present on immune cells and are involved in dampening an immune response due to the presence of ITIM domains. In order to determine which Siglecs bind to sialic acids added by ST6Gal-I, we used human and murine pancreatic cancer cell lines with either overexpressed or knocked down ST6Gal-I. Our flow cytometry data revealed that unlike Siglec 3, 7 and 9, Siglec 2 binds specifically to α±2-6 linked sialic acid. We validated this data using pancreatic cells isolated from SC mice which corroborated our cell line results. Given that Siglec 2 is selectively expressed on B cells, we evaluated the effect of sialic acids added by ST6Gal-I on the B cell response. Murine WEHI-231 B cells were co-cultured with murine pancreatic acinar cancer cell line, 266-6, with ST6Gal-I overexpression or knock-down. From

preliminary studies, we demonstrate enhanced phosphorylation of the B cell receptor and activation of the key downstream mediator, Syk, in the presence of hypersialylated 266-6 cells, suggesting that ST6Gal-I may regulate B cell response through Siglec 2. Collectively, these results reveal a potential mechanism for suppressing B cell response in an ST6Gal-I mediated hypersialylated tumor microenvironment.

Abstract Number 308
Oncometabolite L-2 Hydroxyglutarate Creates Serine Metabolic Liability in ccRCC
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Introduction: Renal cell carcinoma (RCC) is among the ten most common neoplasias in the US and is known to undergo extensive metabolic reprogramming. Previous work by our lab has identified high levels of the oncometabolite L-2 Hydroxyglutarate (L2HG) in RCC. It is currently unknown if we can utilize metabolic liabilities created by oncometabolites for personalized RCC therapy.
Objectives: The primary objective of this study is to understand the mechanisms of L-2HG induced Serine Dependence and how we can utilize this information for both therapy and imaging.
Methods: This project analyzed normal renal cell line HK2 and renal cancer cell lines (RXF-393, OS-RC-2, A498, 786O, 769P, Caki1, Sn12Pm6, and A704) using lentiviral transgene or knockdown expression. Proliferation assays were counted over 4 day periods and inhibitor experiments were done at 10mM. Data was analyzed via real-time PCR and western blot. Patient samples were obtained through proper procedures at UAB.
Results: Lysine Demethylase 4C (KDM4C) and Activating Transcription Factor 4 (ATF4) are known to play roles in the amino acid starvation response. Here we identify that L-2HG inhibits both KDM4's ability to demethylate H3K9me3 at Phosphoglycerate Dehydrogenase (PHGDH) and inhibit translation of ATF4, transcription factor for PHGDH. Loss of both lead to decrease transcription of PHGDH. PHGDH, the first and rate-limiting step in the serine synthesis pathway, is commonly reduced in both RCC patient samples and several RCC cell lines. Serine and glycine starvation significantly decreased proliferation in RCC cell lines with reduced PHGDH but not in RCC cell lines with higher basal PHGDH. Additionally, RCC xenograph tumors show decreased proliferation when mice are given chow without Serine and Glycine. Serine is significantly taken up by SNAT transporters, and thus MEAIB can be used to inhibit serine uptake and MeFAMP can be used to image RCC tumors. Finally, these findings are able to be reversed when the enzyme L2HGDH is restored in these cells, lowering L-2HG levels.
Conclusion: L2HG controls de novo serine synthesis in RCC cell lines via KDM4C and ATF4. Targeting serine and glycine looks to be a promising direction for novel, personalized RCC treatments.

Abstract Number 309
Immune Modulation in Ovarian Cancer by FGF18/NF-kB/TAM Axis
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Epithelial ovarian cancer, the most lethal of all gynecological malignancies, is characterized by a unique TME that enables specific metastatic routes, impairs immune surveillance, and mediates therapy resistance. Tumor-associated macrophages (TAMs) represent the major subpopulation

of myeloid cells in ovarian cancer, and high TAM infiltration has been linked to metastasis, immunosuppression, angiogenesis, and poor prognosis. We identified FGF18 overexpression as an independent predictor of patient prognosis in patients with advanced stage, high-grade serous ovarian cancer and is strongly associated with enhanced angiogenesis and increased infiltration of M2 macrophages. In vitro, we show that FGF18 promotes migration, invasiveness, and tumorgenicity of ovarian tumors through FGFR4-dependent activation of NFkB. In vivo, we found that knockdown of either FGF18 or FGFR4 in SKOV3 or treatment with a pan-FGFR inhibitor significantly suppressed the growth of i.p. xenografts in SCID mice. FGF18-induced activation of the NF-kB pathway promotes production of pro-inflammatory and pro-angiogenic cytokines responsible for recruitment and subsequent polarization of monocytes into a M2-like pro-inflammatory phenotype. Targeting macrophages or IKKβ reduces inflammatory response and inhibits FGF18 mediated tumorigenesis in vitro and in vivo, suggesting a novel therapeutic approach for ovarian cancer patients with FGF18 amplification and overexpression.

Abstract Number 310
Roberts Syndrome: A Disease of Sister Chromatid Cohesion Compensation
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Roberts syndrome (RBS) is a rare developmental disorder, due exclusively to recessive inactivation mutation in the establishment of sister chromatid cohesion gene ESCO2. RBS patients have a wide range of severities from embryonic lethal to viable, but potentially cancer predisposed. Phenotypes include microcephaly, mental retardation, limb deformities, growth retardation and craniofacial defects. To better understanding this disease, we have identified a zebrafish Esco2 null allele. Homozygous loss of Esco2 in zebrafish is embryonic lethal, resulting in phenotypes consistent with the more severe RBS phenotypes, such as microcephaly and growth retardation, partially due to p53-dependent neuronal tube apoptosis. At the cytogenetic level, homozygous loss of Esco2 results in non-paired chromatids due to sister chromatid cohesion loss. In vivo, single-cell imaging revealed chromosome segregation defects, spindle rotation, and micronuclei formation. Most surprising is that while heterozygous loss of Esco2 is viable, they display mild cohesion defects (appearing cytological similar to RBS patients) and a predisposition to tumor formation (described in a few RBS patients). Toward the tissue-specific nature of the RBS phenotypes, we observed in Esco2 null embryos that some cells compensate for Esco2 loss and have intact SCC, suggesting a compensatory mechanism for establishing SCC. We postulate that these compensation mechanisms create the variable tissue-specific phenotypes in RBS patients and are actively pursuing identification of these compensation genes. We generated zebrafish frameshift mutations in candidate modifiers in the esco2 homolog esco1, as well as modifiers based on yeast genetics, Pds5a and b, Wapal, and Hdac8, to determine if they alter the Esco2 loss phenotypes.

Abstract Number 311
Neurofibromin Binding Partners
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Background: NF1 is caused by mutations in the NF1 gene which encodes neurofibromin, a large multi-domain protein with several known binding partners. NF1 binding partners may play a key role in the pleomorphic NF1 phenotype. The BioGrid database summarizes NF1 interacting partners. Despite 37 independent studies, only 12 actually look at NF1 specifically. Although many

studies utilize HEK293 or HEK293T cell lines, Biogrid studies do not overlap except at 3 of the 118 total unique proteins: FAF2, HTR6, and SPRED1. Lack of consistency makes it difficult to define relevant NF1 binding partners that might modulate its function. Reagents for studying neurofibromin and the affinity of neurofibromin antibodies has been limited.
Methods: We have created a tandem affinity purification (TAP) tag composed of a TEV cleavage site followed by a Strep II Tag and a 6X His Tag cloned in frame to the end of the full-length mouse Nf1 cDNA. We have also created an empty vector incorporating this TAP tag system as a control. We transfected these into HEK293 cells and binding partners were purified utilizing Strep-Tactin™ XT beads and identified via mass spectrometry.
Results: We show that our TAP tagged clones are functional and express His-Tag, neurofibromin, and can correct p-ERK/ERK ratios in NF1 null cells. Initially, we were able to pull down over 800 different proteins in HEK293 cells. We were able to refine the combined protein lists to discover approximately 22 proteins with high confidence of interaction with neurofibromin. We are able to compare this list of protein-protein interactions with the literature, global HEK293 proteomics data, and pseudo-validation approaches such as Reactome and Metacore.
Conclusions: We identify several novel binding partners for neurofibromin. These proteins suggest that neurofibromin may take part in cellular processes other than inactivating Ras. Further characterization of these binding partners could aid in discovering new neurofibromin functions and drug targets.

Abstract Number 312

Changes in Employment and Insurance for Patients with Cancer Receiving Safety Net Services

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Background: Under-resourced patients with cancer often face financial burdens due, not only to costs of treatment, but also from side effects that prevent individuals' ability to work, which impact employment status and may cause insurance coverage loss. Financial assistance may be sought from safety net programs, which provide both material support and financial counseling. However, knowledge of the impact of cancer on employment and insurance in a population seeking safety net services is limited. **Methods:** This observational, cross-sectional study uses data on safety net services from a nationwide survey conducted in July 2017 and distributed by the Patient Advocate Foundation (PAF). The survey respondents included patients with cancer who received services from PAF from July 2016 to June 2017. Descriptive statistics were calculated using frequencies for categorical variables.
Results: A total of 508 patients with cancer completed the survey. Most patients had a diagnosis of breast cancer (47%), followed by myeloma (13%), and prostate cancer (8%). The majority of patients reported that their illness affected their employment (67%); by either job loss (13%), income loss (24%), or inability to work as usual (27%). Of these patients, 27% lost their insurance coverage. Those able to enroll in a new insurance plan reported having more expensive rates (40%) and fewer covered services (36%) compared to their previous coverage. The most commonly utilized governmental safety net services were Social Security Disability Insurance (19%) and Medicaid (12%). Non-governmental safety net services such as financial assistance from non-profits (27%) and free medication from drug companies (13%) were also frequently used. Beyond their insurance coverage, cancer patients still needed assistance paying for diagnostic tests (18%), clinic visit fees (23%), and prescription drugs (15%) from the safety net program.

Conclusions: Cancer patients commonly experience financial burden due to losses in employment and insurance, resulting in need for safety net programs. Further work is needed to identify approaches to reducing the adverse financial impact of cancer care.

Abstract Number 313
Analysis of Variations within 20S Proteasome Subunit beta-5 Gene in PI-resistant Myeloma
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Multiple myeloma (MM) is the 2nd most common hematological malignancy in the United States. Proteasome inhibitors (PI) like Bortezomib (Bz) are widely used drugs in the treatment of MM, alone or in combination with other anti-cancer agents and showed remarkable response rates in both relapsed/refractory MM and newly diagnosed MM. Bz, a boronic dipeptide, specifically inhibits the ATP-independent chymotryptic activity of the 26S proteasome through reversible binding to the β5-subunit (PSMB5) of the 20S multi-catalytic protease core. This interferes with tumor progression primarily by accelerating unfolded protein response (UPR) in cancer cells which triggers apoptosis. However, despite an increasing number of approved therapies, MM remains an incurable disease. Bz treatment often achieves only very short-duration responses and drug resistance develops rapidly. Mutations in PSMB5 gene have been proposed as a possible mechanism behind Bz resistance, although the evidences have been conflicting. Bz-resistant human myeloma cell lines (HMCLs) have been shown to harbor PSMB5 mutations that cause conformational changes in the Bz-binding pocket of proteasome resulting in impaired PI binding and decrease in chymotrypsin-like catalytic function resulted into PI resistance. Only recently, clinical studies have reported that the mutation frequency for PSMB5 genes in primary MM increases with drug treatment. However, there is substantial gap in observation between in vitro vs clinical studies pertaining to the discovery of pharmacogenomically relevant variants in PSMB5 vis-à -vis PI resistance. In this study, we used a large panel of human myeloma cell lines (HMCLs; >60 cell lines) representing innate and acquired PI resistance to discover PSMB5 mutations associated with drug resistance in MM. High quality DNA was extracted from the HMCL panel and all the three exons of PSMB5 gene (Genomic location: chromosome 14:23,016,543-23,035,230 (GRCh38/hg38); size: 18,688 bases; orientation: -ve strand; Accession ID: NC_000014) were amplified by PCR following standard protocol. The PCR amplicons were sequenced using high throughput bi-directional Sanger DNA Sequencing, sequencing results were quality checked and multiple sequence alignment (MSA) was performed using SeqMan Pro module of DNASTAR Lasergene v16.0 software to discover de novo and reported variants. We identified a total of 24 genetic variants within the PSMB5 gene. This includes the reported variants rs11543947 (chr14:23034812: G>A), rs769612712 (chr: 23034874: A>C), rs1470676654 (chr: 23034944C>T). Rest of the variations are de novo. Currently, we are performing functional genomics analysis on the variants of undermined significance (VUS) to understand the role of these mutations in the drug resistance of multiple myeloma.

Abstract Number 314
Development of Titanium-45 PET Imaging Agents for PSMA
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The development of new agents for Positron Emission Tomography (PET) allows for imaging of many biological parameters in research studies and patient care. In particular, Prostate Specific Membrane Antigen (PSMA) is overexpressed on prostate cancer cells, and can be targeted for imaging. Titanium-45 (t1/2 = 3.08 h) is a PET imaging radioisotope with excellent nuclear and

chemical properties, including decay characteristics of 85% positron emission with Eβ+max = 1.04 MeV, meaning the images can be expected to have a resolution similar to those of F-18, used routinely for PET imaging with [18F]FDG. Development of Ti-45 PET imaging agents specific for PSMA would allow for more precise imaging techniques for prostate cancer. Ti-45 was produced on the UAB TR-24 cyclotron through the 45Sc(p,n)45Ti nuclear reaction by bombardment of scandium foils. Post-irradiation, the target was dissolved in 6M hydrochloric acid and the solution was added to 175 mg of hydroxamate resin from which the Scandium target material was eluted in 2M hydrochloric acid and the Ti-45 isotope was eluted in 1M citric acid. Recovery yields were up to 61.34% ± 11.00% of the initial activity produced with minimal scandium breakthrough. Next, we investigated the radiolabeling and radiochemical yields (RCY) of several PSMA targeting ligands including DFO-DUPA, LDFC-DUPA, and DFO-KFF-DUPA which showed radiolabeling efficiencies of 90-100%. Cell uptake studies were also conducted with PSMA positive and negative cell lines to assess the specificity of the imaging agent. PSMA+ 22Rv1 prostate cancer cells showed a much higher uptake of [45Ti]DFO-DUPA than PSMA- AR42J cells (P0.005). Preliminary imaging studies have been conducted to investigate uptake of free Ti-45 in normal mouse models. We aim to build upon these imaging studies with tumor mouse models using our PSMA targeting ligands. Current studies demonstrate the promise of Ti-45 labeled PSMA targeting agents and future studies will involve small animal imaging of PSMA+ prostate cancer.

Abstract Number 315

Daily Tips to Enhance a Web-based Diet/Exercise Intervention for Rural Cancer Survivors

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Purpose: Older, rural cancer survivors often struggle to find reliable health information and may benefit from distance delivery of health behavior interventions from credible sources. Aim, Plan, and Act on Lifestyles (AMPLIFY) Survivor Health delivers evidence-based guidance to cancer survivors through interactive technology with the aim to improve quality-of-life and reduce health risk through exercise, healthy diet, and weight management. The Daily Tips portion of AMPLIFY is designed to increase participant engagement by posting a new, encouraging tip about implementing healthy behaviors each day.

Methods: The 48-week multiple behavior web-based intervention will deliver exercise and diet information in sequenced or combined modules which will be tested in a randomized controlled trial. A comprehensive matrix outlines the exercise, diet, and behavior change topics presented each week of the intervention. Using an iterative process involving staff, content experts, and webpage design team, a daily tip was written for each week and logic for webpage presentation determined. Integration with the matrix reinforces evidence-based information given to them in their web-based program. For example, an exercise tip during the resistance-training portion of the matrix might say, “Calling all Do It Yourself-ers (DIY)! Make your own free weights out of full water bottles. Try extending them above your head.” Special attention was paid to ensuring the tips were appropriate for our target population; when appropriate, gender-specific tips were tailored. For example, tips related to manicures as a reward for meeting goals would be of less interest to some male participants.

Results: 168 diet tips and 168 exercise tips were compiled, reflecting 48 themes such as increasing produce intake and fitting exercise into busy schedules. New Daily Tips will be

available for view each day on the website homepage, when users log in to complete weekly assigned tasks and track diet and/or exercise behaviors.
Conclusions: Daily Tips may be a helpful inclusion to web-based interventions to boost website user engagement and motivation for behavior change, as they are easy to develop and integrate, and the participants may be more likely to log in if daily motivation is present, which can increase behavior change. Few logistical issues occurred, and investment of the development team was minimal. Beta testing in the fall of 2019 may provide insight into the benefits of this simple enhancement to websites aimed at improving health behaviors in older, rural cancer survivors.

Abstract Number 316

Prostate Biopsy Time of MRI Fusion Target Only versus with Concurrent Systematic Sampling

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Purpose: To determine if there is a statistically significant difference in operation times between MRI-US fusion-targeted prostate biopsy and MRI-US fusion-targeted biopsy with concurrent systematic biopsy sampling. Factors such as patient demographics or prostate related factors were also analyzed to determine any factors associated between target-only versus targeted and concurrent systematic sampling patient cohorts.
Methods: Patient procedural data from biopsies performed by two fellowship trained urologic oncologists at an academic institution between June 2014 and January 2019 was retrospectively analyzed. Procedure time was collected from nursing logs documenting procedure start and end times in the electronic medical record (EMR) from patients undergoing both target and concurrent systematic biopsies (n=224) and patients receiving target-only biopsies (n=215). Other prostate related factors such as pre-biopsy PSA, prostate volume, PSA density, MRI number of lesions, and number of targeted biopsy cores obtained were also collected from the EMR. Patient demographic information including age at time of biopsy, race, and BMI were also collected. The patients were then split into cohorts of both target and concurrent systematic biopsy sampling versus target-only biopsies and were analyzed for statistical significance when compared.
Results: There was no statistically significant difference in procedure time between target-only and concurrent biopsy sampling schema (10+/- 6 min vs. 10 +/- 6 min, p= 0.27). There was a statistically significant difference in both pre-biopsy PSA level (9.9 +/- 10.6 vs. 7.4 +/- 6.9 ng/mL, p= 0.004) and PSA density (0.22 +/- 0.27 vs. 0.16 +/- 0.19, p= 0.008) comparing the target only to the combined target and systematic biopsy sampling cohorts, respectively. Additionally, the number of MRI targeted lesions (4.2+/- 1.4 vs. 3.5+/- 1.4, p=8.8E-07), needles sampled per target (2.7 +/- 0.8 vs. 2.3+/- 0.6, p0.0001), and total number of target cores obtained (1.6 +/- 0.7 vs. 1.5 +/- 0.6, p= 0.03) were higher in the target-only biopsy cohort.
Conclusions: In conclusion, there was not a statistically significant difference in the total procedure time between target-only biopsies based on MRI-US fusion guidance and concurrent target and systematic biopsy sampling, which may be a result of a significantly higher number of targeted lesions, biopsy cores sampled from each lesion, and total targeted biopsy cores sampled in the target-only biopsy cohort. Future directions include analyzing both the target-only biopsy cohort and concurrent target and systematic biopsy sampling cohorts to a systematic biopsy only cohort of men biopsied by the same urologists.

Abstract Number 317
Targeting Oncogenic non-coding RNA in Lung Cancer Suppresses Tumor Growth
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Lung Cancer is the leading cause of cancer related death in the United States and the world. While great strides have been made in identifying many of the genetic events necessary to drive the development of lung cancer, this has not led to major improvements in patient outcome, highlighting the need to further understand the genetic and molecular mechanisms of this disease. We investigated the role of non-coding genes and recently demonstrated that microRNA-31 (miR-31) is overexpressed in patient lung adenocarcinoma compared to normal lung, high miR-31 levels correlate with decreased survival, and overexpression of miR-31 alone in the mouse lung epithelia initiates lung tumorigenesis and adenocarcinoma development. These data indicate miR-31 could be an important potential therapeutic target for lung cancer. To test this, we have genetically engineered loss of miR-31 in human lung adenocarcinoma cell lines using CRISPR/Cas9. Reduced miR-31 levels resulted in decreased cell growth and colony formation in culture. To determine if Cas9 targeting of miR-31 affected tumor growth, we used an orthotopic xenograft model and observed that loss of miR-31 expression almost completely suppressed tumor growth in vivo. We have determined that loss of miR-31 allows for the reactivation of multiple negative regulators of the RAS/MAPK signaling cascade (RASA1, SPRED1/2, SPRY1/3/4) resulting in decreased phosphorylation of ERK. We are continuing to evaluate other pathways miR-31 might be impacting by kinomic analysis. To thoroughly vet miR-31 as a potential therapeutic target, we examined the effect of loss of miR-31 in normal lung cells. Perhaps most surprisingly, loss of miR-31 does not seem to affect the growth nor viability of non-transformed lung epithelial cells in culture, indicating a potentially cancer specific role. We thus hypothesize that this gene is necessary for the growth and progression of lung adenocarcinoma and may serve as a potentially important therapeutic target.

Abstract Number 318
Disparities in End-of-life Care in Children Dying of Cancer in Alabama
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Institute for Cancer Outcomes and Survivorship, UAB

Background: Nearly 2,800 children die from cancer every year, yet few studies have examined the care that pediatric cancer patients receive at the end of life, particularly in the Deep South. Adult studies demonstrated that adults dying of cancer do not want medically intense interventions like mechanical ventilation, intensive care unit (ICU) admission, or cardiopulmonary resuscitation (CPR) at the end of life and instead prefer to enroll in hospice and have a home death.
Objective: The present study sought to determine rates of and disparities in medically intense end-of-life (EOL) care for children dying of cancer in Alabama.
Methods: We used a retrospective cross-sectional study design of patients who received their primary cancer directed therapy at Children's of Alabama and subsequently died between 2012 and 2018. We included patients who died between 0 and 35 years of age. We collected patient demographics, clinical details, and EOL care information (invasive procedures, chemotherapy, hospice enrollment, palliative care involvement, and location of death) using electronic medical record (EMR) review.
Results: Of the 144 patients, the average age of death was 11 years (STD: 6.8), 35% had a primary diagnosis of brain tumor, and 63% were white. Results demonstrated that 80% of patients received had at least one intensity marker (mechanical ventilation/intubation, CPR, renal dialysis, tracheostomy or gastronomy tube placement, chemotherapy, ICU admission, or hospital death)

in the last 30 days of life, 39% received medically intense care (mechanical ventilation/intubation, CPR, renal dialysis, or ICU admission), only 60% received a palliative care consultation, 38% received hospice care, and 45% died in the hospital. Additionally, patients with hematologic malignancies (e.g. leukemia & lymphoma) had greater odds of receiving cancer directed therapy in the last 30 days of life (Adjusted OR: 5.37, 95% confidence interval: 1.5-19.7) and dying in the hospital (3.7, 1.3-10.6) compared to patients with central nervous system (CNS) tumors. Further, patients with non-CNS solid tumors were more likely to receive a palliative consult than patients with CNS tumors (2.4, 1.0-5.5). Non-white patients were more likely to receive medically intense care compared to white patients (2.3, 1.0-5.1).
Conclusion: This is an important first step in determining if children dying of cancer at Children's of Alabama are receiving goal concurrent end-of-life care. Further research needs to be conducted in order to determine whether these rates and disparities are consistent with patient and caregiver goals and how to better serve these patients and their families.

Abstract Number 319
Leveraging Available Institutional Resources to Identify Study Populations
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Background: Informatics for Integrating Biology and the Bedside (i2b2) is an NIH-funded, clinical database query tool developed primarily for identifying study populations. We sought to use i2b2 to identify patients with recurrent and non-recurrent ductal carcinoma in situ (DCIS) treated at the University of Alabama at Birmingham (UAB) who fit eligibility criteria for a Translational Breast Cancer Research Consortium tissue banking study.
Methods: Women age 40-75 years diagnosed with DCIS from 1998-2016 were eligible. Within i2b2, diagnosis and billing codes were used to identify patients with DCIS recurrence. Age, sex, and recurrence date were also gleaned from i2b2. Chart reviews were performed for patients identified from i2b2 to confirm DCIS recurrence. Sensitivity, specificity, positive predictive value, and negative predictive comparing patients' status as determined by algorithms used in i2b2 and chart review were calculated. Additionally, patients were individually referred by oncologists.
Results: UAB oncologists referred 4 patients with recurrence from December, 2018 to January, 2019; 1 patient was eligible. We identified 532 patients from i2b2 with non-recurrent DCIS and 49 patients with a recurrence. Chart review was performed on all recurrent cases and 200 non-recurrent cases. Of those identified as having a recurrence, 10 were confirmed by chart review (PPV: 20%). Among non-recurrent patients, 1 recurrence was identified (NPV: 99%). Sensitivity and specificity for DCIS recurrence identified by i2b2 were 91% and 84%, respectively. For confirmed recurrent cases, reasons for further exclusion were total lack of tissue (n= 2) and insufficient tissue (n= 1).
Conclusions: Clinical database resources like i2b2 can be used to expedite the process of study population identification. However, this approach requires manual review to ensure accurate findings. Additionally, strict inclusion criteria can lead to significantly reduced sample sizes and can be a burden on identification and recruitment.

Abstract Number 320
Evaluation of Radiolabeled Peptides for HER2 PET Imaging of Breast Cancer
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Breast cancer is the most frequently diagnosed cancer in women and ranks second among causes for cancer related death in women. Treatments targeting the Human Epidermal Growth Factor Receptor 2 (HER2) such as Trastuzumab and Pertuzumab have improved patient outcomes significantly. As patient response relies heavily on the expression of HER2, the development of imaging strategies for HER2 to aid in patient selection for targeted therapies is urgently needed. One solution is the use of peptides as radiolabeled imaging agents. A modified peptide reported by Huawei et al. from Journal of Fluorescence, DOTA-Bn- GSGKCCYSL, was synthesized and radiolabeled with 68Ga to give [68Ga]P5. The combination of labeled peptide and phosporamidon (PA, an enzyme inhibitor to prevent degradation described by Nock et al. in Journal of Nuclear Medicine) was added to three cell-lines: HER2-negative MDA-MB-231 and HER2 positive SKBR3 and BT474 to confirm specificity and uptake of the peptide. Cells were incubated in media with 300nM of peptide and 200nM of PA present. Cells were counted on a Gamma Counter and the %uptake/mg of radioactivity/peptide per protein was determined. Female nude mice were implanted with BT474 cells and were given 4-6 weeks to grow into ~ 5x5 mm tumors aided by an estrogen pellet. [68Ga]P5 was radiolabeled as described above, suspended in sterile saline to have 150 µCi (3.7MBq) per 100 µL of total solution and injected into mice via tail vein. A separate group of mice were also injected with 300 µg of PA. Mice were imaged on the UAB small animal PET/CT at 15 minutes or 60 minutes post-injection. The mice were sacrificed, select organs and tumors were removed, weighed and assessed for radioactivity via gamma counter (%ID/g). Cell-studies showed HER2 positive SKBR3 cells had significantly more %uptake/mg of the radiotracer (0.020±0.006s.e.) similar to BT474 cells (0.026±.008s.e.) compared to the non-HER2 expressing MDA-MB-231 (0.010±.004s.e.). There was a sizeable difference between tumor %ID/g with the co-injection of PA compared to [68Ga]P5 injection alone at both time points. At 15 minutes, the difference was 0.567±0.276s.e. without PA and 1.802±1.414s.e. with PA. At 60 minutes, was 0.176±.035s.e. and 1.730±0.196s.e. respectively (n=2). These preliminary studies show promising results for the development of targeted peptides for HER2 imaging. Other peptide sequences are under investigation as well as control studies. This promising data sets the stage for our proposed experiments for additional peptides to be evaluated.

Abstract Number 321

"I Had Already Made Up My Mind." The Impact of Prior Experience and Healthcare Perceptions on Decision-making in Women with Early Stage Breast Cancer

Rebecca England, MS; Valerie Lawhon, BS; Audrey Wallace, MD; Stacey A. Ingram, MEd; Courtney P. Williams, MPH; Gabrielle Rocque, MD, MSPH

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Introduction: Shared decision-making (SDM) occurs when informed patients partner with their oncologists to incorporate personal preferences into treatment. Even before engaging with an oncologist about treatment options, patients may have personal experiences or knowledge of others' experiences with breast cancer that frame their decision-making. This study sought to understand how prior experiences and knowledge drive preferences in early stage breast cancer treatment approaches. **Methods:** This qualitative study included early stage breast cancer (BC) patients at an academic medical center in the Deep South. Women age >18 with an AJCC stage I-III BC diagnosis were invited to complete semi-structured interviews with a trained interviewer. Interviews were audio-recorded, transcribed, and analyzed by two independent coders utilizing a

constant comparative method from an a priori conceptual model based on the Ottawa Framework. Major themes and exemplary quotes related to decision-making preferences were extracted.
Results: Women (n=33) interviewed were an average age of 74 (4.2 SD), and 19% of participants were African American. Many women were given the option to omit treatments, such as chemotherapy or radiation therapy, based on hormone receptor status and axillary node involvement. Major themes related to a desire for more treatment were past experiences with family members having cancer or an impression that additional treatment would be more effective. For women that opted out of treatments, prior knowledge of potential physical side effects from friends, family, and other cancer survivors were cited as a major deterrent. Perceptions of low recurrence risk also influenced desire to forgo treatments.
Discussion: Women presenting with early stage BC had varied healthcare experiences, which resulted in preconceived ideas about receiving breast cancer treatments. Consideration of these themes may aid physicians' ability to address individual concerns to further personalize patient care, thus enhancing the patient-physician partnership. These findings will ultimately assist in improving patient engagement in SDM.

Abstract Number 322
Differential Outcomes in pNET Resections Among Caucasians and African Americans
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Background: Pancreatic neuroendocrine tumors (pNET) are a rare neoplasm arising from specialized neuroendocrine cells. The incidence of pNETs have increased over the last decade, with African Americans reported decreased overall survival compared to Caucasians. This study explores for a probable cause explaining the decreased survival rates found in African Americans.
Methods: Retrospective review and analysis of all Black or Caucasian patients with resected pNETs with curative intent from UAB Hospital were identified by billing code between 2010-2019. Clinical pathological variables were compared used via SPSS using P=0.05.
Results: There were 127 pancreatic resections identified, with 25% as African Americans and 75% as Caucasians. Results showed no statistically significant difference in survival outcomes between African Americans and Caucasians. However, we did find statistical significance in pathological size of the tumor. African Americans had an overall larger tumor size at the time of the surgery comparatively to Caucasians. No difference between demographics such as age, sex, BMI, and smoking, with exception of African Americans tended to be single while Caucasians were more likely to be married. When looking at morbidity, readmission status, and regards to their operation, no difference was found in post-operative morbidity, mortality and readmission rate. Their length of stay and disposition was also not different. When looking at resected specimens, we found no difference in margin status, lymph node positivity, perineal invasion, lymphovascular invasion, tumor grade or differentiation. Interestingly, African Americans had larger tumors (three 3.2 cm vs 2.5 cm; p = 0.011) than Caucasian counterparts.
Conclusions: African Americans tended to present with larger tumors. We found no difference in post-operative outcomes or recurrence free survival compared to Caucasian counterparts, suggesting published differences in survival maybe more socioeconomic than genetic.

Abstract Number 323
RNA-seq Analysis of HP1 Knockouts in Drosophila melanogaster
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The Heterochromatin Protein (HP1) family is a family of proteins that is highly conserved across eukaryotes, occurring in small gene families in the genomes of species from yeast to humans. As chromatin proteins, they have a variety of functions, and various HP1 proteins are described as involved in centromere stability, telomere maintenance, DNA repair, and regulation of gene expression. Loss of HP1 proteins leads to errors in gene regulation and in some cases to problems with mitotic chromosome segregation. Thus, HP1 loss can lead to lethality, and misregulation of HP1 proteins have been linked to cancer progression in humans. The mammalian HP1 homologs HP1α, HP1β, and HP1γ are related most closely to Drosophila melanogaster's HP1B protein. HP1B, along with HP1a and HP1C are the somatically expressed HP1 family members in Drosophila, which are encoded by the Su(var)205, HP1b, and HP1c genes, respectively. We investigated gene expression changes occurring in animals with mutations in Su(var)205, HP1b and HP1c using RNA-seq data to identify genes regulated by the HP1 family. We found that knocking out HP1a and HP1B proteins lead to increased upregulation of genes whereas knocking out HP1C had varied effects on expression that requires further analysis. Gene ontology analysis further revealed that the affected genes were involved in development, cell growth, and other biological pathways that require careful regulation to prevent aberrant growths. By investigating which pathways are affected by the removal of HP1 proteins in Drosophila, we can further our understanding of how its removal can contribute to genomic instability and cancer progression.

