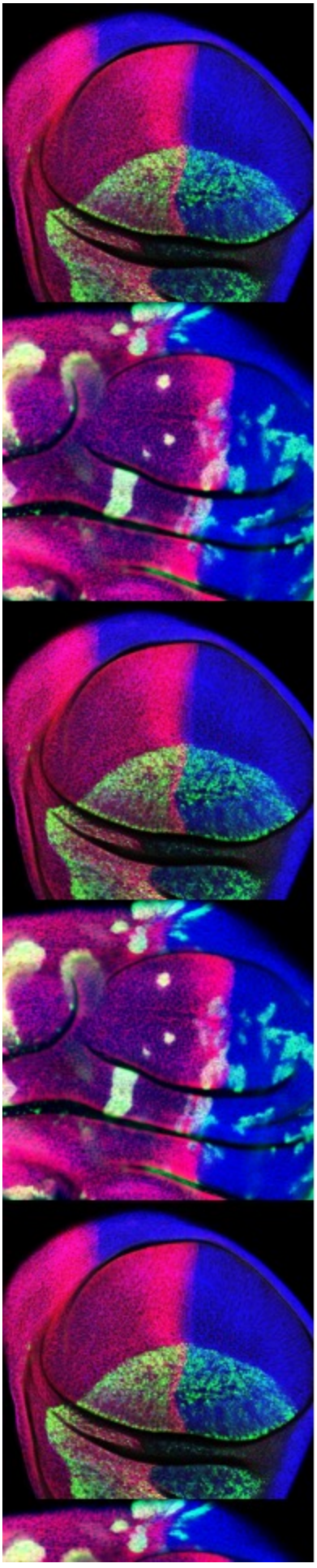

HealthCare
MARKEY CANCER CENTER
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MARKEY CANCER CENTER
RESEARCH DAY

PRESENTED BY THE MARKEY CANCER FOUNDATION

Discover the Latest Advances in Cancer Research

May 12, 2023 | 8 a.m. – 5 p.m.
UK Singletary Center for the Arts

HealthCare
MARKEY CANCER CENTER
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May 5, 2023

Dear Colleagues and Friends,

This has truly been a momentous year for the Markey Cancer Center as we celebrate our 10th year as an NCI designated cancer center. We have received approval to move forward with the new Markey Cancer Center Advanced Ambulatory Building which will be located across from Chandler Hospital Pavilion A and next to Shriners on Limestone. This building will be transformative for cancer care throughout our state by co-locating all cancer services, imaging, laboratory services and including a greatly expanded Oncology Precision Medicine facility. In addition, as many of you are well aware, we submitted our renewal application for our Cancer Center Support Grant requesting consideration as an NCI-designated Comprehensive Cancer Center, which culminated with a very successful site visit in February. We are awaiting final word regarding our application which should come later this month.

The event that I look forward to every year is our annual Markey Cancer Center Research Day that is currently in its 13th year. For this year, we will be back in the Singletary Center for Research Day. Although venues may change, the one thing that remains constant throughout the years is that Markey Cancer Center Research Day remains the one-day event that showcases the impactful work of researchers from myriad disciplines and colleges at the University of Kentucky and is truly the highlight event of the year for our cancer center. This year, you can see 117 reasons why "Markey Makes a Difference" as you peruse posters representing all aspects of cancer research, prevention and control, treatment and clinical care.

The Faculty Oral Presentations will feature two of Markey's leading scholars, and the morning Oral Presentations offer one graduate student, one research associate and one resident physician the opportunity to share their work.

Two keynote speakers highlight the day: Edward Chu, MD, MMS, Director, Montefiore Einstein Cancer Center, Professor and Interim Chair, Oncology, will present the Susan B. Lester Memorial Lecture; and Marvella E. Ford, Professor and Director, Community Outreach and Engagement Hollings Cancer Center, will present the Gilbert H. Friedell, MD, Memorial Lecture.

Please join me in thanking our exhibitors, advertisers and the UK Markey Cancer Foundation for their support, not only of this event but for our educational mission across the University of Kentucky campus.

Enjoy the day, and please let me know your comments regarding this year's event as well as suggestions for future Markey Cancer Center events.
Sincerely

B. Mark Evers

B. Mark Evers, MD

MARKEY CANCER CENTER RESEARCH DAY

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About the Cover

The artwork for the cover is composed of two immunofluorescence images taken from Drosophila wing discs expressing different Ptc mutant forms. The expression of Smo (blue), Ci (red), and HA-Ptc (green) are detected using confocal microscopy to show that the phosphatidic acid binding sites of Ptc dramatically decreased its ability to inhibit Smo accumulation.

Compliments of Jianhang Jia, PhD

Markey Cancer Center Research Day 2023
Agenda

Morning

8:00 – 8:45	Registration and Breakfast
8:45 – 9:30	Student Oral Presentations 1. **Caitlin Miller**: "Production of extracellular vesicles containing mitochondria is a selective survival mechanism for prostate cancer upon radiation treatment" 2. **Vilma Bursac**: "Acceptability of patient components of mPal, a multilevel intervention to improve palliative care integration into advanced lung cancer treatment" 3. **Fengyi Mao**: "PLK1 promotes melanoma progression via phosphorylating BACH1"
9:30 – 10:30	Faculty Oral Presentations 1. **Krystle Kuhs, PhD**: "The promise of biomarker-based screening for oropharyngeal cancer" 2. **Christine Brainson, PhD**: "Inhibiting EZH2 to modulate lung cancer therapy responses"
10:30 – 11:45	Poster presentation #1
11:30 – 1:00	Lunch break/Exhibit Hall

Afternoon

12:15 – 1:30	Poster presentation #2
1:30 – 2:30	**Marvella E. Ford, MD**: Gilbert H. Friedell, MD, Memorial Lecture: "Recruitment and retention studies with black adults: Lessons learned"
2:30 – 2:40	Break
2:40 – 2:45	Markey Women Strong Awards and Patient Presentation by Markey Cancer Foundation
2:45 – 3:05	**Mark Evers, MD**: "State of the Cancer Center"
3:05 – 4:05	**Edward Chu, MD, MMS**: Susan B. Lester Memorial Lecture: "The Chinese herbal medicine PHY906 as a modulator of chemotherapy in the treatment of metastatic colorectal cancer: Where east meets west
4:10 – 4:20	Poster and Mentor Awards Presentation
4:20	Reception

Morning Oral Presenters

Caitlin Miller , BS
Ph.D. Candidate
Toxicology and Cancer Biology

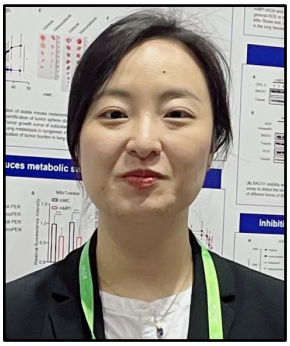
"Production of extracellular vesicles containing mitochondria is a selective survival mechanism for prostate cancer upon radiation treatment"

Caitlin Miller is a 4th year Ph.D. candidate in Dr. Luksana Chaiswing's laboratory in the Department of Toxicology and Cancer Biology in the College of Medicine at the University of Kentucky. Her project focuses on elucidating a novel mechanism by which prostate cancer acquires therapy-induced resistance. More specifically, her project focuses on how prostate cancer can survive post-radiation treatment through mitochondrial transfer via extracellular vesicles. Her main goal in this project is identifying a radiation resistance mechanism that could help improve the effects of radiation treatment and mitigate cancer recurrence in prostate cancer patients. Caitlin obtained her undergraduate degree in biochemistry at the College of St. Rose in Albany, New York.

Vilma Bursac, MS
Senior Research Associate

"Acceptability of Patient Components of mPal, a Multilevel Intervention to Improve Palliative Care Integration into Advanced Lung Cancer Treatment"

Vilma Bursac works primarily with Dr. Laurie McLouth in managing cancer care delivery and cancer survivorship research from inception to completion, including clinical trial protocol development, study personnel management, patient recruitment, data analysis, and manuscript preparation. She earned her MS in Kinesiology and Health Promotion at UK. She is currently seeking a PhD in health services research at the University of Kentucky College of Public Health with a concentration in health outcomes. Her interests lie in advancing health equity, closing gaps in health disparities, and utilizing evidence-based interventions to promote behavior change.



Fengyi Mao , PhD
Post-Doctoral Scholar
Toxicology and Cancer Biology

"PLK1 promotes melanoma progression via phosphorylating BACH1"

Fengyi Mao is a post-doc scholar working in Dr. Xiaoqi Liu's laboratory in the Department of Toxicology and Cancer Biology in the College of Medicine at the University of Kentucky. Her research focuses on Polo-like kinase 1 (PLK1) and its role in promoting melanoma progression. Specifically, she aims to understand the mechanism of how the PLK1/BACH1 axis triggers metabolic reprogramming, leading to accelerated tumor growth, metastasis, and treatment resistance. Through investigating the intersection between kinase activity and cancer metabolism, Fengyi hopes to develop an innovative pharmacological combination targeting both BRAFV600E and PLK1 to suppress tumor growth, which may potentially pave new avenues for melanoma therapies. Fengyi holds an undergraduate degree from Zhejiang University in China and a Ph.D. from Purdue University.

Faculty Oral Presenters

Krystle A. Lang Kuhs, PhD, MPH
Co-Leader, Cancer Prevention and Control Research Program
Associate Professor, Epidemiology and Environmental Health

"The Promise of Bio-Marker Based Screening for Oropharyngeal Cancer"

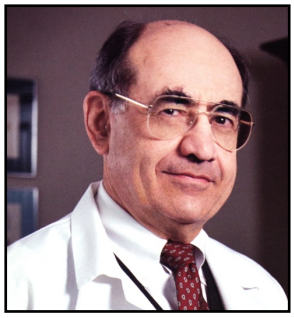
Dr. Kuhs is an Associate Professor in the Department of Epidemiology and Environmental Health. She is also Co-Leader of the Cancer Prevention and Control Research Program at the Markey Cancer Center. Dr. Kuhs is a molecular epidemiologist. Her program focuses on developing novel molecular predictors of head and neck cancer risk, response to treatment, and risk of recurrence, with a particular focus on head and neck cancers caused by infections such as human papillomavirus (HPV). Dr. Kuhs received her PhD in biomedical sciences from the University of Pennsylvania in 2011. Her thesis focused on the development of novel cancer vaccines to prevent hepatitis C infection which resulted in four patented vaccines. Following her PhD program, Dr. Kuhs was selected for the National Cancer Institute (NCI) Cancer Prevention Fellowship Program. She received a Master of Public Health in 2012 from Johns Hopkins University, where she concentrated on Epidemiology and Biostatistics. In 2012, Dr. Kuhs joined the NCI Division of Cancer Epidemiology and Genetics, Infections, and Immunoepidemiology Branch where she conducted her post-doctoral research focused on human papillomavirus (HPV). Dr. Kuhs has earned several awards for research, including the NIH Fellows Award for Research Excellence, American Society of Prevention Oncology New Investigator Award, NIH Intramural Research Award, and the Markey Women Strong Research Award. Dr. Kuhs previously held an NCI K07 Mentored Career Development Award and currently serves as the principal investigator on an R01 from the National Institute of Dental and Craniofacial Research and an R21 from NCI.

Christine Fillmore Brainson, PhD
Assistant Professor, Toxicology and Cancer Biology

"Inhibiting EZH2 to Modulate Lung Cancer Therapy Responses"

Dr. Brainson's laboratory at the University of Kentucky integrates studies of epigenetic programs, lung stem cell biology, and precision medicine. Her group has shown that inhibition of EZH2, the methyltransferase of the Polycomb Repressive Complex 2, can potentiate response to therapies including etoposide, osimertinib, copanlisib, BET inhibitors, and anti-PD1 immunotherapies, but that responses are often both cancer genotype- and subtype-specific. Her group uses specialized three-dimensional air–liquid interface cultures that allow lung cell propagation while maintaining epigenetic programming and specializes in accurate autochthonous models of lung cancer using genetically engineered mice. The laboratory's current funded studies focus on combining EZH2 inhibition with immunotherapy in squamous lung cancers, understanding how methionine metabolism influences initiation and therapy response in lung cancer, and unraveling the role of Polycomb Repressive Complex 2 in lung cell lineage fate and drug responses.

Gilbert H. Friedell, MD, Lecture



About Gilbert H. Friedell, MD

In 1983, Dr. Friedell became the first director of the UK Markey Cancer Center, beginning a legacy of cancer care that continues to grow and make a difference in the lives of Kentuckians every day. At Markey, he co-founded the Kentucky Cancer Registry – now one of the premiere SEER databases in the country – and served as the principal investigator of the National Cancer Institute's Mid-South Cancer Information Service, a cancer education program that provides easy-to-understand information for cancer patients, survivors, health care providers and more.

Dr. Friedell was a passionate advocate for programs that provided education and increased access to healthcare for the medically underserved, particularly in Appalachian Kentucky. He famously believed that "If the problems are in the community, the solutions are in the community." With this in mind, he helped launch Kentucky Homeplace, an initiative that has linked tens of thousands of rural Kentuckians with medical, social and environmental services since it began in 1994.

Though he retired from UK in 2000, Dr. Friedell's influence is still felt strongly in the overarching mission of our cancer center: to conquer cancer in the Commonwealth. We at Markey are proud to uphold the vision and values of Dr. Friedell, building upon his contributions to public health as we continue to care for Kentuckians with cancer.

Marvella E. Ford, PhD
Professor, Public Health Sciences
Associate Director, Population Sciences
Director, Community Outreach Engagement
Hollings Cancer Center/Medical University of SC

Dr. Ford is a tenured professor in the Department of Public Health Sciences at the Medical University of South Carolina (MUSC), where she is the Associate Director of Population Sciences and Community Outreach and Engagement at the NCI-designated Hollings Cancer Center, where Dr. Raymond DuBois is Director. She completed her undergraduate training at Cornell University, and she completed her graduate and postdoctoral fellowship training at the University of Michigan.

Dr. Ford leads several federally funded cancer disparities-focused research grants, including an NCI Partnerships to Advance Cancer Health Equity (PACHE) U54 grant titled "South Carolina Center to Reduce Cancer Health Disparities (SC CADRE)" with Dr. Judith Salley from South Carolina State University, a historically Black university. Dr. Ford is also a multiple principal investigator (MPI), with Drs. David Marshall and Craig Lockhart, of an NCI-funded Minority and Medically Underserved Community Oncology Research Program grant. She is an MPI of the MUSC Hollings Cancer Center's first Stand Up To Cancer grant, with Dr. Robert Winn, Director of the Virginia Commonwealth University Massey Cancer Center. She previously co-led an NIMHD-funded R01 grant with Dr. Nestor Esnaola to test the effectiveness of a multi-level patient navigation intervention in increasing receipt of lung cancer surgery in Black people diagnosed with early-stage lung cancer.

Dr. Ford is the author/co-author of more than 115 peer-reviewed scientific papers. She co-edited the 2017 and 2020 *Advances in Cancer Research* volumes titled "Cancer Disparities" and "Cancer Health Equity," respectively, and has published nine book chapters.

Susan B. Lester Memorial Lecture

About Susan B. Lester

The family and friends of Susan B. Lester endowed a lectureship in her honor, and it is this generous donation that makes Markey Cancer Research Day possible. Mrs. Lester left an indelible mark on her world, serving as a clinical dietician for Eastern State Hospital and for nursing home patients in Eastern and Central Kentucky. This symposium benchmarks recent advances in cancer research and thus honors Mrs. Lester by underscoring both her generous life and the brave battle she fought against this disease.

Edward Chu, MD, MMS

Professor and Interim Chair, Oncology
Professor, Medicine; Professor, Molecular Pharmacology
Director, Montefiore Einstein Cancer Center

Dr. Edward Chu is Professor of Oncology, Medicine, and Molecular Pharmacology, Carol and Roger Einiger Professor of Cancer Medicine, Director of the Albert Einstein Cancer Center (AECC), Interim Chairman of the Department of Oncology, and Vice-President of Cancer Medicine, Montefiore Medicine. He previously served as Chief of the Division of Hematology-Oncology and Deputy Director of the UPMC Hillman Cancer Center (HCC) for 10 years, and prior to that, for 15 years, he had served as Chief of Medical Oncology and Deputy Director of Clinical and Translational Research at the Yale Cancer Center.

Dr. Chu is a medical oncologist and specializes in the treatment of colorectal and GI cancers. His clinical and translational research efforts have focused on identifying novel drugs and combination regimens for colorectal cancer and other GI cancers. In particular, he has focused on developing early-phase I/II clinical trials. He also has a very strong interest in integrating Chinese herbal medicine with standard cancer chemotherapy with the goal of enhancing clinical activity and reducing the toxicity associated with chemotherapy.

His basic research interests have focused on characterization of the molecular mechanisms underlying the development of cellular drug resistance in the treatment of colorectal cancer, especially as it relates to the fluoropyrimidine class of anticancer agents. His research group was the first to identify translational autoregulation as a novel regulatory mechanism in eukaryotes for controlling the expression of the folate-dependent enzymes, thymidylate synthase, and dihydrofolate reductase.

Dr. Chu is a member of several professional and scientific associations including American Society of Clinical Oncology, European Society of Medical Oncology, American Association for Cancer Research, American Association for the Advancement of Science, and American College of Physicians. He serves on the scientific advisory boards of several NCI-designated cancer centers, including Yale, Harvard-Dana Farber, Dartmouth, Case Western Seidman Cancer Center, Duke Cancer Institute, University of Southern California, University of Michigan, University of Chicago, University of Kentucky, University of Arizona, University of Indiana Simon Cancer Center, and Wake Forest Cancer Center.