Abstract Number 324

In Vitro Inhibition of Glioblastoma Growth via Novel Analogs of Glucose Transporter

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Glioblastoma (GBM) is one of the most deadly and aggressive cancers in both children and adults. The ongoing standard of care comprises surgical resection, radiation and chemotherapy. Despite available treatments, GBM is incurable with rapid recurrence and low life expectancy of just 14 months. Development of more effective treatments is difficult because GBM is highly heterogeneous. One aspect of that heterogeneity involves brain tumor initiating cells (BTICs) that have stem cell-like ability to self-renew. BTICs can readily alter their metabolism and survive in low nutrient environments due in part to increased GLUT3 expression. We believe that the higher expression of GLUT3 in cancer cells compared to non-tumor cells makes it a therapeutic target, although the potential for toxicity must be considered. In recently accepted studies by Libby et al., we reported on two novel GLUT inhibitors identified utilizing a GLUT3 homology model by structure based virtual screening (SBVS). We are creating a structure-activity relationship profile and intend to increase the potency, selectivity and stability of the GLUT inhibitors. In this study, we have tested eleven novel analogs and identified three that have maintained efficacy against BTICs in vitro. Importantly, these compounds display minimal toxicity against human astrocytes making them ideal for therapeutic utilization. The novel derivatives have increased stability compared to the lead compounds and are efficacious in the nanomolar range. In the future, we intend to utilize our anti-GLUT compounds alone and in combination with radio- and chemotherapy with the hope of clinical translation.

Abstract Number 325

Obesity Impairs Immunotherapeutic Efficacy in Pre-clinical Breast Cancer

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Background: Obesity has long been known to worsen prognosis and survival for breast cancer patients. Recent reports further indicate that obesity negatively impacts response to targeted anti-VEGF therapy and efficacy of chemotherapeutics. However, no studies have yet investigated the impact of obesity on response to immunotherapy in the context of breast cancer. Although paradoxically, obesity has been found to improve response to immunotherapy in a subgroup of patients with melanoma.

Methods: Wildtype C57BL/6 female mice were randomized to a high-fat (60%) or low-fat standard chow (14%) diet for 16 weeks to generate diet-induced obese (DIO) or age-matched lean controls, respectively. Animals were then challenged with the syngeneic E0771 mammary carcinoma cell line. Tumor outgrowth was quantified by caliper measurements, bioluminescent imaging (BLI) via firefly luciferase-expressing E0771 (E0771-fLUC) cells, and endpoint tumor weights. Once tumors were palpable, animals were randomized to receive no therapy or immunotherapy consisting of intratumoral CpG co-administered with non-replicative adenovirus (Ad) encoding murine TNF-related apoptosis inducing ligand (TRAIL; AdT). Whole tumor immunogenetic gene expression profiles were evaluated using nanoString and immune populations were assessed via multi-parameter flow cytometry. T cell cytokine production was evaluated via flow cytometry following ex vivo CD3/CD28 stimulation.

Results: DIO mice had significantly increased body weights at tumor challenge versus lean controls (45 versus 25 grams, p 0.0001) All methodologies demonstrated that obesity significantly increases primary mammary tumor outgrowth and alters cellular and immunogenetic profiles within the tumor microenvironment. Notable alterations include significant reductions in the frequency of CD4+ T cells, CD8+ T cells, and CD19+ B cells; with a simultaneous increase in the frequency of granulocytic myeloid-derived suppressor cells (MDSCs). Following immunotherapy administration, lean animals controlled tumor growth whereas DIO animals experienced progressive tumor growth. Despite these differential tumor outcomes, both lean and DIO animals displayed robust intratumoral effector CD8+ T cell accumulation and ex vivo function. In contrast, immunotherapy reduced the intratumoral accumulation of monocytic and granulocytic MDSCs only in lean animals. Both MDSC populations persisted in the tumors of animals with DIO, resulting in less favorable effector CD8+ T cell to MDSC ratios.

Conclusions: Our data implicate obesity as a causal factor in impairing immunotherapeutic efficacy in a pre-clinical model of breast cancer, potentially via accumulation of MDSCs. Our data suggest that clinical investigation and consideration is needed for factors such as body composition and body mass index when treating breast cancer patients with immunotherapy.

Abstract Number 326

Overexpressing SSTR2 in Pancreatic Neuroendocrine Tumors to Improve Ga68-DOTATATE Imaging

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Neuroendocrine tumors (NETs) are found throughout the body, including the pancreas (pNETs). These tumors are phenotypically and genetically heterogeneous, and can be difficult to accurately image using current imaging standards. However, PET/CT with radiolabeled somatostatin analogs has shown clinical success, as many NETs overexpress somatostatin receptor subtype 2 (SSTR2). Unfortunately, patients with poorly-differentiated NETs often have a diminished level of SSTR2. We found that histone deacetylase (HDAC) inhibitors can upregulate the functional expression of SSTR2. We evaluated the effect of HDAC inhibitors on SSTR2 expression at the mRNA and protein level in NET cell lines. The effect of HDAC inhibitors on surface-SSTR2 was also investigated by fluorescence-activated cell sorting (FACS) analysis. Changes in SSTR2 expression in NET xenografts after treatment were imaged using Ga68-DOTATATE PET/CT. The functional increase of SSTR2 in NETs after HDAC inhibitor treatment was confirmed through in vitro experiments and small animal Ga68-DOTATATE PET/CT imaging. HDAC inhibitors increased SSTR2 transcription and protein expression in NET cell lines. Small animal Ga68-DOTATATE PET/CT imaging confirmed the enhancement of radiopeptide uptake after HDAC inhibitor administration. This study demonstrates a new method to potentially improve imaging and treatments that target SSTR2 in NETs.

Abstract Number 327

Prostate Cancer-on-a-chip Platform for In Vitro Anti-cancer Therapeutic Evaluation

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The tumor microenvironment (TME) is an intricate network characterized by spatial and temporal heterogeneities in cell populations, tissue microarchitecture, and angiogenic development, resulting in an induced oxygen and nutrient gradient. Each of these characteristics directly contribute to the pathophysiology of cancer; therefore, it is imperative to accurately recapitulate the TME when engineering a biomimetic cancer model for use in clinically-translatable therapeutic development. This study reports the production of a microfluidic prostate cancer-on-a-chip platform that employs primary and ancillary 3D tumor chambers, surrounded by a complex microvascular network derived from computerized tomography images of tumors in vivo. The microvasculature channels were initially perfused with gelatin and fibronectin to aid in cell adhesion and seeded with HUVEC cells held under physiological flow conditions to form a lumenized endothelium. Tunable engineered prostate cancer tissue (EPCaT), comprised of PC-3 prostate cancer cells and BJ-5ta fibroblasts encapsulated in poly(ethylene glycol)-fibrinogen (PF), was crosslinked within the primary tumor chamber (PTC), while ancillary tumor chambers remained empty to monitor for on-chip cell migration. To ensure the EPCaT maintained a high level of physiological relevancy, macrotissues were fabricated off-chip for several characterization studies, including a novel in vivo and in vitro tissue microarchitectural stiffness comparison. In vivo prostate tumors were generated by subcutaneously injecting PC-3 cells into the flank of athymic NCr mice, and both in vivo and in vitro tissue samples were subjected to parallel plate compression. The in vivo tumor stiffness was found to range from 230 -5,500 Pa, whereas the EPCaT stiffness range achievable through PF matrix modulation was 80-12,600 Pa. On-chip, the drug diffusion profile from the microvascular network into the EPCaT was characterized via perfusion of TRITC-dextran (avg. molecular weight 4,400 Da). Initial drug studies were performed using 10 μM doxorubicin perfused over 24 hours. During long-term culture, PC-3 cells were found

to intravasate from the PTC, circulate through the microvasculature, and extravasate into the ancillary tumor chambers. Additionally, PC-3 cells preferentially colonized within the vascular network at areas of low shear. Vascular geometry-dependent diffusion into the PTC was achieved, thus mimicking the differential drug distribution observed in native tumors. Non-uniform cell death was observed post-doxorubicin exposure, thereby confirming limited drug diffusion. Future investigations will monitor on-chip cell behavior, including migratory patterns and drug response, using LNCaP cells to provide an androgen-sensitive versus androgen-insensitive comparison study. Drug studies will also be extended to evaluate size-based drug distribution within the PTC using nano-drug carriers.

Abstract Number 328

Production of Colorectal Cancer Tissue-Engineered Organoids for High-throughput Screening

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Colorectal cancer (CRC) remains the third most prevalent diagnosed cancer among men and the second among women worldwide. Over 80% of anti-cancer drugs fail to be approved by the FDA, partially due to inadequate preclinical testing. While current preclinical models result in promising outcomes, they are labor-intensive, costly, and unable to closely recapitulate both the native tumor microenvironment (TME) and patient-specific variations in drug response. To overcome these challenges, we have established a microfluidic system that can rapidly produce CRC tissue-engineered organoids by encapsulating both the HT29 cell line and cells from a patient-derived xenograft (PDX) of stage II CRC within PEG-fibrinogen (PF) hydrogels. To fabricate the microfluidic system, a jig was 3D printed to hold Teflon tubes and metal wires forming a T-junction and channels. The jig was then utilized as a mold to engineer a PDMS-based encapsulation device with two inlet and one outlet ports. The cell-laden (HT29 or PDX) PF precursor solution and mineral oil were injected to the inlet ports of the encapsulation device and crosslinked using high-intensity visible light to create the organoids. To assess cell viability post-encapsulation, a Live/Dead assay was performed. To demonstrate cell growth within the organoids, temporal changes in cell colony area were quantified. Immunostaining and flow cytometry were performed using Ki-67, CD44, B2M, and CK20 to observe and quantify proliferative cells, cancer stem cells, human cells, and CRC cells, respectively. The mechanical stiffness of the organoids was measured using a MicroSquisher and compared to that of in vivo PDX tumor tissue. Vitality assays revealed that the cells survived the encapsulation process and maintained high viability over 29 days of culture (>90%). Analysis of phase contrast images demonstrated an increase in cell colony area over time. Immunostaining of HT29 organoids revealed that a larger number of cells located on the periphery of the organoids were positive for Ki67, mimicking the highly proliferative periphery of native tumors. The percentage of human (70%) and CRC (30%) cells within the PDX organoids was maintained over time and similar to the original PDX tumor. Finally, the mechanical stiffness of the PDX organoids exhibited a similar order of magnitude (103 Pa) to the original tumor. In conclusion, we have established a microfluidic system for rapid production of tissue-engineered tumor organoids. The encapsulated CRC cells remained viable, colonized, presented similar cell populations and stiffness to the original tumor, and demonstrated potential for high-throughput screening applications.

Abstract Number 329

In Vitro PDX Engineered Tumor Model to Mimic In Vivo Obesity-Promoted Colorectal Cancer

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Epidemiological studies have revealed that obesity is linked with up to 70% increased risk of colorectal cancer (CRC). However, the mechanisms of obesity-promoted CRC are still anonymous at the molecular levels, in part, due to the lack of relevant experimental models. Here, we have established an in vitro model of the obese tumor microenvironment by engineering cancer tissues utilizing patient-derived xenograft (PDX) CRC (PDXCRC) cells and investigated the ability of our model to recapitulate an in vivo obese model of orthotopically implanted PDXCRC. Briefly, PDXs were established through subcutaneous propagation in NOD-SCID mice, excised, and the cells were isolated and encapsulated within PEG-fibrinogen to generate 3D engineered PDXCRC tumors (3DePCCTs). To determine if the PDXCRC cells could maintain their viability in the 3DePCCTs, cell viability assays were performed. To determine how closely the 3DePCCTs mimic the in vivo PDX tumor, cell subpopulations by flow cytometry and H&E staining of the 3DePCCTs were carried out and compared to those of in vivo PDX tumor. To demonstrate model responsiveness to the growth-promoting effects of obesity, the 3DePCCTs were co-cultured with insulin-sensitive (IS) or -resistant (IR) adipocytes (model of leanness or obesity, respectively), differentiated from 3T3-L1 cells. Tumor cell colony area within the co-cultures was quantified over 29 days and compared to our in vivo model in which Rag1tm1Mom mice were fed either a high-fat Western diet + 4% sugar water or chow diet for 12-weeks; PDXCRC tumor fragments were then orthotopically implanted; diets were continued, and tumor growth assessed after seven weeks. Based on viability assays, PDXCRC cells within the 3ePCCTs remained viable over 29 days. Flow cytometry revealed that the percentage of human (β2 microglobulin+) cells within the 3DePCCTs was similar to the original tumor. Based on H&E staining, the cell colonies within the 3DePCCTs were similar to those of the in vivo PDX tumor. Most notably, co-culture of the 3DePCCTs with IR adipocytes resulted in a higher density of PDXCRC cell colonies after 8 days of co-culture as compared to co-culture with IS adipocytes and this difference increased through day 29. Similarly, the weight of orthotopic PDXCRC tumors in obese mice was over 2-fold higher than that of tumors in lean mice. In conclusion, we have established a novel in vitro obesity-mimetic colorectal cancer model which is responsive to the growth-promoting effects of obesity that can potentially be used to study the mechanistic link between obesity and colorectal cancer.

Abstract Number 330

Inhibition of Acid Ceramidase by Carmofur as a Treatment for Glioblastoma

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Glioblastoma (GBM) is the most common primary, malignant brain tumor with a median survival of 15 months. Current standard of care includes maximum surgical resection, chemotherapy with temozolomide (TMZ), and radiotherapy, but these therapies fail to eradicate a subset of cells called brain tumor initiating cells (BTICs). BTICs have properties of neural stem cells and contribute to chemo- and radio-resistance of this aggressive disease. To target BTICs and reduce GBM therapeutic resistance, we are inhibiting the activity of acid ceramidase (ASAH1). ASAH1 is

responsible for the conversion of ceramide to sphingosine and free fatty acids in the lysosome. From the lysosome, sphingosine can be converted to sphingosine-1-phosphate (S1P) which allows cells to evade apoptosis and continue proliferating. ASAH1 inhibitors have been shown to block the production of S1P and increase levels of ceramide, which induces apoptosis and stops the progression of some cancer types. ASAH1 positively correlates with worse survival of GBM patients and is upregulated after irradiation of GBM cultures. One inhibitor of ASAH1, Carmofur, which is a derivative of 5-fluorouracil, is already in clinical use in Japan for colorectal cancer and is known to cross the blood brain barrier. Our work suggests that Carmofur is effective at decreasing growth of neurospheres isolated from patient derived xenografts (PDX) in parental and TMZ-resistant PDXs at concentrations below 30μM and has an additive effect when combined with TMZ in vitro. Furthermore, ongoing in vivo experiments will compare the efficacy of TMZ in combination with Carmofur against TMZ-resistant GBM cells in a nude mouse model. Overall, our data suggest that Carmofur is a potential new therapy that can overcome GBM therapeutic resistance and future studies will define its biodistribution and efficacy against TMZ-resistant GBM cells.

Abstract Number 331
Hedgehog Signaling Alters the Macrophage Phenotype through Eliciting Metabolic Adaptations
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Breast cancer is the most common cancer among women, and there is a 1 in 8 chance that a woman will develop breast cancer in her lifetime. The current standards of care used to treat different types of breast cancer offer little relief to patients with advanced, metastatic disease. Thus, it is imperative to develop treatments that target metastatic cancer. The aberrant activation of developmental signaling enables cancer cells to survive, proliferate, and metastasize. The Hedgehog (Hh) developmental pathway is upregulated in breast cancer and our lab has demonstrated a role for Hh signaling in promoting tumor growth, angiogenesis, and metastasis. Effective metastasis significantly depends upon the surrounding immune microenvironment. Macrophages, innate, antigen presenting immune cells, are highly prevalent in the tumor microenvironment and can make up to up to 50% of the tumor mass. We have recently discovered that Hh signaling programs macrophages towards alternative (M2) polarization. These alternatively polarized macrophages create an immune-suppressive, tumor-permissive environment that enables tumor progression and metastasis. Pharmacological inhibition of Hh signaling in an immune-competent 4T1 mouse model of metastatic mammary carcinoma enhanced the inflammatory tumor infiltrating immune portfolio. Specifically, we have registered an increase in M1, tumor eradicating macrophages, with a concomitant decrease in M2, tumor supporting macrophages. Furthermore, it is widely accepted that macrophage metabolism is predictive of macrophage phenotype. We will pursue efforts to elucidate the impact of Hh signaling on the metabolism of macrophages and how this promotes a change in their phenotype. We are following leads to discover how Hh signaling alters macrophage bioenergetics, UDP-GlcNAc production, and O-GlcNAcylation and how alterations of these metabolic pathways determines the macrophage immune-suppressive phenotype. We anticipate that this investigation will enable us to better understand how to target and diminish the pro-tumorigenic phenotypes of macrophages in the tumor microenvironment and at the metastatic site.

Abstract Number 332
Targeting DNA-damage Inducible 1 Homolog 2 (Ddi2) for the Treatment of Cancer

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DNA-damage inducible 1 homolog 2 (Ddi2) is a novel aspartic protease, known for its function as protein shuttle that delivers the ubiquitinylated proteins to the proteasome for degradation and for the activation of the transcription factor Nrf1 responsible for the restoration of proteasome activity after treatment with proteasome inhibitors. We have made a serendipitous observation that knockout of Ddi2 dramatically delays the growth of xenograft tumors in mice. Ddi2 is amplified in many solid tumors, and overexpression of Ddi2 correlated with poor prognosis in pancreatic cancer. Gene expression profiling of murine tumors revealed that Ddi2 deletion down-regulates two pathways, STAT3 and Gli1/Hedgehog, which play an important role in pancreatic cancer development. Ddi2 is involved in cell recovery from DNA replication stress. Knockdown of Ddi2 sensitizes cells to DNA-damaging agents and DNA replication inhibitors, including gemcitabine, the most common treatment for pancreatic cancer. Therefore, we hypothesize that Ddi2 is a novel molecular target for pancreatic cancer.

Abstract Number 333

Immunoproteasome Inhibitors for the Treatment of ALL

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A build up of misfolded proteins in cancer cells can lead to cell death, and they heavily rely on the proteasome to break down damaged polypeptides. Proteasome inhibitors are approved by the FDA for the treatment of multiple myeloma and mantle cell lymphoma. There are two types of proteasomes, constitutive and immunoproteasomes. Constitutive proteasomes are found in all tissues while immunoproteasomes are expressed predominantly in the lymphoid tissues including hematologic malignancies. FDA-approved Inhibitors block both types of proteasomes. B-ALL leukemia with the MLL-AF4 translocation has been the primary focus of this project. This leukemia is a product of a fusion between chromosome 4 and 11. The MLL-AF4 is an infant leukemia with a poor prognosis and no current targeted treatment. However, it has been shown to be highly sensitive to bortezomib, an FDA-approved inhibitor of constitutive and immunoproteasomes. We found that the overwhelming majority of proteasomes in this subtype of ALL are immunoproteasomes. We asked the question whether bortezomib can be replaced with immunoproteasome inhibitors for the treatment of this leukemia. Immunoproteasome inhibitors should also reduce off-target toxicity of proteasome inhibitors caused by the inhibition of constitutive proteasome in non-lymphoid tissue, i.e. cardiac and gut toxicities. We found that MLL-AF4 cell lines are highly sensitive to a 1-hour pulse treatment with pharmacological relevant concentrations of immunoproteasome inhibitor ONX-0914. However, the activity of the immunoproteasome recovers thereafter providing a potential escape route. The recovery of immunoproteasome occurs by a novel mechanism, distinct from the recovery of constitutive proteasomes, and can thus be potentially targeted to further enhance efficacy of immunoproteasome inhibitors. In summary, these studies demonstrate that immunoproteasomes are therapeutic targets in ALL and suggest that clinical trials of selective immunoproteasome inhibitors in ALL should be conducted.

Abstract Number 334
Role of the ST6Gal-I glycosyltransferase in regulating tumor cell metabolism
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An emerging trend in cancer biology is that surface glycosylation can play important role in the regulation of cancer development and progression. Our group and others have shown that ST6Gal-I, a sialyltransferase that adds α2-6-linked sialic acids to N-glycosylated proteins, is upregulated in many cancer types including ovarian cancer. Furthermore, data has indicated that ST6Gal-I acts as a pro-survival factor in a variety of settings, including resistance to chemotherapeutic drugs. In the current study we describe a novel function for ST6Gal-I in regulating tumor cell metabolism. Using Seahorse technology, we found that cells with high expression of ST6Gal-I had higher rates of both oxidative phosphorylation and glycolysis. Additionally, we found that when we treat with the hypoxia mimetic DFO that cells with forced expression of ST6Gal-I were able to maintain higher metabolic rates overall. These data indicate that cells with high expression of ST6Gal-I are better able to adapt to hypoxic stress by altering their metabolic rates. Furthermore, we demonstrate that the increased glycolytic rate was accompanied by increased activity of the rate limiting glycolytic enzymes hexokinase and phosphofructokinase. Taken together, these data highlight a novel glycosylation dependent mechanism for the regulation of tumor metabolism.

Abstract Number 335
Synergistic Anticancer Effects of 3A.1 and Cabazitaxel Against Prostate Cancer
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Prostate cancer (PCa) is the second leading cause of non-cutaneous cancer deaths in the United States. The 5-year survival rate for local or regional disease is high, while survival for metastatic disease is 28.7%. Many patients eventually develop the more aggressive form “metastatic castration-resistant prostate cancer” (mCRPC). Cabazitaxel (CBZ) and docetaxel (DXT) are FDA approved drugs for mCRPC. However, clinical effectiveness of DTX and CBZ has been hampered by serious adverse effects and emergence of drug resistance. Alternating treatments with first line chemotherapy docetaxel (DTX) or cabazitaxel (CBZ) alone or in combination with new drugs may improve efficacy in PCa. Andrographolide analogue 19-tert-butyldiphenylsilyl-8,7-epoxy Andrographolide (3A.1) shows anticancer drug against various types of cancer. Previously our lab reported its effectiveness and proposed mechanism of action in colon cancer. In this study, we investigated the effects of 3A.1, andrographolide alone or in combination with DTX and CBZ against a variety of aggressive human prostate cancer cell lines (PC-3, PC-3M, Du145 and 22Rv1). Cells were treated with CBZ, DTX, andrographolide and 3A.1 as single agent and as well as in combination for 48 and 72 hr over a broad concentration range; the effect of drug exposure and concomitant therapy was determined used SRB and MTT, classical assays of in vitro cytotoxicity. The combination study for both drugs was analyzed by Calcusyn software to determine combination index (CI) and dose reduction index (DRI) to identify synergism and reduce dose for first line chemotherapy drug. Further, we evaluated the effect of treatment on changes in protein expression of important cancer pathway genes by immunoblotting for single dose vs combination to understand mechanism of action of 3A.1. Results showed that the 3A.1 alone exhibited dose- and time-dependent antitumor activity in PC3. Co-treatment of 3A.1 with CBZ (at its IC50) for 48 hr significantly increase activity (9.5-fold), whereas co-treatment of CBZ

with 3A.1 (at its IC50) for 48 hr significantly reduced antitumor activity (18-fold). All CI values of drug combination were less than one, indicating that co-treatment of 3A.1 and CBZ inhibited PC-3 in a synergistic manner. DRI values also indicated that concomitant drug concentration required to achieve estimated potency was reduced ~2-fold for CBZ. Overall, our in vitro testing revealed that co-treatment of 3A.1 and CBZ had synergistic growth inhibitory effect against prostate cancer. These results suggest that the 3A.1 may be useful at increasing anticancer efficacy of CBZ for treatment of prostate cancer.

Abstract Number 336
Evaluation of the GCH1 Pathway Members PTS and SPR in Glioma
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Introduction: Glioblastoma (GBM) is a deadly primary brain tumor of which there is an imminent need for novel treatment strategies and predictors of therapeutic response. This is dependent on improving our understanding of inter- and intratumoral heterogeneity, including the highly tumorigenic and therapy-resistant brain tumor initiating cell (BTIC) subset. We recently reported that BTIC maintenance is regulated by GTP cyclohydrolase 1 (GCH1), which is the rate limiting step in the tetrahydrobiopterin (BH4) pathway. This pathway also includes 6-pyruvoyltetrahydropterin synthase (PTS) and sepiapterin reductase (SPR), but their levels and role in GBM biology have not been well investigated.
Objectives: The overall goal of this project is to determine if elevation of GCH1 and/or the BH4 pathway is a feed forward loop in GBM, which correlates with poor patient prognosis. The immediate aim of the project was to evaluate the expression of the GCH1 pathway members PTS and SPR to define a potential role in GBM biology.
Methods: We examined the expression of the BH2/BH4 pathway enzymes PTS and SPR at the mRNA and protein level in patient-derived xenografts (PDX) and in glioma gene expression datasets.
Results: In silico analysis of available datasets showed increased expression of PTS and SPR with increased glioma grade. Elevated expression of PTS and SPR also correlated with decreased patient survival. Immunohistochemistry analysis of xenograft tissue slices showed increased protein expression in regions of hypoxia.
Conclusions: Enzymes of the BH2/BH4 pathway are elevated with increasing glioma grade and may be elevated in the hypoxic tumor microenvironment to promote tumor progression. Therapeutically targeting these proteins may be a viable treatment option in combination with radio- and chemotherapy.

Abstract Number 337
The Role of Hedgehog Signaling in the Double-strand Break Repair Pathway in TNBC
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Among the different subtypes of breast cancer, triple-negative breast cancer (TNBC) subtypes are hard to treat because they do not express hormone receptors. Therefore, treatment options for TNBC include genotoxic chemotherapy agents and ionizing radiation (IR) that induce DNA damage such as double-strand breaks (DSBs). DSBs can lead to cell death; however, an enhanced DSB repair pathway in the cancer cell can allow resistance to radiotherapy. One of the abnormally expressing developmental signaling pathway, hedgehog (Hh) signaling pathway, has been reported to confer resistance to radiotherapy in several cancers like prostate, medulloblastoma, and colorectal cancer, including breast cancer. Additionally, GLI1, a

transcription factor that is a terminal effector of the Hh pathway, have been reported to interact with RAD50 and MRE11. RAD50 and MRE11, along with NBS1, comprise the MRN complex, which detects DSB and lead the recruitment of DNA repair proteins to DSBs. The mechanism by which Hh pathway mediates the DSB in cancer cell leading to radio-resistance is not fully understood yet. We sought to understand how Hh signaling affects the primary DSB, homologous recombination (HR) and non-homologous recombination (NHEJ) repair pathways in breast cancer and determine if the combination of Hh inhibitor with radiotherapy would be beneficial in tumor elimination. We hypothesized that Hh signaling promotes repair of DSBs in TNBC through potentiating primary DSB repair pathways. Our results indicate that Hh inhibitor combination with IR significantly reduces spheroid growth of TNBC cell lines in a 3D model compared to IR and Hh inhibitor alone. TNBC cell lines show increased Hh signaling after IR, and its inhibition delays DSB repair due to higher DNA damage accumulation. Specifically, we were able to assign a role for Hh signaling in impacting the NHEJ repair pathway. Ongoing investigations are designed to assess further the mechanistic role of Hh signaling in NHEJ DNA repair and the functional benefits of combining Hh inhibitor with radiotherapy in TNBC. This study will be crucial to improve the outcomes of TNBC patients with acquired resistance to radiotherapy.

Abstract Number 338

Systematic Review: Cost-Effectiveness of Technological Interventions for Cancer Survivors

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Objectives: This is a systematic review of current evidence on the cost-effectiveness of technology-based diet and physical activity interventions among cancer survivors.
Methods: The PRISMA systematic review statement was used. Databases were searched for English-only articles between 2000-2019 for any technological interventions focused on modifying diet, physical activity, and weight. 108 articles were identified. Only 3 met the selection criteria; completed clinical trials involving fully technological interventions for cancer survivors. Design, protocol, and feasibility studies were excluded.
Results: All studies were carried out in developed nations (2 in USA, 1 in Australia), and used lower technology (telephone calls) to deliver group conference sessions, motivational interviewing, and health coaching. 2 analyses included intervention and participant costs only. 1 analysis included intervention and healthcare costs only. Effectiveness was measured by weight regain avoided, and increase in fruit and vegetable consumption, metabolic equivalent (MET) factor, and health-related quality of life. 2 studies suggested their fully telephone-call intervention was worth adopting to promote health behavior change; the analysis for the telephone motivational interviewing compared to printed health information resulted in an Incremental Cost-Effectiveness Ratio (ICER) of $115.1. A sensitivity analysis was not reported. The analysis of the telephone group conference compared to a newsletter resulted in an ICER of $422 per 1 kg of weight regain avoided, and $3,155 to keep 1 more person 5% or more below their baseline weight. The authors performed bootstrapping to evaluate the uncertainty around the ICER, with no further sensitivity analysis. A cost-consequence analysis was used for the telephone health coaching study which was compared to printed health information because there was no intervention effect on health utility, although there were significant improvements in health behaviors (e.g., increased physical activity). The authors wanted to provide evidence of health benefits while describing the costs and consequences for healthcare decision-makers. Their findings suggested that their intervention's mean cost of $303 per participant (21% of the overall healthcare costs in their analysis) was a small cost for generating the physical activity benefits observed, especially since

the benefits were sustained 6 months post-intervention. They performed bootstrapping with no further sensitivity analysis.
Conclusion: Telephone-based interventions have some evidence of cost-effectiveness in improving health behaviors and quality of life for cancer survivors. However, there is no current evidence to support the cost-effectiveness of newer technological interventions. Future studies should perform economic evaluations of higher-end technologies (e.g. web applications) and of older cancer survivors.

Abstract Number 339
Compensatory Ligand Recognition Forms the Basis of Polyspecificity by P-glycoprotein
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The American Cancer Society predicts 1.7 million new cancer cases and 606,880 deaths in the US for 2019. Of these cases, approximately 90% of the patients with metastatic cancer are expected to develop multidrug resistance (MDR). Many mechanisms of MDR have been described, including MDR arising from different sub-families of ABC (ATP-binding cassette) transporters. P-glycoprotein (P-gp) is of pharmacological interest and perhaps the most important MDR transporter expressed in the human body. P-gp represents a major barrier to effective cancer treatment, since it enables cancer cells to develop a resistance to most chemotherapeutic drugs. We have made selected mutations and show mediated drug binding. Through X-ray crystallography, we provide a structural definition of polyspecificity. This work will give insight into the mechanism of cancer multidrug resistance and aid in the development of P-gp evaders.

Abstract Number 340
Tumor Cells on the Move
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Glioblastoma (GBM) is a highly invasive tumor with inevitable recurrence typically occurring within centimeters of the original tumor site. Recurrence is largely believed to be due to the presence of a subset of tumor cells that are highly tumorigenic, stem-like cells. These cancer stem cells are also highly invasive and therapy resistant. We previously found glucose transporter 3 (GLUT3) to be upregulated in GBM cancer stem cells (CSCs) and to promote survival in the low nutrient microenvironments often found within these tumors. Low glucose conditions have also been found to increase GBM invasion. Additionally, reports investigating 343metastatic lung, colon and breast cancers have indicated a role for GLUT3 in mediating their metastatic potential. With this in mind, we utilized the IVYGAP data set to elucidate if there was a potential relationship between GBM invasion and GLUT3 expression. GLUT3 expression was elevated at the leading invasive edge of GBM tumors, an upregulation not seen with GLUT1. When GLUT3 was overexpressed in GBM CSCs, their invasive capacity was significantly increased with no elevation in growth or migration. To define potential mechanisms for GLUT3 regulation of invasion in GBM we used proteomics to identify multiple binding partners of GLUT3 that differ from GLUT1. Additionally, we have mutated GLUT3 by swapping in the corresponding GLUT1 sequence at regions of low homology to identify which region is important for this binding activity as one of these mutants significantly attenuates the GLUT3 invasive phenotype. Understanding mechanisms of GBM invasion will assist in the

identification of novel opportunities to develop therapies to improve patient prognosis beyond the current median of 15 months.