Singletary Center for the Arts

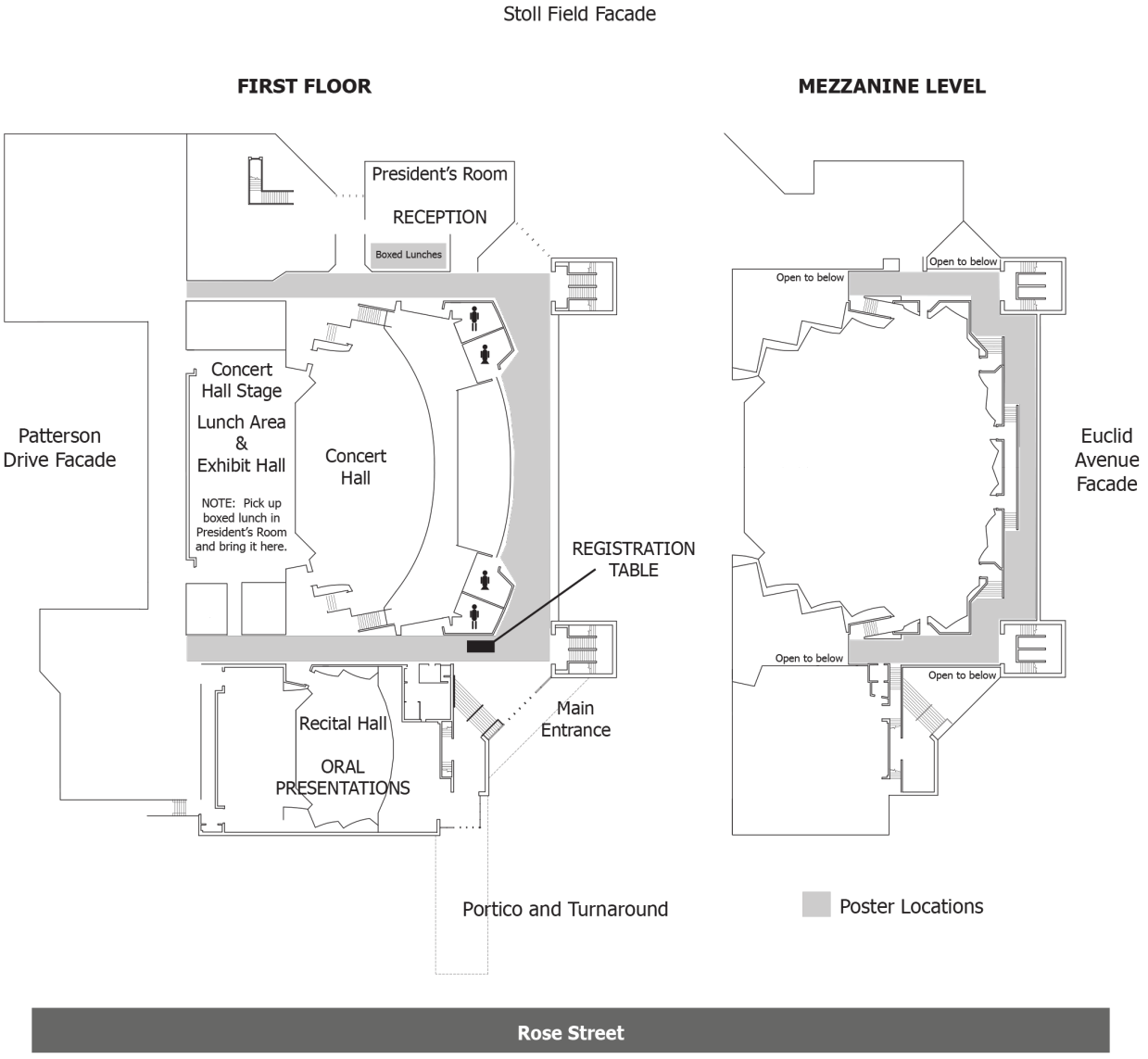
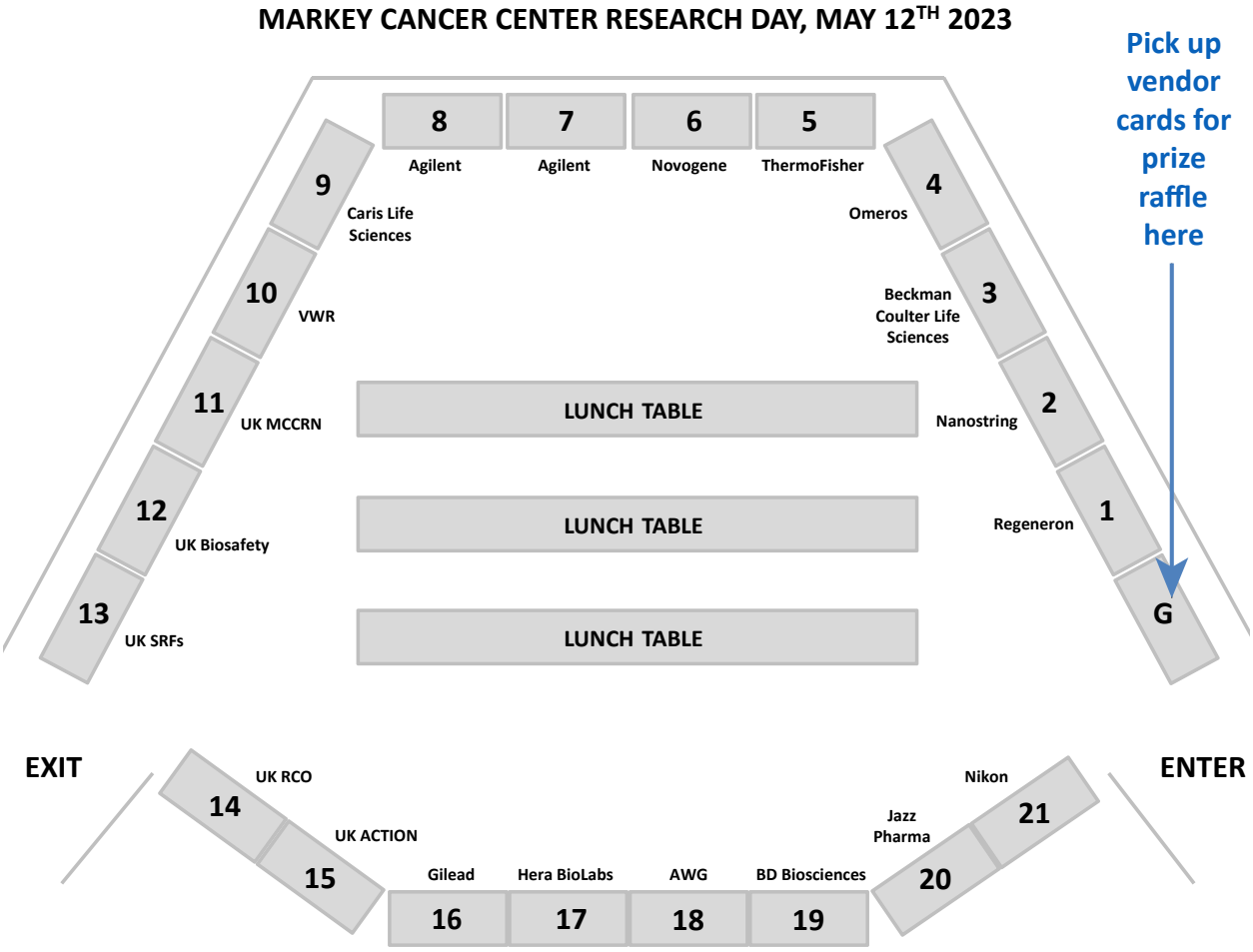


Exhibit Hall

Visit our exhibitors in the Exhibit Hall between 11:30 a.m. and 1 p.m.

For Exhibitor and Advertiser contact information, see next page.



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16	Gilead	gilead.com	Gretchen Koett	270-302-5617	gretchen.koett@gilead.com
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6	Novogene	novogene.com	Cassidy Hardaway		cassidy.hardaway@novogeneusa.com
4	Omeros Corporation	omeros.com	Kevin Strahla	812-240-3014	kstrahla@omeros.com
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10	VWR	vwr.com	Riley Sigler Mary Ella Hamm	859-321-7764 859-321-4741	riley.sigler@avantorsciences.com ella.hamm@avantorsciences.com
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15	UK ACTION Program		Nathan Vanderford Holly Burke		nathan.vanderford@uky.edu holly.burke@uky.edu

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Visit the Exhibit Hall for a chance to win one of the great prizes listed below!

Instructions:

1. Go to Booth "G" to receive up to four tickets per person.
2. Print your name clearly on the ticket(s).
3. Visit the vendor booths and receive one stamp per visit per ticket.
4. Fill up all five boxes with stamps from five different vendors on a ticket and get a chance to win any of our fantastic prizes.

Drawings from all entrants will be announced during the Wine and Cheese Reception in the President's Room immediately following the Awards Presentation.

One prize per person. Must be present to win.

Prizes:

- **(1) RTIC 15 Can Everyday Cooler**
- **(1) Ghirardelli Chocolate Gift Basket**
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- Check at Booth "G" for any last-minute additions!

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Markey Cancer Center Research Day Prize Ticket
Get stamps from FIVE different vendors for a chance to win!

Exhibitor Stamp Goes Here	Exhibitor Stamp Goes Here	Exhibitor Stamp Goes Here	Exhibitor Stamp Goes Here	Exhibitor Stamp Goes Here

PRINT name: _____

Return stamped ticket to Booth "G" at the Exhibit Hall Entrance.

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Abstract 1

Translational

Porcupine Inhibition via LGK974 Enhances Drug-Resistant Prostate Cancer to Enzalutamide Therapy

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UK College of Medicine, Toxicology and Cancer Biology

Androgen receptor (AR) signaling continues to participate as a vital component of castration-resistant prostate cancer (CRPC). Subsequently, this has led to the development of Androgen Signaling Inhibitors (ASI), specifically enzalutamide (ENZ), which is a direct inhibitor of AR, to clinically manage CRPC. Inevitably, ENZ treatment only provides improvement for approximately two months before advancing to an incurable form, ENZ-resistant CRPC. With prostate cancer (PCa) ranking as the second leading cause of cancer-related deaths in American males, there is an urgency and necessity for the discovery and development of novel therapeutic approaches for CRPC. Wnt signaling has been extensively documented in its involvement in PCa and the tumor microenvironment (TME), however the mechanism of how the Wnt signaling cascades contribute to ENZ resistance is still ambiguous. Recently we have published that the activation of the canonical Wnt pathway contributes to the progression of ENZ resistance in CRPC and using a combination of β-catenin inhibitor with ENZ resulted in the synergistic inhibition of patient derived xenograft (PDX) tumor growth. Regarding the non-canonical Wnt pathway, we confirmed its contribution to invasion and migration which leads to metastasis in ENZ-resistant CRPC, and when the downstream effector ROCK is depleted or depleted ROCK cells are treated with ENZ, there is a significant hindering of cell migration and invasion. Also, utilizing a combination therapy of ROCK inhibitor with ENZ synergistically inhibited the growth of PDX tumors. Hence the reasoning that by simultaneously inhibiting both the canonical and non-canonical Wnt signaling cascade will result in the inhibition of cell proliferation, migration, and invasion. The goal of this study was to define the mechanism of Porcupine (PORCN) in ENZ-resistant CRPC and develop a therapeutic approach to combat this disease. My research has determined that PORCN is associated with CRPC progression to ENZ-resistance, and that an inhibition or loss of PORCN has resulted in the regain of ENZ sensitivity in ENZ-resistant models. This model has also demonstrated that PORCN and Wnt signaling engage in a paramount role contributing to AR activation, promoting CRPC progression, and the development of ENZ resistance.

Abstract 2

Basic Science

Role of Exonuclease 1 Protein-Protein Interactions in Human Mismatch Repair

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DNA repair is the process by which cells identify and correct mutations present within the genome. These correction mechanisms maintain genomic stability and prevent the development of cancer. The DNA mismatch repair (MMR) pathway identifies and corrects small insertions, deletions, and misincorporations that arise within the genome as a result of errors during DNA replication. MMR is also critical for inducing apoptosis after chemically induced mispairs, such as those from environmental alkylating agents. The process by which MMR corrects these mispairs includes recognition of the mispair by the MutS complex, recruitment of the MutL complex to the mutation site, excision of the mispair, and gap filling by DNA polymerase. Exonuclease 1 (Exo1) is the protein primarily responsible for the excision step of MMR. In Exo1-dependent MMR, Exo-1 binds to both the MutS heteroduplex (MSH2-MSH6) and MutL heteroduplex (MLH1-PMS2). The MutL interaction with Exo1 in budding yeast is facilitated by an Mlh1 interaction peptide (MIP) box. We recently identified a Msh2 interaction peptide (SHIP) box in yeast Exo1. This project aims to understand how human MIP and putative human SHIP box motifs influence human Exo1 recruitment to MMR processes. We have created point mutations within the predicted binding domains of human Exo1. We observe changes in localization of Exo1-mutant proteins within the cell, suggesting that changes in the overall MMR process may be present. We also observe changes in MMR-mediated apoptotic response when a subset of Exo1-mutations are expressed in the presence of endogenous wild-type Exo1, indicating a potential for a dominant negative interaction. Alterations in Chk1 phosphorylation suggest the Exo1 mutations alter DNA damage response pathways. Our ongoing studies are expected to shed more light upon how the mechanisms of human MMR process and overall genomic stability. This study will have important implications on human cancer development and treatment.

Abstract 3

Basic Science

SHP2-DDX3X Complexes May Control the Translation of the PD-L1 mRNA in KRAS-Active Non-Small Cell Lung Cancer

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UK College of Pharmacy, Pharmaceutical Science

Background: Recent development of immune checkpoint inhibitors (ICIs) have significantly shifted the landscape in management for non-small cell lung cancer (NSCLC) by improving treatment outcomes and allowing approximately 20% of patients with late-stage NSCLC to have long-term survival. One of the predictive responses for immune checkpoint inhibitors is the patient's PD-L1 tumor expression level. Targeting immune checkpoints has shown substantial clinical benefits. However, around 80% of the patients did not respond well to treatment. In order to improve patient treatment outcomes, an effort has been made to identify a potential therapeutic target that could overcome ICIs resistance.

Unpublished work from our group demonstrated that SHP2, a tyrosine phosphatase, negatively regulates PD-L1 expression, a target of ICIs. Using immunoprecipitation of tagged-SHP2 ectopically expressed in H460 cells, mass spectrometry identified DDX3 as a potential partner of SHP2 along with eukaryotic translation elongation factor 1a (eEF-1a), which suggested SHP2 participated in a protein complex that can regulate translation elongation. DDX3x also harbors a tyrosine residue at position 104 that could serve as an interacting site for SHP2. We hypothesize that SHP2 forms a complex with DDX3x and eEF-1a on newly transcribed mRNA to negatively regulate PD-L1 expression.

Materials and Methods: Tagged-SHP2 was transfected into H460 cells and subsequently immunoprecipitated with an anti-Myc antibody. The immunoprecipitated complexes were separated by SDS-PAGE. Excised gel slices were treated with dithiothreitol, iodoacetamide, digested with trypsin, and subjected to liquid chromatography-mass spectrometry (LC-MS/MS) analysis. MS data sets were searched in MASCOT software against database from UniProt to determine interacting proteins. To confirm SHP2 interactions, a pull-down assay was performed using both A549 and H460 extracts with immobilized his-tagged, DDX3X purified from bacteria, and pull-downs were also completed using immobilized His-tagged SHP2. Immunoprecipitation of both endogenous DDX3x and SHP2 was conducted in A549 and H460 cell extracts. Western blot analysis was performed to evaluate interacting proteins.

Results: DDX3X is the highest-ranked protein by percent coverage from the IP-MS assay following SHP2 immunoprecipitation. Purified DDX3x from bacterial expression systems was able to pulldown SHP2 protein from both A549 cells and H460 cells, and vice versa. Immunoprecipitation of endogenous SHP2 were able to co-precipitated DDX3 and vice versa.

Conclusion: DDX3X has the potential to form a complex with SHP2 that may regulate the expression of PD-L1 in concert with eukaryotic translation elongation factors. It is possible the phosphorylation of either or both DDX3X and SHP2 are necessary for complex formation given the results from DDX3X immunoprecipitation. Further studies will be needed to identify the components and activity of this complex in conjunction with the PD-L1 mRNA.

Abstract 4

Basic Science

Plk1 Acts as an Anti-Ferroptotic Regulator in Cancer

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Cancers acquire insensitivity after therapy and advanced cancer usually resists treatment, while the mechanisms involved in the insensitivity and resistance remain elusive. Previously, extensive reports identified that Polo-like kinase 1(Plk1) is highly elevated in the therapy resistant cancer and advanced cancer. Here, we find that Plk1 contributes to the treatment resistance in cancer by regulating ferroptosis, an iron-dependent programmed cell death which is morphologically, biochemically, and genetically distinct from other forms of cell death. Ferroptosis specific protein 1 (FSP1), a parallel regulator of GPX4, protecting the cells from ferroptotic cell death, is phosphorylated by Plk1 at Ser363 and its phosphorylation promotes FSP1 stabilization as well as protein expression. ACSL4, a negative regulator of ferroptosis causing lipid ROS, is phosphorylated by Plk1 at Ser569 and its phosphorylation leads to the degradation of ACSL4. We also find that the combination of Plk1 inhibitor and ferroptosis inducer is a promising therapeutic strategy for Plk1-overexpressing cancers. Our findings provide the novel mechanisms of therapy resistance and potential therapeutic approaches for advanced cancer.

Abstract 5

Biostatistics/Bioinformatics

In Silico Pipeline to Discover Small Molecules Overcoming Mutation Induced Drug Resistance for EGFR

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Mutation-induced therapeutic resistance is one of the central challenges to overcome when developing efficient treatments for non-small cell lung cancer (NSCLC). For instance, certain mutations in the epidermal growth factor receptor (EGFR) gene within patients' tumors could lead to resistance to available kinase inhibitor therapeutics. Such resistance could be due to the mutation-induced conformational changes in the targeted EGFR protein. This poster presents our effort to develop an in-silico pipeline to predict the mechanisms of mutation-induced drug resistance. We built a structure prediction, conformational exploration, and molecular docking pipeline that combines three computational approaches. The first is AlphaFold2 which can predict 3D conformation of a protein based on its sequence. The second is Molecular Dynamics (MD) simulations performed by Gromacs that can generate possible conformational ensembles for a protein in explicit solvent. The third is Molecular Docking performed by Autodock Vina which can identify potential small-molecule inhibitors. Using our pipeline, we investigated how the mutations of the EGFR gene may result in changes in the binding affinity of small molecules. We first used alphaFold2 to predict the 3D conformation of wild-type and mutant EGFR kinase domain. Then we conducted molecular dynamics simulations to generate multiple possible conformations for these proteins in explicit solvents. Lastly, we carried out molecular docking to screen small molecules that can bind to these conformations. We benchmarked the performance of our in-silico pipeline using a pool of known EGFR kinase inhibitors and known decoy molecules. Our results show the potential of this in-silico pipeline in discovering potential small molecule candidates for structural variants of EGFR.

Abstract 6

Basic Science

Peroxiredoxin IV Promotes Prostate Cancer Malignancy through the Activation of NF-κB Signaling

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Background: The peroxiredoxin (Prx) family of proteins functions as major cellular antioxidants to maintain redox homeostasis as well as mediate oxidative signaling in both physiological and pathological conditions. Our previous studies show that Prx4 is highly expressed in prostate cancer and promotes tumor growth in vitro and in vivo. But how Prx4 contributes to prostate cancer cell invasion and metastasis remains to be elucidated. The purpose of this study is to understand the oncogenic signaling pathways that are mediated by Prx4 in prostate cancer cells.

Methods: Bioinformatic analysis of existing database were used to examine the expression of Prx4 in primary and metastatic prostate cancer samples. CRISPR/Cas9 technique was used to establish Prx4 knockout in cultured human prostate cancer cell lines. Matrigel invasion assays were performed to evaluate cell migration and invasion. RNAseq was performed to examine the differences of gene expression between control and Prx4KO cells. Gene Set Enrichment Analysis (GSEA) and Gene Oncology (GO) enrichment analysis were performed using RNAseq results. RT-PCR and immunoblotting were used to validate findings from RNAseq and enrichment analysis.

Results: Compared with normal prostate, transcript levels of PRDX4 are found to be upregulated in specimens of patients with prostate cancer, and those with bone metastasis show even higher levels. Knockout of Prx4 in cultured human prostate cancer cells leads to significantly reduced ability of cell migration and invasion. Gene expression profiling reveals that loss of Prx4 leads to the upregulation of epithelial as well as downregulation of mesenchymal markers, and multiple signaling pathway changes including the inhibition of cellular response to inflammatory factors such as tumor necrosis factors and interleukins. These changes are also associated with a variety of cellular activities such as wound healing, interferon signaling, and antigen processing and presentation. Among known target genes downstream of NF-κB signaling, E-cadherin, vimentin, and matrix metalloproteinase 14 (MMP14) were found to be significantly affected by the loss of Prx4. Therefore, Prx4 plays a critical role in human prostate cancer cell malignancy through regulation of intracellular cell signaling pathways.

Conclusions: A combination of bioinformatic, cellular, and molecular methods reveals that Prx4 plays a critical role in promoting prostate cancer metastasis. Prx4 is a potential therapeutic target to reduce prostate cancer metastasis.

Abstract 7

Population-Based/Behavioral

Genetic Variations Contribute to Cancer Disparities in Appalachia

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Background: Individuals residing in Appalachian regions have significant health disparities, including higher cancer incidence and mortality rates. Previous studies have addressed the impact of socioeconomic status and environmental risk factors on Appalachia cancer disparities, while few studies have evaluated genetic risk factors.

Methods: Germline whole exome sequencing samples from 7,078 individuals with cancer (759 Appalachian) were evaluated. Demographics and relatedness were assessed using KING. Ethnicity was verified by principal component analysis using TRACE, which included 6,034 individuals (85%) of European genetic ancestry. After QC filtering, 5,980 individuals were analyzed. To assess overall predisposition of hereditary disease, gene level frequency of likely pathogenic or pathogenic variants (PGVs) located in ACMG genes were compared between Appalachian and non-Appalachian regions. Assessment of overall alternative allele frequencies and pathway analysis were also performed.

Results: Appalachian individuals were predominantly non-Hispanic Caucasian ($p < 0.001$) with a significantly higher rate of current smoking ($p < 0.001$) compared to non-Appalachian individuals. We identified PGVs in 637 individuals, most commonly SNPs followed by deletions, duplications, microsatellites, insertions, and indels. The most frequently mutated genes were BRCA2 (83 [13.0%]), BRCA1 (56 [8.8%]), and LDLR (47 [7.4%]). In addition, we identified 43 (6.6%) individuals with a heterozygous PGVs in a mismatch repair gene. When comparing Appalachian to non-Appalachian individuals, the OTC gene was more frequently mutated (0.85% vs. 0.043%, FDR = 0.001) in Appalachian individuals. An additional 4,580 germline variants with significantly different alternative allele frequencies in Appalachian individuals were identified, which were enriched in functional networks, including RNA regulation, cancer development, cell proliferation, and regulation of adhesion.

Conclusion: More than 4 million germline variants in 5,980 cancer individuals with cancer were evaluated, identifying a clinically relevant gene with a significantly higher gene level mutation rate in the Appalachian region, as well as 4,580 germline variants with significantly different alternative allele frequencies that enriched in cancer relevant pathways. This suggests the Appalachian population has a unique genetic background, which may predispose them to cancer and contribute to disparities in cancer development.