Abstract Number 341
TCR Sequencing Reveals Recruitment of Tregs from the Periphery into Omental Tumors
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The omentum is an apron-like fatty tissue that connects the spleen, stomach and pancreas, and contains lymphoid tissues termed milky spots. In the advanced stages of ovarian cancers, tumor cells frequently metastasize to the peritoneal cavity and invade the omental adipose tissue. This is accompanied by an increase in CD4+ CD25+ FOXP3+ regulatory T cells (Tregs) in the omentum, which suppress antitumor immunity. Despite the increasing understanding of the roles Tregs play in the context of cancer, how tumor-associated Tregs accumulate in the omentum requires further study. To address this, we intraperitoneally injected EG7 thymomas into syngeneic mice and sorted Tregs for T cell receptor (TCR) sequencing. Although omental Tregs and their splenic counterparts from naïve animals have low degrees of overlap in their TCR repertoires, the similarity between them becomes increased by tumors in the omentum. Due to the phenotypic characteristics shared by Tregs in the omentum and colon, we also examined their repertoire similarity and found it elevated by tumors as well, albeit more moderately. This indicates Treg recruitment from the periphery during cancer progression. Moreover, since we did not observe dominant Treg clones in the omenta of tumor-bearing mice, the augmentation of Tregs may not be primarily due to local clonal expansion. In fact, we found tumor development increases the repertoire diversity of omental Tregs. Given that Treg clonotypes in the spleen are highly diverse, these data suggest Tregs from secondary lymphoid organs may contribute to the omentum compartment during tumor progression. In line with these findings, the frequency of Ki67+ proliferating Tregs is higher in the tumor-free omentum than in the spleen, but it is significantly reduced in the omentum with tumors. Interestingly, FTY720 treatment does not affect the number of omental Tregs in tumor-bearing mice, indicating Treg trafficking into tumors may be independent of the sphingosine-1 phosphate receptors. Collectively, these results suggest that regulatory T cells from the periphery are recruited to the omentum during tumor development. Specific mechanisms by which Treg traffic to tumors may thus be targeted to enhance antitumor immunity and treat omental tumors.

Abstract Number 342
Cyclotron Production of Scandium Radionuclides from Titanium Dioxide Targets
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Background: Theranostic agents, labeled with radionuclides suitable for diagnostic imaging and radionuclide therapy, provide personalized medical care and may improve patient outcomes. The radioisotopes of scandium include two matched pairs that may be used for imaging and therapy, 43Sc/47Sc (T1/2 = 3.89 h and T1/2 = 3.35 d) and 44Sc/47Sc (T1/2 = 3.97 h). Unlike pseudo matched pairs (e.g. 68Ga/177Lu), these radionuclides guarantee identical chemistries and pharmacokinetic profiles for both imaging and therapeutic agents. Thus, the aim of this study at UAB was to optimize production, and investigate the in vitro and in vivo properties of PSMA-617, a prostate cancer targeting agent, labeled with these radionuclides.

Methods: Scandium-43, 44Sc, and 47Sc were produced at proton energies up to 24 MeV using titanium metal and TiO2 targets. Targets were digested and purified using the chromatographic resin N,N,N',N'-tetra-2-ethylhexyldiglycolamide. The yield and radionuclidic purity of activities were characterized using gamma ray spectroscopy. Titanium was recovered from the separation eluate via alkali precipitation. The purified scandium radionuclides were used to develop a method for the radiochemical synthesis of [43,44,47Sc]Sc-PSMA-617, which was used in in vitro studies with LNCaP (PSMA+) and PC3 (PSMA-) prostate cancer cell lines to show specific uptake to PSMA receptors.
Results: Targets were durable at beam currents of 40 uA (foil) and 20 uA (TiO2) at proton energies up to 24 MeV. Irradiated titanium metal and TiO2 were digested with an average percent dissolution of (95 ± 6)% and (96 ± 7)%. Complete digestion took 45 (foil) and 75 minutes (TiO2). Scandium was purified from titanium and vanadium radionuclides with an average recovery of (92 ± 5)%. PSMA-617 was labeled with 43,44,47Sc in 0.25 M ammonium acetate at 95 °C for 30 minutes. A >99% radiochemical yield was measured by HPLC and >95% of the radioligand was still intact at 168 hours in PBS, human serum, and mouse serum. The percent of [43,44,47Sc]Sc-PSMA-617 associated with LNCaP and PC3 cells after 4 hours of incubation at 37°C was (22 ± 1)% and (0.30 ± 0.01)%, respectively.
Conclusion: In this work, 43,44,47Sc were produced in titanium metal and TiO2, rapidly digested in 45-75 minutes, and separated from titanium with high percent recovery and radionuclidic purity. Titanium target material was recovered via alkali precipitation. In vitro studies with [43,44,47Sc]Sc-PSMA-617 showed specific targeting of the PSMA receptors on LNCaP cells. Future studies will focus on in vivo imaging using PSMA labeled with radioisotopically pure scandium activities.

Abstract Number 343
Evaluating the Order of Treatments to Enhance Efficacy of TNBC with Quantitative Imaging
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Introduction: The purpose of this study is to determine if order of combination chemotherapies (paclitaxel, doxorubicin) alters response in triple negative breast cancers (TNBCs). Locally advanced TNBC primarily relies on combined chemotherapy, however the optimal treatment strategy is still unknown. Thus, there is a need to explore how the order of regimens effects tumor response. Currently, one of the most commonly used regimens for locally advanced TNBCs is administration of doxorubicin (DRB) followed by paclitaxel (PTX). Our goal is to evaluate and compare tumor response of the order of dosing of PTX and DRB with quantitative molecular imaging, which is a noninvasive and highly sensitive approach that is able to seize the early signal of drug response prior tumor size changes.
Experimental Design: MDA-MB-231-FUCCI (Green fluorescence during S/G2/M phase; Red fluorescence during G1 phase) TNBC cells (2x10^6) were subcutaneously injected into nude mice (n = 12) and randomly assigned into three treatment groups: PTX (10 mg/kg)→DRB (10 mg/kg), DRB→PTX (same drug dosage), and saline control. 3'-Deoxy-3'-[18F]fluorothymidine ([18F]-FLT) positron emission tomography (PET)/computed tomography (CT) (SOFIE preclinical PET/CT) was performed before treatment (day 0), and on days 3 and 6. Treatment occurred on days 0 and 3. GFP and RFP fluorescence were measured through in vivo imaging system (IVIS) on baseline and on day 6. In vivo cell proliferation was quantifying normalized [18F]-FLT standard uptake value (SUV) (tumor mean/muscle mean) and normalized GFP signal (tumor/muscle). In vivo cell viability was determined by RFP signal. Statistical significance was evaluated with a t-test.

Results: [18F]-FLT PET and fluorescence imaging showed that PTX→DRB treatment significantly decreased cancer cell proliferation and viability. From day 0 to 6, overall RFP signal of control increased 14%, while DRB→PTX decreased 36%, and PTX→DRB decreased 50% (p=0.060). GFP fluorescence imaging showed DRB→PTX group increased by 3 fold (p=0.029) compared to control, while PTX→DRB treatment was similar to control (p>0.05). [18F]-FLT PET SUV revealed from day 0 to 6, control had a 110% increase, DRB→PTX showed 60% increase, while PTX→DRB showed about 10% increase (p=0.010).
Conclusion: PTX prior to DRB significantly improves tumor treatment in this TNBC model by decreasing tumor cell viability and proliferation. Imaging allows us to visualize and quantitate these cellular changes prior tumor size changes and may help guide clinical decision making for the treatment for TNBC patients.

Abstract Number 344
Structure of an Insecticidal Toxin and Engineering for Clinical Applications
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We have solved the structure of the insecticidal toxin, XptA2, at 3.2 Angstrom resolution utilizing both cryo-EM and x-ray crystallography. XptA2 contains cell surface targeting IgG-like domains constructed around a pentameric toxin delivery channel and transmembrane piercing domains. A flexible linker domain in a similar tripartite toxin ortholog was previously proposed to be the driving force for conformational change into the pore forming state after binding to cell surface targets and internalization. Conversely, our structures reveal a high degree of disorder and a lack of strain on the linker region in the pre-pore state suggesting a low amount of potential energy. We therefore challenge the idea that the linker represents a spring initiating the transition from the pre-pore state into the pore state. We first tested the hypothesis that a continuous linker is not needed for XptA2 to fold and assemble into the pre-pore pentamer. XptA2 was expressed as two halves in which the linker was truncated by a stop codon introduced into “frag1”, and translation of “frag2” was initiated by an artificially introduced Methionine start codon. Two-fragment (“2-frag”) XptA2 was able to form a mature pre-pore pentamer by negative stain electron microscopy. Experiments to convert “2-frag” to the pore state are in progress. In parallel work, we have explored the idea of engineering XptA2 for targeting mammalian cells for clinical use. Importantly, wild type XptA2 is non-toxic to mice and is cleared in a manner that is similar to other control non-toxic proteins.

Abstract Number 345
Using 3D Perfusion Bioreactor System to Study Cell Biology in Ovarian Cancer
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Background: Ovarian cancer is the most common cause of death among gynecological malignancies and the fifth leading cause of death from cancer in women. Historically, cancer research has relied on 2D in vitro models, which do not accurately mimic tumor microenvironment. Recently developed 3D perfused models (bioreactors) have the ability to recreate tumor microenvironment, which allows us to study the tumor biology and anti-tumor drug testing in more in vivo-relevant conditions. Studies in breast and neuroendocrine cancer using the bioreactor shown that histological results recapitulate the architecture of histological sections derived from

patients. Moreover, mouse tumors and xenografts cultured in the bioreactor responded positively to treatments with therapeutic agents.
Methods: The bioreactor consists of a scaffold (Matrigel and collagen type I) and is connected to a perfusion system that allows the media to flow. Ovarian cancer cell line, Skov3, was used to assess the suitability of this system for ovarian cancer studies, including the cell density and cell organization analyses. Histological and bioluminescence imaging were used as analyzing tools.
Results: We evaluated the importance of media flow for cell growth and concluded that the growth in perfuse system was higher compare to non-perfusion or solid systems.
Conclusion and future directions: 3D perfusion system is a relevant model to study cancer cells biology. In the future we will evaluate the usage of our bioreactor for analyzing tumor biology of ovarian cancer cells co-cultured with fibroblast. In addition, we will evaluate its effectiveness to support tumor tissue growth from ovarian patient-derived samples.

Abstract Number 346
Altered Differentiation of Mesenchymal Stromal Cells during Infection and Inflammation
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The bone marrow microenvironment (BMM) is a complex network of hematopoietic and non-hematopoietic cells. These cells form the stem cell niche to aid in regulation of hematopoietic stem cell self-renewal and differentiation. Within the non-hematopoietic compartment, subpopulations of mesenchymal stromal cells (MSCs) give rise to osteoblasts and adipocytes. These cells communicate with the hematopoietic system through adhesion molecules and cytokines to maintain homeostasis. Inflammation is an insult to the homeostatic environment. Inflammation's disruptive impact on the hematopoietic system has been greatly studied; but how inflammation impacts the BMM is poorly understood. During inflammatory conditions, bone loss has been noted; therefore, we hypothesize an increase in the MSC population to compensate for the defect in bone differentiation. Using widely studied models of infection, LCMV or direct Toll-like receptor stimulation, we will assess phenotypic changes by flow cytometry and functional changes by lineage differentiation. Additionally, we use lineage-tracing models for an unbiased assessment of altered stromal lineages. The lineage-specific Cre models mark stroma (Prrx1), adipocytes (AdipoQ), and early (Osx) and late (OCN) stages of osteoblasts. Our data shows increased MSCs with decreased osteoblasts and adipocytes just days after Toll-like receptor stimulation. One week later, the osteoblasts are still decreased, while the MSCs are now unchanged. However, differences in stromal cell maturation potential persist from in vivo stimulated versus in culture stimulated when differentiated into their respective lineages. In vivo stimulated cells show increased osteoblasts differentiation while cells stimulated in culture have the opposite impact on bone formation. During adipocyte differentiation, there is no change in the number of adipocytes present; however, the adipocytes are more mature from in vivo stimulation and less mature in culture. Likely, there is crosstalk between the hemtopoeitic and non-hematopoietic system that impacts the stromal compartment leading to these alterations in differentiation bias. Understanding changes in the BMM during inflammation will allow for therapeutic intervention in inflammatory diseases such as arthristis, osteomyelitis, and leukemia.

Abstract Number 347
Exosomal Markers (CD63 and CD9) Expression and Their Prognostic Significance Using IHC
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Background: Embryologically, the right-colon is derived from the midgut, whereas the left-colon is derived from the hindgut. There are clinical, pathological and molecular differences between patients with right-sided colon cancer (RSCC) and left-sided colon cancer (LSCC). Exosomes mediate intercellular communications and impact cancer progression, metastasis and chemoresistance. CD63 and CD9 are widely accepted exosomal markers, but patterns of expression and prognostic significance in RSCC and LSCC are unknown. We explored CD63 and CD9 expression and prognostic significance in RSCC and LSCC using immunohistochemistry (IHC).
Methods: Between 2015 and 2018, 63 patients underwent surgical resection of colon cancer for whom we had available tissues for IHC staining. Two pathologists independently scored the CD63 and CD9 expression in tumor and adjacent normal mucosa (ANM). Staining intensity was graded 1-3 and staining was estimated in 10% increments. Mean Quick-score (Q-score) (intensity*percentage of staining) was calculated.
Results: Median age was 64 (range 33-78). Females represented 60% of our cohort. Caucasians, African Americans and other ethnicities represented 55%, 40% and 5%, respectively. The sidedness was designated as RSCC (cecum, hepatic flexure, ascending and proximal two-thirds transverse colon) in 52% and as LSCC (splenic flexure, sigmoid, descending and distal third transverse colon) in 48%. The ANM and Tumor CD63 Q-scores were 225 vs 191 (p=0.009) in RSCC and 224 vs 154 (p=0.0001) in LSCC, respectively. The ANM and Tumor CD9 Q-scores were 134 vs 152 (p=0.142) in RSCC and 135 vs 154 (p=0.137) in LSCC, respectively. In patients with RSCC and LSCC, the mean Tumor CD63 Q-score was 191 vs 154 (p=0.024), while the mean ANM CD63 Q-score was 225 vs 224 (p = 0.920). The mean Tumor CD9 Q-score was 152 and 154 (p=0.883), and the mean ANM CD9 Q-score was 134 vs 135 (p=0.926). In our cohort, there was no difference in progression free survival (PFS) between patients with RSCC and LSCC (p=0.2349). In all patients, there was no difference in PFS with CD63 expression 100 and ≥100 (p=0.8284). Among patients with RSCC, there was a significantly lower PFS with CD63 expression 100 vs. ≥100 (p=0.0259). However, among patients with LSCC, there was no difference in PFS with CD63 expression 100 vs. ≥100 (p=0.3494).
Conclusions: This is the first study to show a difference in exosomal marker expression pattern and its prognostic significance in RSCC and LSCC. There was a significant positive correlation between progression free survival in RSCC with higher exosomal expression.

Abstract Number 348

Relationship Between Physical Activity and Insulin Levels Among African Amercian Women

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African American women are disproportionately affected by cancer, diabetes, and obesity. Regular moderate-to-vigorous physical activity (MVPA) has been associated with reduced risk of cancer, diabetes, and obesity and can help regulate insulin, which plays a major role in the

development of these conditions. However, African Americans have been underrepresented in research to date in this area, despite their related health disparities. Thus, our study examines the relationship between changes in self-reported minutes of MVPA and insulin levels among African American women. Secondary data analyses were conducted from a pilot randomized controlled trial of a 6-month physical activity intervention for African American women in the Deep South (N=84). Blood draws and physical activity assessments were conducted at baseline, 6 months, and 12 months. As previously reported, larger increases in physical activity from baseline to six months and 12 months were found in the intervention vs control arm. Insulin levels increased, on average, from baseline to 6 months in both arms (by 2.81 ± 8.01 μM/L for intervention and 3.53 ± 9.34 μM/L for control) and slightly declined from 6 to 12 months (-2.30 ± 6.94 μM/L and -0.81 ± 5.10 μM/L, respectively). There was no relationship between changes in insulin and MVPA from baseline to 6 months but trends in the data from 6-12 months indicated a slight inverse relationship between changes in insulin and physical activity controlling for condition (P= 0.078), with decreases in insulin levels associated with increased physical activity. Findings help confirm physical activity's potentially ameliorating effect on insulin levels over the long term. Moreover this study extends this line of work to an undeserved population and has implications in terms of risk reduction and the elimination of health disparities.

Abstract Number 349
Merlin Tumor Suppressor Loss Induces Redox Imbalance in Breast Cancer
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Merlin is encoded by the NF2 gene and as a tumor suppressor stalls cell proliferation by contact-dependent growth inhibition. Our laboratory has also observed that Merlin-deficient breast cancer cells became more glycolytic revealing Merlin's role in metabolic phenotype. After conducting an untargeted metabolomics analysis, we registered that the levels of glutathione (GSH), the most abundant antioxidant cofactor, were decreased with concomitant increase in the accumulation of reactive oxygen species (ROS) upon lack of Merlin, indicating a defect in ROS clearance mechanisms. Therefore, Merlin may play a role in the redox system as well. In order to acquire a comprehensive panel of the expression levels of different redox-associated genes in Merlin-deficient breast cancer cells, I conducted a Human Oxidative Stress RT^2 array. Interestingly, it was detected increased gene expression of NOX4, DUOX1 and DUOX2, members of the NADPH oxidase (NOX) family, in Merlin-deficient breast cancer cells compared to their control counterpart. Concordantly with the upregulation seen in the RT^2 array, the protein levels of NOX4, DUOX1 and DUOX2 were validated by immunoblotting. STAT3 signaling has been reported to activate NOX4 expression and we have previously detected elevated STAT3 activity upon Merlin loss. By treating Merlin-deficient breast cancer cells with STATTIC, a STAT3 signaling inhibitor, not only NOX4 but also DUOX1 and DUOX2 protein levels were decreased, suggesting STAT3 as the mechanistic pathway by which these protein expression are upregulated. The next steps are to check ROS accumulation in Merlin-deficient cells upon STATTIC or NOX inhibitor treatment. This will further support ROS generation by the STAT3-NOX node in Merlin-deficient cells. In conclusion, ROS accumulation in Merlin-deficient breast cancer cells may be resultant of defects on ROS clearance and increased ROS production.

Abstract Number 350

AMPLIFY: A Multimedia Diet and Exercise Intervention for Cancer Survivors

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The "Aim, Plan, and Act on Lifestyles," or AMPLIFY (P01 CA22997), program is a randomized controlled clinical trial of two computer-based diet and exercise interventions for cancer survivors. The study will enroll 652 cancer survivors over the age of 50 across four states to investigate how these two interventions affect health behaviors and conditions (including weight status and comorbidity), medical costs, quality of life, muscle mass, and other factors over a two-year period. The intervention is currently under development and entering beta testing, set to be implemented in 2020. Under funding from the CaRES program (5R25 CA076023), the focus has been to develop a number of video modules for the diet intervention educating the participants on healthy diet habits. The aim is to provide engaging and interactive modules to allow the participants to take a more active role in health education and behavior modification.

Abstract Number 351

Characterizing Novel EGFR Mutations driving Cetuximab Resistance in Head and Neck Cancer

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Purpose: Epidermal growth factor receptor (EGFR) is a protumorigenic receptor tyrosine kinase. Head and neck squamous cell carcinoma (HNSCC) are treated with Cetuximab (CTX), a therapeutic monoclonal antibody, which binds to the extracellular domain of EGFR and inhibits EGFR signaling. CTX has been successfully used as monotherapy and in combination with radiotherapy in HNSCC, however treatment failure occurs frequently due to the acquisition of resistance. In this study, we investigated potential mechanisms underlying CTX resistance in HNSCC.

Methods: Human HNSCC cells (UMSCC-1) were developed as Cetuximab-resistant (CTX-R) cells after several months of continuous exposure to CTX. The DNA sequence and status of EGFR gene was established by DNA sequencing. The levels of total and activated EGFR, and downstream signaling events (Akt, mTOR, NF-κB, STAT3) were assessed by immunoblotting. EGF- and CTX-binding were assessed using labeled EGF and CTX, respectively, with flow cytometry and EGFR sorting and degradation was observed using immunofluorescence assays. Mutational analyses was done by standard Sanger sequencing.

Results: We determined that persistent CTX significantly increased EGFR gene copy numbers, and levels of EGFR mRNA and activated EGFR in CTX-R cells. We identified three mutations in the EGFR extracellular domain in CTX-R cells, which were non-synonymous producing amino acid substitutions (G33S, N56K, and A313V). PyMOL structural modeling analyses predicted that G33S and N56K mutations possibly drive a physical interaction between domain I and III in the inactive state thereby sterically hindering the potential binding site for EGF and CTX. This was confirmed by the reduced affinity for EGF and CTX binding in the CTX-R cells. The A313V

substitution in domain II appears to trap the receptor in an active confirmation in the absence of ligand, leading to constitutive activation of EGFR and its downstream pathways. CTX resistant cells also exhibited impaired sorting and degradation of EGFR contributing to elevated EGFR levels.
Conclusions: Our results demonstrate that upon prolonged exposure to CTX, cells selected for novel activating mutations specific to the EGF and CTX binding domain as well as the dimerization domain. These missense mutations not only reduced the receptor's affinity for both EGF and CTX, but possibly trapped the receptor in an active confirmation that rendered EGFR constitutively active. These data suggest that prolonged exposure to CTX may increase monoclonal antibody therapy resistance in head and neck squamous cell carcinoma.

Abstract Number 352
Comparison of Panitumumab-IRDye800CW and 5-ALA for Fluorescence Guided Surgery
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Introduction: Maximal safe resection of malignant tissue is associated with improved progression-free survival and better response to radiation and chemotherapy for glioblastoma (GBM) patients. 5-aminolevulinic acid (5-ALA) is the current standard for intraoperative brain tumor visualization. Unfortunately, autofluorescence in diffuse areas and high fluorescence in dense tissues significantly limit discrimination at tumor margins. The present study is the first to compare 5-ALA, an FDA-approved agent, to an investigational new drug, panitumumab-IRDye800CW, in the same animal model.
Methods: A patient-derived xenograft (PDX) model was created by orthotopically injecting GBM (JX6) cells from a human patient into female nude mice (n = 16). Two weeks post-implantation, mice received tail-vein injections of 5-ALA, panitumumab-IRDye800CW, IRDye800CW (a nonspecific control), 5-ALA and IRDye800CW, or 5-ALA and panitumumab-IRDye800CW. Brains were harvested and prepared for multi-instrument fluorescence imaging, immunohistochemistry (IHC), and quantitative analysis of tumor-to-background ratio (TBR) and tumor margin accuracy. Statistical analysis was compared with Wilcoxon rank-sum or paired t-test.
Results: Panitumumab-IRDye800CW was found to have a 30% higher comprehensive TBR compared to 5-ALA ($p = 0.0079$). Standard deviations for core and margin regions of interest in 5-ALA-treated tissues were significantly higher than those outlined in tissues treated with panitumumab-IRDye800CW ($p = 0.0240$ and $p = 0.0284$, respectively). Panitumumab-IRDye800CW specificities for tumor core and margin were over 10% higher than those of 5-ALA. Higher area under the curve for panitumumab-IRDye800CW indicated strong capability to discriminate between normal and malignant brain tissue when compared to 5-ALA.
Conclusion: This work demonstrates that panitumumab-IRDye800CW shows potential as a targeting agent for fluorescent intraoperative detection of GBM. Improved margin definition and surgical resection using panitumumab-IRDye800 has the potential to improve surgical outcomes and survival in GBM patients compared to 5-ALA.

Abstract Number 353
Glucagon Regulates Energy Balance via FGF21 Signaling in the Brain
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Glucagon is an essential regulator of glucose and lipid metabolism that also promotes weight loss. Thus, novel therapeutics that stimulate glucagon-receptor (GCGR) signaling are promising targets for treatment of obesity and diabetes; however, the mechanism(s) underlying these effects are yet to be fully elucidated. We previously identified that hepatic glucagon signaling increases the secretion of another fasting hormone, Fibroblast Growth Factor 21 (FGF21), also known to be involved in regulating energy balance. We have recently observed that mice deficient for liver Fgf21 ($Fgf21^{\Delta liver}$) are partially resistant to the anti-obesity effects of GCGR agonism, clearly implicating hepatic FGF21 as an essential component of glucagon's weight-loss effects. FGF21 signals through the canonical FGF-receptors (FGFR-1c, -2c, and -3c) coupled with an obligate co-receptor (Beta Klotho, Klb). FGFR-1c, -2c, and -3c populate a vast array of tissues. However expression of KLB, and therefore FGF21 signaling, is limited to adipose tissue, liver, and brain, specifically within the suprachiasmatic nucleus (SCN) of the hypothalamus and the hindbrain. As the hypothalamus has known roles in regulating energy balance, we hypothesized that the anti-obesity action of the glucagon-FGF21 system signals through a central mechanism. To test this hypothesis, we generated mice with neuronal Klb deficiency (Klbflox x Synapsin1Cre: $Klb^{\Delta CNS}$). $Klb^{\Delta CNS}$ mice are less susceptible to diet-induced obesity than control mice (p=0.01), with no observed differences in food intake or energy expenditure. Following chronic GCGR activation via the selective GCGR agonist IUB288, $Klb^{\Delta CNS}$ mice exhibit a partial reduction in body weight (11%) in comparison to control mice (18%) (p=0.001), suggesting that FGF21 mediates glucagon's anti-obesity properties through central action. Similar with the congenital knockout, wildtype mice treated with a selective KLB antagonist via intracerebroventricular administration also exhibited partial reductions in body weight with chronic GCGR agonism. Consistent with GCGR-stimulated, neuronal FGF21 signaling, we found that neuronal activation, measured via immunohistochemical analysis of cFos expression, was increased in the SCN following IUB288 injection. Future studies will aim to interrogate SCN-specific FGF21 signaling via targeted adenoviral Cre recombinase induction. Taken together, these data suggest that glucagon mediates part of its anti-obesity properties through FGF21-KLB signaling in the CNS and has implications for future treatments against obesity and the metabolic syndrome.

Abstract Number 354

Adapting an Evidence-Based Physical Activity Intervention for Web-based Delivery

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Purpose: Cancer survivors may reduce their risk of mortality and enhance recovery by implementing healthy lifestyle changes, such as regular physical activity. Therefore, our goal was to translate the interactive learning and group-based support components of an evidence-based physical activity intervention into a web-based platform, specifically targeted towards older, rural cancer survivors. This objective was part of a larger intervention development entitled Aim plan and act on lifestyles: AMPLIFY Survivor Health.

Methods: Intervention component translation was based on focus group input, evidence related to the core components (or "active ingredients") of the original evidence-based physical activity intervention, an a priori comprehensive matrix (or rubric) of weekly intervention topics, and an iterative process involving staff, content experts, and webpage design team.

Results: The eLearning course development software Articulate Storyline 360 was chosen to translate the educational aspects of the intervention because it facilitated interactive learning (an intervention core component) accomplished in the original intervention through in-person discussion. Interactive elements prioritized in the session development included opportunities for

self-report to dictate the path of the user experience and knowledge games. Interactive sessions were limited to no more than 15 minutes per week. Team level decisions were made regarding colors, characters, music, etc. to facilitate cohesive appearance of sessions throughout the multi-week intervention. Significant staff time was required to gain proficiency in software use and integrate iterative input. To translate the group support aspect of the original intervention, a Business Facebook account was created to oversee pages and groups related to the study. Secret Facebook groups, exclusive based on intervention receipt, were created as outlets for giving and receiving social support for study participants. A moderator protocol was developed based on a literature review to facilitate group support while also keeping the conversations on topic and without any explicit content being posted. The moderator protocol includes responsibilities for the moderator/s, such as scheduling weekly posts for the group discussion boards, reviewing the threads daily for inappropriate comments and taking the necessary follow up actions, and replying to Facebook messages from participants.
Conclusions: Web-based delivery may be a viable strategy to improve physical activity patterns and health outcomes among rural cancer survivors. However, there are several challenges to the translation and implementation of virtually-delivered behavioral interventions including time required to learn the web-based platform, adapt the content, and build the sessions for dissemination in a manner that optimizes engagement.

Abstract Number 355
Anti-proliferative Effect of Cannabinoid in Canine Lymphoma
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Biomedical sciences, Auburn University

Non-Hodgkin lymphoma (NHL) is the fifth leading cause of human cancer death and is the second fastest growing cancer with regard to mortality in people. Likewise, lymphoma is one of the most common neoplasms encountered in dogs. It accounts for about 20% of all canine cancers and about 85% of blood cancers, with an annual incidence up to 134 per 100,000 dogs. Canine and human lymphoma are generally characterized by a high rate of initial remission following conventional CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone) based therapies; however, 95% of dogs and 30% of humans will succumb to drug-resistant relapse. To date, lymphoma is still a serious condition, it is essential to develop novel strategies to improve the outcome of patients suffering from aggressive or therapy-resistant lymphoma. Cannabinoids have been used in human patients with cancer for their palliative effects (e.g., inhibition of chemotherapy- induced nausea, vomiting, apatite stimulation and pain) since the early 1970s. However, in addition to palliative interventions, cannabinoids have demonstrated anticancer effects on various xenograft animal models of cancer, Cannabinoid appear to decrease tumor progression by at least two mechanisms: potentiating apoptotic death of tumor cells and inhibition of tumor angiogenesis. The dog may be an extremely useful model for the study of lymphoma in humans, owing to striking similarities in histology, biology and gene expression. Objective of our study was to evaluate cannabinoid receptors (CB1 and CB2) expression and anti-proliferative effect of cannabinoid in Canine lymphoma. We used two canine lymphoma B cell lymphoma cell lines (17-71 and CLBL1) and normal canine lymphocytes to characterize the expression of cannabinoid receptors using real time PCR. We found positive expression of cannabinoid receptor CB1 and CB2 in both lymphoma cell lines and normal canine lymphocytes. To find the anti-proliferative effect of cannabinoid we used endocannabinoids (2AG and AE), phytocannabinoids (CBD and THC) and synthetic cannabinoid agonists and antagonists. Cells were incubated for 24 and 48 hours with the drugs in five different concentrations, (100nM, 5000nM, 1 µM, 25 µM and 50 µM). We used untreated cells in media as a control and looked at the anti-proliferative effect of cannabinoids using MTT assay. We found significant time and dose dependent anti-

proliferative effect with AEA, CBD, synthetic agonists WIN55-212-22 and HU-210. Our results suggest that cannabinoids could be developed as novel therapeutic agents for the treatment of canine and human lymphoma.

Abstract Number 356

Characterization of Low-Density Neutrophil Subpopulations in Chronic Inflammation

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Neutrophils represent the first innate immune cell population recruited to the sites of trauma and infection. Recent studies have demonstrated that neutrophils represent a heterogeneous population consisting of subpopulations with distinct phenotype and function. A subset of low-density neutrophils (LDNs) were identified co-purifying in the peripheral blood mononuclear cell (PBMC) layer following density gradient centrifugation in patients with inflammatory conditions. While the origin and function of LDNs are unclear, immunosuppressive functions have been ascribed to this population in multiple diseases including cancer and chronic infections. Increased frequency of LDNs has been associated with the progression of vascular dysfunction. Multiple Myeloma (MM) is associated with an increased risk for developing secondary complications including cardiovascular disease and heart failure. We hypothesize that chronic inflammation leads to an induction of specific subpopulations of neutrophils contributing to the development of thrombosis and CVD in individuals with MM. We have identified two distin357ct LDN subpopulations within the PBMC layer that display significant phenotypic, morphologic, and functional differences. The first subpopulation is characterized as CD16+CD64low suggestive of a mature neutrophil phenotype (mLDN); the second population is characterized as CD16-CD64high consistent with an immature neutrophil phenotype (imLDN). The imLDN subset exhibit elevated levels of LOX-1 and CD66b and a lower level of CD14 compared to the mLDN subset. We have optimized a method for the identification of the immature neutrophil subset (imN) in whole blood using CD64 and CD16 surface markers without the need for density gradient centrifugation allowing for characterization of imNs close to their in vivo state. Sorted imLDNs have a banded morphology consistent with immature neutrophil maturation states. Sorted mLDNs have a similar morphology to whole blood neutrophils evidenced by multi-lobular nuclei. Functional assessments demonstrate that the mLDN subset have a higher capacity to produce reactive oxygen species compared to imLDNs, consistent with their immature phenotype. Determination of the characteristics of the two subpopulations will be instrumental in the elucidation of their role in the development and treatment of secondary complications in MM.

Abstract Number 357

Acarbose Promotes Anti-tumor Immunity and Improves Outcomes in Preclinical Kidney Cancer

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Despite advances in treatments for metastatic renal cell carcinoma (RCC), response rates remain suboptimal. Immune-based therapies such as anti-PD-1 checkpoint blockade generate durable responses in 20-30% of patients, while targeted therapeutics including mTOR inhibitors (temsirolimus, everolimus) achieve response rates of less than 10%. Recent findings demonstrate that caloric restriction and its mimetics (CRMs), including glucoregulatory agents, can augment

chemotherapy responses in murine models of fibrosarcoma and other subcutaneous cancer models by enhancing anti-tumor immunity. However, the effects of these agents on therapeutic efficacy in metastatic kidney cancer are unknown. Here, we studied acarbose, an alpha-glucosidase inhibitor and anti-diabetic with known CRM properties, in a preclinical model of kidney cancer. BALB/c mice were orthotopically injected with syngeneic luciferase-tagged renal carcinoma (Renca) cells and randomized to either a control diet or an isocaloric acarbose-containing diet. As expected, acarbose blunted post-prandial glucose spikes in mice. Therapeutic administration of acarbose impeded primary renal tumor growth at Day 21 (p=0.02) and Day 25 (p=0.0003) after tumor challenge. This protective effect was dependent on CD8 T cells. Furthermore, whereas tumor burdens from control-fed mice increased by 77% between Days 21 and 25, tumors from the acarbose group increased by only 34%. We also observed a trend towards reduced spontaneous lung metastases with acarbose at Day 21 (p=0.07), which was not maintained at Day 25. Notably, mice on acarbose had increased frequencies of intratumoral CD8 T cells at Day 21 (p=0.03); these T cells possessed equivalent IFNg, TNFa, perforin, and granzyme B production as counterparts from control-fed mice. At Day 25, intratumoral CD8 T cells from mice on acarbose were more activated (CD44+CD62L-), exhibited an early-effector phenotype (CD127-KLRG1-), and expressed higher frequencies of PD-1. Based on these observations, we hypothesized that acarbose would synergize with targeted mTOR inhibitors and immune checkpoint-based therapies for advanced-stage RCC. Combining acarbose administration with either anti-PD-1 or Rapamycin resulted in significantly reduced lung metastases, and trending reductions in primary tumors, relative to control-fed mice given the same therapies. Acarbose-induced reductions in lung metastases with Rapamycin were dependent on CD8 T cells. Future studies will focus on elucidating the mechanisms by which acarbose improves CD8 T cell immunity to renal tumors. Although more studies are needed, our findings suggest that combining acarbose with RCC therapeutics is feasible and may improve responses in advanced renal cancer patients in a cost-effective manner.

Abstract Number 358

STAT3 Signaling Pathway Mediates Drug Resistance in Chronic Myeloid Leukemia through AMPK

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Drug resistance is one of the major problems confronting cancer therapeutics. One ideal model to understand the mechanism of drug resistance is chronic myeloid leukemia (CML). In this disease, hematopoietic stem cells acquire a single translocation between chromosome 9 and 22, forming BCR-ABL, a constitutively active tyrosine kinase. The standard regimen for CML is hence tyrosine kinase inhibitors (TKI), but only some patients can be taken off TKI without the risk of recurrence due to CML stem cells being drug resistant. Our preliminary data and previous studies have shown an increase in active STAT3 in drug resistant CML cells. Inhibition of STAT3 using a small molecule inhibitor induced apoptosis of the K562-resistant CML cells, but had minimal effect on the TKI-sensitive cells. Furthermore, single cell RNA-sequencing of the stem and progenitor cells from CML mice reveals a unique transcriptional signature, of which Fos and Dusp1 have already been proven important for resistance, also known to be regulated by STAT3. Interestingly, ChIP-seq using phosphorylated STAT3 revealed localization to unique sites upon TKI treatment,

mostly genes regulating metabolic pathways. Functional analysis using seahorse showed that TKI treated CML cells are more glycolytic compared to CML cells that have more oxidative phosphorylation. This metabolic switch could be due to dysregulation of mitochondrial activity in the resistant cells as demonstrated by reduced electron complex chain protein expression as well as activity. Increased glycolysis suggests reduced production of energy equivalents, proved by an increase in AMP to ATP ratio via untargeted metabolic profiles. Protein microarray and western blots corroborate this result by an increase in the activated AMPK (5' AMP-activated protein kinase). Moreover, inhibiting STAT3 also reduced AMPK activation, suggesting STAT3 regulation of AMPK for metabolic reprogramming towards glycolysis conferring the CML cells resistant to TKI. Understanding this mechanism will help develop novel drug therapies specifically targeting drug resistant leukemic cells in a BCR-ABL independent manner.

Abstract Number 359

MEK1/2 Inhibition as a Therapeutic Target in a NRAS-mutated Pediatric Neuroendocrine Tumor

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Introduction: RAS is a commonly mutated proto-oncogene and regulates downstream cell signaling pathways such as PI3K and MAPK. Our lab encountered a pediatric high-grade neuroendocrine tumor with a NRAS mutation. Trametinib, a MEK1/2 inhibitor, has been shown to effectively impede cell and tumor growth in adult NRAS-mutated malignancies but has not been demonstrated in NRAS-mutated pediatric solid tumors.
Objectives: Our aim was to provide preclinical evidence for Trametinib as targeted therapy for a NRAS-mutated pediatric solid tumor.
Methods: Next-generation sequencing (NGS) was performed using the patient's sample. A patient derived xenograft (PDX, COA109) was established. COA109 cells were treated with increasing doses of Trametinib. Cell viability, proliferation, growth and motility were measured with alamarBlue®, CellTiter 96®, trypan blue exclusion, and Boyden chamber assays, respectively. Immunoblotting examined the effects of MEK1/2 inhibition on downstream cell signaling protein expression. Mice bearing COA109 tumors were treated with Trametinib and tumor growth examined.
Results: A NRAS mutation was identified by NGS. DNA sequencing and histology validated the establishment of the COA109 PDX. In vitro studies of COA109 cells treated with Trametinib demonstrated a LD50 of 7.8 nM and IC50 of 4.5 nM. With 1 nM of Trametinib, cell growth and motility were significantly inhibited. Immunoblotting, following MEK1/2 inhibition with Trametinib, showed decreased phosphorylation of ERK and AKT and decreased c-Myc expression, signifying decreased cell proliferation; and increased cleaved caspase 3 and cleaved PARP, indicating apoptosis. In vivo tumor growth was diminished in the Trametinib treated mice.
Conclusions: Trametinib, a MEK1/2 inhibitor, significantly decreased tumor cell viability and growth both in vitro and in vivo through downregulation of cell proliferation signaling and an upregulation of apoptotic signaling. Decreased cell motility highlights an investigable role for Trametinib in preventing metastasis. These studies suggest the effectiveness of Trametinib and provide preclinical justification for targeting MEK1/2 in NRAS-mutated pediatric solid tumors.

Abstract Number 360

The Role of the Long Noncoding RNA Gas5 in the Clonogenic Potential of Hematopoietic Stem

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Long noncoding RNA (lncRNA) is a class of long untranslated transcripts that can regulate the interactions between DNA, RNA, and proteins to regulate gene expression. Growing evidence has demonstrated that lncRNAs play critical roles in alteration of gene expression in human diseases including cancer. Growth arrest specific 5 (GAS5) is a lncRNA that functions as a tumor suppressor by inhibiting cell cycle progression and inducing apoptosis in solid tumors. Recent studies also revealed that GAS5 has a novel role in stem cell self-renewal. However, its role in the cell cycle and stemness of hematopoietic cells is unknown. Database analyses indicated that Gas5 in highly expressed in undifferentiated hematopoietic cells and leukemic cells compared to terminally differentiated cells. Therefore, we hypothesize that Gas5 is critical for the stemness of hematopoietic stem and progenitor cells. To examine the expression level of Gas5, we determined the expression of Gas5 in different mouse tissues using qPCR and Northern Blot. Our result confirmed that Gas5 is highly expressed in the bone marrow and spleen compared to other tissues. To examine Gas5 expression level throughout hematopoiesis, we measured Gas5 expression level in hematopoietic subpopulations isolated from mouse bone marrow. The results demonstrated that Gas5 is highly expressed in undifferentiated hematopoietic cells, and is decreased in differentiated cells. Furthermore, to examine the function of Gas5 in hematopoietic stemness, we overexpressed and knocked down Gas5 in mouse bone marrow by lentiviral transduction followed by serial replating assay. Our results indicate that Gas5 overexpression increases, while Gas5 knockdown decreases the colony number. These findings suggest that Gas5 is critical to maintain the clonogenic potential of hematopoietic stem and progenitor cells. To determine the mechanism by which Gas5 increases clonogenic potential, we overexpressed Gas5 in Lin-/cKit+ mouse hematopoietic progenitor cells, and performed RNA sequencing. Interestingly, we found that Gas5 overexpression decreases the expression of E2F transcription factor target genes. Since E2F is known to promote cell cycle progression, we speculate that Gas5 may antagonize E2F function to induce quiescence, prevent stem and progenitor cell exhaustion, and maintain repopulating capacity. Further studies will provide insight on the significance of lncRNAs in hematopoietic stem and progenitor cell functions.

Abstract Number 361

DOCK3 is a Novel Regulator of Myoblast Fusion and Normal Muscle Function

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DOCK (dedicator of cytokinesis) proteins are an 11-member family of typical guanine exchange factors (GEFs) expressed almost exclusively in the brain and sprain cord. We previously identified DOCK3 as being strongly upregulated in Duchenne muscular dystrophy (DMD), specifically in the skeletal muscles of DMD patients and mice. Guanine nucleotide exchange factors (GEFs) are direct positive regulators that mediate GDP/GTP exchange and promote the binding of Rho proteins to effectors. Rho GTPases play a regulatory function governing differentiation, proliferation, morphogenesis, and cell migration via the activation of Rac1 and its subsequent rearrangement of the actin cytoskeleton. Here, we aimed to characterize the function of DOCK3

in normal and dystrophin-deficient mice. We performed RNA-sequencing on Dock3 KO muscles and found a critical fusogen, Myomixer (Mymx), to be significantly downregulated. Dock3 KO mice on the dystrophin-deficient background exacerbated skeletal muscle and cardiac phenotypes. Dock3 KO mice appeared to have poor muscle architecture, locomotive activity, and impaired skeletal muscle regeneration. Additionally, Dock3 KO mice had significant cardiac defects similarly observed in DMD mouse models. Together, these studies demonstrate a functional role for DOCK3 in muscle function and DMD disease progression.

Abstract Number 362
Dual Kinase Inhibition to Combat EGFR-inhibitor Resistance in Glioblastoma
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Glioblastoma (GBM) is an aggressive primary brain tumor with a poor survival rate. One of the most common molecular alterations seen in GBM is amplification and/or mutation of the Epidermal Growth Factor Receptor (EGFR), which has made it an attractive therapeutic target. However, several EGFR tyrosine kinase inhibitors have been tested clinically in GBM with minimal success. One reason for this lack of efficacy could be due to acute, adaptive resistance via alternative pathway activation. To investigate this mechanism of tumor resistance, we used RNA-seq and multiplex inhibitor bead/mass spectrometry (MIB-MS) to analyze the transcriptomes and kinomes of genetically engineered murine astrocytes with common GBM genotypes. We have previously shown that 38% of the expressed kinome varied among a panel of diverse nGEM astrocytes harboring Cdkn2a deletion (C) plus Pten deletion (CP), wild-type human EGFR (CE) or EGFRvIII (CEv3) overexpression or both EGFRvIII overexpression and Pten deletion (CEv3P). Although CE have a similar transcriptional profile to C cells at baseline, when treated with the EGFR inhibitor afatinib, CE respond more similarly to CEv3 cells. When cells containing endogenous murine EGFR (C and CP) were treated with afatinib, fewer than 0.5% of kinases showed differential expression. In cells with EGFR overexpression alone, more than 6% of kinases were differentially expressed upon afatinib treatment, including Ntrk3, Fgfr2 and 3, Lyn, Bmx, Epha2 and 5, Fn3k, a kinase involved in fructosamine processing, and Nrbp2, a kinase involved in regulation of apoptosis. This effect was blunted in cells with EGFRvIII, but lacking Pten (CEv3P), resulting in less than 2% of kinases being differentially expressed. The only kinase upregulated in all three EGFR-overexpressing cell types was Coq8a, which is involved in electron transport and response to DNA damage. Given this overlap in response, Coq8a could be a potential dual treatment target for GBM.

Abstract Number 363
Mitochondrial Regulation of Cyclin E Controls Cell Cycle of Ovarian Tumor Initiating Cells
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Self-renewing, therapy resistant ovarian tumor initiating cells (ovTICs) clearly contribute to development of epithelial ovarian cancer (EOC) and its recurrence, which is incurable. In certain tumors and contexts, ovTICs are dependent on mitochondrial energetics, specifically the production of ATP and reactive oxygen species (ROS). Mitochondrial energetics can be repressed through high fragmentation of mitochondria through elevated activity of mitochondrial

fission protein Dynamin-related protein 1 (Drp1). Conversely, repression of Drp1 activity can augment mitochondrial energetics and upregulate stemness markers. Drp1 activity is regulated over the cell cycle and is lowest at the G1-S transition, governed by the cell cycle regulator and EOC oncogene Cyclin E. We and others demonstrated that Drp1 repression increases levels of Cyclin E while disruption of mitochondrial energetics can lead to its degradation and activation of a G1-S checkpoint. In differentiated cells, Cyclin E is rapidly degraded after S-phase entry, but in normal and neoplastic stem cells it is often expressed throughout the cell cycle. The Mitra lab have previously reported a novel pool of Cyclin E localized to mitochondria. This pool can be ablated by inhibition of the ATP synthase, which also selectively kills ovTICs. We hypothesize that regulation of this mitochondrial pool by Drp1-mediated fission and ROS production governs the self-renewal of tumor initiating cells. We identified a Cyclin E fragment which exhibits higher mitochondrial localization than the full length protein, which is primarily nuclear. We also established that Cyclin E protein abundance and localization are dependent on ROS levels. We then studied cell cycle and mitochondrial properties in ovTICs after FACS-sorting based on a TIC marker (Aldh). OvTICs showed lower Drp1 activity and increased levels of Cyclin E protein than the bulk tumor cells. Additionally, media designed to maintain TICs increased mitochondrial localization of Cyclin E specifically in a population of ovTICs with low Drp1 activity and high mitochondrial ROS production. These data suggest that Drp1-mediated mitochondrial fission paired with ROS production regulates Cyclin E to maintain the cell cycle of ovTICs and likely TICs of other tumors, as we have found in transformed keratinocytes. Understanding this regulation will lead to more effective targeting of TICs. We will now establish whether Cyclin E turnover regulation is the mechanism by which Drp1-dependent modulation of ROS production impacts Cyclin E protein abundance and activity.

Abstract Number 364

The Evaluation of 89Zr-Pt@TiO2-SPHINX Nanoconjugate as an Anti-Angiogenic Radio/Photo Sensitizer

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Prostate cancer is the most common cancer malignancy in men and is an important cause of cancer deaths. Thus, the development of novel strategies for performing combined prostate cancer imaging and therapy is important and would have a significant impact on patient care. A novel nanoconjugate was designed for use in PET imaging, optical imaging and as a radio/photo sensitizer. The nanoparticle includes Pt@TiO2 nanoparticles conjugated with SPHINX which has known anti-angiogenesis properties. The PET imaging potential of Pt@TiO2-Spx was evaluated by 89Zr radiolabeling. Characterization of the nanoconjugate was conducted with HPLC, XRD, EDS, FTIR, TEM and DLS analyses. Furthermore, the potential of Pt@TiO2-Spx as an anti-angiogenic radio/photo sensitizer was evaluated by fluorometric and radiochromatographic methods, cytotoxicity, cell uptake/internalization, ELISA assays and photodynamic therapy/radio therapy combinations. In vitro assays showed that Pt@TiO2-Spx could be radiolabeled with 89Zr efficiently with sufficient stability for future studies. Pt@TiO2-Spx binds specifically to prostate cancer cells (PC3 and LNCaP), and was found to be non-toxic with lower binding to normal prostate cells (RWPE-1). ELISA results illustrated that Pt@TiO2-Spx strongly increases the anti-angiogenic VEGFA165b. Furthermore, Pt@TiO2-Spx behaves as a radio/photo sensitizer and decreases the cell viability of prostate cancer cells when subject to photodynamic and radio therapy. In vitro results are promising for future in vivo studies of Pt@TiO2-Spx as a PET imaging agent and anti-angiogenic radio sensitizer.

Abstract Number 365

Identification of Amino Acids Incorporated During Suppression of CFTR Nonsense Mutations

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A nonsense mutation is a single nucleotide substitution in a genomic DNA sequence that generates an in-frame premature termination codon (PTC). PTCs account for ~11% of all disease-associated mutations and ~10% of cystic fibrosis (CF) patients carry a PTC on at least one CFTR allele. Translation of a PTC-containing mRNA terminates at the PTC and produces a truncated, usually nonfunctional, polypeptide. PTC suppression therapy, termed readthrough, utilizes small molecules that suppress translation termination at a PTC to restore synthesis of a full-length polypeptide. Readthrough is mediated by the base pairing of a near-cognate aminoacyl-tRNA with the PTC at two of the three nucleotide positions, allowing its associated amino to become incorporated in the nascent polypeptide chain. However, little is known about the identities of the amino acids incorporated upon readthrough or the functionality of the full-length CFTR proteins generated. To investigate this, we created reporter constructs that express CFTR nonsense mutations in their native mRNA contexts. After readthrough was induced using the aminoglycoside G418, we used mass spectrometry to identify and quantitate the amino acids that were incorporated. At the G542X mutation (a UGA PTC), we found cysteine, tryptophan, and arginine incorporated upon readthrough. At the W1282X mutation, also a UGA PTC, we found cysteine, tryptophan, and a novel residue leucine, but no arginine. As the reporter constructs were identical except for the endogenous mRNA sequences flanking each PTC, these results indicate that the PTC sequence context influences which amino acids become incorporated upon G418-mediated readthrough. Frequently, the amino acids inserted upon readthrough of CFTR nonsense mutations are different from what is encoded in wildtype CFTR. Some of these proteins can exhibit reduced maturation and activity. However, both a CFTR corrector and a potentiator enhanced the activity of the variant proteins generated by G418-mediated readthrough. This suggests that PTC suppression therapy in combination with CFTR modulators may be beneficial for the treatment of CF patients who carry a PTC. Currently, we are investigating how the mRNA sequence surrounding the PTC influences aminoacyl-tRNA accommodation. We anticipate our mechanistic insights into nonsense suppression can also be applied to diseases other than CF. Germline nonsense mutations in tumor suppressor genes such as TP53, RB, and APC are frequently observed in cancer patients. Cancer-related disorders, such as the RASopathy NF1, are frequently caused by nonsense mutations. Therefore, we believe our study has implications for future cancer therapies for patients harboring nonsense mutations.

Abstract Number 366

Examining the Role of CERUS in Characterizing Indeterminate Renal Lesions in CKD

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Introduction: The prevalence of chronic kidney disease (CKD) in the United States is estimated to be >14%. One difficulty among the many that arise when caring for these patients is accurate diagnosis of renal lesions without use of intravenous contrast. Contrast Enhanced Renal Ultrasound (CERUS) is a diagnostic tool with the potential to allow for more precise imaging without the nephrotoxic effects of standard contrast in patients with indeterminate renal lesions and CKD.

Objectives: To compare the results of CERUS with other imaging modalities in both renal-sufficient and insufficient patients when evaluating indeterminate renal lesions.
Methods: A retrospective chart review of patients who underwent CERUS from 2014 to 2015 at a single institution was performed with data collection focused on renal function, prior imaging of renal lesions, and final clinical and pathological diagnoses. The main imaging modalities patients underwent prior to CERUS included Computed Tomography (CT), Magnetic Resonance Imaging (MRI), and non-contrast enhanced ultrasound. Patients were separated into two cohorts based on renal function with an eGFR <60 defining the CKD cohort. Comparisons were made between the cohorts based on the results and findings of prior imaging modalities and follow-up CERUS performed.
Results: A total of 169 patients had CERUS completed in 2014-2015. There were 104 patients with eGFR <60 classified as having CKD. A comparative analysis of categorical variables was done using chi-squared and Fisher's exact test. CERUS provided specific, new, and/or confirmed diagnoses of renal lesions previously labeled "indeterminant" by other imaging modalities in 22 (81.5%) of CKD patients compared to 17 patients (80.9%) with normal renal function (p=0.96). CERUS also resulted in a change in Bosniak classification of cysts in 12 (25.0%) of CKD patients compared to 11 (33.3%) renal-sufficient patients (p=0.41). Finally, CERUS resulted in a change in the number of lesions in 2 (1.9%) CKD patients compared to 5 (7.7%) renal-sufficient patients(Fisher's-Exact-p=0.11).
Conclusion: CERUS can be used to diagnose and follow renal lesions in patients with CKD who otherwise may not be able to undergo imaging with nephrotoxic contrast. Though US has its limitations, CERUS can help differentiate and further classify indeterminate renal lesions that may be concerning for malignancy. Perhaps patients with normal renal function have better complex cystic lesion characterization and detection of otherwise occult renal lesions based on improved blood flow characteristics. Further studies are necessary to validate these findings and further elucidate the mechanisms for the findings to optimize imaging selection in patients with compromised renal function.

Abstract Number 367
GTP Cyclohydrolase 1 in Brain Tumor Initiating Cell Maintenance
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Glioblastoma (GBM) is a deadly disease due to the high degree of intratumoral heterogeneity that contributes to treatment failures. One cellular subset enriched for the capacity to propagate tumor growth and promote therapeutic resistance consists of brain tumor initiating cells (BTICs). We previously determined BTICs have elevated expression of GTP Cyclohydrolase I (GCH1), the rate limiting enzyme in the pathway for synthesis of tetrahydrobiopterin (BH4). BH4 is an important co-factor utilized by synthetases in nitric oxide and catecholamine production. We have demonstrated that elevated expression of GCH1 correlated with poor patient survival and decreased survival in mice bearing orthotopic xenografts. Mechanistically, GCH1 elevation did not strongly alter nitric oxide levels but did significantly lower reactive oxygen species (ROS) levels. We therefore sought to identify binding partners of GCH1, particularly those associated with oxidative stress/antioxidant pathways, that could mediate GCH1 effects. We have also repurposed FDA approved drugs to target the GCH1 pathway and inhibit the synthesis of BH4 in an effort to extend survival. Our data suggests novel mechanisms for GCH1 effects and demonstrate in vitro efficacy of novel drug combinations against GBM cell growth.

Abstract Number 368

Hematological Disorders in Human Patients with SON Mutations

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Precise regulation of transcription and RNA splicing is critical for controlling hematopoietic cell fate determination and lineage differentiation. Alteration of expression of lineage-specific transcription factors and several core spliceosome components in hematopoietic malignancies highlight the significance of abnormal transcription and RNA splicing as disease-causing factors. Our group previously demonstrated that SON, a large nuclear speckle protein possessing dual abilities to bind both DNA and RNA, functions as a splicing factor as well as a transcriptional repressor. We recently identified heterozygous loss-of-function mutations in the SON gene from children with intellectual disability and developmental delay often with a broad spectrum of other congenital anomalies. The disorder caused by SON haploinsufficiency has been designated as ZTTK syndrome (Zhu-Tokita-Takenouchi-Kim syndrome; OMIM #617140). The majority of the mutations found in these patients are frameshift or nonsense mutations which cause degradation of the mutation-bearing transcript. While the most prominent features of these patients are brain malformations and musculoskeletal abnormalities, we identified various hematological disorders from children with ZTTK syndrome. Notable symptoms include bone marrow failure, severe anemia, thalassemia, polycythemia, polycythemia vera, leukocytopenia, and immunoglobulin deficiency, many of which are often considered as pre-leukemic conditions. To investigate how altered SON expression affects hematopoiesis, we generated a mouse line with the Son gene deleted specifically in the hematopoietic lineage. Homozygous deletion of Son in hematopoietic lineage led to embryonic lethality, indicating that SON expression in blood cells is indispensable during development. Mice with heterozygous deletion of Son in the hematopoietic lineage were viable and born without notable defects or sign of diseases. However, there is a significant decrease in bone marrow cellularity in the mice with heterozygous deletion of Son. Furthermore, Son haploinsufficiency decreased the size of the lineage negative (Lin-) cell population and an increase in megakaryocyte/erythrocyte lineage-biased multipotent progenitors (MPP2) within hematopoietic stem/progenitor cells. These findings suggest that the level of Son expression potentially affects stem cell maintenance and MPP lineage bias, and the distortion of the subpopulation balance within hematopoietic stem/progenitors is possibly linked to multiple hematological disorders. Our ongoing analyses of hematopoiesis and gene expression changes using this mouse model will expand our knowledge about the role of SON in several hematological disorders and benefit clinical practice for ZTTK syndrome patients.

Abstract Number 369

MCL1 Binds and Negatively Regulates the Transcriptional Function of Tumor Suppressor p73

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MCL1 is an anti-apoptotic member of the Bcl-2 family, which has become a popular therapeutic target in cancer research over the past decade due to its propensity to promote cancer cell survival and facilitate chemoresistance. Through a phage display screen, we identified a novel MCL1 binding motif that is the reverse of the canonical BH3-binding helix that mediates interactions amongst Bcl-2 family members. Upon a BLAST sequence analysis of native proteins, we identified a putative reverse BH3 (rBH3) motif in the tumor suppressor protein, p73. Here, we

show that MCL1 and p73 bind through a direct protein-protein interaction. Using fluorescence polarization assay, we characterize the strength of this interaction and reduce binding down to the rBH3-containing alpha helix in the tetramerization domain of p73. To elucidate biological function, we employ electrophoretic mobility shift assay to show that excess MCL1 negatively impacts p73 binding to DNA. Finally, using two p53-/- cancer cell lines, we demonstrate that MCL1 inhibits the transcriptional function of p73 through analysis of p73 target gene expression. In summary, we characterize a novel protein-protein interaction between MCL1 and p73 and show that MCL1 negatively impacts p73 target gene activation. This work establishes a novel function of MCL1 outside of apoptotic regulation at the mitochondria and provides new evidence of cross talk between the Bcl-2 family and the DNA damage response pathway.

Abstract Number 370

NO and H2S on Mitochondrial Bioenergetics After Hypoxia-Reoxygenation

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Ewing Sarcoma is the second most common bone cancer in children and young adults with few available treatments. A hallmark feature of these tumors are pockets of hypoxic tissue which contribute to its aggressive nature. While limitatons on oxygen availability can decrease essential processes in energy metabolism, many cancers have developed distinct metabolic adaptations: the Warburg and the Crabtree Effects. The Crabtree Effect refers to a suppression of oxidative phosphorylation by glycolysis when oxygen becomes limiting while the Warburg effect describes and increase in glucose uptake and lactate production regardless of oxygen availability. Understanding metabolic regulators of these characteristic differences between cancerous and healthy tissues can lead to safer and more targeted therapeutics. It has been observed that both nitric oxide (NO) and hydrogen sulfide (H2S) possess dose-dependent, biphasic effects. NO has potent anti-tumor effects through modulating cancer-related events such as angiogenesis and metastasis at low physiological, inducing cell death at higher concentrations achieved using exogenous NO-donors. Likewise, H2S may both protect from oxidative damage and, at high concentrations, inhibit mitochondrial respiration and suppress cancer cell proliferation. In this study, Ewing Sarcoma cells (A-673) were treated with DetaNONOate (DetaNO, an NO donor) and/or diallyl disulfide (DADS, a H2S donor) in a model of hypoxia (1% O2) and reoxygenation (21% O2). To assess interactions with glutaminolysis and TCA cycle metabolites, L-glutamine was removed from the assay medium prior to evaluating mitochondrial function using mitochondrial stress tests by the Seahorse Extracellular Flux Analyzer. We conclude that NO inhibits oxygen consumption rate (OCR) and induces reoxygenation damage regardless of the availability of glutaminolysis and its metabolites. In the absence of L-glutamine, DADS stimulates mitochondrial function, but, when combined with DetaNO, DADS enhances the inhibitory and damaging effects of NO.


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Abstract Number 400
Health Behaviors among Metastatic Cancer Survivors in the Deep South
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Introduction: Approximately 5 million Americans are living with metastatic cancer. Metastatic cancer survivors (MCS) are at an increased risk for poor health behaviors, which may negatively influence quality of life and survival time. The American Cancer Society (ACS) guidelines suggest that cancer survivors: 1) consume ≥5 servings of fruits and vegetable per day; 2) engage in ≥150 minutes of moderate physical activity per week; 3) achieve/maintain a healthy bodyweight (18.5≥BMI≤24.9); 4) refrain from smoking; 5) and limit alcohol consumption. The aim of this study was to asses ACS guideline adherence among MCS receiving care at UAB.
Methods: MCS were identified via UAB Cancer Registry and I2B2. Using a modified Dillman survey method, eligible MCS (>21 years and physician permission to contact) were mailed questionnaires that assessed fruit and vegetable intake (Eating at America's Table Screener [EATS]), physical activity (Godin Leisure-Time Exercise Questionnaire), height, body weight, smoking status, and alcohol consumption. Returned surveys were double-key entered into REDCap®. Data were analyzed using SPSS. Descriptive statistics were used to characterize the study sample and instrument scores.
Results: To date, 200 surveys have been returned. Respondents (107 female; 93 male; 176 Caucasian; 24 African American; Mage=66 years; Msurvivorship=35 months) were representative of the identified census of MCS receiving care at UAB (34% female cancers; 27% gastrointestinal; 19% genitourinary; 14% pulmonary; 5% melanoma; 1% other). MCS reported low daily fruit and vegetable intake (M=4.11) and weekly moderate physical activity (M=20.62), with 70% of respondents being overweight or obese. Half of respondents (n=100) reported a history of smoking, with 6% currently smoking. MCS who reported alcohol use (33%), consume an average of 3.5 drinks per week.
Discussion: Findings suggest that MCS may not be adherent to the ACS health behavior guidelines. Further research is needed to better understand facilitators and barriers to health behavior change among MCS. **Acknowledgements:** First author was supported by the NCI-funded Cancer Prevention and Control Training Program (T32-CA04788). Study was supported by Health Disparities Research Education Program Pilot Project Award (PI: Bail).

Abstract Number 401
Skeletal Muscle Index Predicts Postoperative Nutrition after Pancreatoduodenectomy
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Introduction: Sarcopenia, defined as the loss of skeletal muscle mass, is a component of cachexia and fatigue among pancreatic cancer patients and has been significantly associated with decreased overall survival and major postoperative complications following pancreatoduodenectomy (PD). Limited research has investigated postoperative nutritional requirements for sarcopenic patients compared to non-sarcopenic patients following PD.
Objectives: This retrospective study investigated the relationship between preoperative skeletal muscle index (SMI) and postoperative supplemental nutrition in patients undergoing PD.
Methods: Study population consisted of all patients who underwent an elective, non-traumatic PD from 2013 to April 2018. The skeletal muscle area was CT-assessed at the third lumbar vertebra (L3) and indexed to patient height to calculate SMI. Sarcopenia was based on sex-specific cutoffs of SMI (≤52.4 cm2 /m2 in men and ≤38.5 cm2 /m2 in women).

Results: We identified 134 patients in the study period undergoing PD. Median SMI of males (n=60) was 50.67 cm2 /m2 and females (n=74) was 37.18 cm2 /m2. The median SMI of female patients who required tube feeds at discharge (dTF) compared to females who did not was lower (31.97 [IQR 28.44-36.83] vs. 38.01[IQR 34.77-45.77] cm2 /m2; p= 0.019). The median SMI of male patients who required dTF compared to patients who did not was also lower (45.11 [IQR 39.92-50.65] vs. 51.85 [IQR 46.52-57.15] cm2 /m2; p= 0.020). Receiver operating characteristic (ROC) curve analysis suggested SMI may be used to predict dTF for both females (AUC = 0.752) and males (AUC = 0.717). Youden's index analysis of these curves suggested a SMI cut-off of ≤32.5 cm2/m2 for females and ≤42.9 cm2/m2 for males as prognostic of dTF. Standard cutoffs for sarcopenia did not show a significant association with dTF by univariate logistic regression; however, the above proposed SMI cutoffs did correlate with dTF for both female (OR: 14.05, 95% CI; p= 0.0013) and male (OR: 8.60, 95% CI; p=.0036) patient populations.
Conclusion: There is a significant association between low SMI and postoperative enteral nutrition requirements, suggesting these patients may benefit from placement of a feeding tube at time of resection.