Abstract 8

Core Resources (Informational and not judged)

MCC Research Communications Office: Helping Markey Researchers with Editing, Graphics and Grants

Mallory Antel, Cathy Anthony, Marcia Ballard, Amy Beisel, J. Bybee, Megan Eder, Donna Gilbreath, Rachel Grace LeComte, Kristin Pratt, Sandra Shepherd, Danielle Story, Phillip Strunk, Lauren Tecau

UK Markey Cancer Center, Research Communications Office

The UK Markey Cancer Center Research Communications Office (RCO) was created in 2009 to help cancer researchers obtain grant funding, publish material in support of their research initiatives and facilitate opportunities for continuing education. In 2020, RCO launched Markey Connect, Markey's employee website—a linkblue-protected site with valuable information available to all Markey Cancer Center employees and trainees

We are a team of professional editors, graphic designers, project managers, grant specialists, event/seminar coordinators, and website specialists available to help all cancer researchers at the University of Kentucky—free of charge—by:

- Editing and submitting grant proposals, journal articles and manuscripts;
- Creating and editing graphics;
- Preparing conference posters and presentations;
- Facilitating surveys and coordinating messages for the Markey listserv;
- Serving as the key point of contact for the Markey website and Markey Connect;
- Planning, promoting and coordinating Markey research meetings, seminars, and special events;
- Tracking, evaluating, and reporting on pilot awards and funded projects;
- Assisting with proposal development, budgeting, ensuring adherence to sponsor guidelines, and coordination of large grants

The RCO works with faculty, administrators, staff, grant agencies, and medical and scientific publishers to assist cancer researchers in effectively communicating their research.

Offices: CC 415, CC 416, and CC 418 of the Ben F. Roach Building
Email: mccrco@uky.edu
Website: https://ukhealthcare.uky.edu/markey-cancer-center/research/rco
Start your project: https://ukhealthcare.uky.edu/markey-cancer-center/research/rco/starting-your-project

Abstract 9

Biostatistics/Bioinformatics

Application of Interim Monitoring Strategies for Targeted and Precision Medicine Clinical Trials

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Background: We present the application of novel interim monitoring strategies for the therapeutic Phase I and Phase II trials of targeted and genomic-driven clinical trials using the BOIN and U-BOIN to assess safety and optimal biologic dose, a basket Bayesian design to assess activity or futility in disease-specific cohorts and a modified one-sample log-rank test (MOSLRT) for a Phase II trial design to assess progression-free survival (PFS).

Methods: A Phase I Trial is performed to determine the biological effects of hydroxychloroquine on PAR-4 Levels in patients with resectable solid tumors. Given the established safety and minimal toxicity of HCQ in several studies, study design is based on determining the dose with the target biological effect as well as acceptable toxicity. A Phase II therapeutic genomic driven trial is performed to assess the progression free survival ratio of patients treated with a targeted therapy based on genomic profile and recommendation by a Molecular Tumor Board. A PFS ratio ≥ 1.3 is considered of clinical relevance. The trial will assess PFS ratio among disease specific cohorts. Interim analysis using the Bayesian basket design (Ref: Simon R, Geyer S, Subramanian J, Roychowdhury S. Seminars in Oncology, 2016) was used to assess activity or futility in different cohorts. Another Phase II trial is a single-arm trial of cabozantinib and pembrolizumab (C+P) to improve PFS in the second line setting for patients with metastatic pancreatic cancer. The primary study endpoint is PFS and interim analysis will be performed based on an optimal two-stage design for Phase II survival trials. Specifically, an exponential distribution with an exact variance estimate of the one-sample log-rank test (OSLT) will be used for the two-stage design (Wu, 2015).

Results: For the Phase I trial assessing safety, application of the BOIN resulted in an estimated MTD of dose level 2 (HCQ 400mg, twice a day) while using the isotonic regression and U-BOIN designs resulted in dose escalation decisions to continue enrolling the next cohort to dose level 2 (HCQ 600mg, twice a day). For the Phase II genomic driven trial, we performed interim monitoring analysis after a total of 100 patients have been enrolled in different disease cohorts. Results indicated a high response rate in all cohorts with high posterior probabilities of activity indicating continuing enrollment in the disease cohorts. For the Phase II C+P trial, the R function, Optimal.rKJ, will be utilized at the time of interim analysis of PFS after 14 patients. If the first stage test statistic $Z1 < 0.088$, we stop the trial for futility. Otherwise, the trial continues to the second stage until a total of 21 evaluable patients is enrolled on the study. If the second stage test statistic $Z < 1.632$, we don't reject the null hypothesis and conclude no efficacy of the treatment, otherwise, if $Z \geq 1.632$, we conclude that the C+P treatment is promising.

Abstract 10

Basic Science

Determining the Effects of Programmed Genome Rearrangement on Germline Development in Lamprey

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Cancers in humans often arise from mutations or epigenetic changes in genes that are involved in embryogenesis. The development of primordial germline cells (PGCs) is particularly relevant to this, as these cells maintain a higher degree of pluripotency than adjacent tissues and are thus heavily associated with protooncogenes. Understanding the connection between germline differentiation and oncogenesis is therefore of great interest to cancer biology. The sea lamprey Petromyzon marinus provides a useful model for addressing this question, as large portions of the genome are purged from somatic cells during early development in a process known as programmed genome rearrangement (PGR). Several of the genes deleted from somatic cells are known protooncogenes in humans, meaning that lamprey may help us further determine the relationship between germline suppression and types of cancer development. To test this, we compared the histology and transcriptomes of early lamprey somatic cells and PGCs around the timing of PGR. We will then compare these profiles in response to changes in WNT signaling, a core component of germline development and the oncogenesis of breast and colorectal cancers. We hypothesized that the evolutionary role of PGR is to remove genes involved in PGC differentiation, this process also indirectly removing potential oncogenes. Our transcriptome analyses have revealed gene expression clusters that can be used as landmarks for identifying cell types at mid-blastula transition, an important first step in this understudied stage of development. Despite this, we have not found any major differences in gene expression between potential PGCs and somatic cells by these stages. On the histological level, we have identified cell types which may correspond to PGCs, sharing features with zebrafish and frog cells at these stages. Together, these findings provide a way to more broadly view early developmental processes like programmed genome rearrangement and how these processes may parallel oncogenesis.

Abstract 11

Basic Science

JNK1 Mediated PUMA Expression Contributes to Platycodin D Induced Apoptosis in Lung Cancer

Qing Wang, Hsin-Sheng Yang

UK College of Medicine, Department of Toxicology and Cancer Biology

Lung cancer is the most devastating disease and leading cause of cancer death in men and women. Platycodin D is one of the major bioactive components of the Platycodon grandiflorum, an herb that is frequently used for the treatment of lung diseases in Chinese medicine. Platycodin D is a triterpenoid monomer with activities of antioxidant, anti-obesity, and anti-inflammatory. Studies have also shown that Platycodin D has an anti-tumor effect on various types of cancer. However, the mechanism by which platycodin D suppresses tumorigenesis remains elusive and some experimental results are controversial. In this study, we aim to understand the effect of platycodin D on the growth of non-small lung cancer cells (NSCLC) and the underlying mechanism. We found that NSCLC cells (H1299, H2030, and A549 cells) treated with platycodin D significantly reduced the cell viability, decreased the number of colonies, impaired the mitochondrial function, and induced apoptosis. To understand the mechanism, the protein levels of the Bcl-2 protein family were examined and p53 upregulated modulator of apoptosis (PUMA) was found elevated by platycodin D treatment. The induction of PUMA expression by platycodin D treatment was through activation of the c-Jun N-terminal kinase (JNK) and AP-1 dependent transcription since JNK inhibitor or over-expression of dominant negative c-Jun abolished the elevated PUMA expression. Moreover, knockdown of JNK1, but not JNK2, significantly decreased the level of Platycodin D-induced PUMA and cleaved caspase 3, indicating the essential role of JNK1 in Platycodin D-induced apoptosis. Furthermore, Platycodin D efficiently suppressed the tumor growth in the H1299 xenograft. Taken together, activation of JNK1 by Platycodin D is essential for induction of apoptosis in suppression of growth of NSCLC, providing a new mechanism of how platylcodin D suppresses NSCLC.

Abstract 12

Core Resources (Informational and not judged)

Biostatistics and Bioinformatics (BB) Shared Resource Facility

Heidi L. Weiss, Chi Wang, Brent Shelton, Donglin Yan, Bin Huang, Li Chen, Jinpeng Liu

UK Markey Cancer Center, Division of Cancer Biostatistics

The Biostatistics and Bioinformatics Shared Resource Facility (BB SRF) is a cancer center-managed facility providing essential data science expertise in biostatistics and bioinformatics to catalyze and enable the Markey Cancer Center's (MCC) basic science, clinical and population research. The BB SRF exhibited continued growth and increased breadth of services over MCC's tenure as an NCI-designated center. BB SRF faculty and staff are integrated into MCC's multi-disciplinary and translational science teams, providing centralized, state-of-the-art and accessible services to ensure rigor in the development and execution of cancer research. The Specific Aims of the BB SRF are to: 1) Provide statistical expertise and consultation in study design, study conduct and analysis across the spectrum of projects from MCC Research Programs; 2) Provide high-quality bioinformatics expertise focused on study design and data analysis across the spectrum of projects from MCC Research Programs; and 3) Enhance MCC research through a team science model along with utilization of unique processes for interfacing across MCC Shared Resources. BB SRF services are coordinated with other MCC SRFs via integrated workflows to ensure comprehensive, seamless and non-overlapping support. Key technical strengths of the BB SRF include: 1) innovative methods and new designs for MCC investigator-initiated trials including MCC-led multi-center NCI Experimental Therapeutics Clinical Trials Network trials; 2) cutting-edge bioinformatics and integration of omics and high throughput platforms; 3) advanced methods for population-based, behavioral and molecular epidemiology research. In the current funding cycle of the NCI Cancer Center Support Grant, the BB SRF supported 165 unique users (79% of whom are MCC members), served as co-investigators on 84 MCC peer-reviewed grants, and are lead statisticians on 69 investigator-initiated trials and clinical studies, resulting in co-authorship on 127 publications. BB SRF faculty engage in training and education initiatives of the MCC. A key strength of the BB SRF is engagement in novel methodological work, evidenced by statistical publications and BB SRF faculty's independent R21, R03, and U grants, ultimately enhancing MCC Research Program science. The BB SRF receives significant personnel and resource investment and is governed by rigorous oversight from the MCC. Its efficient and cost-effective operations are bolstered by strong leadership and significant breadth of faculty technical expertise supportive of MCC's existing and emerging research directions. The BB SRF will pursue methodologies to support MCC Research Priorities in integrative molecular oncology and biomarker discovery for new high throughput platforms, genomic and molecularly driven trials, and community-based precision medicine clinical studies, survivorship and outcomes research. The BB SRF, which received an Exceptional rating in the last CCSG renewal, adds significant value to the execution of scientifically rigorous research and is well positioned to support MCC as a Comprehensive Cancer Center.

Abstract 13

Basic Science

Targeting Leukemia Initiating Cells in T-cell Acute Lymphoblastic Leukemia (T-ALL) through the β-catenin Pathway: Repositioning of Erlotinib

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This study aimed to identify a small molecule that inhibits the Leukemia Initiating Cells (LIC) in T-cell acute lymphoblastic leukemia (T-ALL) by targeting the β-catenin signaling cascade. The Wnt/β-Catenin signaling axis plays an important role in development of the cancer initiating cells in multiple cancers including T-ALL. Since this pathway is involved in normal tissue regeneration, available inhibitors are associated with significant side effects that limit their clinical utility. There is an unmet need of Wnt/β-Catenin pathway inhibitors with an acceptable safety profile. We employed a TCF/LEF transgenic zebrafish with GFP reporter to interrogate a library of over than 770 FDA approved drugs for their effect on this pathway. We identified Erlotinib as a hit compound. Erlotinib is a small molecule tyrosine kinase inhibitor that targets the epidermal growth factor receptor (EGFR), that is commonly expressed in many cancer types. We found that Erlotinib is able to significantly reduce the number of colonies formed in vitro (P value= 0.0076) using the 3D sphere formation assay. Using zebrafish T-ALL models and the limiting dilution method, Erlotinib resulted in three-fold decrease in frequency of T-ALL leukemia initiating cells (P value= 0.0352) and about 60% decrease in leukemia burden in vivo. Erlotinib treatment significantly inhibited the expression of Wnt/β-Catenin target genes possibly through modulating the cell's ATP metabolism.

Abstract 14

Core Resources (Informational and not judged)

Enhancing ROS-Inducing Nanozyme through Intraparticle Electron Transport

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Iron oxide nanoparticles (IONPs) are promising candidates for reactive oxygen species (ROS)-mediated disease treatment due to their excellent biocompatibility and Fenton catalytic activity. However, the low catalytic activity has hindered their clinical translation. To address this challenge, IONPs of different compositions are examined for their Fenton reaction catalysis under pharmacologically relevant conditions. The results show that wüstite (Fe1-xO) nanoparticles exhibit high catalytic activity compared to magnetite (Fe3O4) or maghemite (r-Fe2O3) of matched size and coating, despite all IONPs having a similar surface oxidation state. Analyses of nanoparticle compositions suggest that the high catalytic activity of wüstite nanoparticles is due to the presence of internal low-valency iron (Fe0 and Fe2+), which accelerates the recycling of surface Fe3+ to Fe2+ through intraparticle electron transport. Additionally, ultrasmall wüstite nanoparticles are generated by tuning the thermodecomposition-based nanocrystal synthesis, resulting in significantly enhanced Fenton catalysis. When compared with ferumoxytol, an FDA-approved IONP, wüstite nanoparticles can substantially increase the level of intracellular ROS in mouse mammary carcinoma cells. This study presents a novel mechanism for developing efficient ROS-inducing nanozymes for therapeutic applications.

Abstract 15

Core Resources (Informational and not judged)

Patient-Oriented and Population Sciences Shared Resource Facility

Jessica L. Burris

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The Markey Cancer Center (MCC) has invested in a shared resource that facilitates behavioral, psychosocial, and epidemiologic cancer research. The MCC Patient-Oriented and Population Sciences Shared Resource Facility (POP SRF) supports studies with cancer patients, caregivers, and providers, as well as the general population, regarding cancer-related topics. POP SRF services span the full lifecycle of a project and include consultation, delivery of high-quality accrual, retention and data collection services, assistance with qualitative data analysis, and help with scholarly dissemination. To meet MCC researchers' needs and ensure usage representation across diverse research foci, POP SRF supports population surveys, clinical studies, health services research, community-engaged research, and non-therapeutic clinical trials.

POP SRF staff are adept at navigating clinical workflows, partnering with community organizations, obtaining informed consent, and facilitating surveys, interviews and focus groups. Thus, MCC researchers can seamlessly integrate their studies into clinical and community settings. POP SRF is led by Jessica Burris, PhD, who has 15 years of relevant experience. POP SRF is comprised of four masters-level staff, including manager Ms. Joan Kahl, MS, and one graduate student, all with ample research experience. Regular quality assurance data collection, internally driven audits, and professional development activities ensure that POP SRF staff remain abreast of the latest knowledge, tools, and procedures germane to the conduct of cancer research with a high scientific impact and direct relevance to MCC's catchment area. POP SRF Specific Aims are to: 1) Streamline methods for participant accrual and retention; 2) Collect patient-reported outcomes and other survey data for epidemiological and other observational studies and non-therapeutic clinical trials; 3) Facilitate rigorous qualitative research as part of observational studies, clinical trials, and dissemination and implementation studies; and 4) Coordinate behavioral, psychosocial and epidemiologic studies to accelerate cancer research findings and their dissemination. As the only SRF with procedural expertise in patient-oriented and population-based research, POP SRF adds unique value to MCC's research enterprise and is integral to the MCC Research Programs' future goals that are focused on risk identification, mechanistic intervention targets, clinical trials, and implementation science.

Abstract 16

Basic Science

Addressing hERG Activity of Inhibitor of DCN1-Mediated Cullin Neddylation

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Neddylation is a post-translational modification that conjugates the neural precursor cell expressed, developmentally downregulated 8 (NEDD8) protein to protein substrates. The best-known neddylation substrates are the cullins, which upon neddylation join a multiprotein complex to form cullin-RING ubiquitin E3 ligases (CRLs). The CRLs play a key role in controlling proteasomal degradation and are activated by neddylation. The NEDD8-activating enzyme (E1) inhibitor Pevonedistat prevents neddylation of the CRLs and has clinically validated the neddylation pathway for oncology. We recently discovered a small molecule anticancer agent (NAcM-OPT) that potently inhibits the interaction of the Defective in Cullin Neddylation 1 (DCN1), a CRL neddylation co-E3, and UBE2M, a neddylation E2. However, this inhibitor possesses off-target affinity to the human Ether-a-go-go Related Gene (hERG) channel with IC50 in the micromolar range. The hERG is one of the most important antitargets to be addressed in the early drug discovery process to avoid more costly failures in the development phase. Blocking hERG channel has the potential to cause QT prolongation, which could lead to Torsades de pointes, a life-threatening ventricular tachyarrhythmia. Herein, we performed the structural modifications of NAcM-OPT to identify small molecule inhibitors of DCN1-UBE2M interaction devoid of hERG activity.

Abstract 17

Translational

The Role of Plk1 in Pulmonary Fibrosis

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UK College of Medicine, Toxicology and Cancer Biology

Idiopathic pulmonary fibrosis (IPF), the most common and aggressive form of interstitial pneumonia, stems from an unknown cause that results in respiratory failure within two to five years of diagnosis. Increasing evidence suggests that the alveolar epithelium may be uniquely vulnerable to pathogenesis of pulmonary fibrosis (PF); however, the mechanism behind pathogenesis is not fully understood. The TGFβ-1 pathway is considered the major mechanism for several fibrotic diseases, including ones resulting from both environmental exposures and genetic factors. Therefore, our objective is to discern how TGFβ1 exposure leads to activation of alveolar epithelial type 2 (AT2) and/or fibroblast differentiation and eventually progression of IPF by utilizing Polo-like Kinase 1 (Plk1) inhibition. Plk1 plays many roles in cell cycle and mitotic progression, where Plk1 activity in is abolished upon DNA damage and acts as a transcriptional target of p53 to cause cell cycle arrest. This interaction with p53 inhibits its apoptotic function and drives stress responses. Plk1 also plays a major role in homologous recombination which contributes to its anti-apoptotic activity. Overexpression of Plk1 is well established in several cancers where it contributes to tumorigenesis and metastasis via abnormal cell cycle regulation. Several recent studies have established efficacy of Plk1 inhibition as a cancer therapeutic with clinical trials ongoing.

I hypothesize that that polo-like kinase 1 (Plk1) acts as a critical molecule to link ROS, NFκB, and p62/Nrf2 signaling, and that this kinase can be activated by TGFβ1 exposure as it would it be human IPF. This hypothesis has been formulated on a key observation made by the Liu laboratory, that Plk1 activates the p62/Nrf2 signaling via direct phosphorylation of p62 and Nrf2. Therefore, Plk1 may be a novel and efficient therapeutic target to prevent aberrant AT2 differentiation, fibroblasts, and accumulation that leads to ECM (extracellular matrix) deposition characteristic of IPF. Additionally, by utilizing Plk1 inhibition/activation, a unique opportunity exists to investigate the contribution of aberrant cell cycle progression of AT2 cells to impact disease development mechanistically.

Based on this knowledge, experiments with human alveolar epithelial and lung fibroblasts, a 3-D murine lung organoid, and novel mouse model of bleomycin-induced PF, Spc-Cre;Plk1-KO/KI, will be utilized alongside a model of familial IPF, SpcI73T;Plk1-KO/KI, and compared. Using western blotting, immunofluorescence, qPCR, flow cytometry, and immunohistochemistry, these models will be evaluated after induction of pulmonary fibrosis, for in vivo/ex vivo experiments. Preliminary results in a bleomycin-induced model of WT mice yielded significantly reduced hydroxyproline and Ashcroft scoring with Plk1 inhibition by GSK461364, supporting the TGFB1-induced pro-fibrotic state seen in human BEAS-2B and normal human lung fibroblasts.

The rationale for this research is that once the mechanism of Plk1-associated kinase contribution to disease progression is established, the Plk1 can be manipulated pharmacologically, offering a novel multi-mechanism drug target that may overcome limitations of single-mechanism therapeutics for this disease.

Abstract 18

Basic Science

CDK1-PLK1 Axis Impedes DNA Damage Repair and Radiation Resistance Through Inducing ASF1A Degradation by Phosphorylation

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UK College of Medicine, Department of Toxicology and Cancer Biology

Radiation therapy (RT) or radiotherapy is used to treat up to 50% of cancer patients and to manage 40% of patients who are cured. However, improvement in the efficacy of radiotherapy is still needed and required, including strategies for improving radiotherapy with the aim to increase the killing of tumor cells and to decrease damage to surrounding normal tissues. ASF1A (Anti-Silencing Function 1A Histone Chaperone) is well-known as a histone chaperone that is involved in nucleosome assembly, DNA damage repair, and cell division. Accumulating evidence and our previous study showed that PLK1 overexpression suppresses the DNA damage response (DDR) pathway and enhances the sensitivity to ionizing radiation. Herein, we identified ASF1A as a novel substrate of polo-like kinase 1 (PLK1), which is a critical regulator of cell cycle-related events, such as G2/M transition and sister chromatid segregation. Moreover, we showed that PLK1 phosphorylates S166 of ASF1A and this process can be created and promoted by CDK1 phosphorylation at Ser16. The phosphorylation leads to ASF1A degradation in a proteasome-dependent way through FBW7 recognition. Furthermore, our result suggested that ASF1A degradation or inactivate mutagenesis will abolish the regulation of it-mediated DNA damage repair, increase chromosomal instability, and attenuate the resistance to DNA damage. These findings provide a mechanism for PLK1-induced DDR pathway suppression and chromosomal instability, and importantly, highlight potential therapeutic opportunities for improving the efficacy of radiotherapy and other DNA damage-induced treatment in cancer therapy.

Abstract 19

Translational

ICAM-1-suPAR-CD11b Axis Mediates Tumor Cell-Neutrophil Binding to Promote Triple-Negative Breast Cancer Metastasis

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Accumulating evidence demonstrates that circulating tumor cell (CTC) clusters have higher metastatic ability than single CTCs and negatively correlate with cancer patients' outcomes. In addition to homotypic CTC clusters, the heterotypic CTC clusters such as neutrophil-CTC clusters were recently identified in both cancer mouse models and cancer patients, leading to more efficient metastasis formation and worse patient outcome. However, the mechanism by which neutrophil binds to CTC remains elusive. In this study, we found that the intercellular adhesion molecule (ICAM-1) on triple-negative breast cancer (TNBC) cells and CD11b on neutrophils mediate tumor cell-neutrophil binding. Consequently, CD11b deficiency inhibited tumor cell-neutrophil binding and TNBC metastasis. Furthermore, CD11b mediated hydrogen peroxide (H2O2) production from neutrophils, which suppresses natural killer (NK) cell-mediated tumor cell killing. Moreover, we found that ICAM-1 in TNBC cells promotes tumor cells to secret suPAR, which functions as a chemoattractant for neutrophils. Knockdown of uPAR in ICAM-1+ TNBC cells reduced lung-infiltrating neutrophils and lung metastasis. The bioinformatics analysis further confirmed that uPAR is highly expressed in TNBCs, which positively correlates with higher neutrophil infiltration and negatively with breast cancer patients' survival. Collectively, our findings provide new insights into how neutrophil binds to CTC to facilitate metastasis, and a novel potential therapeutic strategy by blocking ICAM-1-suPAR-CD11b axis to inhibit TNBC metastasis.

Abstract 20

Population-Based/Behavioral

A Longitudinal Study of the Association between the Teachable Moment Heuristic and Tobacco Use of Head and Neck Cancer Patients

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Introduction: The health consequences and risks associated with smoking after a cancer diagnosis include early mortality, new primary cancer, cancer recurrence, treatment failure, and poor quality of life. The Teachable Moment Heuristic (McBride et al., 2003) posits that change in its three components (i.e., affective response, risk perception, and social role or self-concept) could increase motivation for health promoting behavior change, such as smoking cessation. This intensive longitudinal study with cancer patients aimed to evaluate the link between Teachable Moment Heuristic constructs and key outcomes pertinent to the process of smoking cessation.

Method: The sample consisted of 42 newly diagnosed head-neck cancer patients (Age: M, SD = 57.64 g 7.34; 71% male, 98% white, non-Hispanic, 24% employed) who reported smoking 12.35 (SD = 11.27) cigarettes per day at enrollment. Participants were recruited from an outpatient oncology clinic. A 20-item survey was administered via interactive voice response technology on 30 consecutive days. Participants answered single-item questions about their smoking, general distress, cancer worry, perceived benefits and risks of smoking, social support, and intention.

Results: Between-persons, only participants who perceived more benefits of smoking abstinence reported greater intention to abstain from smoking, $p < .01$. Participants who perceived more benefits of smoking abstinence and who reported less cancer worry smoked fewer cigarettes than those perceiving fewer benefits of abstinence or more cancer worry, $ps < .05$. Within-persons, less cancer worry than one's personal average predicted increases in next-day intentions to abstain, $p < .01$, whereas both perceiving greater benefits to abstaining and less cancer worry predicted decreases in the number of cigarettes smoked the next day, $ps < .02$. The other Teachable Moment Heuristic variables were not associated with the outcomes ($ps > .05$).

Conclusions: Some constructs derived from the Teachable Moment Heuristic (specifically, cancer worry, an aspect of affective response, and perceived benefits, an aspect of risk perception) are associated with favorable smoking cessation outcomes in adults recently diagnosed with head-neck cancer. Interventions to aid cancer patients with smoking cessation should focus on reducing cancer-related worry and capitalize on the benefits of abstaining.

Abstract 21

Basic Science

ABL1/2 and DDR1 Drive MEKi Resistance in NRAS-Mutant Melanomas by Stabilizing RAF Proteins and Promoting RAF Homodimerization

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Melanomas of the skin are among the most prevalent cancers in the U.S., and, unlike other cancers, their incidence rates have continued to rise over the past 30 years. Melanomas harboring NRAS mutations are a particularly aggressive and deadly subtype. First-line treatment for patients with metastatic NRAS-driven melanomas is immunotherapy, but only a subset of patients can tolerate the treatment and respond to their effects. Second-line regimens are limited to cytotoxic agents that are ineffective and have many adverse effects. Importantly, there are no FDA-approved targeted therapies for these patients because drugs that target the downstream RAF/MEK/ERK pathway, which are effective for patients with mutant BRAF-driven melanomas, do not increase progression-free survival (PFS) in patients with NRAS-driven melanomas due to intrinsic and acquired resistance. Here, using both loss-of-function and gain-of-function approaches, we demonstrate that ABL1/2 and DDR tyrosine kinases cooperate to drive acquired resistance to MEK inhibitors (MEKi) in NRAS-mutant melanomas. Previous research shows that ABL1/2 play a critical role in driving the development of leukemia, and both ABL1/2 and DDR1/2 have oncogenic roles in melanoma. Using cell lines that we engineered to develop resistance to the MEKi trametinib, we show that ABL1/2 and DDR1 cooperate to stabilize RAF proteins, repress p27/KIP1 expression, and promote RAF homodimerization to drive MEKi resistance. Importantly, targeting ABL/DDR kinases in combination with trametinib prevents survival and clonogenicity of resistant cells, induces apoptosis, and efficiently blocks BRAF/CRAF activation of MEK/ERK/MYC signaling during resistance. Significantly, targeting ABL1/2 and DDR1 with an FDA-approved anti-leukemic drug, delays acquisition of acquired resistance and doubles the survival time in a NRAS-mutant mouse model. These data indicate that repurposing FDA-approved drugs targeting ABL1/2 and DDR1 may be a novel and effective strategy for treating patients with treatment-refractory NRAS-driven melanomas.

Abstract 22

Basic Science

Prx4 Depletion Enhances Ferroptosis Susceptibility in Colorectal Cancer

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Ferroptosis is form of cell death that is characterized by the accumulation of lipid peroxides. Dysregulation of ferroptotic cell death has been linked to the development of cancer. Several important regulators involved in ferroptosis have been discovered, but the molecular mechanism behind this process has yet to be completely understood. Members of the peroxiredoxin (Prx) family function as leading cellular antioxidants that react with hydrogen peroxide to keep redox homeostasis as well as contribute to oxidative signaling under both physiological and pathological conditions. In this study, we investigated the role of Prx family members in the regulation of ferroptosis in colorectal cancer cells (CRC) using loss-of-function and gain-of-function experiments. Firstly, immunoblotting was used to examine endogenous levels of Prx family members in different colorectal cancer cells and cells with high or low expression of Prxs were selected for study. Secondly, each of the endogenously expressed 2-Cys containing Prxs including Prx1, Prx2, Prx3, and Prx4 in representative CRC cell lines was depleted by stably expression of ShRNA targeting their coding regions or knockout using CRISPR-Cas9. The consequences of Prx knockdown in these cells in response to ferroptosis inducers, including erastin and RSL3, were examined by cell viability and colonogenic assays. Cell death due to ferroptosis in these cells was demonstrated by the measurement of lipid peroxidation using the sensor BODIPY 581/591 C11, levels of MDA and 4-HNE. The mode of cell death was differentiated from other cell death types by the combined application of various inhibitors. In addition, overexpression was used to validate the results observed in Prx-depleted cells. Finally, Prx4 depleted cells were submitted for RNA sequencing and the data were subjected to GSEA analysis to identify enriched pathways. In conclusion, we found that 2-Cys containing Prxs are widely expressed in CRC cell lines, and depletion of Prx1, Prx2 or Prx4 but not Prx3 sensitizes CRC cells to erastin induced cell death. However, only depletion of Prx4 but not other Prxs sensitizes CRC cells to ferroptotic cell death that is characterized by the accumulation of lipid peroxidation and can be rescued by the treatment with inhibitors of ferroptosis. Moreover, Prx4 depleted cells show higher levels of MDA and 4-HNE. Consistently, overexpression of Prx4 also leads to the resistance of CRC cell to ferroptosis. Importantly, according to gene set enrichment analysis (GSEA) of (KEGG) pathway, pathways including P53 pathway and arachidonic acid (AA) metabolism, which have a well-known significant role in ferroptosis regulation, were activated in Prx4 depleted cells. Thus, our findings reveal an essential role of Prx4 in protecting colorectal cancer against ferroptosis and provide a potential target to enhance the antitumor activity of ferroptosis-based treatment.

Abstract 23

Basic Science

Anticancer Activity of Artesunate Combined with Navitoclax is Synergistic in Ovarian Cancer

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UK Markey Cancer Center

Introduction: Ovarian cancer is the deadliest gynecologic malignancy in the United States yet modern ovarian cancer treatment has not substantially improved outcomes. The five-year overall survival rate remains below 50% and superior therapeutic strategies are needed. Artesunate belongs to a class of medications derived from the sweet wormwood plant (Artemisia annua) known as artemisinins. Artesunate has traditionally been used as a frontline treatment for severe malaria but has also demonstrated antineoplastic activity against various malignancies, including ovarian cancer. Data suggests artesunate exacerbates cellular oxidative stress, triggering apoptosis. Moreover, our prior work demonstrated KEAP1, a regulator of cellular oxidative stress response, plays a key role in mediating anticancer activity of artesunate in lung cancer. In the current study, we extend these findings to ovarian cancer models and investigate the ability of the Bcl-2 protein family inhibitor navitoclax to enhance artesunate efficacy in ovarian cancer cells in vitro.

Methods: Five immortalized human ovarian cancer cell lines (CAOV3, OVCAR3, OV-90, SKOV-3, and UWB1.289) and one novel human ovarian tumor organoid model (UK1254) were treated with navitoclax and artesunate, alone and in combination. Cell viability was measured following 72 h treatment using CellTiterGlo Cell Viability Assays (Promega). Dose response curves were fit using 4-parameter logistic regression models to determine IC50. Drug synergy was assessed using the Loewe additivity model. Protein expression was analyzed using Bis-Tris SDS-PAGE and western blots.

Results: Artesunate IC50 values for ovarian cancer cell lines ranged from 5.95uM to 61.0uM with OVCAR3 being the most sensitive cell line and OV-90 the most resistant. Navitoclax IC50 spanned from 3.3uM (CAOV3) to 13.3uM (OVCAR3). For the UK1254 tumor organoid model, the artesunate IC50 was 4.15uM and navitoclax IC50 was 3.80uM. Combining navitoclax with artesunate enhanced drug efficacy in every ovarian cancer model tested, including the artesunate-resistant SKOV3 and OV-90 cell lines. According to the Loewe additivity model, combination treatment was synergistic in CAOV3 (mean synergy score=8.97; p=3.5x10-20), OVCAR3 (mean synergy score=5.38; p=8.5x10-4), UWB1.289 (mean synergy score=4.04; p=2.8x10-8), SKOV-3 (mean synergy score=3.46; p=9.7x10-6), OV-90 cells (mean synergy score=7.30; p=4.5x10-4) and UK1254 tumor organoids (mean synergy score=19.13; p=1.4x10-7). To better understand the mechanism of action for the drug combination, expression of regulators of oxidative stress (e.g., KEAP1, NFE2L2/NRF2, NQO1) and apoptosis (e.g., Bcl-2, Bcl-w, Bcl-xL, CHOP/DDIT3, MCL1) were examined. KEAP1 and anti-apoptotic MCL1 expression decreased with artesunate treatment while pro-apoptotic CHOP/DDIT3 expression increased. Notably, artesunate-resistant OV-90 cells were devoid of KEAP1 expression. The correlated expression changes among CHOP/DDIT3, MCL1 and KEAP1 suggest a coordinated response to artesunate exposure, leading to initiation of apoptosis. Together these data show, regardless of KEAP1 status and/or intrinsic artesunate resistance, artesunate-induced apoptosis can be augmented by the addition of navitoclax.

Conclusion: Navitoclax enhances the anti-cancer efficacy of artesunate in ovarian cancer cells. This drug combination could help fulfill the urgent need for new therapeutic options in ovarian cancer and warrants further pre-clinical investigation.

Abstract 24

Basic Science

Phosphorylation of WRN by PLK1 Affects DNA Damage and Repair

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Regulation of end-processing is very important for precise repair and to switch between non-homologous end joining (NHEJ) and homologous recombination (HR). End resection has a key role in double-strand break repair and DNA replication. Malfunctional end resection leads to defective DNA repair and replication, causing greater genomic instability, especially in humans. End resection is a two-stage process. WRN participates in one of the two alternative long-range resection pathways. Here, we demonstrate that PLK1 phosphorylates WRN by in vitro Kinase assay and in vivo specific antibodies. The phosphorylation of S435/S462 on WRN is essential to perform long-range end resection at replication-related double strand breaks (DSBs). Collectively, we unveil a PLK1-dependent regulation of the WRN-DNA2 mediated resection.

Abstract 25

Population-Based/Behavioral

Tobacco Use, Secondhand Smoke Exposure and Infant Feeding Practices Among Mothers in Rural Kentucky

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The purpose is to examine tobacco use, secondhand smoke exposure (SHS) and infant feeding practices including breastfeeding (BF duration) in mothers living in rural Kentucky. Aim 1: Determine the association of tobacco use status and SHS exposure status with infant feeding status among mothers living in rural Kentucky, controlling for occupational status and strength of smoke-free laws in county of residence. Aim 2: Determine the association of tobacco use status and SHS exposure status with BF duration among mothers living in rural Kentucky, controlling for occupational status and strength of smoke-free laws in county of residence. Smoking rates in adults 18 and older are higher in Kentucky (23.4%) compared to the national average (16.1%). Conversely, rates of BF in KY are lower than the national average with only 44.5% of mothers BF their infants at six months and 23.2% at 12 months of age compared to 58.3% and 35.3%, respectively. Women in rural communities have higher rates of tobacco use and SHS exposure as well as lower rates of BF initiation and duration compared to their urban counterparts. Prior research has shown an association between the strength of municipal smoke-free laws and various health outcomes such as lower incidence of adult smoking, rates of lung cancer and preterm birth. However, research is lacking on the association of tobacco use, SHS exposure and infant feeding status in rural communities. This study uses a cross-sectional retrospective design with participants completing a one-time survey (online, phone, or paper copy options are available). Purposive cluster sampling will be utilized with stratification by strength of municipal smoke-free laws and tobacco use and/or exposure status). Women between 18-45 years of age currently residing in one of the six identified rural Kentucky counties: Bath, Menifee, Morgan (absent smoke-free laws) and Knott, Owsley and Perry (strong smoke-free laws), who have given birth to a live infant within the past two years and speak English are eligible to participate in this study. Recruitment methods include ResearchMatch, Craigslist and parenting groups on Facebook. The projected sample size is 280 mothers (140 in each county cluster) with 40% of participants from each county cluster with self-reported tobacco use and/or secondhand smoke exposure. Measures include demographics; infant feeding practices (prior BF history, infant feeding plans during prenatal period for first 6 weeks of life, infant feeding status at various time points, depending on current age of child); tobacco use (previous and current use including cigarettes and e-cigarettes); SHS exposure (home, workplace, and vehicle); lung cancer (prior screening, personal and family history, and worry); depression (Patient Health Questionnaire-2); anxiety (General Anxiety Disorder-7) and alcohol and substance abuse (prenatal and current). Participants completing the survey will receive a $15 Amazon gift card. Data analysis will include descriptive statistics, bivariate (associations among tobacco use, SHS exposure, infant feeding and BF duration) and multi-level modelling (evaluate factors associated with BF and BF duration). IRB approval through the UK ORI has been obtained, survey items have been refined and grant funding has been procured. Data collection will begin once the Procard for purchasing participant incentives has been received. Preliminary study findings will be available in May.

Abstract 26

Basic Science

Triapine and BAY1895344 as a Cisplatin-Sparing Therapy in the Treatment of Pancreatic Neuroendocrine Neoplasms

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Introduction: Despite clinical advances, pancreatic neuroendocrine neoplasms (pNEN) remain a difficult clinical entity to treat and can carry a poor prognosis. Systemic therapy is used to treat pNENs which are not amenable to surgical resection. Prior work from our laboratory demonstrated triapine, a ribonucleotide reductase inhibitor (RNRi), and BAY1895344, an ataxia telangiectasia and Rad3-related protein inhibitor (ATRi), were efficacious in combination to sensitize pNENs to radiation therapy through inhibition of the de novo and salvage pathways for deoxynucleotide triphosphate (dNTP) synthesis, respectively. Cisplatin is another form of DNA-damaging therapy, similar to radiation therapy, currently used to treat more aggressive pNENs, but is limited in efficacy with dose-limiting toxic side-effects. This study sought to increase the sensitivity of pNENs to cisplatin therapy through coadministration of triapine and BAY1895344.

Methods: (i) The CellTiter-Glo (Promega) luminescent cell viability assay established drug sensitivities of two pNEN cell lines (BON and QGP-1). Assays were performed with at least three replicates. Data were fit using a four-parameter log-logistic model, and IC50 values were calculated with R statistical software. (ii) Loewe synergy models were used to calculate the two-drug combination effects in both cell lines with synergy scores greater than 0 indicating synergistic effects and scores less than 0 representative of antagonistic effects. (iii) Clonogenic assays were performed and analyzed via colorimetry with sulforhodamine B to assess the effect of each drug alone and in combination. (iv) Immunoblots were used to assess apoptosis and cell cycle arrest following individual and combination drug treatments. (v) γH2AX immunofluorescent assay and enzyme-linked immunosorbent assay (ELISA) were used to quantify DNA damage and cell death.

Results: (i) BON cells show greater resistance to cisplatin therapy with IC50 of 19.5 ± 1.8μM compared to QGP-1 (IC50 4.49 ± 1.21μM; $p<0.001$). (ii) Whereas triapine and cisplatin demonstrate a neutral to potentially antagonistic drug interaction in both BON and QGP-1 with synergy scores of -4.54 and -12.2, respectively, BAY1895344 and cisplatin demonstrate potential synergism with synergy scores of 15.5 and 16.1 respectively. (iii) Combination therapy of triapine, BAY1895344, and cisplatin leads to significantly less colony formation than cisplatin therapy alone in both cell lines with reduced doses of all three agents ($p<0.001$). (iv) Increased apoptosis and cell cycle arrest is appreciated with triple drug therapy compared to single agent and double agent treatments in both cell lines at reduced doses of all three agents. (v) Triple drug therapy creates significantly greater DNA damage based on γH2AX immunofluorescent staining compared cisplatin therapy alone in BON ($p<0.001$) and QGP-1 ($p<0.05$) cell lines. Similarly, triple drug therapy resulted in greater cell death compared to cisplatin therapy alone based on ELISA in both cell lines ($p<0.001$).

Conclusion: Combination therapy of triapine and BAY1895344 sensitizes pNEN cells to cisplatin therapy allowing for efficacious treatment at reduced doses of all agents. This represents a novel potential treatment regimen for metastatic and/or poorly differentiated pNENs.