Abstract Number 402

Racial/ethnic Differences in Healthy Lifestyles Promotion in Older Cancer Survivors

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Background: Cancer care provider recommendations are critical in promoting healthy lifestyles (HLS) among cancer survivors. To better understand potential gaps in HLS counseling from medical providers this analysis investigates racial/ethnic differences in the occurrence of HLS discussions between older cancer survivors and their providers.
Methods: This secondary data analysis utilized cross-sectional survey data from older cancer survivors (≥65 years) seen at 12 Southeastern cancer centers at the University of Alabama at Birmingham (UAB) Health System Cancer Community Network (CCN) during 2013-2015. The main outcome was self-report of HLS discussions regarding exercise, diet, or weight management with providers (oncologists, nurses, and/or another doctor). Race/ethnicity was categorized as white and minority (African-American, and other). Descriptive statistics were calculated for survivor demographic and clinical data. Bivariate comparisons were calculated. Odds ratios (OR) and 95% confidence intervals (CI) compared odds of HLS discussions by race/ethnicity using multivariate logistic regression.
Results: This sample included 1,460 cancer survivors of mean age 74 years (SD 6). Our sample was majority white (81%), female (60%), college-educated (62%), >1 year post-diagnosis (84%), and overweight and/or obese (64%). Compared to white survivors, minority survivors were less often college-educated (51% vs. 65%), more often younger (67% vs. 56%), obese (40% vs. 25%), and more often reported pain (66% vs. 58%) and distress (59% vs.49%). A higher proportion of minority survivors reported discussing exercise (59% vs. 47%), healthy diet (61% vs. 52%), vegetable consumption (38% vs. 26%), and weight loss (41% vs. 31% respectively) with their providers than white survivors. After adjusting for demographic and clinical characteristics, minority respondents had higher odds of HLS discussions regarding exercise (OR 1.4, 95% CI

1.08-1.90), vegetable consumption (OR 1.5, 95% CI 1.12-2.03), and on all three main HLS topics (exercise, diet, and vegetable consumption) (OR 1.4, 95% CI 1.04-2.07). Similar to white respondents, minorities reported discussing HLS with another doctor more often than with oncologists and nurses. **Conclusion:** Additional studies are needed to understand reasons for differences in HLS discussions in each racial/ethnic survivor group. Strategies are needed to increase oncologists' and nurses' promotion of HLS discussions in older adults.

Abstract Number 403

Role of Prognostic Information in Shared Decision Making among Women with Metastatic Breast Cancer

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Context: Increasing emphasis on patient-centered care has led to highlighted importance of shared decision making, which better aligns medical decisions with patient care preferences. Effective shared decision-making in metastatic breast cancer (MBC) treatment requires prognostic understanding, without which patients may receive treatment inconsistent with personal preferences.
Objectives: To assess MBC patient and provider perspectives on the role of prognostic information in treatment decision making.
Methods: We conducted semi-structured interviews with MBC patients and community oncologists and separate focus groups involving lay navigators, nurses, and academic oncologists. Qualitative analysis utilized a content analysis approach which included a constant comparative method to generate themes.
Results: Of 20 interviewed patients with MBC, 30% were African American. Academic oncologists were mostly women (60%), community oncologists were all Caucasian, and nurses were all women and 28% African American. Lay navigators were all African American and predominately women (86%). Five emergent themes were identified. (1) Most patients wanted prognostic information but differed in when they wanted to have this conversation, (2) Emotional distress and discomfort was a critical reason for not discussing prognosis, (3) Religious beliefs shaped preferences for prognostic information, (4) Healthcare professionals differed on prognostic information delivery timing, and (5) Providers acknowledged that an individualized approach taking into account patient values and preferences would be beneficial. **Conclusion:** Most MBC patients wanted prognostic information, yet varied in when they wanted this information. Understanding why patients want limited or unrestricted prognostic information can inform oncologists' efforts towards shared decision-making.

Abstract Number 404

Biomarkers Associated with Tumor Cathepsin L Gene Expression and Ki67 in Prostate Cancer Patients Participating in a Weight Loss Trial

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Our previous presurgical weight loss trial among men with prostate cancer found that rapid (but not slow) weight loss resulted in increased tumor Ki67, as well as increased Cathepsin L (CTSL) expression. In follow-up analyses, we strove to better understand these unexpected findings. Serum free fatty acids (FFA) and inflammatory cytokines were analyzed, and studied in relationship with Ki67, body composition, physical activity (PA), fecal microbiome and tumor gene expression data for both the weight-loss intervention (WLI) and control study arms. Cross-sectional and longitudinal associations between biomarkers were assessed with Spearman correlations. Paired sample t-tests compared within group changes in biomarkers and analysis of covariance was used to assess between group changes in the subsample of participants (n=12) with gene expression data. Positive associations were observed between changes in percent body fat and serum FFA (p=0.026) as well as Interleukin-6 (IL-6, p=0.041). WLI lost more weight (-6.8kg vs. -0.3kg, p=0.002) and lean mass (-1.8kg vs. 0.2kg, p=0.029), and had increased Ki67 (+5% vs. -8.1%, p=0.001), with no differences between groups in physical activity, diet, and inflammatory cytokines. Change in Ki67 was inversely associated with change in lean mass (p=0.001) and CTSL (p=0.148). CTSL was positively associated with change in PA (p=0.015) and change in lean mass (p=0.102). Relative abundance of Bacteroidetes was inversely associated with CTSL (p=0.016) and change in PA (p=0.019); relative abundance of Firmicutes was positively associated with CTSL (p=0.021) and change in PA (p=0.003). Greater fat loss was accompanied by greater decreases in FFA. Negative energy balance driven by increased physical activity was associated with increased tumor proliferation rate and decreased Cathepsin L protease activity accompanied by distinct differences in the two primary bacterial phyla of the fecal microbiome. These findings warrant future studies in larger cohorts.

Abstract Number 405
Pilot Trial of a Web-based Lifestyle Intervention for Cancer Survivors
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Background: Cancer survivors need effective home-based healthy lifestyle interventions, like RENEW (tailored workbook, 4 tailored quarterly newsletters, 8 tailored telephone prompts, and 15 telephone counseling calls). The prevalence of Internet-delivered lifestyle interventions have been increasing and show potential for reaching large numbers of survivors. Thus, formative research was conducted to adapt RENEW for web delivery and the resulting site, SurvivorSHINE, was beta tested. The purpose of the current study is to examine changes in physical activity, knowledge, and BMI from a small pilot trial of SurvivorSHINE (www.survivorshine.org).
Methods: This 3-week single-arm pilot trial of SurvivorSHINE assessed healthy lifestyle knowledge (e.g., recommendations for diet and exercise)), levels of physical activity, and anthropometrics at baseline and follow-up (roughly 3-weeks later). Paired T-tests were used to measure changes in knowledge, physical activity (self-report), and weight status.
Results: Forty-one participants completed baseline assessments. The average age of participants was 61.8 years, 61% (N=25) were White, 58.5% (N=24) were women, 58.5% (N=24) were married, and 48.8% (N=20) were retired. Most women (N=20, 83.3%) were breast cancer survivors, while most men (N=13, 46.5%) were prostate cancer survivors. To date, 34 participants have completed follow-up. Data show significant improvements in both knowledge ($t = 5.31$, $p = 0.000$) and physical activity ($t = 2.23$, $p = 0.033$). However, participants did not experience significant changes in BMI.
Conclusions: Preliminary results from this pilot trial support further investigation of web-delivered lifestyle interventions among cancer survivors. Significant increases in healthy lifestyle knowledge

and physical activity are promising, but future directions should include larger studies with control conditions to help determine efficacy.

Abstract Number 406

Targeting GLI1-mediated NBS1 Transcription Overcomes 5-FU Resistance

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Resistance to radiation and chemotherapy in colorectal patients is considered one of the major contributors of refractory disease and disease progression. Dysregulation or aberrant activation of the SHH/GLI1 signaling pathway, common to both colorectal cancer and other cancer subtypes, influences the integrity of the DNA damage response (DDR) pathway, impacting tumor response to chemo- and radiation therapies. The DDR pathway is initiated in part by the recognition of a DNA lesion by a sensor protein and the recruitment NBS1 to that lesion. Subsequent repair is initiated by the Mre11, Rad50, NBS1 (MRN) complex formation. In this study, we investigated the expression of GLI1 and NBS1 in tissues samples from 188 patients diagnosed with colorectal cancer by immunohistochemistry and analyzed the clinical significance and prognostic relevance. GLI1 expression was positively associated with the NBS1 expression, and high expression of both in the biopsied specimens significantly correlated with poor patient survival. The result of a series of biochemical experiments directly links GLI1 transcriptional activity to the DNA damage repair pathway. First, NBS1 mRNA levels and protein were found to be reduced upon treatment with GANT61, a GLI inhibitor, indicating that GLI activity was required for NBS1 transcription. Second, overexpression of NBS1 in HT29 cells rescued GANT61-induced cell death by measure of cleaved caspase-3, indicating a significant role of induced DNA damage in the mechanism of GANT61-induced cell death in oncogenic GLI1 cancers. Third, this was verified by ChIP analysis of GLI1 binding to the NBS1 promotor region containing a putative GLI binding sequence. The efficacy of GLI1 inhibition as therapeutic strategy was then tested in parallel with 5-Fluorouracil (5-FU). In vitro, colorectal cell lines with GLI1-driven NBS1 expression demonstrated strong 5-FU resistance. These cells were re-sensitized to 5-FU by silencing the expression of NBS1 with siRNA, or with combination therapy involving 5-FU and GANT61. This suggests that a first-in class chemotherapeutic strategy may provide additional treatment options for patients demonstrating 5-FU resistance during the course of their chemotherapy. While GANT61 is commonly used to inhibit GLI in vitro, it exhibits poor pharmacokinetic properties and is not a viable drug for the clinic. To that end, we present a novel GLI1 antagonist SR38832, which exhibits a more stable pharmacokinetic profile than GANT61 in xenograft mouse models and lower IC50 in colon cancer cell lines. In a human colorectal cancer xenograft mouse model, SR38832 significantly inhibited both tumor growth and proliferation.

CHAUDHURI
AWARD

Abstract Number 500
Non-competitive Off-target Inhibition of Proteasome by BTK Inhibitors
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Inhibitors of Bruton's Tyrosine Kinase (BTK) and inhibitors of the proteasome are currently in use for treatment of hematologic malignancies. While the proteasome is necessary for protein homeostasis in all mammalian cells, BTK is unique to B-cell malignancies. However, BTK inhibitors and LU-102, a specific inhibitor of the beta site of the proteasome have previously been shown to synergize in hematologic malignancies which do not express BTK, at a 100-fold higher concentration than is needed for complete inhibition of BTK, suggesting an off-target effect of these BTK inhibitors. Triple Negative Breast Cancer (TNBC), a cancer with poor prognosis and no current targeted therapy, also does not express BTK. We found that LU-102 and a specific BTK inhibitor, CGI-1764, are de-facto synthetically lethal to TNBC cells, and that effect of other BTK inhibitors varied from similar synergy to no synergy. This data further supports the idea that synergy is due to off-target effects of BTK inhibitors. We found that CGI-1764 is a non-competitive, allosteric inhibitor of all catalytic subunits of the proteasome 20S proteolytic core and exerts its effect in a unique, dose-dependent manner. Furthermore, CGI-1746 and LU-102 synergize in inhibiting intracellular protein degradation. These findings may pave the development of more potent allosteric inhibitors of the proteasome, and suggest that kinase inhibitors should be screened for inhibition of the proteasome as potential off-target effect.

Abstract Number 501
In Vivo Breast Tumor Stiffness Recapitulated in A Microvascularized Tumor-On-A-Chip
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In vitro recapitulation of the native tumor microenvironment (TME), including stiffness, can be achieved by leveraging tissue engineering toolsets, thereby overcoming the limitations of 2D cell culture systems. However, in addition to providing cell-cell and cell-matrix interactions, an endothelialized, physiologically relevant and perfused vascular network is required for engineered cancer tissues for examining anti-cancer drug targeting and delivery. Here we established engineered breast cancer tissues within a vascularized, tumor-mimetic microfluidic chip model and modulated the stiffness of these on-chip engineered cancer tissues to replicate in vivo tumor stiffness. To assess in vivo and in vitro engineered tumor mechanical stiffness, breast tumors (MDA-MB-231 flank xenografts in athymic nude mice) and engineered breast cancer tissues were subjected to parallel plate compression testing. Tumor mimetic (SynvivoTM) microfluidic chips were employed to recapitulate the tumor vasculature; The chips vascular network was designed to provide differing flow regions that recapitulate the geometry of in vivo tumor vasculature. Breast tumor-associated endothelial cells (hBTECs) were seeded in the vascular network and formed a lumenized vascular lining. Metastatic breast cancer cells MDA-MB-231 and human foreskin fibroblast BJ5ta (ATCC) cells were mixed (5:1) with PEG-fibrinogen polymer precursor solution, loaded into the primary tumor compartment and crosslinked for 2 minutes under visible light. To recapitulate the different in vivo tumor regions (periphery, midpoint, core), engineered breast cancer tissue stiffness was modulated using PEG-diacrylate. In vivo breast tumor stiffness at the tumor periphery, midpoint and core was found to be within the range of the 3D engineered breast tumor tissues with time in culture through day 29. In the vascularized microfluidic chip, cancer cells from the engineered breast cancer tissue were observed to intravasate, circulate in the

endothelial vascular channel, adhere and migrate to the secondary chamber, recapitulating the process of metastasis. In the native TME there are known to be regional differences in drug diffusion; TRITC dextran (4.4 kDa) was administered at a constant flow rate through the chips' vascularized networks and found to have vascular network geometry and engineered tumor construct stiffness dependent differences in diffusion into the primary tumor chamber, mimicking this in vivo phenomena. Overall, we have developed a novel tumor mimetic microfluidic chip with an intricate vascular design that can be used for demonstrating a high degree physiological relevance of the native breast TME which facilitates the understanding of complex tumor heterogeneity in an in vitro platform with real-time visualization capabilities for potential anti-cancer drug screening.

Abstract Number 502

Sox2 Drives ST6Gal-I Expression to Promote Cancer Stem Cell Traits in Ovarian Cancer

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This study elucidates a novel, glycosylation dependent pathway driven by stem cell transcription factor, Sox2, to promote cancer stem cell (CSC) characteristics. Herein we identify Sox2 as a transcriptional regulator of ST6Gal-I, a sialyltransferase that adds α2-6-linked sialic acid to N-glycosylated proteins. ST6Gal-I is upregulated in many cancers, including 98% of ovarian tumors, and high ST6Gal-I expression correlates with reduced survival in high-grade serous ovarian carcinoma. However, the transcriptional drivers of ST6Gal-I expression in stem-like cells remain largely unknown. In the current study we determined that Sox2 and ST6Gal-I are both located on one of the most commonly amplified chromosomal segments in cancer, amplicon 3q26. Analyses of TCGA databases revealed that 48 out of 73 cancer cohorts, including ovarian cancer, display an increase in SOX2 and ST6GAL1 gene copy number. To determine whether Sox2 and ST6Gal-I are co-expressed, we performed immunoblotting experiments across a range of ovarian cancer cell lines and found a strong correspondence between the levels of Sox2 and ST6Gal-I. In addition to being genetically co-amplified, the presence of Sox2 response elements in the ST6GAL1 promoter suggested that Sox2 may induce transcription of ST6Gal-I. Indeed, chromatin immunoprecipitation (ChIP) assays confirmed that Sox2 binds to the ST6GAL1 promoter. Using ovarian cancer cell model systems, we forced overexpression (OE) or knockdown (KD) of Sox2, and consistently found that high expression of Sox2 directly induces expression of ST6Gal-I. In cells with Sox2 OE, ST6Gal-I mRNA and protein levels rise, and there is an increase in overall α2-6 surface sialylation, as measured by SNA labelling. Contrarily, Sox2 KD causes a decrease in ST6Gal-I mRNA and protein, as well as surface α2-6 sialylation. Finally, given the relationship between Sox2 and ST6Gal-I in the promotion of a CSC phenotype, we profiled the expression of stemness markers, Nanog and Oct4. Sox2 OE increases, while Sox2 KD decreases, expression of Nanog and Oct4. Importantly, differential ST6Gal-I expression can reverse the effects of Sox2 manipulation on stem cell markers. Specifically, ST6Gal-I KD in cells with Sox2 OE inhibits the Sox2-induced expression of stem cell markers, whereas ST6Gal-I OE in cells with Sox2 KD restores marker expression. These collective results suggest that Sox2 drives ST6Gal-I expression, and that this relationship contributes to the promotion of a CSC phenotype.

Abstract Number 504

Characterization of MTHFD1L Expression and Role in Breast Cancer Progression

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Breast cancer (BrCa) remains the one of the most common epithelial malignancies and is a common cause of cancer-related death for women in the United States. Despite advancements in diagnosis and treatment options, it remains a major cause of cancer related death. For 2019, there are an estimated 268,600 new cases of BrCa projected, with approximately 41,000 estimated deaths. Recent advances, including experiments using high-throughput technologies, suggest that diverse genetic, epigenetic, and environmental factors influence BrCa initiation and growth, which can lead to incurable metastatic disease. Molecular events such as BRCA1/2 mutations are well known for their involvement BrCa initiation, unregulated growth, invasion, and metastasis, but the steps involved are not fully known. Breast cancers are sub-classified into various molecular subtypes: Her2-positive, luminal A/B (estrogen and progesterone receptor positive), and triple negative (TNBC). In many cases, patients respond initially to treatments, but the heterogeneity of the tumor allows for the disease to re-emerge. Thus, there is an urgent need to understand the molecular events and signaling pathways that contribute to BrCa growth, metastasis, and therapy resistance in order to develop new therapeutic options. Characterizing cancers by gene expression profiling and next-generation sequencing has led to the identification of tumor-specific signatures and oncogenic targets. Several groups have implemented microarrays and next-generation sequencing to analyze BrCa specimens. Our analyses of breast cancer datasets identified that the methylenetetrahydrofolate dehydrogenase (NADP+ Dependent) 1-like (MTHFD1L) is overexpressed in breast cancer. Furthermore, our investigation identified that overexpression of these enzymes predicts poor outcome in breast adenocarcinoma patients. Our functional studies using breast cancer cell lines in vitro showed that MTHFD1L is critical for various breast cancer functions. Thus, MTHFD1L, being an enzyme involved in a critical metabolic pathway required for cancer cells and amenable to small molecule inhibition, can serve as a therapeutic target.

Abstract Number 505

Health Insurance Literacy and Financial Hardship in Women Living with Breast Cancer

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Background: In patients with metastatic breast cancer (MBC), low health insurance literacy may be associated with adverse material conditions, psychological response, and coping behaviors due to financial hardship (FH). This study explores associations between FH and health insurance literacy in women receiving MBC treatment.

Methods: This cross-sectional study utilized data collected from 84 women receiving MBC treatment at two cancer centers in the Southeastern US. FH was defined by lifestyle changes due

to medical expenses, financial toxicity, and medical care modifications due to cost. Low health insurance literacy was defined as not knowing premium or deductible costs. Mean differences were calculated using Cramer's V. Associations between FH and health insurance literacy were estimated with adjusted generalized linear models.
Results: Half of surveyed patients had low health literacy, 26% were underinsured, 45% had private insurance, 39% Medicare, and 15% Medicaid. Patients with low health literacy more often reported borrowing money (19% vs. 4%, V=0.35), inability to pay for basic necessities like food, heat, or rent (10% vs. 4%, V=0.18), and skipping a procedure (8% vs. 1%, V=0.21), medical test (7% vs. 0%, V=0.30), or treatment (4% vs. 0%, V=0.20) when compared to patients with high health literacy. Median Comprehensive Score for Financial Toxicity was 23 (IQR 17-29). In adjusted models, health insurance literacy was not associated with financial toxicity.
Conclusion: Low health insurance literacy was common in women receiving MBC treatment. Further research to increase health insurance literacy could lessen undesirable material and unnecessary behavioral financial hardship associated with cancer-related care.

SOONG
AWARD

Abstract Number 600
Detecting Glioblastoma Multiform Survival Markers: Finding the Needle in the Haystack
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Informatics Institute, UAB

In this work, we report the GBM patient survival modes built upon expression of three genes: LEF1, PPBP, and RPL39L. These models achieve the 1-year average survival prediction accuracy/AUC of 0.65/0.68 in the TCGA-GBM microarray gene expression and 0.67/0.71 in the RNA-seq gene expression. The predictive performance is closely comparable to the performance of state-of-the-art GBM survival models on gene expression. The interesting point in this report is the process to detect these three genes by the integration of expression dataset, GBM-annotated gene set collection from the domain knowledge, customized statistics, and machine learning techniques. We compared the LEF1-PPBP-RPL39L model with three baseline models. First, the fully literature-based model, built from the comprehensive 1200 GBM-associated genes from the literature collected in the PAGER database yields the low accuracy/AUC of 0.52/0.58. Second, the fully statistical-based model, built from 3990 genes detected by the Cox regression p-value (0.05), achieves the accuracy/AUC of 0.51/0.54. Third, the fully machine-learning-based (Random Forest) model, built from 370 genes, achieve the accuracy/AUC performance of 0.67/0.70. The number of genes overlapping among the three baseline models is 25. The 3-genes (LEF1, PPBP, and RPL39L) belong to the intersection of the three baseline-model genes. These results suggest that although applying machine learning would significantly increase the survival prediction accuracy and return a significantly small set of marker candidates, the domain knowledge are still very helpful to filter out potential noise in the machine learning models to discover GBM survival markers.

Abstract Number 601
Acquired Resistance to Tyrosine Kinase Inhibitors in EGFR-driven Glioblastoma
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Glioblastoma (GBM) is a devastating primary brain tumor with limited treatment options. Extensive molecular characterization has revealed two particularly frequent mutations: CDKN2A deletion (50-60%) and EGFR (40-50%). EGFRvIII (~35%) is a constitutively active truncation mutant with exons 2-7 deleted. EGFR is a particularly attractive therapeutic target due to frequent activating mutations, such as EGFRvIII, and ready availability of multiple targeted inhibitors. Several EGFR tyrosine kinase inhibitors (TKI) have failed clinically, due in part to acquired resistance. To mechanistically examine this type of resistance, we used genetically-engineered mouse astrocytes harboring homozygous deletions of Cdkn2a, as well as EGFRvIII (CEv3). CEv3 astrocytes were made intrinsically resistant to the EGFR TKI gefitinib or erlotinib via long-term exposure, both in vitro and in vivo. We found that long-term gefitinib or erlotinib exposure conferred variable levels of cross resistance to a panel of second- and third-generation EGFR TKI (Δ IC50 1.12-36.1-fold), relative to non-resistant parent lines. We have previously shown that dynamic kinome reprogramming may be responsible for TKI resistance in glioblastoma. Therefore, we used a chemical proteomics method, multiplexed inhibitor beads and mass spectrometry (MIB-MS), to examine changes in the expressed and functional kinome, in both the

presence or absence of one of several EGFR TKI known to penetrate the blood-brain barrier. Additionally, we performed RNA sequencing (RNA-seq) to inspect transcriptomic alterations in response to these drugs. RNA-seq showed that resistant CEv3 mouse astrocytes clustered separately from their non-resistant in vitro and in vivo counterparts. Acquired resistance also induced transcriptome alterations governing cellular metabolism, including upregulation of metabolic pathways and downregulation of RNA processing genes. Importantly, the kinase transcriptome was rewired, as 67 kinases (of 422 expressed in an RNA-seq dataset) were differentially expressed across parental and resistant cell lines (Q<0.001). Probing the dynamic kinome response to the EGFR TKI afatinib, using combined results from RNA-seq and MIB-MS experiments, identified multiple potential kinases involved in acute, adaptive resistance. Integrated kinome profiling using RNA-seq and MIB-MS in murine models of GBM with defined mutational profiles provides a powerful framework to define novel therapeutic targets that could significantly alter current treatment paradigms.

Abstract Number 602

Host Genetics Impact Glioma Progression in a Collaborative Cross-based Mouse Model

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Gliomas are diffusely invasive brain tumors with fatal outcomes and few effective treatments. Precision medicine focuses on targeting the genetics of individual tumors, but not host genetics, despite studies that have linked germline polymorphisms with glioma risk. Accordingly, glioma survival studies in mice utilize genetically variable tumors on identical host genetic backgrounds, which fails to differentiate between cancer cell-autonomous (CCA) and tumor microenvironment (TME) effects on glioma progression and host survival. The Collaborative Cross (CC) is a panel of genetically diverse mouse strains derived from both wild- and traditional inbred laboratory strains that facilitates high-resolution genetic mapping in models of complex disease. Here, we implement a novel platform to discover genetic modifiers of both CCA and TME phenotypes using genetically defined orthotopic murine allograft gliomas and CC hosts. We stereotactically injected Nf1;Trp53-/- oligodendrocyte progenitor-derived mouse tumor cells into syngeneic C57BL/6 control mice and 14 different CC strains. Seven strains survived significantly longer than controls (P=0.05), suggesting slower tumor growth (Gs, growth slow). The remaining 7 strains survived similarly to controls, suggesting fast growth (Gf, growth fast). Variable tumor growth in CC mice suggests that genetic background influences molecular processes in the TME that inhibit or potentiate tumor growth, respectively. To identify candidate genes, we performed RNA sequencing on 36 tumors from 3 Gf strains, 4 Gs strains, and controls. 134 genes were differentially expressed among Gf, Gs, and control tumors (P=0.05). Hierarchical clustering on these genes revealed that Gs strains clustered separately from Gf and controls. Gene ontology analysis using GOrilla showed 30 enriched processes, (FDR q0.001), all of which were involved in immune responses or extracellular matrix biology. These results suggest that Gs strains activate immune and TME processes that slow tumor growth. Quantitative trait locus (QTL)

analyses of host genetics and tumor data are pending and will facilitate identification of genetic variants that influence TME effects on tumor progression.

Abstract Number 603

DR1 Associated Protein 1 Overexpression in Hepatocellular Carcinoma Portend Poor Prognosis

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Background: Hepatocellular carcinoma (HCC) is a primary malignancy of the liver. It is the sixth most common malignancy worldwide and the third most frequent cause of cancer related death. Higher rates in African Americans vs whites (4:1). HCC occurs predominantly in patients with underlying chronic liver disease and cirrhosis. To improve patient outcomes it is essential to find diagnostic and prognostic biomarkers and target for treatment. There are many classes of genes that can be targeted for treatment, including genes that code for proteins. DR1 Associated Protein 1 (DRAP1), also known as negative cofactor 2, is known to be involved in transcriptional repression. Our comprehensive analysis identified DRAP1 overexpression in HCC. Furthermore, HCC patients with higher DRAP1 expression show poor survival, suggesting it is a prognostic marker. As a critical regulator of gene expression, it could be a valuable target in developing inhibitors to treat HCC.
Methods: UALCAN was used to study gene expression level of DRAP1 in HCC. UALCAN uses TCGA RNA-sequencing data to present RNA level expression of 33 different cancers, including HCC. From UALCAN's data, DRAP1 expression for 574 patients was evaluated for normal samples and primary tumors, the four stages of HCC, and different histological subtypes. Kaplan-Meier plots depicting the relationship between DRAP1 expression and HCC patient survival indicate that DRAP1 is a prognostic marker and high expression of DRAP1 predicts poor survival. Positive and negatively correlated gene expression was analyzed using UALCAN. Immunohistochemical analysis was obtained from the Human Protein Atlas.
Results: Pan-Cancer analysis of DRAP1 expression using TCGA data shows overexpression of DRAP1 in Hepatocellular Carcinoma. TCGA data also shows that DRAP1 expression increases with the grades (1 to 4) of HCC, and predicts poor patient survival with high expression by Kaplan-Meier plot (p=0.00075). DRAP1 expression is positively correlated to BANF-1 expression in HCC (Pearson correlation = 0.72), and conversely, negatively correlated to MLYCD (Pearson Correlation = -0.39)
Conclusions: DRAP1, a negative regulator of gene expression is overexpressed in HCC. DRAP1 expression decreases the probability of patient survival. DRAP1 is a possible oncogene and a possible therapeutic target. DRAP1 expression is positively correlated to BANF1 expression, and negatively with MLYCD suggesting a potential mechanism of DRAP1 action.

Abstract Number 604

The Novel Genetic Candidates and Drug Associated to Glioblastoma (GBM) Discovery using BEE

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Gliomas comprise the great majority of malignant brain tumors and are one of the deadliest cancers, having a median survival of 14 months. High grade gliomas, of which the most common

is glioblastoma (GBM), are characterized by extensive dispersal throughout the brain, indicative of their highly invasive nature. The genetic variation study sheds lights on discovery of the GBM cohorts by yielding the genetic phenotypes. However, there still two puzzles for the conventional hypothesis-driven researchers to solve. Which extended knowledge and insights including the gene, diseases, drugs, phenotypic features and clinical attributes from the list of the variations we can reveal? Are there any novel functional genes cohesively interact to the driver variants we can investigate? We motivated by the need to address the two types of recurring questions and develop a web server called Biomedical Entity Expansion, Ranking, and Explorations (BEERE). BEERE aims to prioritize user-provided biomedical entities for detailed investigations of the related concepts, known associative relationships among them, supporting literature evidence, their relative significance to one another, and the relationship network context that they reside in. In the conjunction of genes and terms, BEERE provided sophisticated analysis to validate the associations between genes and diseases with semantic relationships mined in PubMed articles. In the GBM study, the input was 200 Glioblastoma (GBM) genetic candidates from OMIM database (https://omim.org/). BEERE expanded and performed ranked order in result of 1,962 genes. 98 among them were significant using p-value <= 0.05. 14 out of 98 genes were expanded candidates. BEERE validated all the 14 novel genes by revealing the directly or indirectly semantic relationships to GBM. In the drug discovery, BEERE reported 47 drugs in drugBank to be significant in BEERE ranking (p-value <= 0.05). 35 out of 47 drugs interact to GBM and GBM candidates with semantic relationships, and those drugs covered all 4 GBM specific drugs indicated in drugBank, which are temozolomide, bevacizumab, carmustine and irinotecan. 12 out of 47 drugs were indirectly related to GBM through interfering the GBM candidates. BEERE further reveals the mechanisms interacted by the 12 novel drugs.

Abstract Number 605

A Batch Effect Adjusted Simon's Two-Stage Design for Cancer Vaccine Clinical Studies

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In cancer vaccine clinical studies, immune responses are commonly used as the primary endpoint for assessing vaccine efficacy. Simon's two-stage design is a popular clinical trial design in Phase II cancer studies. Nonetheless, it is not straightforward to apply it when performing immune assays in batches, as outcomes from multiple patients may be correlated with each other in the presence of batch effects. This violates the independence assumption of Simon's two-stage design and correspondingly, may affect the clinical study in an unexpected way. We numerically explored the impact of batch effects on Simon's two-stage design, proposed a batch-effect adjusted Simon's two-stage design, and demonstrated the proposed design by both a simulation study and a therapeutic human papillomavirus vaccine trial example. In the simulation study, outcomes (immune responses) were added with additive and multiplicative batch effects. Four different batch effects were considered: large or small, and normally distributed or skewed. Type I error rate and power from Simon's two-stage design, adjusted Simon's two-stage design, and Sargent's method were compared. When batch effects are neglected, trials have inflated type I

error rates and deflated power. This negative impact worsens as the batch size increases. The skewness of batch effects does not affect type I error rates and power much. Sargent's method controls the type I error rate inflation. However, it only provides appropriate power when the batch size is very small. Adjusted Simon’s two-stage design controls both the type I and II errors well, suggesting itself as a promising solution for dealing with batch effects in Phase II cancer vaccine clinical studies.

OPEN
CATEGORY

Abstract Number 700

Targeting AR and cFLIP Greatly Reduces Proliferation and Invasion of Prostate Cancer Cells

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Prostate cancer (PCa) is the second leading cause of cancer-related deaths in American males. Despite impressive advances in surgical and/or radiation treatment, the 5-year survival of patients with advanced stage PCa remains very low. Androgen deprivation therapy or blockage of androgen at the level of androgen receptor (AR) remain the treatment options for advanced disease. However, the emergence of androgen resistance limits their therapeutic usefulness and most patients with PCa progress from an androgen-dependent status to invasive castration-resistant PCa (CRPCa). CRPCa continues to express AR and depends on functional AR signaling for growth. Studies have shown that cFLIP, an anti-apoptotic protein, reduces the efficacy of AR-targeted therapy. In addition, the expression of cFLIP correlates with progression to CRPCa. Presently, the management of CRPCa poses a great challenge, and there is no beneficial therapeutic treatment. Therefore, finding agents that can disrupt AR function, especially in conjunction with other small molecules that target cFLIP, could dramatically improve the benefits of therapeutic intervention of CRPCa. We found that fisetin, a naturally occurring flavonoid, is a potent inhibitor of AR signaling and interacts with the ligand binding domain of AR. Additionally, vorinostat, a HDAC inhibitor, increases the acetylation of Ku70 by disrupting the interaction of endogenous cFLIP and Ku70 leading to degradation of cFLIP. We next determined whether fisetin in combination with vorinostat could offer therapeutic benefit over an individual agent for the management of CRPCa. Combination treatment (fisetin plus vorinostat) of CRPCa cells more effectively reduced cell growth and colony formation than individual agents. Combination treatment also resulted in increased apoptosis as revealed by cleaved PARP and modulation in Bcl2 family proteins. Additionally, combination treatment more effectively reduced the protein expression of HDAC6, cFLIP, and procaspase 8 than individual agents. Activation of AR signaling and induction of HDAC6 expression play an important role in cell invasion by inducing epithelial-to-mesenchymal transition (EMT). We found that combination treatment more effectively reduced invasion and modulated EMT in CRPCa cells. In summary, our data indicate that combination treatment exhibits more potent anti-proliferative, pro-apoptotic, and anti-invasive activities in CRPCa cells.