Abstract 27

Basic Science

ABL1 Mediates MLH1 Regulation and DNA Mismatch Repair

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The DNA mismatch repair (MMR) pathway and its regulation are critical for genomic stability. Mismatch repair (MMR) repairs misincorporated bases and small insertions or deletions that are not recognized and removed by the proofreading polymerase during DNA replication. MMR is also critical for initiating apoptosis after certain classes of DNA damaging agents that create mispairs that are recognized by but not repaired by MMR. Germ line mutations in MMR are causative of the hereditary cancer syndrome Lynch syndrome. MMR defects are also seen in a large portion of sporadic colorectal, endometrial, and gastric cancers. Cells deficient in MMR exhibit an increased overall mutation rate and also unrepaired expansion and contraction of repetitive sequences termed, microsatellite instability (MSI). MSI high/MMR-defective tumors respond well to immunotherapy, presumably due to a high tumor mutation burden and increased neoantigen production. MLH1 is an endonuclease that is essential for MMR. Loss or mutation of MLH1 subsequently leads to defective MMR, increased mutation frequency, and MSI-high phenotypes. In this study, we report that tyrosine kinase inhibitors (TKIs), imatinib and nilotinib, lead to decreased MLH1 protein expression but not decreased MLH1 mRNA levels. Of the seven cellular targets of imatinib and nilotinib, we show that silencing of the ABL1 kinase reduces MLH1 protein expression. TKI treatment or silencing of ABL1 both result in decreased apoptosis after treatment with alkylating agents, suggesting the level of MLH1 reduction is sufficient to disrupt MMR function. We demonstrate that MLH1 downregulation by ABL1 knockdown or TKI treatment requires chaperone protein Hsp70. MLH1 degradation can be abolished by Hsp70 inhibition or lysosomal inhibition. Taken together, we propose that ABL1 prevents MLH1 from being targeted for degradation by the Hsp70 chaperone and that in the absence of c-Abl activity, a subset of MLH1 is degraded through the lysosome. We observe that MLH1 can be directly tyrosine phosphorylated by ABL1. MLH1 mutants disrupting a predicted phosphorylation site exhibit decreased apoptosis after alkylating damage and are under further investigation to determine if they are the site of MMR phosphorylation. This study represents an advance in understanding regulation of MLH1 and the MMR pathway. This study also has interesting and important clinical implications as given the rise of immunotherapy use in MSI-high/MMR deficient tumors. We are currently pursuing this work at the pre-clinical stage as a strategy for sensitizing MSI-low/MMR proficient tumors to immunotherapy, especially as tyrosine kinase inhibitors are FDA-approved drugs with a known safety profile.

Abstract 28

Basic Science

Targeting the FASN/VEGF Axis to Improve Efficacy of BRAF-Targeted Therapy in Colorectal Cancer

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Aberrant lipid metabolism is a hallmark of cancer associated with poor prognosis in colorectal cancer (CRC). Fatty acid synthase (FASN), a key enzyme of lipid synthesis, is overexpressed and potential therapeutic target in CRC. Our studies have shown the crucial importance of lipid synthesis and FASN in CRC progression and metastasis. BRAFV600E mutation occurs in 10-15% of CRC cases. BRAF-targeted therapy is effective, but quickly developed resistance is a serious problem leading to cancer relapse. Our preliminary data show that the development of resistance to BRAF-targeted therapy is associated with an increase in the expression of FASN, accumulation of triglycerides (TGs), and mitochondrial respiration. Therefore, our central hypothesis is that inhibiting de novo lipogenesis can increase the efficacy of BRAF-targeted therapy in CRC patients with BRAFV600E mutation.

Methods: We have established HT29 cells and primary PT130 and PT2449pt cells resistant to PLX8394, a novel BRAF inhibitor, and confirmed it by measuring IC50. PrestoBlue viability assay, CytoSelect™ Cell Invasion assay, Triglyceride Assays, Seahorse XF analysis, Western blot, and confocal microscopy were used to evaluate differences between parental and resistant cells. RNA-seq, lipid analysis, and multiplex cytokine array were used to evaluate changes in gene expression, lipids levels, and secreted cytokines, respectively. The efficacy of PLX8394 was determined in cells with shRNA-mediated knockdown of FASN and TVB3664 (FASN inhibitor) treated cells.

Results: CRC cells resistant to PLX8394 have higher cellular proliferation and invasion compared to parental cells. Using Western blot and confocal microscopy, we show that an increase in invasion of resistant cells is associated with loss of e-cadherin. The multiplex cytokine array shows an increase in VEGF secretion in resistant cells as compared to parental cells. The RNA-sequencing and KEGG pathway analysis show a significant increase in genes and pathways associated with lipid metabolism. Western blot analysis of resistant cells confirms upregulation of FASN and other lipogenic markers. Moreover, resistant cells have an increase in levels of triglycerides as compared to parental cells. Seahorse XF Cell Mito Stress Test shows that resistant cells have higher mitochondrial respiration as compared to parental cells. shRNA knockdown of FASN in parental HT29 and PT130 makes the cells more susceptible to PLX8394 treatment compared to control. Moreover, the combination of PLX8394 and TVB3664 has a synergetic on cell viability in parental cells, but not in resistant cells. Consistently, using TVB3664 along with PLX8394 before resistance occurs slows the development of resistance. The addition of Axitinib, a VEGFR inhibitor, decreases viability of the parental cells compared to control.

Conclusion: Our study demonstrates that resistance to BRAF inhibitors is associated with a significant increase in proliferation, metastasis, and upregulation of lipid metabolism. We show that a combination of FASN and BRAF inhibitors has a combinational effect on inhibiting cell viability in parental but not resistant cells, suggesting that the addition of a FASN inhibitor to the standard regimen for BRAFV600E mutation positive patients can improve the efficacy of these therapies. Furthermore, adding Axitinib to TVB3664 and PLX8394 therapy may be a promising therapeutic approach in CRC with BRAFV600E mutation.

Abstract 29

Basic Science

Delineating the Effects of Perfluorooctanesulfonic Acid in Normal Intestinal Tissues and Adenoma Organoid Models

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PFOS, members of a chemical group called per- and polyfluoroalkyl substances (PFAS), are well-known contaminants in the environment and have shown to disrupt multiple biological pathways and negatively affect human health. As a contaminant in drinking water, PFOS can accumulate in the intestine, thus, modulating intestinal homeostasis under physiological and pathological conditions. Although some studies have highlighted the potential mechanisms of PFOS in tumor promotion, there is not much known about PFOS exposure in normal and pre-malignant intestinal tissues and its contribution to carcinogenesis. Therefore, the goal of this study is to investigate PFOS effects on normal (I) and pre-malignant intestinal epithelium (II).

Methods: The effect of PFOS and/or diets supplemented with inulin or pectin on gene expression profiles was assessed by RNA-Seq analysis in intestinal tissue of C57BL/6 mice. Tumor organoids were established from Apc/Cre and ApcMin (adenoma models). Organoid cultures have been treated with 1ug/ml of PFOS for 4, 8 or 15 days. PFOS, and qRT-PCR, western blot and immunofluorescence analysis have been performed to determine PFOS-induced changes. Colorectal cancer (CRC) cell lines, HCT-116 and HT-29, and human CRC tissue slices were used to assess the mechanisms involved in PFOS-induced intestinal alterations.

Results: Using RNA-Seq analysis, we have identified that PFOS exposure resulted in transcriptome alterations with exacerbated changes in pathways involved in lipid metabolism and immune system regulation. The volcano plot analysis highlighted the decrease in HMGCS2, an important enzyme of ketogenesis pathway, and the increase in VEGFR expression in intestinal cells exposed to PFOS. Also, PFOS-induced upregulation of FASN (a key gene of de novo lipogenesis) and PDL-1 (a protein that allows malignant cells to escape from being attacked by the immune system) mRNA and protein expression levels in Apc/Cre and ApcMin organoids. Using FASN knockout cells and TVB-3664, a FASN inhibitor, we identified the possible role of FASN in PDL-1 regulation.

Conclusion: In summary, our data suggest that PFOS may contribute to upregulation of de novo lipid synthesis and mediate tumor immune escape to promote transition from adenoma stage to colorectal cancer and, thus, contribute to initiation of CRC. Further studies are warranted to determine the effect PFOS on HMGC2 and VEGFR expression and its functional significance. In addition, investigation of changes in lipidomic, metabolomic, and immune cell profiles after PFOS exposure will help to elucidate its role in CRC promotion. Delineating the effects of PFOS on intestinal epithelium may contribute to development of interventional strategies to eliminate harmful effects of these environmental pollutants.

Abstract 30

Basic Science

NT Contributes to Defective Mitochondrial Function of Small Intestinal Epithelial Cells Associated with Obesity

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Obesity is associated with elevated intestinal nutrient absorption and excessive accumulation of lipids in the liver, adipose tissue, skeletal muscle, and other organs, which contributes to metabolic diseases such as type 2 diabetes, non-alcoholic fatty liver disease (NAFLD), cardiovascular disease, and certain types of cancer. The effect of obesity on intestinal lipid metabolism is currently unclear. We previously demonstrated that the obese phenotype and its associated insulin resistance and NAFLD were ameliorated in mice deficient in the intestinal hormone neurotensin (NT) by inhibiting small intestinal fat absorption and preserving the activity of AMPK, an enzyme that plays a key role as a master regulator of cellular energy homeostasis. However, how NT/AMPK signaling regulates this process remains unknown. The purpose of the current study was to evaluate the genes related to small intestinal lipid absorption in the context of obesity and the regulation of these genes by NT/AMPK signaling.

Methods: NT wild type (WT) (Nt $^{+/+}$) and knockout (KO) (Nt $^{-/-}$) mice, fed standard control diet (CD, 10% kcal from fat) or high fat diet (HFD, 60% kcal from fat) were used. i) Total RNA was isolated from mouse jejunal mucosal scrapings and RNAseq analysis performed to profile gene expression; ii) Jejunal crypts were isolated for 2-D monolayer culture; total RNA or protein was isolated for qPCR and western blot analyses, respectively, to confirm gene or protein expression.

Results: RNAseq analysis of female mice fed CD or HFD for 28 weeks showed that genes involved in lipid absorption (Fabp1, Fabp2, Cd36, Alpi, and Plin2) were upregulated (FDR <0.05) in Nt $^{+/+}$ mice fed HFD vs. CD; interestingly, these alterations were not noted in Nt $^{-/-}$ mice fed HFD vs. LFD; qPCR or western blot further confirmed these results. Concurrently, phosphorylation of AMPK (p-AMPK) was decreased in HFD-fed Nt$^{+/+}$ mice, which was rescued by NT deficiency; consistently, palmitic acid (PA) treatment decreased p-AMPK and increased FABP1 and FABP2 protein expression.

Conclusions: These findings suggest that HFD increases small intestinal lipid absorption by upregulating FABP1 and FABP2 expression. HFD feeding or PA treatment decreases p-AMPKe activity, suggesting that AMPK mediates HFD-upregulated FABP1 and FABP2 expression. NT deficiency preserves AMPK signaling and prevents HFD-upregulated FABP1 and FABP2 levels, thus reducing increased lipid absorption. NT signaling may represent a therapeutic target to inhibit intestinal lipid absorption associated with obesity.

Abstract 31

Basic Science

A Potent Small Molecule that Inhibits RPS6KB1 and Survival of Diverse Treatment-Resistant Cancer Cells

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Lung cancer is the leading cause of cancer-related deaths worldwide. Oncogenic mutations in KRAS and EGFR are the most common drivers of lung cancer. Mutations in the tumor suppressor gene TP53 often co-occur with mutant KRAS or EGFR, and contribute to therapy resistance and poor prognosis. To identify small molecules that can overcome therapy resistance, we screened an FDA approved drug library of about 1,400 compounds for inhibition of a mutant-KRAS / TP53-null lung cancer cell line. This unbiased screen led to the identification of Ebastine (EBS), a second-generation anti-histamine reported in literature to exhibit anti-cancer effects. However, the IC50 of Ebastine is upward of 10 M and the exact molecular mechanism by which it induces cell death is not clearly elucidated. Toward the goal of developing more potent analogs of Ebastine, we generated an aminoguanidine derivative that is far more potent than EBS in diverse tumor cell lines. This analog exerted growth inhibition in cancer cell lines resistant to standard-of-care treatments at concentrations significantly lower than Ebastine. We designated this analog as Super-Ebastine. PRISM analysis of 900+ cell lines at the Broad Institute, MA, indicated that diverse cancer cell types are sensitive to Super-Ebastine. Importantly, analysis of the PRISM data predicted the Ser/Thr kinase RPS6KB1 (S6K1, ribosomal protein S6 kinase B1) as a prospective target of Super-Ebastine at a concentration of 4nM. S6K1 as well as 4E-BP1 are the two key substrates of the upstream kinase complex mTORC1 that regulate cancer cell survival. Interestingly, our validation studies indicated that Super-Ebastine inhibited phosphorylation of S6K1 but not 4E-BP1 in 2 h. Consistently, in silico molecular docking studies independently confirmed S6K1 as a target of Super-Ebastine with binding affinity far superior than the conventional inhibitor of this protein. S6K1 is generally associated with therapy resistance in ER-positive breast cancer, and with aggressive forms of prostate cancer and lung cancer with poor prognosis. Conversely, inhibition of S6K1 results in inhibition of cell survival proteins, such as survivin and BAD, and induction of apoptosis. Super-Ebastine was also effective inhibiting the growth of tumors in in vivo mouse models. Thus, our studies identified a novel small molecule that inhibits the critical cell survival kinase RPS6KB1 in therapy-resistant cancer cells and induces cell death.

Abstract 32

Basic Science

Upregulation of Fatty Acid Synthase Increases Expression of Notum and Stemness in Colorectal Cancer

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Upregulation of lipid synthesis has been associated with a poor clinical outcome in colorectal cancer (CRC). Fatty acid synthase (FASN) synthesizes 16-carbon fatty acid palmitate which can be utilized for multiple functions in a cell including post-translational modifications of various proteins. Notum, a palmitoleoyl-protein carboxylesterase, is involved in the negative regulation of Wnt signaling pathway via its role in de-palmitoylation of Wnt ligands and has been identified as a marker for poor prognosis in CRC. However, the crosstalk between FASN and Notum has not been reported. Our preliminary data suggest that overexpression of FASN activates β-catenin signaling and increases Notum expression. Moreover, an increase in FASN expression leads to an increase in expression of stem cell markers and stemness. Therefore, the purpose of this study is to elucidate (I) the mechanisms of how FASN regulates expression of Notum and (II) the contribution of FASN/Notum axis to stemness in CRC.

Methods: Tumor and normal intestinal organoids were established from transgenic mice models, ApcMin and Apc/VillinCre-ERT2, with inducible hetero- and homozygous deletion of FASN. The effect of genetic deletion and pharmacological inhibition of FASN on organoid growth and viability was assessed by 4-hydroxytamoxifen (4-OHT) and TVB-3664 (a FASN inhibitor) treatments, respectively. LIVE/DEAD™ Viability/Cytotoxicity Cell Viability Kit and Cell Titer-Glo® 3D Cell Viability Assays were used for quantitative analysis of growth and viability. Human NOTUM/Protein notum homolog ELISA Kit was used for quantitative analysis of Notum secretion. HCT116, NTC and FASN shRNA, and SW480, control and FASN overexpression cells were used for analysis.

Results: ERT2-mediated deletion of Apc leads to upregulation of FASN and Notum expression in mouse intestinal tissues and organoids. RNA-seq analysis of adenomas from Apc/VillinCre mice showed that hetero- and homozygous germline deletion of FASN is associated with a significant decrease in number of adenomas, expression of Notum and CRC stem cell markers. Using qRT-PCR and western blot, we confirmed that FASN downregulation is associated with a decrease in active β-catenin, Notum, and stem cell markers. Consistently with Apc/VillinCre model, downregulation of FASN results in a decrease in bud formation in Apc/VillinCre-ERT2 organoids, and viability and size in ApcMin organoids. Interestingly, pharmacological inhibition of FASN decreases spheroid formation efficiency in HCT116 and HT29 cells. Furthermore, overexpression of FASN increases the levels of active and total β-catenin, Notum, and stem cell markers expression in SW480 cells. In contrast, shRNA-mediated deletion of FASN decreases expression and secretion of Notum in HCT116 cells.

Conclusion: In summary, downregulation of FASN leads to a decrease in expression and secretion of Notum and is associated with morphological changes and significant decrease in viability in organoid models. Conversely, FASN overexpression upregulates Notum expression suggesting a potential cross talk between de novo lipid synthesis and Notum. FASN inhibitors are currently tested in clinical trials and Notum inhibitors are in pre-clinical development. Delineating the role of FASN regulation of stemness via alteration in β-catenin signaling and expression of Notum will provide the rationale for targeting the FASN/Notum axis as a preventative or early-stage therapeutic approach in CRC.

Abstract 33

Clinical

Tobacco Cessation and Radon Risk Messaging Practices during Lung Cancer Screening Shared Decision-Making in Kentucky

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Lung cancer screening (LCS) is recommended for high-risk individuals and has been shown to decrease mortality. Co-exposure to tobacco and radon has a synergistic effect on the development of lung cancer. Kentucky leads the nation in lung cancer mortality. Widespread use of tobacco is a major factor in this burden, yet exposure to radon is undoubtedly also a contributing factor as 93% of Kentucky counties have moderate-to-high radon risk potential. Lung cancer screening shared decision-making (SDM), which involves counseling high-risk individuals on the risks and benefits of LCS, is an ideal teachable moment to promote smoking cessation as well as home radon testing and mitigation. Using stratified random sampling by ADD, we invited 1,000 PCPs from across Kentucky to participate in a mailed survey assessing beliefs and practices related to lung cancer prevention and explore current tobacco and radon risk messaging during LCS SDM visits. A total of 149 (14.9%) PCPs responded to the survey, 78% APRNs, while the remaining 22% were MD/DOs. Providers frequently reported counseling patients on smoking cessation during LCS SDM, while 70% reported never recommending home radon testing; 77% reported never recommending radon mitigation. Providers who reported ever testing their homes for radon, and those who had greater self-efficacy in counseling patients on tobacco cessation reported higher frequencies of tobacco and radon risk reduction counseling during lung cancer screening shared decision making. Lung cancer screening does not prevent most lung cancer deaths; thus, risk reduction remains essential.

Abstract 34

Population-Based/Behavioral

Codesign Comprehensive Connected Cancer Care Program: A Qualitative Analysis of Participant Notes

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Introduction: Cancer patients in Kentucky experience a variety of unique challenges. The implementation of technology for psychosocial support in cancer care is an interest whose implications for enhanced patient outcomes and access to support resources are promising. A co-design studio connects the experiences of patients, caregivers, and providers alike to align ideals in developing an intervention to address barriers to obtaining resources.

Methods: This analysis is based on participants' (n=24) responses in a co-design studio at Markey Cancer Center in December of 2022. Participants were purposefully assigned tables to foster interaction among patients, community members, and providers. During the technology development phase of the studio, participants were assigned to different tables indicated by role, either as a patient or as a caregiver or provider, to allow like participants to focus on creating a similar technology. Each table contained a facilitator from the research team who guided participants through several structured activities. Written responses to co-design activity questions were coded and summarized.

Results: Participant responses were used to analyze common themes stretching patient access to resources, benefits and drawbacks of technology, and technological adaptations for cancer care. Participants proposed that a personalized, central database of resources would be beneficial for utilization, and that navigator referral and easy access to technology-based resources was best. Accessibility and knowledge deficiencies were cited as barriers to technology incorporation; however, increased provider accountability and flexibility, and enhanced patient autonomy, security, and assistive communication relieved associated stress. Participants identified an ideal application as being accessible, easily navigable, personalized, and visually simple. The significance of "closing the loop" of referrals was also emphasized. Participants stressed the need for enhanced patient-provider communication in an app compatible with existing EHR systems.

Conclusion: The co-design studio enables program development aligning with patient ideals and capabilities, and provider interests. Future studios will focus on improving recruitment and participation, and soliciting ideas organized from this studio.

Abstract 35

Population-Based/Behavioral

Withdrawn

Abstract 36

Basic Science

NAC1 Drive the Dual Role of Neutrophils in Primary and Metastatic TNBC through Modulating the JAK/STAT3 Pathway

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One of the important features of triple-negative breast cancer (TNBC) is the enrichment of stem-like cells that contribute substantially to the malignant phenotype of this disease. Nucleus accumbens associated protein 1 (NAC1), a member of the BTB-POZ family, is implicated in tumor development and progression of various types of cancer. The objective of this study was to test the hypothesis that NAC1 plays a critical role in maintaining stemness of TNBC. Bioinformatic analysis and hematoxylin-eosin staining of human breast cancer tissues revealed high NAC1 expressions in TNBC clinical samples compared to the normal tissues. Depletion of NAC1 in TNBC cells decreased their mammosphere formation, migration, and invasion ability, and altered their EMT phenotype. The stemness markers CD44 and ALDH1A1 as well as aldolase activity were downregulated in TNBC cells subjected to NAC1 depletion. NAC1 depletion in TNBC cells led to a decreased tumorigenicity in nu/nu mice. Interestingly, we observed an increased tumor initiation capability of low NAC1 cells in natural killer (NK) cell-deficient NSG mice. Similarly, tumor xenografts from NAC1 depleted cells in nu/nu mice led to reduced tumor growth while xenografts of NAC1 low TNBC cells in NK cell-deficient mice led to increased tumor growth. Depletion of neutrophils in nu/nu mice using Ly6G antibody increased tumor initiation and growth of NAC1 depleted cells compared to the controls. Allografts from NAC1 low 4T1 cells demonstrated low tumor growth in immunocompetent BALB/c mice. Analysis of the Timer2.0 database to determine immune cell infiltration in TCGA clinical samples revealed an increased NAC1 expression with increasing myeloid-derived suppressor cells (MDSCs). Additionally, neutrophils from tumor bearing mice had increased expression of NAC1 as compared to the control. In vitro co-culture assays of EO771 tumor cells and NAC1-KO Gr1/Cd11b+ cells associated with immunosuppression in murine model demonstrated the ability of these cells to inhibit tumor cells growth suggesting the tumoricidal ability of NAC1 low neutrophils as compared to neutrophils from control mice. Intriguingly, knockout of NAC1 in Gr1/Cd11b+ cells decreased CD44 expression and aldolase activity in EO771 cells. RNA sequencing uncovered the enrichment of JAK/STAT3, PI3K/AKT, WNT and hypoxia pathways in TNBC upon depletion of NAC1. IHC analysis revealed increased expression of phospho-STAT3 (Y705), IL-6, β-catenin, PDK1, cyclin D1, cMYC, and phosphorylated PTEN in TNBC cells in agreement with the RNA sequencing data. Immunoprecipitation assays demonstrated a physical association of NAC1 with STAT3 and pulse-chase experiments depicted that the proteasome-mediated turnover of STAT3 was faster in NAC1-depleted cells compared to control cells (half-life: 5 h vs 24 h, respectively). The increased proteasomal degradations of STAT3 was rescued by treatment of NAC1 depleted cells with proteosome inhibitor MG132. Collectively, we demonstrate a novel role of NAC1 in regulation of tumor stem-like cells and their crosstalk with immune cells in the tumor microenvironment (TME). The results suggest that targeting NAC1 could be exploited as a new approach to reverse immune-suppressive TME and to improve treatment of TNBC.

Abstract 37

Clinical

Implementation of Nurse Navigation Improves Rate of Molecular Tumor Testing for Ovarian Cancer in a Gynecologic Oncology Practice

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The National Comprehensive Cancer Network recommends somatic tumor testing for all patients with epithelial ovarian cancer, as harboring specific mutations has implications for therapy. Targeted panel testing (TPT) includes some of these genes, however Next Generation Sequencing (NGS) is preferred for the complete gene complement. The rate of guideline concordant molecular tumor testing is low; therefore, we implemented a nurse navigator (NN) to improve rates. The purpose of this study was to assess the impact of a NN on molecular testing rates and timeliness of testing.

This is a single institution evaluation of the impact of education sessions, consensus formation, and NN implementation for molecular tumor testing in patients with epithelial ovarian cancer. The NN responsibilities included attending tumor board, and ensuring NGS is ordered, reviewed, and coordinated for appropriate patients.

Nurse navigation significantly improved NGS testing rates from 35.29% to 77.27%, p=0.002. Ordering a TPT was the most common reason for not ordering NGS in the pre NN cohort (13/22, 59%). The total turnaround time for testing was reduced after NN from 145.2 days to 42.8 days, p<0.0001. The post NN group had a significantly higher rate of actionable mutations identified for the recurrent setting [67.6% versus 20.8% (p=0.0005)] and a trend towards a higher rate of actionable mutations identified in the frontline setting [41.2% versus 33.3% (p=0.54)].

Nurse navigation significantly improved guideline concordant somatic tumor testing rates and timeliness for patients with epithelial ovarian cancer. Discontinuing TPT in favor of NGS revealed higher rate of actionable tumor mutations that would have been missed with TPT alone.

Abstract 38

Basic Science

Artesunate Acts through Cytochrome C to Inhibit Growth of Pediatric AML Cells

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Background: Artesunate is a derivative of artemisinin, an active compound isolated from Artemisia annua which has been used in Traditional Chinese Medicine and to treat malaria worldwide. Artemisinin derivatives have exhibited anti-cancer activity against both solid tumors and leukemia. The direct target(s) of artesunate are controversial; although, heme-bound proteins in the mitochondria have been implicated.

Methods: We utilized computational modeling to calculate the predicted binding score of artesunate with heme-bound mitochondrial proteins. After identifying potential binding partners for artesunate, we used UV-visible spectroscopy to determine if artesunate was able to induce any changes in the protein in vitro. Finally, we tested if the predicted binding partner for artesunate could explain the effect of artesunate on cell viability, apoptosis, and mitochondrial membrane potential in MV4-11 and THP-1 pediatric AML cell lines.

Results: Computational modeling identified cytochrome c as potential artesunate target. UV-visible spectroscopy showed changes in the absorbance spectrum, and thus protein structure, when cytochrome c was incubated with artesunate in vitro. Additionally, artesunate induces apoptosis, disrupts mitochondrial membrane potential, and is antagonized by methazolamide, an inhibitor of cytochrome c release, in pediatric AML cells indicating a probable mechanism of action involving cytochrome c.

Conclusion: We utilized a multi-disciplinary approach to show that artesunate can interact with and is dependent on cytochrome c release to induce cell death in pediatric AML cell lines.

Abstract 39

Translational

FASN Inhibition Potentiates the Efficacy of Chemotherapeutic Drugs in Colorectal Cancer by Inhibiting DNA Repair

Moumita Banerjee[1], Yekaterina Zaytseva[2], Sumati Hasani[3], Izumi Tadahide[2], B. Mark Evers[4], Tianyan Gao[3]

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Abstract: Fatty acid synthase (FASN) a key enzyme for fatty acid metabolism, plays significant role in colorectal cancer progression. Our previous studies in patient and PDX (patient-derived xenograft) models show that TVB-2640 and TVB-3664, a class of potent small molecule inhibitors of FASN, have excellent on-target effect and manageable safety profile but exert modest effect on tumor growth. The goal of this study was to determine if FASN inhibitors can enhance the efficacy of chemotherapeutics such as FOLFIRI (FOlinic acid, Fluorouracil and IRInotecan) in colorectal cancer.

Methods: Colorectal cancer cell lines were treated with TVB-3664 and Irinotecan combination and assayed for DNA damage (vH2AX foci formation), apoptotic cell death (cleaved PARP and Annexin V staining), and cell survival by colony formation assay. Total protein malonylation was used as a biomarker for TVB-3664 efficacy. Epigenetic changes were assessed by measuring histone acetylation marks such as acetyl H3K9. DNA repair efficiency was assessed by analyzing ATM/ATR expression, Chk1/Chk2 phosphorylation (Western blot), and BRCA1 or 53BP1 recruitment to vH2AX foci in response to Irinotecan or radiation by immunostaining. For in vivo study, HT29 tumor xenograft in athymic nude mice (6/group) were treated with TVB/Irinotecan combination. Tumor growth and relapse were analyzed by measuring tumor volume weekly.

Results: TVB-3664 treatment of colorectal cancer cell lines increased total protein malonylation, potentiated DNA damage and cell death, and significantly decreased cell survival synergistically with Irinotecan. TVB-3664 treatment also increased histone acetylation but reduced the efficiency of DNA repair by decreasing ATM levels and reducing Chk1/Chk2 phosphorylation. TVB-3664 further blocked BRCA1 or 53BP1 recruitment to vH2AX foci as induced by Irinotecan or radiation treatment. Mechanistically TVB-3664 exerts its effect through ACLY activation, as ACLY knockdown cells were resistant to TVB-3664 induced DNA damage. In accordance with previous studies, TVB-3664 modestly decreased tumor xenograft growth but significantly delayed tumor relapse in combination group after treatment withdrawal.

Conclusion: TVB-3664 potentiates the effect of chemotherapy-induced DNA damage in colorectal cancer by reducing the efficacy of DNA repair through an epigenetic mechanism.

Abstract 40

Translational

M1 Macrophage Engineered Vesicles as Ovarian Cancer Treatment in a Mouse Xenograft Model

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Background: Ovarian cancer is the deadliest gynecologic cancer in women in the United States. The tumor microenvironment, which includes tumor-associated macrophages, plays an important role in cancer progression and resistance to treatment. Two major macrophage phenotypes include pro-inflammatory M1 macrophages and anti-inflammatory M2 macrophages. The ovarian cancer tumor microenvironment contains a predominance of M2 macrophages, which contributes to its lack of response to immunotherapy. Extracellular vesicles are cell membrane derived structures that are endogenously released by cells or engineered from various cell types. Prior studies have shown that M2 macrophages can be converted to a pro-inflammatory M1 phenotype via M1 macrophage derived engineered vesicles (MEVs) and that these M1 MEVs have therapeutic potential alone and potential to serve as a nanoparticle drug delivery vehicle.

Methods: CAOV-3 luciferase expressing ovarian cancer cells were cultured and injected into BALB/c scid mice acquired from the Jackson Laboratory. Bioluminescence imaging performed using a Spectral Instruments Imaging Lago X in vivo imager was used to track progression of tumor in the mouse xenograft models. RAW 264.7 cells, a mouse macrophage cell line, were cultured and stimulated with IFN-Ɣ and LPS to the M1 phenotype. Nitrogen cavitation was used to generate MEVs, which were then isolated by a series of centrifugation steps. Mass spectrometry was used to quantify the amount of cisplatin encapsulated in the cisplatin loaded MEVs. MEV preparations were characterized with a nanoparticle tracking assay (NanoSight NS300). CAOV-3 luciferase expressing mice with tumor uptake were randomized to four treatment groups. Control mice received DPBS (vehicle) and free cisplatin mice received doses of cisplatin equivalent to cisplatin loaded MEVS. Empty MEVs and cisplatin MEVs mice received matched particle number. All mice received assigned treatment via weekly intraperitoneal injections. Mice were followed with bioluminescence imaging, weights, and assessments for adverse events. Statistical test including Mann-Whitney tests and ANOVA were performed using GraphPad Prism.

Results: M1 MEVs are similar in size to standard exosomes; cisplatin MEVs mean particle size is 162.75 +/- 17.55 nm (n=12) and empty MEV mean size is 155.84 +/- 7.13 nm (n=12), which are not significantly different (p=0.37). MEV preparations yielded consistent particle numbers; cisplatin MEV preparations yielded a mean of 2.59x1012 +/- 3.56x1011 particles/ml (n=12) and empty MEV preparations yielded a mean of 4.11x1012 +/- 7.58x1011 particles/ml (n=12), which are significantly different (p<0.0001). M1 MEVs were consistently loaded with cisplatin, mean 394.28 +/- 317 µg/mL (n=6). Both cisplatin loaded and empty vesicles demonstrate potential treatment efficacy by luminescence signal when compared with control. Mouse treatments are ongoing. After six weeks of treatment, mice in all four groups showed minimal toxicity and adverse events. Mean weights after six weeks of treatment include free cisplatin group 22.98 g, control group 24.26 g, empty MEV 25.91 g, and cisplatin MEV 24.63 g. The mean weights of the four groups are not the same (p=0.0017). No group of mice has met criteria for humane endpoints.

Conclusion: M1 MEVs are a therapeutic strategy and novel nanoparticle drug delivery vehicle that can be generated with consistent numbers, size, and concentrations. This experiment is ongoing.

Abstract 41

Translational

Engineered M1 Macrophages for Targeted Delivery of Cisplatin Drug and Its Localization for the Treatment of Osteosarcoma: An In Vitro and In Vivo Study

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Introduction: Osteosarcoma (OS) is the most common primary bone malignancy that affects children and young adults. In the U.S., it is anticipated that 3,970 new cases will be diagnosed with 2,140 deaths in 2023. Treatment for the disease includes surgery and neoadjuvant chemotherapy including methotrexate, doxorubicin, and cisplatin.

Rationale: Lung metastasis is the most significant prognostic marker in OS and can only be eliminated with a chemotherapeutic drug like cisplatin but because it is highly toxic, its usage is limited. Therefore, a strategy to deliver these drugs with minimum toxicity would be clinically useful. The tumor microenvironment has tumor-associated macrophages (TAM) whereas M1 macrophages show anti-inflammatory properties. M1 macrophage-based engineered vesicles to target cancer cells and TAM could be an advanced and alternative approach to deliver cisplatin to the targeted cancer cells.

Materials and Methods: Human PBMCs were isolated from blood and stimulated to M1 macrophages and were used to prepare the empty and the cisplatin-loaded vesicles (E-MEVs and C-MEVs) using N2 cavitation. The characterization of the vesicles was performed using electron microscopy (EM). These vesicles were used to check their effect on HOS, 143B, and HEK cells. The dose response and DNA damage using ɣH2AX detection were examined on all treated cells to monitor the efficiency of various drugs. The OS humanized mice model was established using nude mice and was injected with 0.25 million luciferases 143B cells into each tibia of the mice and was given the treatment of different vesicles in order to study their localization.

Results: The EM of the vesicles showed a round morphology with a size range of 150-200nm. The mean IC50 value for free cisplatin and C-MEVs of 4.033±0.35 and 1.492±1.13μM was found on HOS cells and in the case of 143B cells, 3.741±0.5 and 1.198±1.0 μM was found for 143B cells, respectively. HEK cells showed mean IC50 values of 2.514±2.1 and 1.570±1.3 for free cisplatin and C-MEVs, respectively. The DNA damage was also found to be equally induced by the free cisplatin and C-MEVs for both 143B and HOS cells despite the high IC50 value of free cisplatin as compared to C-MEVs. The orthotropic OS tumor model was developed in nude mice and the development of the primary tumor was observed by two weeks post-injection, followed by the development of lung metastasis by four weeks and liver metastasis was found to be developed by six weeks post-injection. The mice survived till seven weeks post-injection as they reached their tolerable tumor burden. The vesicle treatment was started after four weeks of tumor cells injection and the localization of the vesicles was found to be in the primary tumor and the liver of the mouse, these results suggest our next step to analyze the efficacy of E-MEVs and C-MEVs in the mouse model.

Discussion: Cisplatin is the most versatile drug used to treat cancer patients but is toxic at higher doses. Our results had shown the efficacy of cisplatin-loaded vesicles on OS cells inducing equal DNA damage despite low IC50 values as compared to free cisplatin. Development of a mouse model also suggests promising targeted delivery of engineered vesicles into the tumor of the mouse.

Abstract 42

Translational

Identification of Target Protein Molecules in M1 Macrophage-Based Vesicles Responsible for M2 to M1 Repolarization

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Introduction: Macrophages (MØ) are dynamic immune cells that exist in different phenotypic states such as M1 (pro-inflammatory) and M2 (anti-inflammatory). The M1Ø are known to protect against various infections and inhibit the development of tumor growth, whereas the M2Ø induce tumor growth and metastasis.

Rationale: The tumor microenvironment (TME) consists of both M1Ø and tumor-associated macrophages (TAM), which have an M2Ø-like phenotype. Therefore, it is of utmost importance to identify various proteins and their associated complexes that signal the repolarization from M2 to M1 Ø. We have engineered M1 macrophages to become M1 vesicles which can easily deliver drugs inside the cell and can show repolarization efficiency.

Materials and Methods: Human blood monocytes were isolated from the fresh granulocyte blood and stimulated with macrophage colony-stimulated factor (MCSF) to induce M0 Ø. M0 Ø were further stimulated with LPS and IFN-gamma to make M1Ø and with IL-4 and IL-13 for making M2Ø. These M1 and M2 Ø were dissociated using N2 cavitation with a cold pressurized chamber and further processed with an ultracentrifugation process to collect the vesicles. The collected vesicles were lysed with RIPA buffer and run on SDS-PAGE gel. The protein bands on SDS-PAGE were subjected to proteomics analysis by LC-MS to identify the proteins present on the vesicles.

Results: Proteomic results were analyzed using Proteome Discoverer 2.4 and were searched using the human protein database from UniProt. A total of 3,777 proteins were identified from M1 and M2 vesicles and were filtered based on at least two peptides matched with the database and which had at least three protein spectrum matched (PSM). These filtered proteins were quantified based on the abundance in each sample and a total of 30 and 19 proteins were identified only in M1 and M2 vesicles, respectively. The 30 proteins that were only found in M1 vesicles were associated with two major complexes: Interferon gamma signaling, and NF-kappa beta signaling pathway when studied using the online String database. A total number of 690 proteins were found to be higher in M1 than in M2 vesicles. The number of abundant proteins in M1 vesicles was further filtered out on the basis of at least a 10-fold ratio difference of M1 vs M2 and a total of 44 and 19 were found in M1 and M2 respectively. The 44 proteins found in M1 vesicles consisted of three major complexes; 1) Type 1 interferon gamma signaling, 2) death-inducing signaling complex assembly, 3) negative regulation of DNA damage response. These results suggested that the proteins responsible for M2 to M1 repolarization are mainly associated with the interferon-gamma signaling complex and can be further subjected to knockdown studies and immunoprecipitation to identify the specific protein complexes.

Discussion: TME consists of both M2Ø and TAM which are targeted by a number of various immunotherapies, however, not all patients will respond to immunotherapy. Therefore, having a strategy to repolarize these anti-inflammatory M2Ø populations to pro-inflammatory M1Ø is an essential strategy to control tumor and metastatic progression. Our M1Ø based vesicles are the carrier of these proteins and may exert anticancer activity by repolarizing the M2Ø to M1Ø phenotype.

Abstract 43

Population-Based/Behavioral

Lung Cancer Prevention among Appalachian Kentucky Women: A Community-Engaged Mixed Method Study

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Introduction: Residents of Appalachian Kentucky (KY) experience the highest lung cancer rates in the U.S. Although lung cancer has declined steadily among men since the 1990s, such decreases have not been seen among Appalachian women. While high smoking rates may account for some of this high prevalence, 20% of women with lung cancer are lifelong non-smokers. Promoting lung cancer prevention requires understanding of culturally targeted and gender-specific primary and secondary prevention efforts for Appalachian women. This study utilizes concept mapping, a community-engaged mixed method, to: 1) uncover the range of perceived barriers and facilitators to lung cancer prevention and 2) identify community-specific intervention ideas among Appalachian KY women.

Methods: We recruited 71 Appalachian KY women to participate in concept mapping activities, including a series of online sorting and rating activities and qualitative group discussions. We used multidimensional scaling to create a point map representing perceived similarities and hierarchical cluster analysis to create a cluster map illustrating thematic categories. We also generated comparisons of average cluster ratings through bivariate comparisons across importance and feasibility. In the qualitative discussions, we shared the generated maps and comparisons with participants to obtain insights on potential lung cancer prevention interventions.

Results: Participants listed 70 perceived barriers and facilitators across individual, interpersonal, community, and environmental levels, which grouped into 8 thematic areas, including: 1) Community Programs and Resources; 2) Availability and Access to Healthcare; 3) Barriers to Seeking Healthcare; 4) Health Conditions and Genetics; 5) Community Influences and Social Norms; 6) Smoking and Tobacco; 7) Physical Environment; and 8) Environmental Concerns and Pollution. Participant ratings indicated two potential intervention areas: community exposures and policies (e.g., public smoking/second-hand smoke, education programs on lung health for women) and healthcare access. Environmental factors, comorbidities, and complex cultural beliefs/social norms women experience were seen as less feasible intervention targets.

Conclusions: Participants suggested multilevel or multicomponent interventions for lung cancer prevention, including efforts to improve local policy, education, and access to screening for women in Appalachian KY. Overall, this research contributes novel understanding of local barriers, gender-specific risk factors, and community-driven intervention ideas among Appalachian KY women and provides a platform for future studies to understand gender-related lung cancer prevention needs throughout the heavily affected Appalachian region.

Abstract 44

Translational

Increased Incidence of DACH1 Mutation in Appalachian Women with Uterine Cancer and Altered Chemotherapy Sensitivity

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Objective: DACH1 is a novel transcriptional repressor and tumor suppressor gene with an increased prevalence in the Appalachian region of Kentucky. We evaluated data from the Oncology Research Information Exchange Network (ORIEN) to determine the frequency of DACH1 mutations in patients with endometrial cancer and evaluate its impact on RNA expression, clinical correlates and outcomes, and the impact of DACH1 knock-outs on DNA repair capacity and drug sensitivity.