Abstract Number 701

Fatigue is Independently Associated with Functional Status Limitations in Older Adults with Cancer – Results from the Cancer and Aging Resilience Evaluation (CARE) Registry

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Introduction: Fatigue is an indicator of frailty among older adults, compromising independent living. The burden of fatigue and its impact on functional well-being in older adults with cancer remains understudied. We addressed this gap in older adults with cancer seen at a single center.

Methods: Patients completed a modified geriatric assessment (GA) survey (CARE) that included the PROMIS® global health 10 (assesses fatigue). We examined the prevalence of

fatigue, bivariate associations between those with/without moderate/severe fatigue, and logistic regression of the association between moderate/severe fatigue and limitations in Instrumental Activities of Daily Living (IADL) and Activities of Daily Living (ADL) adjusting for age, sex, race/ethnicity, education, cancer type and stage, pain, comorbid conditions, and time from cancer diagnosis.
Results: A total of 495 participants completed the CARE survey at a mean age of 70y; 56.7% were male; 23.3% Black. Tumor types included colon [22.1%], pancreatic [17.8%], rectal [8.7%], other [51.4%]; mostly advanced stages (70% stage III/IV). Overall, 289 (58.4%) patients reported moderate/severe fatigue. Patients with moderate/severe fatigue were more likely (p0.0001) to report IADL and ADL limitations (66.1 vs. 27.6% and 25.1 vs 6.5%, respectively), >=1 fall (29.6% vs. 12.2%), limitations in walking one block (71.4% vs. 26.0%), limitations in social activities (60.9% vs. 20.6%), depression (27.1% vs. 6%), moderate/severe pain (63.2% vs. 24.1%), >=3 comorbid conditions (41.3% vs. 24.1%). In multivariable analyses (adjusting for factors in methods), the odds of IADL and ADL impairment in those with moderate/severe fatigue were 2.7-fold (95%CI 1.7-4.4, p0.001) and 2.8-fold (95%CI 1.4-5.5, p=0.004), respectively, compared with those with no/mild fatigue.
Conclusions: Over half of older adults with cancer report moderate/severe fatigue that is associated with numerous GA impairments and independently associated with functional status limitations. Further understanding of the multifaceted aspects of fatigue and development of targeted interventions combating fatigue are needed.

Abstract Number 702
N-Myc Interactor (NMI) Regulates STAT5A in Mammary Development and Metastasis
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It is well documented that the dynamics of developmental processes are used by cancer cells to support cancer initiation and progression. Disruption of the delicate balance between cellular plasticity and differentiation during mammary development leads to breast cancer initiation and metastatic progression. Our recent findings show that Nmi is expressed in the mammary ductal epithelium at the onset of puberty and is induced in pregnancy and lactation. We have found that in breast cancer, NMI protein is decreased in about 70% patient specimens with metastatic dissemination. Mammary specific Nmi knock out mouse model revealed that conditional Nmi loss disrupts luminal differentiation in the mammary gland resulting in increased alveologenesis and prompted the progression of tumors with aggressive metastatic characteristics. STAT5a is one of the downstream effector of prolactin and is essential for differentiation of secretory alveolar epithelium. Moreover active Stat5 characterize breast cancer patients with favorable prognosis. However, functional relationship of NMI and STAT5a is still unknown. Here we present our finding that Nmi and Stat5a expression has a direct relationship in normal mammary development. We demonstrate that silencing Nmi caused a decrease in STAT5a activity and a subsequent failure of differentiation upon prolactin stimulation of HC11 murine mammary epithelial cells. Loss of Nmi in vivo caused a decrease in STAT5a activity and a subsequent transcriptomic shift in mammary epithelial and breast cancer cells. Detailed examination of mammary specific genetic program controlled by STAT5a in the context of transcription profiles of NMI knockout and overexpressing cell lines as well as mammary tumors revealed ISG20, interferon stimulated exonuclease gene 20, as a unique negatively regulated transcript. Here we show that expression of ISG20 is increased in metastatic patient’s specimen compared to its matched primary breast cancer

tissues. We elucidated that ISG20 is kept in check by NMI and STAT5A through miR17-92 cluster and that ISG20 has a positive influence on tumor progression and metastasis.

Abstract Number 703

ABT-263 Induces Apoptosis by Upregulating Caspase-3 and Inhibition of Bcl-2 Family in CRC

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Colorectal cancer (CRC) is the third most common cancer in the United States. Most CRC patients receiving therapeutic agents are resistant to apoptotic stimuli. Two protein families, caspase enzymes and B-cell lymphoma-2 (Bcl-2) family proteins are involved in apoptosis. Caspase enzymes promote apoptosis, functioning in a cascade, of which caspase 3 is the pivotal protein. Members of the Bcl-2 family, Bcl-2, Bcl-xL, and Bax are functionally opposed: Bcl-2 and Bcl-xL inhibit apoptosis, whereas Bax counteracts this effect. Members of the Bcl-2 family that have only BH3-domain trigger apoptosis by binding to the pro-survival proteins and neutralizing their functional activity. This “BH3 mimetic” concept has prompted the discovery and development of ABT-263, a small-molecule that mimics BH3-only proteins and thus induces apoptosis. Despite advances made in targeted therapy, the mechanism of action of ABT-263 is less studied. To bridge this gap in the cellular mechanism of ABT-263 on apoptosis, we quantified the gene expression in CRC tumor samples with corresponding controls and observed several-fold higher expression of Bcl2, Bcl-xL, and Mcl-1. These anti-apoptotic proteins were overexpressed in various tumor samples, supporting cell death avoidance, a hallmark of cancer. Since ABT-263 is a classical drug for Bcl-2 inhibition, we studied the effect of ABT-263 (2.5 μM for 24 hr.) in RKO CRC cells, which has increased endogenous expression of Bcl-2, by measuring gene expression profiles of Bcl-2 family members, along with other gene targets involved in apoptosis. ABT-263 treatment resulted in down-regulation of anti-apoptotic proteins, Bcl-2, Bcl-xL, and Bcl-w, by 27%, 29%, and 13%, respectively; these effects were concomitant with two-fold higher expression of caspase 3. Further, ABT-263 (2.5 μM) decreased cell proliferation by 15% after 24 hr. Similar decreased gene expression patterns of Bcl-2 family members were evident in LS174T CRC cells, which also exhibits high endogenous Bcl-2 expression, exposed ABT-263. These results indicate that, for CRC, ABT-263 promotes apoptosis by upregulating caspase 3 through inhibition of Bcl-2 family members. These studies are supported by the Elkus Eminent Scholar Program in Gastro-Intestinal Cancer Research

Abstract Number 704

Topical Therapeutic Drugs are Essential to Reducing Global Disparities in HPV Diseases

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HPV diseases are responsible for 0.8% of all human deaths and over 5% of cancer deaths. HPV vaccines are safe and effective at blocking new infections by 9 viral genotypes. However, anticipation that prophylactic vaccines would substantially reduce the staggering burden of HPV diseases has been unfulfilled. Although the first FDA-approved HPV vaccines have been heavily marketed since 2006, less than 1.5% of the world’s population is vaccinated, primarily in high income countries. Behind the disappointing uptake are bottlenecks in manufacturing and delivery, high cost, lack of public health infrastructure, anti-vaccine efforts, and competing economic and medical issues. Despite philanthropic and WHO initiatives in low and middle income countries where 85% of life-threatening HPV diseases occur, population growth is outpacing vaccination by

10-fold. 80% of people eventually acquire and can pass along the sexually transmitted HPVs, with a 2% lifetime risk that lesions will undergo neoplastic progression. Globally, 15,000,000 incident HPV cancer cases will emerge over the next 20 years. Over-zealous support for HPV vaccination has crippled efforts at alternative approaches to HPV management. Pathological evaluations and surgical interventions are out-of-reach in much of the world. Immunotherapies have proven elusive, likely due to the immune escape functions of the HPV oncoproteins E6, E7 and E5. Elimination of active lesions and long-term suppression of persistent infections ideally involves periodic screening for both non-oncogenic and oncogenic HPV infections using sensitive and specific molecular tests following sexual debut; however, observational diagnosis should be adequate when resources are limiting. Positive cases should immediately be provided with self-administered topical treatments using effective, safe, well-tolerated and universally affordable small molecule antivirals. Our conceptual approach to timely, efficient and economical discovery is to repurpose existing drugs that have cleared Phase I clinical trials for completely different indications. A decade ago, our lab created a 3-dimensional organotypic epithelial tissue culture model that supports a robust productive infection cycle of high-risk HPV-18. With this informative system, we characterized and validated a number of anti-HPV agents of very different pharmaco-chemical classes, several of which have already moved into clinical trials. This is the most promising path forward to diminish the health disparities in HPV diseases that disproportionately impact economically challenged communities and countries.

Abstract Number 705

Nitric Oxide--NVN1000 Inhibits HPV-18 by Inactivating Oncoproteins in Organotypic Cultures

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Human papillomaviruses (HPVs) are highly prevalent small DNA tumorviruses that cause epithelial hyperproliferation called warts, papillomas or condylomas. There are no reliably effective therapeutic agents. Our laboratory mission is to identify, develop and validate safe, well-tolerated and affordable inhibitors of HPV infections with which to prevent progression of lesions to the 750,000 HPV-associated cancers of women and men that arise each year around the world, particularly in low and middle income countries. (See our companion poster concerning the epidemiological challenges and extreme public health care disparities in HPV prevention and management.) Persistent infections by the high-risk (HR) HPV genotypes are causally associated with high-grade cervical and anal intraepithelial neoplasias (CINs and AINs) and carcinomas attributed to inappropriate over-expression of the viral oncoproteins E6 and E7. The coordinated functions of E6 and E7 are to recondition differentiated epithelium to support the viral reproductive phase by destabilizing host cell tumor suppressors p53 and the pRB family of cell cycle regulatory proteins. Exogenous nitric oxide is known to inhibit DNA replication of large DNA viruses. NVN1000, a polymeric macromolecule that releases nitric oxide gas upon application to warm, moist tissues, was applied topically for 1 hour daily over 6 consecutive days to 3-dimensional epithelia raft cultures developed from uninfected primary human keratinocytes or from PHKs harboring oncogenic HPV-18 genomes. Viral DNA copy number was quantified by real time qPCR. At 2 mg/ml, NVN1000 significantly reduced HPV DNA amplification and abrogated progeny virus production. E6 protein was reduced and p53 was stabilized. The E7 protein and activities were compromised and suprabasal S-phase progression was impaired. Based on gamma-H2AX and TUNEL signals in suprabasal strata of the HPV-18 infected cultures, NVN1000 induced DNA damage and apoptosis, whereas gamma-H2AX signals were much lower in uninfected control rafts. NVN1000 also caused DNA damage and apoptosis in raft cultures of

PHKs transduced with recombinant retroviruses expressing HPV-16 E6 and E7 genes. Uninfected tissues became thinner, indicative of reduced basal cell proliferation. Terminal differentiation of the keratinocytes was inhibited in both cultures. Ongoing investigations are focusing on biomarkers that will reveal details on the modes of action of NVN1000 and nitric oxide inhibition. Our results indicate that topically applied NVN1000 is a promising candidate for treating premalignant HPV infections before the lesions progress to high-grade dysplasias and cancers. (Supported by Novan Inc., Morrisville, NC, USA 27560 and an Anderson Family Endowed Chair to LTC)

Abstract Number 706

Humanized Mouse Model to Dissect the Role of T-cells in EBV Type 2 Lymphomagenesis

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Epstein-Barr virus (EBV) has been classified into two major strains, EBV Type-1 (EBV-1) and EBV Type-2 (EBV-2) based on genetic variances and differences in transforming capacity. EBV-1 readily transforms B-cells in culture while EBV-2 is poorly transforming. The differing ability to immortalize B-cells in vitro suggest that in vivo these virus strains likely use alternative approaches to establish latency. Indeed, we recently reported that EBV-2 has a unique cell tropism for T-cells, not only infecting T-cells in culture, but also infecting T-cells of healthy Kenyan infants, strongly suggesting EBV-2 infection of T-cells is a natural part of the EBV-2 life-cycle. However, limitations of human studies hamper further investigation into how EBV-2 utilizes T-cells to establish latency and/or persist. Thus, we developed an EBV-2 humanized mouse model, utilized BALB/c $Rag2^{tm1Fwa}$ $IL\text{-}2R_{\gamma C}{}^{tm1Cgn}$ $SIRP\alpha^{NOD}$ (BRGS) mice engrafted with CD34+ hematopoietic stem cells. Infection of humanized BRGS mice with EBV-2 led to infection of both T-cells and B-cells, unlike infection with EBV-1, in which virus was only detected in B-cells. Gene expression analysis demonstrated that EBV-2 established a latency III infection with evidence of ongoing viral reactivation in both B- and T-cells. Importantly, EBV-2 infected mice developed tumors resembling diffuse large B-cell lymphoma (DLBCL). These B-cell lymphomas had morphological features comparable to EBV-1 induced DLBCL and developed at similar rates with equivalent frequency as well as a latency III gene expression profile. Thus, despite the impaired ability of EBV-2 to immortalize B-cells in vitro, EBV-2 efficiently induces lymphoma development in BRGS mice. This new in vivo model can now be utilized to better understand the methods used by EBV-2 to persist and induce lymphomagenesis with specific emphasis on deciphering how EBV-2 infection of T-cells contribute to these processes.

Abstract Number 707

A Novel Oncogenic miRNA in Lung Squamous Carcinoma

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Lung Cancer is the leading cause of cancer related death in the United States and is classified by multiple histological subtypes. Lung adenocarcinoma, squamous cell carcinoma, and large cell carcinoma are all non-small cell lung cancer (NSCLC), and the remainder of lung cancer are small

cell lung cancer (SCLC). Each of these subtypes differs not only histologically, but also drastically in the genetic drivers involved and treatment options available. microRNA (miRNA) are a class of small non-coding RNA molecules that regulate gene expression by binding to the 3'-UTR of mRNA, stalling translation. They are well suited to facilitate the many cellular changes needed for lung oncogenesis as they can regulate hundreds of genes at a time. In addition, miRNA can demonstrate surprising tissue specificity, in that a single miRNA can act as an oncogene in one tissue but function as a tumor suppressor in another tissue, as is the case for miRNA-31 (miR-31). Our lab has previously shown that miR-31 is overexpressed in patient lung adenocarcinoma, the most common histological type of lung cancer, compared to normal lung and that overexpression of miR-31 alone in the mouse lung epithelia initiates lung tumorigenesis and eventual adenocarcinoma development. We now seek to examine the function of miR-31 across the remaining histological subtypes of lung cancer. We analyzed frozen and archived tissue samples collected at VUMC and UAB and determined that miR-31 levels are elevated in lung squamous cell carcinoma compared to normal lung, but not in lung adenosquamous carcinoma, small cell lung carcinoma, nor lung carcinoids, indicating not only a tissue specific role for this miRNA, but potentially a cell-type specific function. Interestingly, ectopic miR-31 did not promote growth in vitro in human lung squamous, large cell, small cell, and mesothelioma cell lines as was previously observed in lung adenocarcinoma cell lines. However, we observed that overexpression of miR-31 in a lung squamous cell carcinoma cell line drastically increased tumor growth in a subcutaneous xenograft model. The cellular pathways miR-31 is regulating may be different in squamous carcinoma than those observed in lung adenocarcinoma, however; we are currently performing kinome analyses to evaluate these pathways. We hypothesize this gene is necessary for the growth and progression of certain histological subtypes of lung cancer, and may regulate diverse pathways within each and thus may serve as a potentially important therapeutic target across lung cancer subtypes, particularly those which have limited treatment options.

Abstract Number 708

Impact of T-Regulatory Cells on Outcome of Breast Cancer in African American Population

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Background: Outcome of breast cancer (BC) in African American females (AA) remains worse than Caucasian females (CA) after accounting for socioeconomic factors and tumor characteristics. Heterogeneity of tumor immune microenvironment (TIME) composition demonstrated varying roles of infiltrating lymphocytes (TILs) in tumor progression and clinical outcome. We hypothesize that different racial TILs composition impacts downstream tumor signaling and worsens AA BC outcome.

Methods: The Cancer Genome Atlas (TCGA) harmonized RNA-Seq HTSeq and 450K Methylation data were accessed from GDC. Clinical, outcome and CIBERSORT immune composition data downloaded from final PANCAN publications. Differential expression and gene set enrichment analysis (GSEA) were performed using R packages limma and ClusterProfiler. FDR corrected p values 0.05 were considered significant. RESULTS. Of 182 AA and 755 CA, TNBC/Basal subtypes were higher in AA (33, 34% vs 14, 13% respectively). OS, DSS, DFI and PFI were worse in AA but did not reach significance. Within TNBC, AA did worse than CA (HR 2.18[1.04–4.56]). Compared to CA, AA had higher TILs, T-Regulatory (Tregs) and T-Follicular Helper cells but lower M2 Macrophages (all p0.01). Within all IHC subtypes, as well as Basal and LumA, Tregs and Treg:TILs ratios were higher in AA (all p0.05). TILs predicted favorable OS in CA only (HR 0.11[0.27-0.46] versus 1.26[0.87-18]). Treg, Treg:TILs and Treg:CD8+ ratio predicted worse DSS (HR 1525[1-5e7], 51[1.8-1.4e3], 1.05[1-1.1]) and worse DFI (HR 3241[1.14-

9e6], 49[1.78-1.3e3], 1.04[1-1.09]) respectively. On GSEA of differentially expressed genes, multiple pathways were enriched in AA including KEGG (IL-17 signaling), MySigDB Hallmarks [H] (IFN-α and IFN-γ), MySigDB Gene Ontology [C5-BP] (CCR Binding), MySigDB Immunological Signatures [C7] (induced Treg signaling) and REACTOME (IL-10 signaling, NF-kB). Notable CA enriched pathways included KEGG (PI3K-Akt, TGF-β, cGMP-PKG) and MySigDB Hallmarks [H] (Hedgehog, early and late Estrogen responses). T-cell exhaustion markers were higher in AA transcriptome, with higher PDCD1, CTLA4, IDO1 and LAG3 but lower TIM3 expression. After correcting for false discovery, AA had lower mean CpG β-value methylation signature of CTLA4, IDO1 and PDCD1. **Conclusion:** AA had overall more immune infiltrates and different composition of BC TIME compared to CA, with higher immunosuppressive fractions. Tregs were persistently higher across all IHC subtypes, predicted worse outcome and generated higher T-cell exhaustion signature, as well as downstream Treg-specific enriched chemotaxis and activation gene sets in AA compared to CA. These data support the hypothesis of adverse TIME in AA and opens venues of personalized TIME modulation.

Abstract Number 709

A Case of T-Cell Large Granular Lymphocyte Leukemia Masquerading as Lupus Nephritis

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Large granular lymphocytic (LGL) leukemias involve the abnormal clonal proliferations of either natural killer (NK) cells (CD 3-) or cytotoxic T-cells (CTLs) (CD3+). LGL leukemias are a rare subset of chronic lymphoproliferative diseases. The clinical presentation of LGL leukemias typically include neutropenia, anemia, splenomegaly, recurrent infections, and various autoimmune disorders, most commonly rheumatoid arthritis. Less commonly reported presentations of T cell LGL leukemia include oral ulcers and membranoproliferative glomerulonephritis. This case report discusses the clinical presentation and evaluation of a 35-year-old Caucasian female who was previously diagnosed with lupus nephritis and presented with vague symptoms of recurrent painful oral ulcers onset over 2 years ago. Following extensive hematologic diagnostic studies, including peripheral blood flow cytometry and bone marrow biopsy, she was diagnosed with T- cell LGL leukemia and was started on methotrexate for treatment of this indolent disease. The purpose of this report is to discuss a rare case of an already rare hematologic malignancy by describing this patient's clinical presentation, laboratory findings, and steps to the diagnosis, and to also discuss the current recommendations for treatment of T-cell LGL leukemia.

Abstract Number 710

Cytotoxic Effects of CFI-402257, a Potent Inhibitor of Dual Specificity Kinase Mps1, is Up

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Background: The spindle assembly checkpoint (SAC) is a signaling cascade that prevents chromosome mis-segregation by arresting (via APC/C) the cell cycle in mitosis until all chromosomes are properly attached to the mitotic spindles. Monopolar spindle 1 (Mps1 or TTK), a dual specificity protein kinase, is a core component of the SAC pathway. It phosphorylates mitotic checkpoint complex (MCC) proteins, Knl1, Bub1 and Mad1 (Ji et al, PMID: 28072388; Faesen etal, PMID: 28102834). Mps1 expression is elevated in many cancers including cervical cancer (analysis of TCGA database). TTK/Mps1 also phosphorylates and activates BLM, Chk2

and c-Abl, hence may have a potential role in regulating DNA damage repair process (Wei e al, 2005, PMID: 15618221; Leng etal, 2006, PMID:16864798). Hypothesis: (1) Mps1 expression is elevated by HPV oncogenes and (2) its inhibition could reduce cell growth and induce apoptosis in cervical cancer cells.
Methodology: (a) Immunoblot assays for Mps1 in cervical cancer cell lines (HeLa, CaSki and SiHa) relative to uninfected primary human keratinocytes (PHK); (b) Assays to show Mps1 protein levels are related to HPV E6 and E7 oncogene expression; (c) Assays to show effects of E6, E7 siRNAs on Mps1 expression; (d) Assays to show effects of Mps1 siRNA or small molecule inhibitor (CFI-402257) on submerged and raft cultures of CaSki, and in raft cultures of HPV-18 immortalized cell lines.
Results: (1) Mps1 is highly expressed in cervical cancer cell lines in comparison to primary human keratinocytes (PHK). (2) HPV E6, and to a lesser extent, E7 of the oncogenic HPV-16/-18 elevate Mps1 transcription and protein in submerged cultures of transduced PHK. (3) HPV-16 E6/E7 siRNAs reduced Mps1 protein in submerged cultures of CaSki cells. (4) MTT and Trypan-Blue assays revealed that Mps1 siRNA inhibited growth and induced cytotoxicity in submerged cultures of CaSki and SiHa cells. (5) CFI-402257 reduced E6 and E7 proteins, and increased pRB, p130, p53 and γ-H2AX in HPV-18 immortalized PHK, CFI-402257 inhibited host DNA replication and induced DNA damage and apoptosis in raft cultures of CaSki and in HPV-18 immortalized cells. (6) CFI-402257 inhibits the growth of cervical cancer xenografts (PDX A001a) in SCID mice.
Implication: Mps1 inhibition induces cytotoxicity by interfering with HPV E6/E7 expression, in addition to potential effect on SAC. Future Direction: (1) Synthetic lethality of CFI-402257 and cisplatin in PDX models of cervical cancers. (2) Mechanism of Mps1 regulation of HPV E6 and E7 expression.

Abstract Number 711
Lost Productivity and Time in Patients with Metastatic Breast Cancer Receiving Treatment
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Background: Costs for cancer patients are not all monetary. For patients with limited life expectancy, such as metastatic breast cancer (MBC) patients, time spent in the hospital or clinic setting can become burdensome. The goal was to evaluate time spent on healthcare among patients receiving treatment for MBC.
Methods: This survey-based, cross-sectional study included women ≥18 years with MBC who received treatment at two academic medical centers in Alabama from 2017-2019. Questions regarding employment status, MBC-related hours missed from work, and time spent on healthcare-related activities were used to quantify lost productivity and time. Descriptive statistics included means and standard deviations (SD) or medians and interquartile ranges (IQR) for continuous variables and frequencies for categorical variables. Effect sizes were calculated using Cohen's d or Cramer's V. Results: We surveyed 100 female MBC patients with a median age of 58 years (IQR 49-66). Among all respondents, 32% were African American, 41% held a college degree, and 51% had a household income of $40,000. Patients spent a median 60 minutes (IQR 30-120) traveling from their home to clinic and a median 120 minutes (IQR 60-210) receiving care at a clinic visit. Though not statistically significant, modest differences were found for patients with differing insurance types in travel time. Patients with Medicare had the shortest travel time (median 45 minutes [IQR 30-90]) compared to Medicaid (60 minutes [IQR 50-90]) and private

insurance (75 minutes [IQR 30-120]; d=.05).). Patients spent a median 30 minutes (IQR 0-60) on cancer care related activities outside of a clinic visit. Most patients were retired (29%); however, 15% worked full-time, 8% worked part-time, and 20% were on disability. For working women, a median of 7.5 hours (IQR 1-11) were missed from work in the week.
Conclusions: This study highlights productivity losses uncaptured by current patient healthcare cost calculations. Further work is needed to identify and minimize these additional patient costs related to lost productivity during cancer treatment.

Abstract Number 712
Antifibrogenic Activities of Novel Vitamin D3 Analogs are Dependent on VDR Expression in HDF
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We have previously discovered new pathways of vitamin D and lumisterol activation by CYP11A1. The vitamin D receptor (VDR) is expressed in dermal fibroblasts and serves as the receptor for active hydroxylated vitamin D analogs. In continuation of our previous studies that showed that non-calcemic 20(OH)D3 has antifibrotic activity in human dermal fibroblasts, we tested the role of VDR in the action of 20(OH)D3, its metabolites including 20,23(OH2D3, 1,20(OH)2D3, or 1,20,23(OH)3D3 in comparison to canonical 1,25(OH)2D3 using human fibroblasts isolated from the skin of white or black donors. Tested vitamin D3 analogs inhibited proliferation of fibroblasts and decreased collagen production with a similar potency in cells isolated from either black or white donors. Antifibrotic activities of the analogs and of 1,25(OH)2D3 were confirmed by inhibition of COL1A1, COL1A3, COL3A1, fibronectin, THBS1, αSMA, PAI-1, SERPINE-1, CTGF, PDGFA and TGFB1 gene expression. All of these compounds increased expression of MMP1 gene and especially TIMP1 gene. We also employed ShRNA transduction of fibroblasts to silence the VDR gene and tested the wild type (+/+) or knock-out (-/-) cells for proliferation when treated with the vitamin D3 derivatives. The CYP11A1-derived vitamin D3 analogs inhibited proliferation of VDR+/+ fibroblasts in a dose-dependent manner with a similar potency to 1,25(OH)2D3, while this effect was abrogated in VDR-/- fibroblasts. These results show vitamin D hydroxy-derivatives have antiproliferative and antifibrotic activities in fibroblasts which are dependent on the expression of the VDR.

Abstract Number 713
Honokiol-loaded Exosomal Preparation for Enhanced Cellular Uptake and Anti-tumor Efficacy
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Exosomes are nanosized membrane vesicles that are shed by nearly all types of cells. They play important roles in intercellular communications through active transfer of biomaterial from one cell to another in a targeted manner. As a result, exosomes are currently being explored as efficient drug carriers. Exosomes derived from mesenchymal stem cells (MSCs) are not only biocompatible, but exhibit no or minimal immunogenicity. We previously reported antitumor efficacy of honokiol (HK), a phytochemical isolated from oriental medicinal plant Magnolia officinalis/grandiflora, against pancreatic cancer. Here we prepared a nanoformulation of MSC-derived exosomes loaded with HK, and compared its therapeutic efficacy against various cancer cell types. HK was encapsulated into exosomes by sonication, and size and integrity of Exo-HK formulation was determined by dynamic light scattering. HK loading in exosomes was evaluated

by HPLC. Equivalent doses of free and exosome-encapsulated HK were used to assess in vitro cytotoxicity against different human cancer cell lines, including MiaPaCa-2 and Colo357 (pancreatic), MDA-MB-231 (breast), SKOV3 (ovarian), HT-29 (colon), and LNCaP (prostate) using WST1 assay. The data revealed that cytotoxicity of HK-loaded exosomes was nearly 4-5 greater than that of free HK at 72 hours. Similarly, increased anti-tumor potency of HK-loaded exosomes was also observed in long-term clonogenic survival assay. Growth inhibitory effects of free HK or Exo-HK were due to cell-cycle arrest and apoptosis induction where the latter induced greater effects at the cellular and molecular levels. Determination of intracellular HK by LC-MS/MS analysis demonstrated an enhanced accumulation in tumor cells treated with Exo-HK as compared to the tumor cells treated with free HK. Further, blocking of exosomal uptake reduced intracellular HK accumulation and tumor cell death. Together, these findings provide support for the utility of exosomes in efficient delivery of HK to tumor cells.

Abstract Number 714
Hexosamine Biosynthetic Pathway in ccRCC
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Protein O-GlcNAcylation is addition of O-linked β-N-acetylglucosamine on serine and threonine residues of various proteins and is altetred in various cancers. In addition to its role as “molecular bricks”, O-GlcNAcylation dictates the protein subcellular localizations, activity, and signaling in response to nutritional status of the cells and regulates fundamental cellular process in responses to nutritional cues. O-GlcNAcylation is dynamically mediated by O-GlcNAc transferase (OGT) and O-GlcNAcase (OGA). Uridine diphosphate-N-Acetylglucosamine, the substrate for O-GlcNAcylation, is generated by hexosamine biosynthetic pathway (HBP). Expression of GFAT1, the first and rate-limiting enzyme of HBP, has been implicated in various cancers with conflicting results. Despite a critical role of HBP and O-GlcNAcylation in cancers, it is not well studied in context to clear cell renal cell carcinoma (ccRCC). In the present study, we investigated the effects of nonessential amino acids in the activation of HBP and protein O-GlcNAcylation in ccRCC. Inhibition of GFAT1 decreased O-GlcNAcylation, decreased stability of EMT markers, and resulted in decreased wound healing and migration. Similarly, inhibition of OGT also resulted in decreased expression of EMT markers, and decreased wound healing. These preliminary data points out a critical role of HBP and protein O-GlcNAcylation in ccRCC.

Abstract Number 715
Detection of 20-Hydroxy-7-Dehydrocholesterol and 20-Hydroxyvitamin D3 in Honey
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20(S)-hydroxyvitamin D3 (20(OH)D3) which can be produced by the action of cytochrome P450scc (CYP11A) on the side chain of vitamin D3, has been detected in vivo in humans, and can be further hydroxylated by CYP11A1, CYP27A1, CYP24A1 and/or CYP27B1 to several di- and tri-hydroxyderivatives. CYP11A1 also hydroxylates vitamin D3 producing 22(OH)D3, which is detectable in the epidermis. 20-Hydroxy-7-dehydrocholesterol (20(OH)-7DHC) can also be produced in human skin and can be phototransformed into 20(OH)D3 following the absorption of UVB energy by the B-ring. 20(OH)D3 and its hydroxyderivatives have anti-inflammatory, pro-differentiation and anti-proliferative effects on many cell types, comparable to 1,25(OH)2D3. Since a cytochrome P450 with 20-hydroxylase activity is found in insects, participating in ecdysone

synthesis from 7-dehydrocholesterol, we tested whether the above hydroxyderivatives are produced by bees and found in honey. Honey was collected during summer in the Birmingham, AL area, extracted with methylene chloride and analyzed by LC-MS. We detected clear peaks of m/z = 383.3 [M+H-H2O]+ with retention times corresponding to 20(OH)D3 and 20(OH)-7DHC standards. We also detected species with m/z = 407.3 (M+Na]+ at the retention times of 7-dehydrocholesterol, vitamin D3 and lumisterol, that were above the background. Similarly, a peak with m/z = 399.3 [M+H-H2O]+ was detected corresponding to the retention time of 1,25(OH)2D3. Species corresponding to 20-hydroxylumisterol, 25(OH)D3, 22(OH)D3, 20,23(OH)2D3, 20,24/25/26(OH)2D3 and 1,20,23/24/25/26(OH)3D3 were not detectable above the background. Our data suggest that bees produce 20(OH)7DHC and 20(OH)D3 although further analysis is required to confirm this, possibly arising from the 20-hydroxylase involved in ecdysone synthesis producing 20(OH)-7DHC and UVB exposure converting this to 20(OH)D3.

Abstract Number 716

PAICS, a Nucleotide Metabolic Enzyme, is Involved in Colon Cancer Growth and Metastasis

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Genomic and expression profiling analysis have revealed a variety of genetic and molecular alterations during the development and progression of colorectal cancer (CRC). Despite the extensive use of 5-fluorouracil (5FU) for treatment of CRC, disease recurrence and metastasis are common and lead to cancer-related deaths. Thus, it is necessary to discover molecular determinants to detect early-stage cancers and markers that identify patients who are at risk of developing recurrent CRC following surgical resection. New and specific targets should be investigated, and development of drugs blocking these targets is needed to improve patient survival. In the present study, to identify a possible target for treatment of CRC, we performed gene expression analyses using publicly available mRNA expression data by UALCAN application. Our analysis identified overexpression of phosphoribosylaminoimidazole carboxylase, phosphoribosylaminoimidazole succinocarboxamide synthetase (PAICS), a de novo purine biosynthetic enzyme that, in most cancer cells, is involved in synthesis of DNA. Here, we established an association between PAICS overexpression and cancer progression. To determine the function of PAICS in CRCs, we performed in vitro and in vivo investigations using CRC cells modulated for PAICS expression. The results demonstrated that PAICS was involved in cell proliferation, cell invasion, and tumor growth. Depletion of PAICS in CRC cells led to reduced metastasis of these cells to lung, liver, and bone. Following PAICS knockdown, there was upregulation of the epithelial-mesenchymal transition marker, E-cadherin. Furthermore, the bromodomain inhibitor, JQ1, decreased expression of PAICS. Thus, these results show that PAICS is involved in and required by CRC cells. Lowering of PAICS expression or blocking its enzyme activity by small molecule inhibitors would be an effective strategy for treatment of CRCs.

Abstract Number 717

Novel Patient-Specific Mutation Rodent Models of Neurofibromatosis Type I

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Background: Animal models of human diseases such as neurofibromatosis type 1 (NF1) are essential for preclinical studies of therapeutics. Development of these models has accelerated greatly due to advances in genome editing technologies. Rodent NF1 models developed to date represent missense, nonsense, frameshift and splicing mutants to create a suite of models amenable to intervention with therapeutics of different classes (i.e., c.1466A>G; p.Tyr489Cys splicing modulation with antisense oligonucleotides, c.2542G>C; p.Arg681X with nonsense suppression). Models were selected from multiple genotype-phenotype correlation groups, including less severe p.M992del and p.Arg1809Cys variants, and more pathogenic variants p.Gly848Arg and p.Arg1276Gln associated with familial spinal NF1.
Methods: Mouse and rat Nf1 genes are targeted by pronuclear microinjection or electroporation of CRISPR/Cas9 and Cpf1 reagents with repair templates to generate founder animals. Both traditional targeting vectors and CRISPR reagents are used to modify mouse Nf1 in embryonic stem cells. Founder animals or chimeras are outcrossed to establish germline transmission and independent colonies. Mutant alleles are tested for viability when homozygous as well as tumor formation when placed in the proper genetic context.
Results: Mouse models developed to date include c.2041 C>T (p.Arg681X), c.2542G>C (p.Gly848Arg), c.2393_2408del16, c.2919_2920insTT, c.2446C>T (p.Arg816X), c.5425C>T (p.Arg1809Cys), c.1466A>G (p.Tyr489Cys), c.2970-2972delAAT (p.M992del), and c.499_502delTGTT. Rat models created include the c.3827G>A (p.Arg1276Gln) mutation and a 14bp deletion model.
Conclusions: Multiple patient-specific alleles have been successfully generated in rodents using ES cell and CRISPR based approaches. Genotyped stillborn pups have shown homozygous knock-in and knock-out mutant alleles, consistent with embryonic lethality from biallelic loss of NF1. The c.2041 C>T; p.Arg681X, c.5425C>T (p.Arg1809Cys), and c.2393_2408del16 mouse models are embryonic lethal when homozygous, whereas the homozygous c.2542G>C; p.Gly848Arg mice are viable with no gross phenotype despite reduced neurofibromin levels. Both the c.3827G>A (p.Arg1276Gln) mutation and 14bp deletion models in the rat appear to be embryonic lethal; however, these results may be confounded by severe mammary gland adenocarcinoma induced by pregnancy.

Abstract Number 718
Mammary Cancer in a Patient Specific Neurofibromatosis Type I Rat Model
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Background: Rat models of neurofibromatosis type I (NF1) were created to fulfill a need for additional preclinical models to allow examination of the efficacy and safety of pharmacological modulators, and more readily assess cognition and behavior. The pathogenic patient missense allele c.3827G>A, p.R1276Q (KI), associated in humans with spinal NF1, as well as a 14 base pair deletion c.3661_3674del, p.P1220fs*1223 (KO) model were generated.
Methods: KO and KI rat models were created using two CRISPR guides and a dually compatible repair template designed to target exon 28 of rat Nf1 gene. Affected animals were euthanized upon tumor mass ulceration or inhibition of normal movement, and tissues were collected for histology, immunostaining and Western blot analysis. Males were analyzed for cognitive and behavioral defects at six months of age by elevated plus maze, open field test, novel object recognition and Morris water maze (n= 6-11).
Results: Three of seven pups born were positive for CRISPR activity from which independent Nf1 knock-out (P1220fs*1223) and missense knock-in (R1276Q) colonies were established. Unmated heterozygous Nf1 R1276Q female rats do not display spontaneous tumors; however,

mated females rapidly developed mammary tumors within two weeks of pregnancy. Heterozygous Nf1 P1220fs*1223 females develop tumors spontaneously at sexual maturity with acceleration of tumor formation post-mating. Homozygous mutant offspring have not been detected in litters born from intercrossing of heterozygous Nf1 mutant rats. Male rats do not display significant cognitive deficits or behavioral differences when assessed at 6 months of age with any of the tasks performed.
Conclusions: Two novel mutant Nf1 alleles, patient mutation c.3827G>A, p.R1276Q and deletion c.3661_3674del, p.P1220fs*1223, have been created in the rat. The mammary tumors are consistent with cribriform mammary gland adenocarcinoma in sexually mature females and carcinoma in situ in young unmated females. Restriction of tumor development to pregnancy in Nf1 R1276Q females suggests hormone induction plays a major role in tumor development. The divergence in phenotype between patient and null alleles may be due to residual function of R1276Q missense NF1 protein. Lack of full-term homozygous mutant pups indicates that these alleles are embryonic lethal, although rapid tumor onset post-mating may confound this result.
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Abstract Number 719
"It's Important to Me": An Analysis of Shared-decision Making in Early Stage Breast Cancer
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Background: Shared decision-making (SDM) occurs when both patient and provider are involved in the treatment decision-making process. SDM allows patients to understand the pros and cons of different treatments while also helping them select the one that aligns with their care goals when multiple options are available. This qualitative study sought to understand different factors that influence early-stage breast cancer (EBC) patients' approach in selecting treatment.
Methods: This cross-sectional study included women with stage I-III EBC receiving treatment at the University of Alabama at Birmingham from 2017-2018. To understand SDM preferences, patients completed the Control Preferences Scale and a short demographic questionnaire. To understand patient's values when choosing treatment, semi-structured interviews were conducted to capture patient preferences for making treatment decisions, including surgery, radiation, or systemic treatments. Interviews were audio-recorded, transcribed, and analyzed using NVivo. Two coders analyzed transcripts using a constant comparative method to identify major themes related to decision-making preferences.
Results: Amongst the 33 women, the majority of patients (52%) desired shared responsibility in treatment decisions. 52% of patients were age 75+ and 48% of patients were age 65-74, with an average age of 74 (4.2 SD). 21% of patients were African American and 79% were Caucasian. Interviews revealed 19 recurrent treatment decision-making themes, including effectiveness, disease prognosis, physician and others' opinions, side effects, logistics, personal responsibilities, ability to accomplish daily activities or larger goals, and spirituality. EBC patient preferences varied widely in regards to treatment decision-making.
Conclusions: The variety of themes identified in the analysis indicate that there is a large amount of variability to what preferences are most crucial to patients. Providers should consider individual patient needs and desires rather than using a "one size fits all" approach when making treatment decisions. Findings from this study could aid in future SDM implementations.

Abstract Number 720

Metal-organic Nanoparticles Prepared SMILE Method for Disulfiram-based Cancer Therapy

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Disulfiram (DSF) is currently tested in several clinical trials for cancer treatment in combination with copper (Cu) ions. Usually, DSF and Cu are administered in two separate formulations. In the body, DSF and Cu ions form diethyldithiocarbamate copper complex [Cu(DDC)2] which has potent antitumor activities. However, the "two formulation" approach often achieved low Cu(DDC)2 concentration at tumor regions and resulted in compromised anticancer efficacy. Therefore, pre-formed Cu(DDC)2 complex administered in a single formulation will have better anticancer efficacy. However, the poor aqueous solubility of Cu(DDC)2 is a significant challenge for its clinical use. Our laboratory developed a novel SMILE (Stabilized Metal Ion Ligand complex) method to prepare a metal-organic nanoparticle formulation of Cu(DDC)2 to address the drug delivery challenges. The prepared Cu(DDC)2 MONs showed high drug concentration, excellent loading efficiency, and desirable physicochemical properties. Our studies also demonstrated Cu(DDC)2 MONs had excellent anticancer activities against prostate cancer and breast cancer, including drug-resistant cancers. Cu(DDC)2 MONs could inhibit poly-Ub protein degradation, result in ER stress, and eventually cause cell death through paraptosis without caspase activation. This nanoparticle formulation provided a method to address drug delivery challenges of DSF/Copper-based chemotherapy and facilitate its clinical translation.

Abstract Number 721

Use of Natural Language Processing to Identify Cancer Clinical Trials Candidates

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Background: Clinical trials are critically important to advance scientific understanding and improve outcomes. However, NCI continues to identify low trials accrual (5%), particularly among underserved populations, as a top priority concern. Barriers may include low awareness among patients and providers of eligible trials, and inefficiencies in identifying clinical trials candidates. For example, pediatric oncologists may not be aware of clinical trials offered by medical oncologists, and vice versa. Additionally, existing recruitment methods such as manual chart review or provider referrals are inefficient. A technology intervention that prospectively identifies candidates for clinical trials may improve accrual.

Methods: In 2013, we successfully deployed CRCP (Cancer Registry Control Platform), a rules-based platform actively used by cancer registrars at the University of Alabama at Birmingham to identify reportable cancer cases. We subsequently deployed "PheDRS" (Phenotype Detection Registry System), a successor platform that integrates Natural Language Processing (NLP) to prospectively identify patients matching a specific phenotype based on structured as well as unstructured data (e.g., clinical notes, pathology reports, radiology reports) in the Electronic Health Record (EHR). We propose extending PheDRS's functionality to identify cancer patients eligible for clinical trials.

Results: We identified 2 active clinical trials to serve as pilot uses cases: a targeted agent trial in patients with advanced head & neck cancer, and a targeted agent trial in patients with advanced breast cancer. We conducted workflow analyses with clinical trials teams to understand cancer

clinical trials enrollment workflow. We analyzed each trial's eligibility criteria for priority and technical feasibility. We conducted iterative modeling of eligibility criteria as a search query that is practically useful for clinical teams. We developed proposed user interface changes and functionality to support discovery of clinical trials candidates. We developed evaluation metrics including total number of patients screened, total patients enrolled in trials, and user feedback on acceptability. Work is ongoing to develop and evaluate a prototype of the re-design before real world implementation.
Discussion: Cancer clinical trials accrual continues to be suboptimal among underserved populations. A NLP-based platform that prospectively identifies cancer clinical trials candidates may enhance accrual.

Abstract Number 722
Real-world Usage of NGS Testing in High Grade Serous Ovarian Cancer (HGSOC)
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Introduction: The Precision Medicine Initiative (PMI) study at the University of Alabama at Birmingham (UAB) was developed to include Next Generation Sequencing (NGS) as part of patients' standard of care and to follow them prospectively. The objective of this study was to analyze how the usage of NGS in patients with high grade serous ovarian cancer (HGSOC) affected their management.
Methods: Between 5/2015 and 2/2019, 224 ovarian cancer patients (167 HGSOC) signed informed consent and were enrolled. Archival tissue was sent to Foundation Medicine for NGS analysis; 324 genes, including genes involved in homologous recombination repair deficiency (HRD genes), microsatellite instability (MSI) status and tumor mutation burden (TMB) were assessed. Demographics including germline BRCA status, treatments, duration of targeted therapy, and platinum sensitivity were collected.
Results: 62% (103/167) of HGSOC patients had at least 1 targetable mutation and 40 patients received targeted therapy. 16% (27/167) were germline BRCA+ (gBRCAm+), 12 (10%) somatic BRCA + (sBRCAm+), and 120 wtBRCA. Of the gBRCAm+ patients, 81% were platinum-sensitive, compared to 66% of all patients. 22% (36/167) of patients underwent LOH testing: 39% (14/36) were LOH-high. 43% (6/14) of LOH-high patients had an alteration in one of the HRD genes. Of the LOH-low patients, 23% (5/22) harbored mutations in HRD genes: (3) BRCA2, (2) CHEK2. 7% (8/114) of platinum-sensitive patients were placed on PARPi maintenance therapy after two or more lines of platinum-based therapy. Of the s/gBRCA+ patients, 24 % (11/39) received PARPi monotherapy, 8 received both maintenance PARPi and monotherapy. 33% (13/167) of patients received a non-PARPi targeted therapy based on their NGS testing. Patients on maintenance PARPi received it for an average of 560 days (range 345-890 days), PARPi monotherapy: 254 days (range 30-750 days), and non-PARP targeted therapy: 186 days (range 24- 435 days.
Conclusions: NGS testing potentially effected the treatment of 23.9% of HGSOC patients. Our division has developed a streamlined mechanism for HGSOC patients to undergo routine NGS testing with limited out of pocket patient cost regardless of insurance status. Given the recent upfront approval of PARPi maintenance for all HGSOC who are s/gBRCAm+, we have now integrated both germline and somatic testing during front-line therapy. More information will be required to further elucidate how LOH status effects treatment decisions and outcomes for patients.

Abstract Number 723

Breaching Barriers in Glioblastoma: Comparison of Currently Available Proteasome Inhibitors

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Glioblastoma multiforme (GBM) is a fast-growing glioma that develops from star-shaped glial cells (astrocytes and oligodendrocytes) that support the health of the nerve cells within the brain. These tumours are highly cancerous with a high cell proliferation level. New therapeutic strategies are being developed worldwide to fight against deadly GBM, which has a median survival time of just 14 months. Proteasome inhibition has shown promising results in cancers such as myeloma. However, this form of therapy has also shown positive results in brain tumours in the form of elevated apoptosis. A head-to-head comparison of clinically available proteasome inhibitors (PIs) shows that in the clinically relevant setting only the co-inhibition of beta-2 with beta-5 activity achieves meaningful functional proteasome inhibition and cytotoxicity. The selective inhibition of beta-5 subunit is sufficient to induce cytotoxicity in PI-sensitive, but not in PI-resistant GBM, and beta-5/beta-2 co-inhibition is the most cytotoxic in PI-resistant GBM.

Abstract Number 724

High Throughput Screening Reveals Novel Regulators of EMT in Breast Cancer Cells

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A delicate balance between cellular plasticity and differentiation is critically maintained during mammary gland development. Disruption of this balance leads to breast cancer initiation and metastatic progression. Recent findings from our lab have revealed that N-Myc and STAT Interactor (NMI) protein is decreased in 70% of primary patient specimens with metastatic breast cancer. Furthermore, we have established NMI as a critical regulator of epithelial-mesenchymal transition in breast cancer, as such; cancer cells that lack NMI consistently demonstrated elevated attributes of a mesenchymal like invasive phenotype. Moreover, these cells, which have undergone EMT, attained enhanced chemotherapy resistance. Sequencing of breast cancers has not yielded any indications of mutations in the NMI gene; however, NMI levels remain low in breast cancer. Effective treatment modalities remains elusive for metastatic disease of triple negative breast cancer. We undertook a high throughput screen (HTS) of 5K- FDA approved compounds, to discover activators of NMI expression. The underlying hypothesis was that by restoring NMI expression, we would be able to successfully curb the invasive, mesenchymal like phenotype of breast cancer cells. We used MDA-MD-231 as a model cell line to understand the impact of NMI restoration via treatment with this class of compounds. Screening of these compounds revealed topoisomerase inhibitors as a top most hit, enabling re-expression of NMI at both the RNA and protein levels. Upon treatment with teniposide, cells displayed a dramatic reversion in their morphology to more epithelial-like structures in 3D culture assays, in addition to alterations in hallmark EMT gene signatures. We sought to determine the underlying mechanism whereby this class of compounds was able to initiate expression of NMI thereby altering the EMT program. Dissection of the NMI promoter element revealed a cluster of Interferon Response Factor regulatory sites in the basal NMI promoter. IRF7, a master regulator of type-I interferon response, was found to be a pivotal effector of topoisomerase inhibitors. Teniposide treatment dramatically upregulated expression of IRF7 in breast cancer cells. Silencing of IRF7 in breast cancer cells

drastically reduced the ability of teniposide to upregulate NMI gene expression. Furthermore, IRF7 has been shown to be a critical regulator in the propensity of breast cancer cells to metastasize to bone, suggesting the importance of IRF7 in regulation of the metastatic cascade. We hypothesize that topoisomerase inhibitors may serve a yet undiscovered function to modulate the EMT cascade via an IRF7-NMI mediated response.

Abstract Number 725

AMPLIFY: Development of Web-Based Lifestyle Interventions for Cancer Survivors

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Background: Among cancer survivors, health risks, such as poor diet and physical inactivity, tend to not occur in isolation, rather they cluster together. These health risk amplify the risk for secondary primaries and cancer recurrence. The AMPLIFY project (1P01 CA22992)will seek to improve both diet and exercise and address gaps in the multiple behavior literature by clarifying best practices in terms of targeting risk factors simultaneously or sequentially in older, rural, and underserved cancer survivors. One of the strategies to promote behavioral change in this study will include interactive online sessions that focus on theoretical constructs important for making health behavior change.
Methods: Sessions were created using Articulate Storyline 360. Extensive research team training in the use of Articulate Storyline 360 and weekly intervention development meetings were held to finalize intervention messaging, select age-appropriate story line characters and music for these user-friendly interactive sessions.
Results: Each week, a new storyline focusing on a specific health behavior theoretical construct was created including stimulus control, time management, stress management, social support, and problem solving. Key features of these educational sessions were session overviews, didactics, cancer survivor testimonials, quizzes/surveys, and interactive activities (e.g. open-ended questions, drag and drop). Sessions were created to allow adaptation to either diet or exercise content.
Conclusions: While sessions created with Articulate Storyline 360 are visually appealing and perhaps more engaging than a traditional PowerPoint or video, limitations of this application included poor quality computerized narration and limited selection of visual aids and characters. Overall, Articulate Storyline 360 was a user-friendly, practical tool for preparing educational web-based lifestyle intervention sessions for cancer survivors.

Abstract Number 726

Accuracy of an AI-assisted Tumor Metrics Image Processing Platform

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Background: Artificial Intelligence (AI) can be used to replicate human tasks including measurement and anatomic labelling of target lesions on computed tomography (CT) images in patients undergoing therapy for advanced cancer. An AI-assisted tumor metrics image processing platform has the potential to reduce errors and improve accuracy, standardization, efficiency, and communication between the radiologist, oncologic providers and patients.

Purpose: To evaluate the accuracy of a custom artificial intelligence (AI)-enhanced tumor metrics image processing platform designed to automate target lesion measurements and anatomic labelling on CT images from patients with advanced cancer.
Materials and methods: For this retrospective study, baseline body CT images from 119 adult patients with advanced cancer and measurable disease were prepared for analysis. A custom AI-enhanced tumor metrics image processing platform (aiMass) was created in collaboration with commercial software engineers (Innolitics) and designed to automate target lesion measurements and anatomic labelling in patients with advanced cancer. The AI algorithms were developed by annotating an independent training set of 12,000 target lesions (masses and lymph nodes). Using aiMass, 3 readers independently selected target lesions from a wide variety of anatomic locations and activated the AI algorithms with a single computer mouse click on each target lesion. The percentage of target lesions correctly measured and anatomically labelled by AI served as a measure of accuracy.
Results: A total of 795 target lesions were segmented from 119 CT studies. The average accuracy for AI to correctly measure the target lesions on first click was 83% (range 74-88%), with measurements presented in 1 second. The 17% of target lesions not correctly measured by AI were manually corrected within a few additional seconds for each. The average accuracy for AI to automatically label the location of the target lesion on the first attempt was 77% (range 75-79%), with an additional 14% corrected with one additional mouse click on an AI-guided display of anatomic locations.
Conclusion: An AI-enhanced tumor metrics image processing platform designed to automate target lesion measurements and anatomic labelling on CT images in patients with advanced cancer had high accuracy and required minimal manual effort.

Abstract Number 727
PGC1 Suppresses Kidney Cancer Progression by Inhibiting Collagen-induced SNAIL Expression
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The transcriptional events that promote invasive and metastatic phenotypes in renal cell carcinoma (RCC) remain poorly understood as much of the known biology of this cancer comes from the study of primary tumors. Herein, we report that decreased expression of the transcription factor peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PGC1α) and increased expression of collagen genes are associated with RCC tumor progression and worsened outcomes. PGC1α expression attenuates invasive phenotypes in RCC cells and suppresses tumor progression in vivo. In contrast, collagens produced by RCC cells promote invasive and migratory phenotypes. We identify a novel role for PGC1α in tumor biology that is estrogen related receptor (ERR) independent.
PGC1α suppresses collagen gene expression and tumor phenotypes via the induction of miR-29a. Furthermore, decreased collagens via the PGC1α/miR-29a axis suppresses collagen-mediated activation of discoidin domain receptor 1 (DDR1)/ERK signaling. The suppression of collagen/DDR1 cascade signaling by PGC1α leads to decreased levels of the known EMT regulators SNAIL1 and 2. Collectively, our results indicate that PGC1α inhibits RCC tumor progression via decreased expression of collagen and subsequent degradation of SNAIL proteins in RCC cells. Taken together, these data provide new insight into the development of aggressive renal cancer that could inform novel therapeutic approaches.

Abstract Number 728
Economic Evaluations in NCI-sponsored Network Cancer Clinical Trials

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Background: The rapid increase in the cost of cancer care in the United States has created a need to consider the efficacy of new treatments in the context of value. We examined the extent to which economic evaluation studies (EEs) were conducted for new treatments evaluated in clinical trials at SWOG, a large NCI-sponsored cancer research network.
Methods: We investigated Phase III cancer clinical treatment trials activated from 1980 onward with primary articles reporting the protocol-designated endpoints published in scientific journals by 2017. Using the PUBMED database, we searched for EE using the trial name, cancer type, information on the comparison arms, and refined key words for EE designs. We reported the overall proportion of trials with associated EE and the time trend of this proportion. We synthesized and analyzed information on funding sources, health outcomes, and sources of quality-of-life and cost data from the EE.
Results: Among 182 examined trials, 15 EEs were associated with 13 (7.1%) trials. Among the EEs, almost half (7/15) were either unfunded or did not report funding information, while nearly half (7/15) were funded by pharmaceutical companies and two EEs (2/15, 13.3%) were supported by federal funding. All EEs reported a healthcare payer perspective. The proportion of trials with an associated EE increased from 1980-1989 to 2000-2009, but never exceeded 11%. Sources for cost and quality-of-life data for the EEs primarily came from outside the clinical trials.
Discussion: Few economic studies of treatments evaluated in NCI-sponsored clinical trials have been conducted. Policymakers, payers, and patients lack economic evidence to consider newly evaluated cancer treatments, despite an urgent need to control healthcare costs.

Abstract Number 729

Targeting Voltage-gated Sodium Channels in Neuroendocrine Tumors with Novel Compounds

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Introduction: Neuroendocrine tumors (NETs) are a heterogeneous group of neoplasms including multiple subtypes found throughout the body. Although they are relatively rare and slow growing, NETs can be highly metastatic. Therefore, a molecular therapy capable of targeting NET cell migration and invasion could be useful for treating patients with metastatic disease. Voltage-gated sodium channels (VGSCs) have been shown to play a role in the spread of multiple different types of cancers, indicating that they could serve as potential drug targets for treating metastasis in NETs. Therefore, we have identified and evaluated two novel small molecule compounds, named SV57 and SV188 that were designed to inhibit VGSC activity.
Methods: Initially, the basal mRNA and protein expression of two VGSC isoforms most commonly reported in various cancers were assessed in NET cells (TT, MZ-CRC-1, BON-1, and QGP-1) and normal, non-cancerous cells (917 and HEK293) using quantitative RT-PCR and Western blot, respectively. Furthermore, the cytotoxicity threshold of the small molecule compounds was

evaluated in NET cells by a cell proliferation assay (MTT). A Boyden chamber assay was used to investigate the impact of the compounds on NET cell migration.
Results: We have discovered that NET cells overexpress at least two VGSC isoforms named Nav1.5 and Nav1.7 in comparison to normal cells. We also determined the cytotoxicity of both small molecule compounds in NET cells. Finally, we demonstrated the ability of the small molecule compounds SV57 and SV188 to reduce NET cell migration.
Conclusions: We have shown that VGSCs may indeed be potential targets for treating metastatic NETs. The results may be translated to future studies focused on controlling metastasis through molecular therapies.

Abstract Number 731

Visualization of the Relationship Between Survival and Sequential Treatments in Metastatic Breast Cancer

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Background: Sequential therapeutic options in metastatic breast cancer (MBC) are disparate. However, clinical trial data on treatments received pre- or post-trial are limited to number of prior therapies and not the prior sequence or duration of treatment. The lack of contextual data on prior treatment history in clinical trials may contribute to challenges evaluating efficacy.
Methods: This retrospective cohort study utilized the nationwide, de-identified electronic health record (EHR)-derived Flatiron Health database to identify women with estrogen receptor (ER) positive, human epidermal growth factor receptor 2 (HER2) -negative MBC diagnosed in 2014 who subsequently received paclitaxel. Visualizations were created with individual patients represented on the Y-axis and time on the X-axis to qualitatively evaluate treatment patterns and survival. Time 0 was defined as initiation of paclitaxel in the metastatic setting. Specific treatments were represented by a color-coded treatment bar, with post-metastatic paclitaxel in black, hormonal therapy in shades of red, chemotherapy in shades of blue, HER2-targeted therapy in shades of green, and other targeted therapies in shades of orange. A Kaplan-Meier curve was generated as a function of time from paclitaxel to death or censoring and was superimposed on the visualization. Separate visualizations assessed progression-free and overall survival (PFS, OS). Hazard ratios (HR) and 95% confidence intervals (CI) from Cox proportional hazard models evaluated the association between prior treatment time and post-paclitaxel OS.
Results: Of 877 ER+/HER2- MBC patients diagnosed in 2014, 234 received paclitaxel. Qualitatively, the visualization graphic sorted by OS demonstrated that patients who received treatments for longer durations prior to paclitaxel had worse survival compared to those who received paclitaxel earlier in their treatment course (i.e., shorter time on treatment prior to receiving paclitaxel). Quantitatively, median survival from paclitaxel initiation was 20 months (IQR 8-53). In adjusted models, every year increase on treatment prior to paclitaxel initiation was associated with a 16% increased yearly hazard of death (HR 1.16, 95% CI 1.05-1.29).
Conclusion: Visualizing paclitaxel in the context of disease course demonstrates an association between length of time on prior therapy and remaining OS, highlighting the potential for an overall OS benefit to be observed merely based on early vs. late receipt of treatment. This study should promote future evaluation of treatment sequencing and duration of prior therapies when studying the impact of new treatments.

Abstract Number 732

Prospective Feasibility Study of a Mindfulness-Based Program for Breast Cancer Patients in the Deep South

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Purpose: Mindfulness interventions are effective in decreasing psychological distress and improving quality of life in cancer patients. However, few mindfulness programs for cancer recovery exist in major cities and surrounding areas throughout the South. This study assessed the feasibility of a mindfulness-based program for breast cancer patients in Birmingham, Alabama. Secondarily, the study determined the effectiveness of the program by examining changes in patients' mindfulness skills and quality of life.

Material and Methods: The trial was a prospective feasibility study conducted over 10 months. Baseline data were compared to follow-up data collected after the 8-week program and at 16 weeks post enrollment. Intervention activities occurred within a university hospital clinic in Birmingham, Alabama. The sample consisted of 12 referred patients. Participants were female; mean age was 53 years. Participants must have been diagnosed and treated for breast cancer, capable of attending weekly sessions, and scored a 4 or above on a validated distress scale. Three groups of 2-5 patients underwent the 8-week program at different times during the length of the study. Program sessions followed a well-known mindfulness-based stress reduction curriculum, including mindfulness techniques like sitting meditation, hatha yoga/restorative yoga, and a body-focused attention practice called body scan. Feasibility would be achieved if 80% of eligible patients chose to enroll in the study and 70% of enrolled patients attended all program sessions. Secondary objectives were assessed by changes in mindfulness skills and quality of life indicators. Variables were measured by validated scales administered at baseline and two follow-ups.

Results: Of eligible patients, 44. 4% (12/27) enrolled in the study and 16. 7% (2/12) attended all program sessions; however, 66. 7% (8/12) of participants completed at least 7 sessions. Between baseline and 16-week follow-up, patients demonstrated statistically significant increases in mindfulness skills (P=0. 033), self-compassion (P=0. 038), spiritual wellbeing (P=0. 033), and overall wellbeing (p=0. 017). There were statistically significant decreases in fatigue (P=0. 011) and distress (P=0. 008).

Conclusions: Program effectiveness was promising for all attendees. Major reasons for the low recruitment and retention numbers were distance from the clinic and length of the study. Future studies should consider the commute and time constraints of attendees to increase program accessibility.

Abstract Number 733

Verrucarin A: A Mycotoxin with Anti-Tumor Activity in Thyroid Cancers

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Introduction: As the most prevalent endocrine malignancy, thyroid carcinomas affect over 50,000 adults annually. Approximately a quarter of patients do not respond well to currently available treatments including radioactive iodine ablation, TSH suppressive therapy, and thyroidectomy, due to their cancer de-differentiating. These cancers become progressively harder to target and treat, underscoring the need for an alternative treatment. Natural compounds remain

to be a fruitful source of potential therapeutic agents. Verrucarin A, a mycotoxin derived from Myrothecium verrucaria, has previously demonstrated anti-cancer properties in breast, renal, and prostate cancer. Our study investigates the therapeutic efficacy of verrucarin A in aggressive thyroid cancer cell lines.
Methods: Papillary (TPC-1), follicular (FTC236), and anaplastic (8505C) thyroid cancer cell lines were treated with verrucarin A for 48 and 96 hours. Lung fibroblast (WI-38) cells were used as control. To determine the IC50 for all cell lines, a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was used. To detect apoptotic cells after treatment with verrucarin A, an Annexin V assay was used. In further confirming apoptosis as the mechanism of cell death, protein was harvested and analyzed via Western Blot to detect marker of apoptosis, including XIAP, cleaved PARP, and MCL-1. Finally, RNA was harvested after treatment to analyze thyrocyte specific differentiation markers (TTF1, TTF2, NIS, and PAX8), assessed by qPCR to determine verrucarin's potential in inducing re-differentiation.
Results: Verrucarin A demonstrated an IC50 of 4.4nM against the papillary thyroid (TPC-1) and the follicular thyroid (FTC236) cancer cell lines, and an IC50 of 10nM against the anaplastic thyroid cancer cell line (8505C). The control, WI-38, was unaffected by verrucarin A at these concentrations. Annexin V and Western Blot results suggested that verrucarin A increased apoptotic events in a dose-dependent manner. Analysis of RNA conclusively indicated that verrucarin A effectively induced thyrocyte specific genes, increasing expression levels of TTF1, TTF2, NIS, and PAX8.
Conclusion: Upon treatment with verrucarin A, proliferation is reduced in papillary, follicular, and anaplastic thyroid cancer cell lines via an apoptotic mechanism, differentiation of thyrocyte specific markers are induced and the migration of cells is attenuated. These findings offer promising potential in using verrucarin A as a targeted therapy in the future.

Abstract Number 734
Gene Expression Profiles in Individual Canine Patient Osteosarcomas
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The study of gene expression in tumors has great promise for elucidating both mechanisms of tumorigenesis and identification of therapeutic targets. As technology has improved, the focus of such studies has shifted from identifying similarities in groups of tumors to teasing out unique details of individual masses. This novel approach requires model systems in which both the tumor and the therapeutic response can be assessed. Canine osteosarcoma represents both a significant health problem in larger breed dogs and a highly correlative model of osteosarcoma in humans. Using this model, gene expression profiles were completed for eight canine osteosarcomas using corresponding patient normal bone RNA for comparison. RNA was extracted from tumor and normal, marrow-depleted, phalanges from amputated limbs of patient dogs and sequenced. Sequence data was examined in the context of similarities between the patients' tumors and normal bone cellular constituents, both as a group and as individuals. Approximately 3,000 genes were found to be differentially expressed in tumors as compared to the matched normal tissue. These genes were expressed in both unique and overlapping patterns when comparing individuals. When expression of cellular receptors was examined specifically, the four most commonly upregulated receptors across all tumors were CXCR4, AVPR1,

ADGRB1, and TNFRSF4. Expression of these genes was increased in most, but not all tumors. When the most overexpressed receptor in each dog was determined, the pattern was less cohesive, with significant inter-individual variation. Ultimately, these data emphasize both the commonalities and differences among different tumors of the same histological type. The latter supports development of therapeutic approaches based on individual tumor gene expression patterns as potentially the best approach to confronting the variability of tumor gene expression.