Methods: We obtained clinical and genomic data for 691 patients with endometrial cancer from nine U.S. institutions within the ORIEN network with whole exome sequencing (WES) and RNA Sequencing (RNA-Seq) and evaluated the frequency of DACH1 mutations, as well as association with clinical and genomics factors. DACH1 knock-outs were created with CRISPR and assessed for impact on non-homologous end joining (NHEJ), drug sensitivity, synergy, and DNA damage.

Results: Appalachian women with endometrial cancer had an increased frequency of DACH1 mutations (6/41 patients, 14.6%) compared to the non-Appalachian population (24/581 patients, 4.1%) with p-value = 0.010, consistent with the rate of DACH1 gene mutation in TCGA at 3.8%. DACH1 mutated patients have a higher tumor mutation burden compared to DACH1 wild-type (32.2 vs. 4.62, p-value = 2.17E-10) though no difference in microsatellite instability between DACH1 mutated and wild-type was present (p-value = 0.35). DACH1 mutations showed significant gene co-occurrence patterns with POLE, MLH1, MSH2, MSH6, and PMS2. DACH1 knock-outs were deficient in NHEJ and were more sensitive to the combination of an ATR inhibitor and cisplatin.

Conclusions: Precious analysis using the TCGA PanCancer Atlas and the current study using the ORIEN multi-institution cohort demonstrate that DACH1 mutations are prevalent in Kentucky patients with endometrial cancer, particularly those from the Appalachian region. In addition, DACH1 mutations are associated with high tumor mutational burden and co-occur with genome-destabilizing gene mutations. These findings suggest that DACH1 is a candidate biomarker for future immunotherapy trials and support further evaluation of cisplatin with an ATR inhibitor for treating DACH1 mutated gynecological cancers.

Abstract 45

Translational

Characterization of TP53 T253I as Likely Pathogenic in a Patient with Adrenal Cortical Carcinoma

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Recently an 8-month-old patient was administered at the Kentucky's DanceBlue Kentucky Children's Hospital Hematology/Oncology Clinic presenting with an Adrenal Cortical Carcinoma (ACC). This type of cancer, when seen in patients this young, is most commonly associated with Li-Fraumeni Syndrome (LFS). About 70% of patients with LFS have a familial mutation in the TP53 gene. A recent initiative known as the Project Inherited Cancer Risk (PICR) was launched at UK to provide screening opportunities to newly administered patients at the clinic, and upon obtaining consent, genetic screening of the patient revealed a heterozygotic p53 mutation identified as p53 T253I. This mutation has been previously reported on ClinVar, an NIH repository for genomic variations and their relationship with human health, as variant of uncertain significance (VUS), and as such has not been positively associated with LFS. Given that, we sought to assay the functional consequences of p53 T253I. We stably transduced p53 CRISPR deleted HEK293 cells with p53 WT or p53 T253I and gauged their ability to complement the p53 deficiency. Whereas WT-p53 cells showed full complementation of p53 functions such as binding to p53 responsive elements, DNA damage dependent replication arrest and apoptotic signaling, p53 T253I was unable to fully complement these cells, suggesting that it is a dysfunctional, pathogenic variant of p53.

Abstract 46

Population-Based/Behavioral

Outcomes of the Markey STRONG Scholars Program in Increasing Diversity in Cancer Research and Health Science Careers

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Participation in research is a widely used tactic for mitigating the challenge of increasing the representation and persistence of minoritized groups in the health sciences. Yet, few undergraduate research training paradigms have programming that collectively address the dramatic inequities in education, opportunity, and proactive mentorship experienced by these students. The Markey Science Training in Research, Oncology, Networking and professional Growth (STRONG) Scholars Program aims to increase the number of individuals who are from underrepresented and/or underserved backgrounds in cancer research through use of inclusive and equitable training practices, as well as boost its participants' scholarly confidence and promote their persistence in their chosen health science careers. Specifically, this summer program provides minoritized undergraduate students with research and clinical experiences, cancer education, personalized mentoring, outreach opportunities, social activities, and personal and professional development. Pre- and post-program surveys and focus groups were developed and employed to assess program outcomes and identify ways to maximize participant interest in cancer and persistence in health science careers. Analysis of mixed methods evaluation data demonstrates that Markey STRONG Scholars Program participation resulted in students' increased understanding of cancer and the scientific process along with pronounced interest in research. Additionally, program participation enhanced students' health science professional identity and sense of belonging to the health science community.

Abstract 47

Biostatistics/Bioinformatics

Mosaic Chromosomal Alterations in Blood Across Ancestries via Whole-Genome Sequencing

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Mosaic mutations in blood are common with increasing age and are prognostic markers for cancer, cardiovascular dysfunction, and other diseases. This group of acquired mutations include megabase-scale mosaic chromosomal alterations (mCAs). These large mutations have mainly been surveyed using SNP array data from individuals of European (EA) or Japanese genetic ancestry. To gain a better understanding of mCA rates and associated risk factors in genetically diverse populations, we surveyed whole genome sequencing data from 67,390 individuals, including 20,132 individuals of African ancestry (AA), and 7,608 of Hispanic ancestry (HA) with deep (30X) whole genome sequencing data from the NHLBI Trans Omics for Precision Medicine (TOPMed) program. We adapted an existing mCA calling algorithm for application to WGS data, and observed higher sensitivity with WGS data, compared with array-based data, in uncovering mCAs at low mutant cell fractions. As in previous reports, we observed a strong association with age and a non-uniform distribution of mCAs across the genome. The presence of autosomal (but not chromosome X) mCAs was associated with an increased risk of both lymphoid and myeloid malignancies. After adjusting for age, we found that individuals of European ancestry have the highest rates of autosomal mCAs, mirroring the higher rate of leukemia in this group. Our analysis also uncovered higher rates of chromosome X mCAs in AA and HA compared to EA, again after adjusting for age. Germline variants in ATM and MPL showed strong associations with mCAs in cis, including ancestry specific variants. Rare variant gene-burden analysis confirmed the association of putatively protein altering variants in ATM and MPL with mCAs in cis. Individual rare variants in DCPS, ADM17, PPP1R16B, and TET2 were all associated with autosomal mCAs and rare variants in OR4C16 were associated with chromosome X mCAs in females. There was significant enrichment of co-occurrence of CHIP mutations and mCAs both altering cancer associated genes TET2, DNMT3A, JAK2, CUX1, and TP53. Overall, our study demonstrates that rates of mCAs differ across populations and that rare inherited germline variants are strongly associated with mCAs across genetically diverse populations. These results strongly motivate further studies of mCAs in under-represented populations to better understand the causes and consequences of this class of somatic variation.

Abstract 48

Informatics/IT

ImagePath: A User-Friendly Tool for Whole Slide Imaging Annotation and Data Management

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Over the past century, microscopic observation of tissue samples has been a crucial tool for quantifying biomarkers and diagnosing diseases. With the advancements in biomedical and Artificial Intelligence (AI) technologies, Whole Slide Imaging (WSI), a high-resolution digital microscopic image of a biopsy, has been approved by the FDA for diagnostic use in the quantification of breast cancer markers and actively used in Computational Pathology (CPATH). The combination of WSI with advanced Next Generation Sequencing (NGS) technology and AI has opened up new avenues for translational medicine and clinical practice, enabling the identification of promising features for disease diagnosis, disease prognosis prediction, and treatment decisions.

However, the annotation, search, and sharing of WSI data is a significant challenge due to proprietary image formats, large file sizes, and the absence of a supporting information retrieval system. Although there exist open-source applications, they are often too complicated for researchers and clinicians with limited programming knowledge and difficulty setting up system environments.

Therefore, we introduce ImagePath, a cloud-based integrated annotation tool and database for WSI. ImagePath provides a user-friendly graphical user interface (GUI) for WSI annotations and a database management system that can securely track and store data via HTTP protocols, supporting all known WSI file formats. Through this poster, we will introduce the system design, functions, and potential applications of ImagePath, addressing the needs of researchers and clinicians seeking a simplified approach to managing and analyzing WSI data.

Abstract 49

Basic Science

PLK1 Promotes Melanoma Progression via Phosphorylating BACH1

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PLK1, a critical cell cycle regulator, is associated with cancer progression and negatively correlates with patient survival in cutaneous melanoma based on clinical database analysis. In a melanoma mouse model induced by BRafCA mutation and Pten-deficiency, we observed that PLK1 overexpression markedly accelerated tumor growth, promoted metastasis, mediated metabolic reprogramming, and shortened mice survival. Mechanistically, PLK1 phosphorylates and stabilizes BACH1, which serves as a crucial transcription factor for genes involved in metabolism and metastasis. Moreover, the PLK1/BACH1 axis renders the resistance to Vemurafenib, a BRAFV600E inhibitor, in melanoma. In light of this, we attempted an innovative pharmacological combination targeting both BRAFV600E and PLK1, identifying the synergistic efficiency of this approach to suppress tumor growth. Overall, we have discovered a novel function of PLK1 that is independent of the cell cycle, which could pave new ways for melanoma therapies.

Abstract 50

Basic Science

Set1 Targets Genes with Identity and Tumor-Suppressing Functions in Planarian Stem Cells

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Regeneration and tumorigenesis share common cellular processes e.g., proliferation and migration, and common molecular pathways e.g., Wnt and NF-κB signaling. Nevertheless, the process of regeneration is strictly controlled, whereas malignant transformation is unrestrained (Charni et al., 2017). Planarian flatworms are the master of regeneration and the key to the regenerative ability of these creatures are neoblasts, a heterogeneous population of multi and pluripotent stem cells distributed throughout the organism (Newmark and Sanchez Alvarado, 2002). However, despite the presence of this large pool of proliferating stem cells, planarians do not generally form tumors, even after experiencing genotoxic stress that causes DNA damage. Planarian stem cells therefore share similarities with cancer stem cells, including the ability to withstand high doses of radiation and recover their stem cell population after radiation exposure. I am interested in understanding the molecular mechanisms that regulate these remarkable functions of planarian stem cells. It is known that epigenetics play an important role in carcinogenesis and that having abnormal epigenetic patterns at tumor suppressor gene loci or oncogenes can lead to carcinogenesis. For example, a study by Chen et al. (Nature Genetic 2015), showed that broad peaks of histone H3 lysine 4 trimethylation (H3K4me3) mark both cell identity genes and tumor-suppressor genes in human cells and that this broad signature is lost in cancer cells. H3K4 methyltransferases are highly conserved from yeast to humans, allowing us to model this phenomenon in planarians. We previously showed that planarian Set1 is responsible for creating broad peaks at the loci of tumor suppressor gene homologs in the planarian species *Schmidtea mediterranea* (Verma et al., 2021). Further, RNAi of set1 resulted in hyperproliferation and abnormal DNA damage response after 2GY radiation, which suggests loss of tumor suppressor gene function. Specifically, many stem cells in set1 RNAi animals failed to arrest their progression through the cell cycle after 2GY ionizing radiation, but this abnormal response was variable across the population. This suggested that the loss of Set1 and its H3K4me3 activity is inducing increased transcriptional and functional heterogeneity in the planarian stem cell population. To test this hypothesis, we performed a single-cell RNA sequencing (scRNA-seq) experiment using cells from dissociated set1-RNAi and control planarian animals. Importantly, data from this experiment supports my immunofluorescence data (H3S10ph staining) that showed an increase in the number of mitotic stem cells in set1 RNAi as compared to control worms. Additionally, preliminary analysis of the scRNA-seq data supports my hypothesis that loss of Set1 leads to increased transcriptional heterogeneity in the stem cell population, as shown by sub-clustering analysis. I also find significant differences in particular differentiated cell type clusters, including neuronal cell types. Overall, my results support my hypothesis that Set1 activity is an important regulator of stem cell heterogeneity and function.

Abstract 51

Basic Science

PTPRF Negatively Regulates EGFR Signaling to Inhibit Cell Migration in Colon Cancer

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UK, College of Medicine, Molecular and Cellular Biochemistry

The spaciotemporal control of cell signaling requires a balancing act of protein kinases and phosphatases. Hyperactivation of signaling downstream of receptor tyrosine kinases (RTKs) is one of the most common mechanisms leading to oncogenic transformation in numerous cancer types. Although the activation process of RTKs has been extensively studied, the inactivation mechanisms mediated by tyrosine phosphatase are less understood. Previously, we have determined the molecular mechanisms by which protein tyrosine phosphatase receptor type F (PTPRF) regulates the Wnt pathway. In this study, we investigated the functional importance of PTPRF in controlling EGFR signaling in colon cancer. Deletion of PTPRF using CRISPR/cas9 in 293T cells led to increased phosphorylation of EGFR and downstream AKT and ERK signaling upon EGF treatment. Similarly, knockdown of PTPRF resulted in an increase in EGFR activation in colon cancer cells. In addition, re-expression of WT, but not phosphatase deficient PTPRF, rescued the phenotype suggesting a phosphatase activity-dependent regulation. Co-Immunoprecipitation experiments indicated that PTPRF interacts with EGFR via its extracellular domain. However, PTPRF-mediated regulation of EGFR phosphorylation had no effect on EGF-induced receptor internalization. Functionally, knockdown of PTPRF promoted cell migration in colon cancer cells. The effect of PTPRF on controlling the specificity of signaling scaffolds downstream of EGFR is being further investigated. Taken together, our study identified PTPRF as an important regulator of EGFR signaling in colon cancer.

Abstract 52

Basic Science

NMR Methods for Determining Lipid Turnover via Stable Isotope Resolved Metabolomics

Penghui Lin[1], Li Dai[2], Daniel Crooks[2], Leonard Neckers[2], Teresa Fan[1,3,4], Andrew Lane[1,3,4]

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Here we report a quick and easy way to estimate the incorporation of 13C into different subunits of complex lipids by NMR using cellular phosphatidylcholine lipids (PCs) as internal standard in stable isotope tracer experiments. The ratios of peak intensities of other species to that of PC methyl groups in both the proton and the HSQC spectrum could be used for enrichment calculation. This method provides a simple tool for generating an overview of 13C incorporation into lipid molecules, which can be utilized as a standalone approach or to complement targeted mass spectrometry-based lipidomics workflows. Further, with the detection limit as low as 2%, it provides very valuable information other techniques cannot easily generate.

Abstract 53

Clinical

A Pilot Randomized Controlled Trial of Smoking Cessation Induction Treatment for Rural, Underserved Cancer Survivors Across the Continuum of Motivation to Quit

William Robert Bowling, Jessica Burris

UK College of Arts and Sciences, Clinical Psychology

The United States' smoking prevalence has decreased substantially, but this public health improvement is unevenly distributed across the population. A promising individual-level approach to cancer control equity is to develop more acceptable and efficacious interventions that are widely disseminated to rural and other disadvantaged cancer survivors. Smoking cessation induction focuses not on long-term abstinence, but on engaging people in the process of making quit attempts and may be ideal for hard-to-reach populations. The aim of this pilot randomized controlled trial was to evaluate the feasibility and acceptability of a smoking cessation induction intervention designed for rural cancer survivors. The treatment group received a free, two-week supply of nicotine replacement therapy and brief advice pertinent to smoking cessation and resources for unmet needs; the control group received no medication. Participants (n=49; 51.0% male) were proactively recruited and procedures were accomplished via mail or phone. Data collection occurred pre-intervention (Day 0) with Days 30 and 60 follow-up. The accrual rate for the primary recruitment source (specifically, cancer registry) was 66.7%. Retention was 75.0% and 72.0% for the treatment and control groups. Across follow-up, the treatment group reported intention and confidence to quit (ps=.24-.55) and instances of 50% smoking reduction (p=.15) that were similar to the control group, though they reported more 24-hour quit attempts (p=.02). Treatment acceptability ratings were favorable; no serious adverse events reported. Future studies should consider alternative community-based recruitment strategies and interventions with greater intensity and more interaction to bolster and sustain early gains in motivation and behavior change.

Abstract 54

Population-Based/Behavioral

Parental Perceptions of Priorities and Features in the Development of an Obesity Risk Reduction Mobile Application

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Introduction: Obesity rates in 2- to 5-year-old children is increasing rapidly in recent decades with a higher prevalence among low socioeconomic status and minority groups. Childhood obesity increases risk of many chronic conditions at a young age, causing a need for accessible interventions for child health and wellness. The purpose of this study was to explore the needs and desires of parents of 2- to 5-year-old children for a health-based mobile app designed to reduce childhood obesity by utilizing a novel mixed method approach.

Methods: We conducted qualitative interviews of primary caregivers of a 2- to 5-year-old children who use a smartphone/mobile phone and smartphone applications to ask about parenting practices, desired areas of improvement, smartphone use, and app design/feature preferences. Additionally, participants completed online concept mapping based on the health and wellness priorities of their child and what app features would help uphold these priorities. Participants then grouped items into thematically similar piles and then rated each item on a five-point scale of importance.

Results: The themes of the interviews fell into two categories: 1) parental priorities and 2) application features. For parental priorities, participants desired features to capture aspects of overall health and wellbeing, to set boundaries and encourage routine behaviors, and to focus on a variety of nutrition and healthy eating features. Participants highly rated features such as healthy recipes, goal tracking, and notifications/tips to improve behaviors. The study team created the corresponding characteristics into the designed app, which will be tested for efficacy in Head Start programs across Kentucky.

Conclusion: This mixed-methods study demonstrates parents' desire to take control of their child's health and eating habits, and the potential usefulness of a goal-tracking app to assist in these priorities. This supports the development of a childhood obesity reduction application. The CHEW (Children Eating Well) mobile application was designed with the features parents highlighted in mind.

Abstract 55

Basic Science

CRISPR/Cas9 Genome Editing Delivered by MNP-BV System

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Introduction: CRISPR/Cas9 technology has great potential for cancer therapy due to its ability to selectively target and edit genomic DNAs. CRISPR/Cas9 can correct or disrupt genes that play a role in tumor growth or drug resistance, as well as modify immune cells to better target and destroy cancer cells. However, the lack of a reliable delivery method hurdles the clinical translation of in vivo genome editing. Viral vectors, such as lentivirus and adeno-associated viruses, lipid nanoparticles, and hydrodynamic injection are the most common methods for in vivo gene delivery. However, low delivery efficiency and the risk of virus gene integration limit the application of these methods in CRISPR/Cas9 delivery. Here we develop magnetic nanoparticle-conjugated baculoviral vectors for site-specific delivery of CRISPR/Cas9 into the targeted tumor cells.

Materials and Methods: Magnetite nanocrystals were synthesized through thermodecomposition of Fe(acac)3 in a mixture of benzyl ether, oleic acid, and oleylamine. As-synthesized nanocrystals were coated with DSPE-PEG2000-methoxy and DSPE-PEG2000-maleimide using a dual solvent exchange method. To conjugate peptides to the surfaces of MNP, freshly coated MNPs were mixed with cys-TAT (CGYGRKKRRQRRR) peptides in 0.2×PBS and incubated overnight. Unconjugated peptides were removed by ultracentrifugation. Conjugated MNPs were characterized by TEM, DLS, and gel shift assays. BV-eGFP, BV-PDL1VB1, and BV-CD47sg4 vectors were constructed by the Bac-to-Bac baculovirus expression system and extracted by the ZR BAC DNA Miniprep Kit. The recombinant bacmids were transduced into SF-9 cells using Cellfectin II. BV of passage 3 was used in the transfection studies.

Results and Discussion: The magnetite nanocrystals we synthesized were 15 nm in diameter. When DSPE-PEG-coated magnetic nanoparticles (MNP-PEG) were conjugated with TAT peptide, the positively charged peptide could facilitate the interaction between MNPs and baculovirus vectors. We further confirmed BV transduction efficiency in MC38 and KPC tumor cells was significantly enhanced when mixed with MNP-TAT. Next, we selected sgRNAs to target PDL1 and CD47, respectively, on MC38 and KPC cell lines by T7E1 assay. We found that VB1 sgRNA knocked out nearly 58.9% of PDL1 in MC38 and 50.6% in KPC, while sg4 sgRNA knocked out nearly 10.2% of CD47 in MC38 and 27.9% in KPC. In addition, it was shown that 11.8% of MC38 infected with BV-VB1 lost PDL1 expression and 12.3% of MC38 infected with BV-sg4 lost CD47 expression. Interestingly, both BV-infected MC38 and KPC grew slower when compared with the uninfected cells. Furthermore, in vitro studies showed 28.9% and 29.5% of cell cytotoxicity when splenocytes cocultured with BV-VB1 and BV-sg4 infected KPC cells. Meanwhile, it showed 11.0% and 19.8% of cell cytotoxicity when splenocytes cocultured with BV-VB1 and BV-sg4 infected MC38 cells. Previous studies showed that BV could activate dendritic cells and induce non-specific NK cell and T cell immune responses and be used as an effective immune adjuvant. In the future, we will further determine the therapeutic efficacy of BV-VB1 and BV-sg4 in vivo.