Abstract Number 735
MYB-AR Cross-talk Promotes Castration-resistance in Prostate Cancer
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Progression of prostate cancer from castration-sensitive to castration-resistant disease is a complex process, which likely involves multiple genetic and epigenetic alterations. Here we report a novel mechanism where MYB acts as a novel binding partner of AR enabling its ligand independent activation to support castration resistance in prostate cancer. MYB and AR interact and co-localize with each other predominantly in the nuclei. Androgen-depletion or enzalutamide treatment does not interfere with MYB-AR interaction, and MYB overexpressing prostate cancer cells retain AR in the nucleus even when cultured under androgen-deprived condition. Transcriptional activity of KLK3 (an androgen-responsive gene encoding PSA) promoter is increased in MYB-overexpressing cells, while sustained under androgen-depleted condition. There is a MYB-binding region in KLK3 promoter in close proximity to the AR binding site as identified by in silico analysis, and MYB cooperatively promotes AR binding to the KLK3 promoter as shown by chromatin immunoprecipitation assay. MYB-overexpressing prostate cancer cells exhibit greater tumorigenicity when implanted orthotopically and quickly regain growth following castration leading to the poorer survival of mice, compared to those carrying low MYB-expressing prostate tumors. Together, these findings establish a novel and significant role of MYB-AR cross-talk in prostate cancer, which could be exploited for its therapeutic management.

Abstract Number 736
Mutations in the TERT Promoter Associate With a Distinct Biological Signature
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TERT promoter mutations (TPM) drive allele-specific expression of the catalytic subunit of telomerase, thereby allowing telomere length maintenance in many cancers. To gain insight into mechanisms that may be operating specifically in cancers with TPM, we characterized the expression profiles of hundreds of tumor-derived cell lines from the Cancer Cell Line Encyclopedia. Transcriptional and proteomic signatures identified a significant association between TPM and enhanced extracellular matrix degradation, altered expression of integrins, MAPK activation and mesenchymal/EMT characteristics. E-cadherin was found to be expressed at lower levels in cell lines and clinical tumor samples with TPM. These results indicate that TPM cancers display distinct cell surface and signaling properties, including enhanced MAPK pathway activation. We show here, by inhibiting canonical Ras-pathway effectors, MEK1 and 2, with the FDA-approved MEK inhibitor trametinib, that mutant TERT promoter function relies on MEK1 & 2 signaling. These TPM-specific signatures may be explained in part by several transcription factors

differentially expressed in TPM cancer types vs wt cancers. Of note, the transcription factor slug, which is a canonical regulator of mesenchymal cell identity, was consistently expressed at high levels in TPM cell lines. Based on shRNA knock-down experiments, slug promotes TERT expression. Our results indicate that mutations in the TERT promoter may be a marker for a biologically distinct subset of cancer cells and that these identified differences could impact the clinical behavior of TPM tumors.

Abstract Number 737
Clinical Trials Time to Activation: The Process, Structure and People
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O'Neal Comprehensive Cancer Center at UAB

Introduction: The accessibility of unique clinical trials attracts new patients to the institution, leading to higher accrual numbers, and better patient access to new, novel agents. However, activating clinical trials in large academic institutions, such as UAB, is a long, arduous, and costly process. This process involves communication and collaboration between many stakeholders across numerous departments. Consequently, many trials do not open soon enough to accrue patients at an optimal rate or the trial closes nationally by the sponsor as soon as it becomes active, leading to loss of time and effort, and a negative financial impact. We aimed to perform a preliminary analysis to describe trials activation process at UABCCC, as well as identify the length of time that a new trial takes to become activated, and determine the rate limiting steps and processes.
Methods: We mapped the current operational/administrative process for activation in our Cancer Center. Using the dates available for industry sponsored protocols activated between 2016 and 2018, the difference between each step in the process was calculated.
Results: Our retrospective analysis showed that, overall, the median complete activation process from WG approval to conducting the study initiation visit (SIV) takes 221 days. However, it took a median and average time of 205, and 218.83 days, respectively, from Protocol Review Committee review to SIV during the same time period. The median time it took from PRC approval to completing all administrative submissions (FAP, Budget, OSP, IRB, WIRB) was 63 days, and the review process from administrative submission to approval was 26.5 days. Finally, the median time from WIRB approval to SIV was 70 days. From 2016 to 2017 and 2017 to 2018, there has been an increase in total time to activation each year, by 9.0% and 26.22%, respectively.
Conclusion: Though the overall process of trial activation is long, there are external factors that influence the first part of the trajectory (CDA receipt, regulatory documents receipt, feasibility assessments by the sponsors, etc.). Since availability of regulatory package is the time when all essential documents required for activation are available, defining the starting point of activation is critical, and the NCI has recommended the PRC review to be the best proxy for the start of activation. This study should provide the framework for future studies, to better understand each process in the activation process, and the current system gaps to re-engineer workflows to improve time to activation.

Abstract Number 738
Psychosocial Wellbeing and Supportive Care among Metastatic Cancer Survivors in the South
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Introduction: Of the 16 million cancer survivors in the U.S., 10-30% are living with metastatic disease. Metastatic cancer survivors (MCS) are at an increased risk for psychosocial distress,

which may negatively influence quality of life. The National Comprehensive Cancer Network guidelines recommend that supportive care begin at diagnosis and continue through end of life. The aim of this study was to better understand psychosocial wellbeing and supportive care needs among MCS residing in the Deep South. **Methods:** MCS were identified via UAB Cancer Registry and I2B2. Using a modified Dilman's method, eligible MCS (>21 years and physician permission to contact) were mailed a survey. Psychosocial wellbeing (i.e., physical and mental wellbeing, anxiety, depression, social isolation, emotional support, and hopefulness) of MCS were assessed via PROMIS® measures. Two survey questions queried supportive care use and interest. Returned surveys were double-key entered into REDCap®. Data was analyzed using Excel. Descriptive statistics were used to characterize the study sample and instrument scores. Between group difference, between female and male MCS, was examined via independent-samples t-test. **Results:** To date, 100 surveys have been returned (female=60; male=40; Mage=67 years; Msurvivorship=3 years) with a broad representation of primary cancer sites (breast=23%; prostate=10%; gynecological=16%; colorectal=13%; lung=10%; kidney=11%; other=17%). Mean instrument scores were within "normal limits" among MCS. However, females reported better physical (45.35 vs 43.89) and mental wellbeing (49.29 vs 47.88), anxiety (47.54 vs 48.60), depression (45.91 vs 47.91), social isolation (40.68 vs 40.79), emotional support (56.90 vs 55.04), hopefulness (57.51 vs 54.89) and history of supportive care use (40% vs 23%), than males, respectively. Both females (65%) and males (58%) reported interest in future supportive care, with the greatest interest in nutrition classes (37%). Gender differences were seen in program preferences (i.e., gardening, yoga, art therapy). Twenty percent of men who reported interest in supportive care desired "other" programs.
Discussion: Findings suggest that female MCS may be more likely to use supportive care, which may enhance psychosocial wellbeing. Gender preferences may play a role in supportive care uptake. Further research is needed to better understand preferences, facilitators, and barriers to supportive care use among male MCS.

Abstract Number 739
Serum Exosome-based Biomarker to Predict Chemobrain
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Background: Chemotherapy for the clinical management of cancers is associated with severe cognitive decline, an effect often referred to as "chemobrain". Despite the benefits of cancer therapy, an increasing number of clinical studies have shown that systemic chemotherapy is associated with long lasting cognitive impairments that can adversely impact quality of life (QOL). Chemotherapy triggers neuroinflammation (microglial activation) that contribute substantially to the underlying neurotoxicity and cognitive dysfunction. Therefore, minimally invasive and easily assayable biomarkers that are predictive of chemobrain are required to identify at risk patients.
Objective/Hypothesis: We propose to identify a serum exosome-based biomarker to predict and link cancer therapy-related cognitive impairment (CRCI) associated with microglia-mediated neuroinflammation in a minimally invasive and clinically relevant manner. Microglia-mediated neuroinflammation is the hallmark of chemobrain. UAB is the only U.S. site with investigational approval of a human imaging agent (PET ligand [18F]DPA-714) targeting the brain inflammation marker translocator protein (TSPO) in activated microglia to detect and quantify neuroinflammation. This proposal harnesses the momentum of this technology to test serum of ovarian and breast cancer patients before and after chemotherapy treatment. Exosomes are now recognized as important regulators of biological processes, with specific cargo capable of controlling cell signaling and target cell function. We hypothesize that patient serum-derived

exosomal cargo (RNA, miRNA or proteins) can be analyzed and profiled to detect a link between cognitive dysfunction and neuroinflammation and predict chemobrain in future patients.
Specific aims: 1) To characterize exosomal miRNA profile from the patient serum; 2) To determine proteomics profile of patient serum-derived exosomes.
Design: Serum from 8 ovarian cancer patients before and after Neo-Adjuvant Chemotherapy Treatment (NACT) will be collected. We will also recruit 4 age-matched, never cancer control participants. Neuroinflammation will be measured before and after treatment using PET with tracer [18F]DPA-714 and, cytokine/chemokine ELISA. Cognitive functioning will be measured before and after treatment with self-report and objective neuropsychological measures. These data will be linked with patient's serum exosomal miRNA and proteomics profiles in search of a reliable biomarker for the CRCI.
Anticipated Impact: Successful accomplishment of the proposed study will allow for the rapid, non-invasive serological diagnosis of chemobrain.

Abstract Number 740
Heparanase Promotes Cancer Stemness and Aggressive Tumor Growth In Vivo
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Heparanase is a heparan sulfate degrading enzyme that is upregulated in most cancers where it promotes extracellular matrix degradation and tumor cell migration thereby facilitating malignant cell exit from primary tumor sites. Heparanase expression is often enhanced in tumors as they become more aggressive; however, the role of heparanase in regulating cancer cell stemness is not well explored. To examine this, heparanase was knocked down in cells from human myeloma cell lines using shRNA. These knockdown cells exhibited significantly reduced expression of pro-tumorigenic genes HGF and MMP-9, and reduced shedding of syndecan-1, consistent with the known influence of heparanase on these cells. When grown in stem cell medium supportive of sphere formation, the heparanase knockdown cells exhibited a dramatic decrease in the number of spheres that formed compared to cells expressing a high level of heparanase. These results clearly suggest that increased heparanase expression in myeloma cells correlates with increased sphere formation, indicating the presence of tumor cells having stem cell characteristics. We also discovered that heparanase high cells exhibited elevated expression of cancer stem cell markers including SOX2, ALDH1A1, and GLI1. A novel paradigm in tumor biology suggests that cancer growth is driven predominantly by stem-like cells within a tumor. Thus, we examined whether this increased stemness potentiated the development of metastatic foci of myeloma tumor cells when injected into SCID mice. The heparanase high cells readily formed metastatic foci in bone. In contrast, both the number and size of metastases were reduced when cells were injected in the presence of Roneparstat, an inhibitor of heparanase enzyme activity. Intravascular injection of heparanase deficient cells revealed that they failed to form aggressively growing tumor, consistent with the finding that they have decreased stemness properties. These studies provide the first evidence that heparanase plays an essential role in establishing cancer stemness, and these properties contribute to increased metastatic tumor growth in myeloma cells.

Abstract Number 741
Transgelin 2 is Overexpressed in Breast Cancer and is a Predictor of Poor Patient Survival
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Introduction: For women in the USA, breast cancer is a common cause of cancer-related death. To improve the survival of breast cancer patients, there is a need to identify new molecular biomarkers and therapeutic targets. Integrative analyses of publicly available cancer transcriptomic data (TCGA datasets) by using the UALCAN cancer transcriptome analysis portal (a UAB web portal, http://ualcan.path.uab.edu) have established that transgelin 2 (TAGLN2) gene is overexpressed in breast cancers compared to normal tissues. TAGLN2, a member of the calponin family of actin-bundling proteins, is involved in the regulation of cell morphology, motility, and cell transformation. In the current study, we analyzed the expression of TAGLN2 in subtypes of breast cancer and assessed its utility as a predictive biomarker of patient survival. **Methods:** With UALCAN, a publicly available portal for analysis of cancer transcriptome data, we searched for overexpressed genes in breast cancers. Our analyses confirmed that TAGLN2 was highly expressed in breast cancers. Furthermore, we assessed the extent and staining patterns of TAGLN2 expression in various stages of breast cancer and evaluated gene expression based on race. We also analyzed survival of those with breast cancers expressing high or low TAGLN2. RESULTS: Sequencing data for breast cancers in TCGA were analyzed for 114 normal breast tissues and 1097 breast cancers. Our analyses demonstrated TAGLN2 RNA overexpression in breast cancers and established that it was overexpressed in all stages of breast cancers, with highest expression in stage IV cancers. Furthermore, TAGLN2 was overexpressed in both African American and Caucasian breast cancers, with higher expression in African American patients. There was elevated expression of TAGLN2 in all types of breast cancers, including luminal (ER- and PR-positive), HER2-positive, and triple-negative breast cancers. Survival analyses showed that high expression of TAGLN2 was associated with poor patient survival. Additionally, our immunohistochemical staining data from The Human Protein Atlas showed high TAGLN2 protein expression and validated the gene expression findings for human breast cancer tissues. In the future, we plan to perform biochemical and functional analyses using breast cancer cell lines and tissues to evaluate protein expression of TAGLN2 and to determine its role in progression of this disease.
Conclusion/discussion: Our preliminary findings suggest that high expression of the TAGLN2 gene in breast cancer is associated with aggressive phenotypes, predicts poor patient survival, and, for this disease, could serve as a biomarker and potential therapeutic target.

Abstract Number 742
Single-cell Transcriptome of Lung Cancer Brain Metastatic Cells in the Cerebrospinal Fluid
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In patients with stage IV non-small cell lung cancer (NSCLC), the incidence of leptomeningeal metastasis (LM) is 3-5%. Although the diagnosis and prognosis of NSCLC-LM patients have improved in recent years, the overall treatment outcomes are still not ideal. This study is to investigate the characteristics of circulating tumor cells (CTCs) in cerebrospinal fluid (CSF) of NSCLC-LM patients, to provide essential knowledge and inform diagnosis and treatment of lung cancer brain metastasis. In NSCLC-LM patients, a small number of CSF-CTCs are present in the lymphocyte background, therefore the standard bulk RNA-seq method is unable to detect these metastatic CTCs. To establish the cell type profile in normal CSF, we investigated diagnostic test samples from patients with other brain diseases that do not affect the CSF cell composition, and performed single-cell RNA-seq (scRNA-seq) on 344 cells in 3 patients to profile the cell type composition in these nearly normal CSF samples. About 10% of the normal CSF cells are monocytes and the rest 90% belong to T cells. In 6 NSCLC-LM CSF samples, we sorted CD45(-

) cells by flow cytometry and performed scRNA-seq in 3,154 individual CTCs following the Smart-Seq2 protocol. After quality control and filtering, transcriptome data from 1,908 cells were used in the subsequent analyses. Clustering analysis revealed that lung adenocarcinoma markers, including SFTPA, SFTPB, SFTPC, NAPSA, TTF-1 and KRT family members, are highly expressed these cells, which is sharp contrast to CSF lymphocytes. These results indicate that these cells are indeed lung cancer metastatic cells in CSF samples (CSF-CTCs). Interestingly, within each patient, the CSF-CTCs form two clusters: one is shared across all tumor samples and the other is patient-specific. These finding suggest a subset of CSF-CTCs from different NSCLC-LM patients share commonality in the transcriptome, and yet there is substantial heterogeneity in scRNA-seq profile among patients. This research is the first systematic and comprehensive characterization of normal CSF cell profile as well as lung cancer brain metastatic CTCs in CSF samples at single-cell transcriptome level.

Abstract Number 743

MDSC-derived Extracellular Vesicles Modulate B cell Function in Lung Cancer

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Introduction: Extracellular vesicles (EVs) are extracellular, membrane-bound cell-derived vesicles that facilitate intercellular communication. Myeloid-derived suppressor cells (MDSCs) are heterogeneous immature myeloid cells that are drivers of tumor associated immune suppression. We demonstrated earlier that MDSCs can suppress B cell responses. However, it is unknown if MDSC-derived EVs regulate B cell responses during tumor progression. In this study, we investigate the role of MDSC-derived EVs on the regulation of B cell differentiation and mitochondrial function.

Materials and Methods: A syngeneic mouse model of lung cancer was used by intravenous or intracardiac transfer of Lewis Lung Carcinoma (LLC) cells into C57BL/6 mice. MDSCs were purified from BM of naïve mice or tumor-bearing mice. MDSC-derived EVs were isolated by differential centrifugation method and quantified by Nanosight. 5 x 105 BM cells or splenocytes from naive or tumor-bearing mice were co-cultured with MDSCs-EVs from tumor-bearing mice or immature myeloid cells (IMC)-EVs from naïve mice at 1:10 cell versus EV ratio. Flow cytometry analyses were performed at 48 h and 72 h after co-culture to assess the percentages of immature and mature B cells.

Results: The percentages of total B220+ cells and B220+IgD+IgM+ mature B cells were decreased while the percentage of B220+IgD-IgM+ immature B cells was significantly increased in the co-culture of BM cells from naïve mice in the presence of MDSC-EVs from tumor-bearing mice compared to IMC-EVs from naïve mice. Interestingly, the percentages of total B220+ cells, and B220+IgD+IgM+ mature B cells were further reduced whereas the percentage of B220+IgD-IgM+ immature B cells was elevated in the co-culture of BM cells from tumor-bearing mice in the presence of MDSC-EVs from tumor-bearing mice compared with IMC-EVs from naïve mice. Additionally, the percentages of total B220+CD19+ cells and B220+CD19+IgD+IgM+ mature B cells were decreased in the co-culture of splenocytes from naïve mice in the presence of MDSC-EVs from tumor-bearing mice compared with IMC-EVs from naïve mice. Moreover, the mean fluorescence intensity (MFI) of MitoView staining on total B220+ cells was reduced in the co-culture of BM cells in the presence of MDSC-EVs from tumor-bearing mice compared to IMC-EVs from naïve mice, indicating that MDSC-EVs inhibited mitochondrial activity during B cell differentiation. **Conclusions:** Together these data indicate that MDSC-EVs may suppress B cell maturation via regulation of mitochondrial function.

Abstract Number 744

Investigation of FCRL Family Member Applications using 89Zr Labeled Antibodies

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Fc receptor-like (FCRL) genes encode type I cell-surface transmembrane glycoproteins primarily restricted to lymphocytes. FCRL proteins harbor cytoplasmic tyrosine-based signaling motifs that regulate lymphocytes, but their ligands are largely undefined. FCRL1-5 are preferentially expressed by B-cells, while FCRL6 is expressed by cytotoxic T and natural killer cells. Because of this preferential expression pattern, we aimed to investigate FCRL family members for applications in immunodiagnostics and immunotherapy in human malignancies. We have developed a series of anti-FCRL monoclonal antibodies and labeled them with the PET isotope Zirconium-89 to monitor localization and biodistribution in animal models. Receptor-specific anti-FCRL1 and anti-FCRL6 antibodies were conjugated to desferrioxamine (Df-Bz-NCS) using a previously reported literature procedure. In brief, the antibody solutions were adjusted to pH 9 with a sodium carbonate buffer, combined Df-Bz-NCS, and incubated at 37°C for one hour. After incubation excess Df-Bz-NCS was removed and the concentration of the antibody was confirmed via BCA assay. Zirconium-89 was produced and purified by the UAB cyclotron facility, labeling was optimized and immunoreactivity was confirmed via the Lindmo Assay. Two groups of RAG1 KO mice were implanted with mouse BW5147 T cells retrovirally transduced with FCRL6 or an FCRL6 negative-vector only control construct. These cell lines formed tumors after three days. Each mouse was injected with 100 µCi of Zirconium-89 labeled antibody and PET/CT images were collected. After the final imaging time point was completed, the animals were sacrificed, organs were harvested and the activity in each organ was measured to determine the distribution of activity. After optimizing the labeling we were able to achieve 100% labeling at a specific activity of 4mCi/mg of the anti-FCRL6 antibody without affecting affinity to the FCRL6 protein. The PET/CT images showed a significant difference in the average Standard Uptake Values (SUV) of the FCRL6 expressing tumors vs the non-expressing tumors (5.64 ± 1.27 vs 1.57 ± 0.28, p=0.0001). Biodistribution studies confirmed the results from the image analysis showing a significant difference in the average %ID/g of the expressing tumors vs the non-expressing tumors (24.7 ± 6.98 vs 6.87 ± 1.86, p=0.0001). Spleen uptake was found to be 13.2-15.4 %ID/g suggesting some immune response. The primary method of clearance was found to be the liver, a common route for antibody clearance. These results show that we are able to successfully target FCRL6 with anti-FCRL6 antibody to acquire PET/CT images. Further studies investigating the efficacy of anti-FCRL1 antibodies as PET/CT imaging agents in B cell malignancies are in progress.

Abstract Number 745

CHK1/2i Suppresses NOTCH Signaling and Enhances Cytotoxicity of Cisplatin-IR in HNSCC

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Platinum-based chemoradiotherapy is a mainstay of organ-preserving treatment for patients with head and neck squamous cell carcinoma cancer (HNSCC). However, the disease eventually becomes refractory to treatment necessitating new therapies. Checkpoint kinase 1 and 2 (CHK1/2) are serine/threonine kinases that activate cell cycle checkpoints and serve as critical regulators of the DNA-damage response (DDR). As resistance to cisplatin and radiation may involve a heightened DDR, we hypothesized that prexasertib, an inhibitor of CHK1/2, may

enhance the cytotoxicity induced by cisplatin and irradiation in HNSCC. In this study, we found that the addition of prexasertib to cisplatin and radiation (IR) significantly decreased the in vitro survival fraction in HNSCC cell lines both with and without radiotherapy. Reduced survival was accompanied by inhibition of DNA repair checkpoint activation which resulted in persistent DNA damage and increased apoptosis. Additionally, genomic analysis revealed that prexasertib downregulated NOTCH signaling target genes (NOTCH1, NOTCH2, NOTCH3) and their associated ligands (JAG1, JAG2, SKP2, MAML2 and DLL1). Prexasertib also recuded NOTCH1, NOTCH3 and HES1 protein expression. Importantly, a significant tumor growth delay was observed in vivo in both HPV-positive UM-SCC47 and HPV-negative UM-SCC1 cell line xenografts receiving prexasertib, cisplatin, and radiotherapy without a concomitant increase in toxicity as assessed by mouse body weight. Taken together, prexasertib reduced NOTCH signaling and enhanced the in vitro and in vivo response of HNSCCs to cisplatin and radiation, suggesting combination therapy may increase clinical benefit. A clinical trial has been ongoing (NCT02555644).

Abstract Number 746

Somatic Moesin E349G Mutation Is An Oncogenic Therapeutic Target in Cervical Cancer

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Moesin (MSN, membrane-organizing extension spike protein) is encoded by a gene on chromosome X, and has significant sequence homology to ezrin, protein 4.1, talin, radixin, and merlin (ERM). The ERM proteins are a family of widely distributed membrane proteins which link membrane proteins and actin and play important roles as cell-shape determination, membrane-protein localization, membrane transport and signal transduction as well as regulating several cellular processes including reorganization of actin cytoskeleton, cell survival, membrane dynamics, cell migration, adhesion and regulation of membrane protrusion. Multiple reports have outlined different factors including localization of ERMs within the cell, their level of phosphorylation as well as expression profile to be responsible for ERM protein-mediated promotion of tumorigenesis. Exome sequencing of malignant cervical tumors revealed significant recurrence of somatic mutations in MSN, including a hot spot of glutamic acid to glycine mutations at position 349 (E349G). Overexpression of wild type MSN in C4-I (CRL1594) cervical cancer cell line represses cell growth suggesting it acts as a tumor suppressor, however, expression of the MSN E349G promotes transduced C4-I tumor cell proliferation. C4-I cells transduced with MSN E349G presented lower mutant protein expression and knock down of wild type MSN with hsRNA both reverted the pattern of cell migration and adhesion in cells with wild type MSN. Western blot analysis also detected lower level of total as well as activated/phosphorylated moesin suggesting that MSN E349G may modulate its ubiqutination/degradation. Confocal immunofluorescence localization analyses demonstrated that E349G mutant moesin localized to the apical surface, concentrated in the membrane borders, and in filopodia-like protrusions of the cervical cancer cell C-4I, in contrast, the WT moesin are enriched in the membrane surface only. Furthermore, E349G moesin mutant cells failed to response to the PMA compare to the WT cells. We also observed changes in β-catenin, E-cadherin levels in C4-I cells overexpressing MSN E349G implicating their possible roles played in cell proliferation, cell adhesion and cell migration. Our data suggest that MSN E349G contributes to cancer development with increasing cellular proliferation and lower motility via changes in its open and closed state.

Index of Presenting Authors

Last Name	First Name	Category	Abstract Number	Page
Afaq	Farrukh	Open	700	103
Agarwal	Sumit	Postdocs	200	23
Akintola	Olasubomi	Chaudhuri	500	92
Alexander	Katie	Postdocs	201	23
Al-Obaidi	Mustafa	Open	701	103
Alsheikh	Heba Allah	Open	702	104
Anbiah	Benjamin	Chaudhuri	501	92
Anderson	Ashlyn	Grad Students	300	39
Anderson	Zoya	Grad Students	301	39
Anderson	Nicholas	Grad Students	302	40
Ankenbauer	Katherine	Grad Students	303	41
Antoine	Lisa	Grad Students	304	41
Bail	Jennifer	Bailey	400	86
Bajpai	Prachi	Open	703	105
Bandari	Shyam Kumar	Postdocs	202	24
Bastola	Soniya	Grad Students	305	42
Beer	Hannah	Grad Students	306	42
Behring	Michael	Postdocs	203	24
Bhalerao	Nikita	Grad Students	307	43
Booker	Burthia	Postdocs	204	25
Bownes	Laura	Residents/Fellows	100	14
Bradley	Kelley	Open	718	114
Brinkley	Garrett	Grad Students	308	44
Broker	Thomas	Open	704	105
Brooke	Dewey	Grad Students	309	44
Brown	Reginald	Grad Students	310	45
Bugide	Suresh	Postdocs	205	26
Carnes	Rachel	Grad Students	311	45
Caston	Nicole E.	Grad Students	312	46
Chakravarti	Sayak	Grad Students	313	47
Chaple	Ivis	Grad Students	314	47
Chow	Louise	Open	705	106
Coleman	Carrie B.	Open	706	107
Curtis	Peyton	Grad Students	315	48
Cutshall	Hannah	Grad Students	316	49
Davenport	Mackenzie	Grad Students	317	50
Davis	Elizabeth	Grad Students	318	50
Davis	Jasmine	Grad Students	319	51

DeAtkine	Andrew	Bailey	401	86
Dhir	Aditi	Residents/Fellows	101	14
Dorsett	Kaitlyn	Chaudhuri	502	93
Ducharme	Maxwell	Grad Students	320	51
Edmonds	Mick	Open	707	107
Elkhanany	Ahmed	Open	708	108
England	Rebecca	Grad Students	321	52
Fang	Hua (Amanda)	Grad Students	322	53
Feng	Justina	Grad Students	323	53
Gahagan	Andrew	Open	709	109
Gc	Sajina	Grad Students	324	54
Gibson	Justin	Grad Students	325	55
Goliwas	Kayla	Postdocs	206	26
Gowda	Pramod	Open	710	109
Guenter	Rachael	Grad Students	326	55
Gupta	Priyanka	Postdocs	207	27
Habbit	Nicole	Grad Students	327	56
Halilova	Karina I.	Bailey	402	87
Hashemi	Mohammadjafar	Grad Students	328	57
Hassani	Iman	Grad Students	329	58
Hawkins	Cyntanna	Grad Students	330	58
Hinshaw	Dominique	Grad Students	331	59
Hossain	Md Emon	Postdocs	208	28
Houson	Hailey	Postdocs	209	28
Ibtisam	Ibtisam	Grad Students	332	59
Ingram	Stacey	Open	711	110
Janjetovic	Zorica	Open	712	111
Jenkins	Tyler	Grad Students	333	60
Jones	Robert	Grad Students	334	61
Kanchanapally	Rajashekhar	Open	713	111
Kansom	Teeratas	Grad Students	335	61
Karki	Suman	Open	714	112
Kim	Tae-Kang	Open	715	112
Kim	Hyung-Gyoon	Open	716	113
Kumar	Harish	Postdocs	210	29
Kundu	Anirban	Postdocs	211	30
Lallani	Shoeb	Grad Students	336	62
Lama-Sherpa	Tshering	Grad Students	337	62
Lambert	Laura	Open	717	113
Lau	Ying Qing Kristal	Grad Students	338	63
Lawhon	Valerie	Open	719	115

Le	Christina	Grad Students	339	64
Li	Feng	Open	720	116
Liang	Wayne H.	Open	721	116
Libby	Catherine	Grad Students	340	64
Lin	Victor	Residents/Fellows	103	15
Liu	Mingyong	Grad Students	341	65
Londono	Angelina	Open	722	117
Loveless	Christopher	Grad Students	342	65
Lu	Yun	Grad Students	343	66
Maksimenko	Andrey	Open	723	118
Marayati	Raoud	Residents/Fellows	104	16
Martin	Cole	Grad Students	344	67
Martinez	Alba	Grad Students	345	67
Matkins	Victoria	Grad Students	346	68
Mazumder	Suman	Postdocs	212	30
McCormick	Benjamin	Grad Students	347	68
McKenzie	Jessica	Grad Students	348	69
Metge	Brandon	Open	724	118
Mitra Ghosh	Taraswi	Postdocs	213	31
Modi	Bhavan	Open	725	119
Moore	Lindsay	Residents/Fellows	105	16
Mota	Mateus	Grad Students	349	70
Mresh	Rafah	Open	726	119
Murphy	Ryan	Grad Students	350	701
Nair	Sindhu	Grad Students	351	71
Nam	Hyeyoung	Open	727	120
Napier	Tiara	Grad Students	352	72
Nason	Shelly	Grad Students	353	72
Nghiem	Van T.	Open	728	120
Nguyen	Thanh	Soong	600	97
Niranjan	Soumya	Bailey	403	88
Odom	L. Victoria	Grad Students	354	73
Omer	Saba	Grad Students	355	74
Ong	Krystle	Grad Students	356	75
Orlandella	Rachael	Grad Students	357	75
Ovaitt	Alyssa	Residents/Fellows	106	17
Patel	Sweta	Grad Students	358	76
Patel	Zeelu	Open	729	121
Quinn	Colin	Grad Students	359	77
Rangarajan	Sunil	Residents/Fellows	107	18
Rao	Tejeshwar	Postdocs	214	32

Rehman	Hasib	Postdocs	215	32
Richard	Alexander	Grad Students	360	78
Robinson	Alyncia	Chaudhuri	504	94
Rocque	Gabrielle	Open	731	122
Salvador	Carolina	Open	732	123
Samani	Adrienne	Grad Students	361	78
Schlappi	Charles	Residents/Fellows	108	18
Shelton	Abigail	Soong	601	97
Shihab	Yazen	Open	733	123
Shimoga Chandrashekar	Darshan	Postdocs	216	33
Sinha	Rajesh	Postdocs	217	34
Skinner	Kasey	Soong	602	98
Smith	Kristen	Bailey	404	88
Smithberger	Erin	Grad Students	362	79
Spurlock	Brian	Grad Students	363	79
Srivastava	Sanjeev Kumar	Open	735	125
Stefan	Joanna	Postdocs	218	34
Stern	Josh	Open	736	125
Suri	Yash	Open	737	126
Tekin	Volkan	Grad Students	364	80
Thrasher	Kari	Grad Students	365	80
Tiesi	Koriann	Open	738	126
Totsch	Stacie	Postdocs	219	34
Triebel	Kristen	Open	739	127
Tripathi	Kaushlendra	Open	740	128
Tucker	Taylor	Grad Students	366	81
Turbitt	William	Postdocs	220	35
Tuy	Kaysaw	Grad Students	367	82
Varambally	Meena	Open	741	128
Vukadin	Lana	Grad Students	368	83
Wall	Jaclyn	Residents/Fellows	109	19
Wang	Zhixin	Soong	605	100
Wang	Xu	Open	742	129
Wang	Yong	Open	743	130
Widden	Hayley	Grad Students	369	83
Williams	Victoria	Bailey	405	89
Williams	Courtney	Chaudhuri	505	94
Wright	Brian	Open	744	131
Xing	Dianna	Grad Students	370	84
Xiong	Meng-Jun	Soong	603	99
Ye	Star	Residents/Fellows	110	20

Yue	Zongliang	Soong	604	99
Zeng	Ling	Open	745	131
Zhang	Ruowen		406	90
Zhang	Jianqing	Open	746	132
Zheng-Pywell	Rui	Residents/Fellows	111	21
Zubair	MA	Postdocs	221	36