Conclusions: This study provides an efficient and reliable method for CRISPR-Cas9 delivery into tumor cells, which can potentially facilitate the development of drug discovery and genome engineering in cancer and diseases caused by single-base mutations.

Abstract 56

Biostatistics/Bioinformatics

An Effective Statistical Framework for Establishing Multivariate Reference Regions to Aid in the Diagnosis of Cancer

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In clinical chemistry and laboratory medicine, a reference interval is a range of values that clinicians use to interpret a patient's test results. Typically, they are based on the results that are seen in 95% of the healthy population as determined through previous analyses of a reference sample. While they are considered the most widely used medical decision-making tool, direct construction of reference intervals in the multivariate context suffers from certain practical deficiencies. For example, in diagnosing cancer, it is to be expected that assessment of more than one tumor marker will be necessary, which would require a multivariate reference region. However, individual reference intervals will fail to take into consideration the correlation between those markers. Moreover, such a reference region for multiple tumor markers may vary between populations, detection systems, and the methods used to obtain their values. This work provides a novel statistical framework to address these shortcomings by developing multivariate hyperrectangular nonparametric tolerance regions for setting the reference regions. The approach utilizes the notion of statistical data depth to provide an ordering of the multivariate data, followed by determining which points to trim. Then, the extremes of the trimmed dataset are used as the faces of the hyperrectangular region, which produces easily interpretable reference intervals for each marker. It is also straightforward to calculate such hyperrectangular regions when having to adjust for covariates in a model-based framework. Excellent performance of this approach is demonstrated with respect to meaningful statistical criteria. We further demonstrate our procedure's efficacy in the construction of a reference region on cancer and carbohydrate antigens to aid in the diagnosis of pancreatic cancer. Comparisons will be made with the reference limits currently used for the antigens in our analysis.

Abstract 57

Translational

Sensitizing Artesunate Anti-Cancer Activity in Non-Small Cell Lung Cancer via Glutaminase Inhibition

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Background: Lung cancer is a global leading cause of cancer related deaths. Artesunate (ART) is an anti-malaria treatment with demonstrated in vitro anti-cancer activity in several cancer types. Despite promising in vitro activity, genomic mutations such as loss of function Kelch-like ECH-associated protein 1 (KEAP1), an anti-oxidative stress response protein, in non-small cell lung cancer (NSCLC) still confers resistance to ART. We previously demonstrated that activity of ART resulted in oxidative stress, cell cycle arrest, and DNA damage. Therefore, we hypothesize that combining ART with drugs targeting oxidative stress, cell cycle arrest, or DNA damage can sensitize ART anti-cancer activity in NSCLC.

Objective: Identify compounds that sensitizes NSCLC cell lines to ART activity and elucidate the mechanism of action of drug combinations.

Methods: A549 and H1299 lung cancer cell lines were utilized as cell models. Cells were seeded in 96 well plates and allowed to adhere for 24 hours. Media were removed and compounds added after 24 hours. Cell viability was assessed after 72 hours of treatment with CellTiter-Glo 2.0 and analyzed on Varioskan microplate reader. Cell viability was normalized to control and dose response analyzed in GraphPad Prism (v5.01). Synergy assays were conducted using a 6 by 6 checkerboard method normalized to control and coupled with ZIP synergy score. ZIP synergy scores were analyzed with synergyfinder (v3.0.13) package in R statistical software (v4.1.1). Protein expression wa evaluated with western blots.

Results: Telaglenastat (CB-839), a glutaminase inhibitor, was one of the compounds with statistically significant synergy with ART with mean ZIP synergy scores of 4.22 ($p=0.02$) and 17.8 ($p<0.0001$) in A549 and H1299, respectively. Next, we assessed if ART anti-cancer activity is sensitized with 3 different concentrations of CB-839 (5, 2.5, and 1.25uM). The mean ART IC50 combined with 2.5uM CB-839 was 4.79uM (95% CI 3.95-5.81) and 0.251uM (95% CI 0.188-0.335) in A549 and H1299, respectively, and were statistically significantly different ($p<0.001$) compared to mean single agent ART IC50 of 15.8uM (95% CI 13.5-18.6) and 2.23uM (95% CI 1.73-2.86), in A549 and H1299, respectively. Mechanisms of ART and CB-839 were demonstrated in previous literature to have associations to the oxidative stress response pathway. Therefore, we assessed whether KEAP1 expression was associated with response to treatments. Cell lines were treated with DMSO control, ART 10uM, CB-839 2.5uM, or ART 10uM plus CB-839 2.5uM, and lysates were collected for western blot. In both cell lines, single agent ART decreased KEAP1 expression compared to control but single agent CB-839 did not result in decreased KEAP1 expression compared to control. ART and CB-839 combination compared to single agent ART demonstrated decreased KEAP1 expression in H1299 but not A549 cell lines.

Conclusion: NSCLC cell lines were sensitized to ART anti-cancer activity when combined with CB-839. Decrease in KEAP1 may be a result of oxidative stress. Therefore, proteomics studies will be conducted to further elucidate mechanism.

Abstract 58

Basic Science

Targeting Spermine Synthase Triggers Lipid Metabolism Reprogramming as a New Therapeutic Option to Combat Colorectal Cancer

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Dysregulation of polyamine metabolism has been linked to the development of colorectal cancer (CRC). Our recent work demonstrates that spermine synthase (SMS), a polyamine biosynthetic enzyme that converts spermidine to spermine, is overexpressed in CRC, which is required for balancing cellular spermidine levels to facilitate CRC tumorigenesis (Nat Commun 11:3243, 2020). Our findings reveal SMS as an attractive therapeutic target in CRC; yet genetic depletion of SMS expression only shows a limited antitumor effect. Using unbiased metabolomics and transcriptomics analyses, we identified a lipid metabolism reprogramming as among the most impacted metabolic change by SMS depletion in CRC cells. Specifically, SMS depletion significantly altered long-chain fatty acid, triacylglycerol and phospholipid metabolism. Furthermore, targeted inhibition of SMS significantly increased the number of lipid droplets and the levels of long-chain fatty acid acylcarnitines for oxidative phosphorylation in mitochondria, and upregulated expression of genes associated with increased mobilization of polyunsaturated fatty acids and the genes associated with lipid peroxidation for induction of ferroptosis, an iron-dependent form of nonapoptotic cell death. The glutathione peroxidase 4 (GPX4) is a key negative regulator of ferroptosis by neutralizing lipid peroxides. Notably, pharmacological inhibition of GPX4 or its upstream regulator system xc- in combination with genetic depletion or pharmacologic inhibition of SMS synergistically caused lipid peroxidation leading to ferroptosis induction and marked suppression of CRC cell growth. Collectively, these findings highlight lipid metabolism reprogramming as an adaptive response to targeted inhibition of SMS to enable CRC cell survival, which represents an Achilles' heel that can be exploited for potential effective therapy for CRC.

Abstract 59

Basic Science

Repurposing PI3K/Akt Inhibitors to Improve Brain Uptake of Anticancer Drugs in Glioblastoma Resection Models

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Background: Our laboratory has shown that drug efflux transporters at the blood-brain barrier are regulated via the PI3K/Akt signaling pathway. We want to repurpose the PI3K inhibitor alpelisib (ALP) and the Akt inhibitor capivasertib (CAP) to downregulate these drug efflux transporters with the goal to increase anticancer drug brain concentrations. This therapeutic strategy holds the potential for translation into the neuro-oncology clinic.

Methods: GL261 Red-FLuc (GL261-RF) and TRP-mCherry-FLuc (TRP-mCF) cells were injected (2.5K cells/µl; 2µl/2min) into 8-week-old female J:NU and 7-week-old female B6(Cg)-Tryc-2J/J mice, respectively. Tumor burden, volume, and invasiveness were assessed with in vivo imaging using IVIS® Spectrum imaging, MRI, and histopathology, respectively. On day 14 post-injection, mice received 5-aminolevulinic acid (200 mg/kg i.p.), and tumors were resected with a 2mm punch biopsy tool using a surgical fluorescence microscope (ex/em: 405/635nm). Drug efflux transporter function of isolated brain capillaries was determined with a functional assay. Cytotoxicity was assessed after 72-hour drug incubation using CyQuant MTT Assays.

Results: IC50 values from MTT assays with ALP were 15.2 and 37.8µM for GL261-RF and TRP-mCF cells, respectively. Median survival of GL261-RF and TRP-mCF mice was 27 d and 24 d, respectively. Tumor resection significantly increased median survival of GL261-RF mice from 27 d to 34 d (p=0.0007). ALP and CAP significantly reduced P-gp and BCRP transport function. Cytotoxicity studies with CAP, resection of TRP-mCF tumors, and in vivo treatment studies in GBM mice are ongoing.

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Abstract 60

Population-Based/Behavioral

The Association Between Obesity, Breast Density, and Cancer Progression in Breast Cancer Patients from Appalachia

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Background: Breast cancer mortality rates in rural Appalachian areas, such as eastern Kentucky, are among the highest in the United States. Epidemiological studies suggest that there is an association between increased breast cancer incidence and BMI>30 in the region. Obesity is also known to be associated with breast cancer progression and poor survival, with a 35%-40% increased risk of breast cancer recurrence and mortality. However, the relationship between obesity, breast density, and cancer progression in the Appalachian region remains to be determined.

Purpose: The aim of this retrospective study is to investigate the association between obesity, breast density, and cancer progression in breast cancer patients from the Appalachian region.

Methods: We utilized data from the University of Kentucky's Markey Cancer Center Research Network (MCCRN) data warehouse for a 20-year period (from 2000-2019). The study has been determined to be exempt from IRB review and consent requirements as it involves the use of existing, de-identified data with minimal risk to patients. We collected data on age at diagnosis, BMI, cancer diagnosis, cancer stage, and treatment follow-up from breast cancer patients from Kentucky Appalachian counties. Mammogram density data is being collected, and we expect to finish data collection and analysis by the end of April. We will use the BIRADS breast density score to estimate breast density from available full film digital mammograms or mammogram reports near the time of cancer diagnosis. The Kaplan-Meier curve and log-rank test will be used to analyze the association between obesity status, breast density, and breast cancer survival rate. The chi-squared test will be used to assess the relationship between breast density and obesity status.

Results: We collected data on age at diagnosis, BMI, cancer diagnosis, cancer stage, and treatment follow-up from 1,478 breast cancer patients. We are in the process of analyzing the breast density data. We plan that all the data collection and analysis will be done in April before the presentation. We anticipate revealing the association between obesity status, breast density, and breast cancer survival rate in the Appalachian population. Our research may potentially provide a novel epidemiological prognostic factor to explain the increased breast cancer mortality rate in the Appalachian region. Understanding the association between obesity, breast density, and cancer progression could have significant clinical relevance in identifying at-risk patients and developing personalized prevention and treatment strategies for breast cancer patients in the region.

Conclusion: This study aims to improve our understanding of the relationship between obesity, breast density, and cancer progression in breast cancer patients from the Appalachia region and to examine its clinical relevance. The results of this research could contribute to the development of more effective prevention and treatment strategies for breast cancer in the region and ultimately improve patient outcomes. The findings could also aid in identifying at-risk populations and implementing targeted screening and prevention programs.

Abstract 61

Population-Based/Behavioral

Racial Disparities in Financial Toxicity, Cost-Related Health Literacy and Healthcare Transitions among Adolescent and Young Adult Cancer Survivors in Kentucky

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Black adolescent and young adult (AYA) cancer survivors (diagnosed between 15 and 39 years of age) are at higher risk for experiencing financial toxicity (FT) and poorer health outcomes as they transition from pediatric/oncology care to adult/primary healthcare settings. The objective of this study is to identify, assess, and examine racial disparities in FT, cost-related health literacy, and health care transitions (HCTs) among AYA cancer survivors in Kentucky. Data collection and analysis is ongoing. To date, surveys from approximately 230 Black and White AYA cancer survivors have been collected through the Kentucky Cancer Registry. Additionally, two key informant interviews were conducted with survivors from a pediatric oncology clinic and analyzed using a case study approach. Case 1: 33-year-old male currently in remission from leukemia; diagnosed in 2015; experienced FT due to loss of assets and subsequent lack of access to proper financial aid. Case 2: 22-year-old non-binary person who currently has relapsed with rhabdomyosarcoma; experienced FT due to costs of cancer care and side effects. Major themes that arose in both interviews were related to FT, mistrust in the healthcare system and providers, cost-related health literacy, and financial navigation. Barriers to successful HCTs resulted in noncompliance with medications and healthcare follow-up after completing cancer treatments. Treatment plans were impacted due to barriers to access, FT, and lack of patient advocacy. Additional findings from quantitative survey data will help further establish racial disparities in outcomes and address barriers to HCTs that could lead to better health/financial outcomes among AYA cancer survivors.

Abstract 62

Basic Science

The Role of HIF-1α in a Tongue Cancer Radioresistance Cell Model

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According to estimations of the International Agency for Research on Cancer, oral carcinomas represent the 12th most common incidence in the U.S. In general, treatment options available for oral cancer patients include combinations of surgery, radiotherapy (RT), and chemotherapy, depending on the stage of the disease, however over 50% of these RT-treated patients are prone to develop recurrence post-treatment. The literature suggests that metabolism remodeling allows tumor cells to survive, grow, and metastasize under nutrient-poor and hypoxic environments, also it seems to endow tumor cells to adapt and escape treatment. Recent in vitro studies indicate that radioresistant Head and Neck Squamous Carcinoma cells (HNSCC), like some breast cancer models, have enhanced glycolysis and decreased oxidative phosphorylation (OXPHOS) once compared to radiosensitive ones. Hypoxia-inducible factor-1α (HIF-1α), a regulator of cellular oxygen sensing and adaptation to hypoxia, plays an essential role in tumor cell survival, growth, and spread. Thus, given the high recurrence and death rates for radioresistant HNSCC patients, it becomes essential to understand the mechanisms involved in the RT-resistance development, to improve radiation treatment for HNSCC patients to prevent tumor recurrence, and improve survival. Using the glucose analog (2-NBDG) and tetramethyl rhodamine ethyl ester (TMRE) to image glucose uptake and mitochondrial membrane potential, respectively, we are applying optical imaging techniques to identify metabolic differences after RT-resistance acquisition, testing radiosensitive and radioresistant HNSCC cell lines under radiation stress with or without Hypoxia-Inducible Factor 1-alpha (HIF-1α) inhibition. At this point, our preliminary results confirm the radiosensitivity differences between the two cell lines, under different radiation concentrations. Rather, the radioresistant cell line RSCC90 shows a higher expression of HIF-1α at baseline conditions (non-irradiated) and after a 2Gy irradiation, comparatively. Those preliminary results suggest the importance of HIF-1α expression/function for the radioresistance differences in this model. Additional experiments must be done to underline the metabolic differences and confirm our hypothesis.

Abstract 63

Population-Based/Behavioral

Recipe Creation for a Mobile Application to Encourage Healthy Food Choices Among Parents of Preschool Age Children

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Introduction: The prevention of obesity and chronic illnesses requires a worldwide shift toward diets that are healthier. Considering that parents have a significant role in influencing their children's food habits, establishing intervention techniques that target families and their practices enhances the potential for achieving positive behavior change among children. This project's objective was to create an array of family-friendly meals with high nutritional value for inclusion in a health-based mobile application designed for parents of preschool aged children to reduce childhood obesity risk.

Methods: We analyzed data from 26 formative qualitative interviews with primary caregivers of 2-to 5-year-old children, to inform development of seasonal, healthy, and easy to create meals for a health-based mobile app. In the creation of the recipe feature, the selection of weekly featured dishes was based on a seasonal food guide. We included conventional seasonal dishes as well as vegan and vegetarian seasonal recipes, recipes without tree nuts or peanuts, and recipes without shellfish or dairy, all of which are searchable by ingredient and include allergy information. In addition, we ensured that the weekly dishes were kid-friendly, simple, and inexpensive. Finally, the nutritional content of each dish was considered.

Results: Recipes were the most sought-after app feature described by interview participants. In our app design, each dish contains the number of servings, the number of calories per serving, and the preparation time. The recipe instructions and tips are easily accessible, including an ingredient list and a nutrition facts label. Users can apply filters for seasonal recipes, dietary preferences, dietary restrictions, and meal type. In addition, a Featured Recipes section highlights two recipes that are prominently displayed at the top of the screen, which rotate each week. To select the featured recipes, we determined four seasonal dishes for each calendar week that accommodate possible filter selections, resulting in approximately 208 seasonal dishes for the entire year. We prioritized featured dishes that can be prepared with children.

Conclusion: Currently, this recipe feature is being incorporated into an efficacy trial for this health-focused mobile app. With this data, we will evaluate the utilization of these recipes, the preferences of participants, and the potential for such an app feature to aid in the prevention of childhood obesity among preschoolers.

Abstract 64

Basic Science

Assessing Potential Interactions Between Epigenetics and Exposure to Environmental Particulates in Lung Cancer in Appalachian KY

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The overarching goal of this research was to determine if interactions between epigenetic changes and inhalation exposure to trace elements can help explain the extremely high incidences of lung cancer in Appalachian KY, which are not explained by smoking alone. Our specific aim was to investigate whether changes in histone methylation and in expression of the genes responsible for these epigenetic modifications are greater in the Appalachian KY than in general KY population and if there is a correlation between these epigenetic changes and concentrations of trace elements in the lung tumor tissues. We obtained 90 frozen samples from lung tumor and non-tumor surrounding tissues from 45 cases with non-small cell lung cancer (23 squamous cell carcinoma and 22 adenocarcinoma) from the Biospecimen Procurement and Translational Pathology Shared Resource Facility (BPTP SRF) at the Markey Cancer Center. Among the cases, 24 were from Appalachian KY and 21 from General KY. All samples were processed to measure global methylation levels at histone methylation marks, H3K4me3 and H3K9me2, and expression of the genes encoding methyltransferases (SETD1A and EHMT2) and demethylases (KDM5A and KDM3A) in tumor and surrounding non-tumor samples. The samples were analyzed for 17 trace-elements using laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS), as well as micro-digestions followed by ICP-MS, which allowed us to work with small tissue samples. The significant differences were detected between adenocarcinoma and squamous cell carcinoma in histone trimethylation at H3K4 and its respective methyltransferase and demethylase. We did not observe any significant differences in distribution of the trace-elements between the two cancer types. Comparisons of Appalachian KY and General KY populations revealed higher concentrations of such potential lung carcinogens as cerium, nickel, and vanadium in males and decrease in histone methylation. Nickel and vanadium are associated with coal mining and coal combustion waste. It has been shown that airborne particulates linked to the mining and transport of coal contain potentially carcinogenic trace elements, and Appalachian KY has been an intensive coal mining area for over two centuries. The data from this project are indicative of the potential interaction between epigenetic changes and inhalation exposure to environmental trace elements in Appalachian KY.

Abstract 65

Basic Science

N-MYC is a Key Regulator of Core Fucosylation in Neuroblastomas

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Neuroblastoma (NB) is the most common extracranial solid tumor in children and accounts for 15% of all childhood deaths. Nearly half of children with high-risk disease have MYCN-amplification, which is associated with 40-50% chance of survival. N-MYC is a key oncogenic transcription factor and metabolic regulator. Glycosylation is the addition of complex carbohydrates to proteins and lipids, and glycosylation patterns are known to be different in adult cancers. Herein, we utilized matrix-assisted laser desorption/ionization mass spectrometry imaging to profile the N-linked glycome of human NB tumors. We identified that core fucosylated glycans are enriched within NB tumors compared with benign controls. Fucosylation is the addition of L-fucose to glycans. Lectin-based analyses demonstrate that core fucosylated glycans are highly expressed and secreted by NB cell lines. These findings led to our central hypothesis that fucosylation may be a critical driver of neuroblastoma progression. We explored whether mediators of de novo fucose production were associated with poor patient outcomes in neuroblastomas using RNA sequencing data from NB tumors. GDP-mannose 4,6-dehydratase (GMDS) is the first enzyme responsible from synthesizing GDP-fucose from glucose and mannose precursors. We found that MYCN-amplified NBs had significantly higher levels of GDP-mannose 4,6-dehydratase (GMDS; 7.44x10-27) than MYCN non-amplified NBs. GMDS knockdown abrogates NB cellular growth, adherence, and migration in vitro. We have also demonstrated that induction and repression of MYCN expression correlates with RNA and protein levels in vitro. Chromatin immunoprecipitation has also demonstrated that N-MYC binding is enhanced in the GMDS promoter region. Taken together, these findings suggest that N-MYC regulates GMDS expression and promotes core fucosylation in NB tumors.

Abstract 66

Population-Based/Behavioral

Feasibility, Acceptability, and Preliminary Outcomes of "Pathways," a Hope-Enhancing Intervention for Patients Undergoing Treatment for Advanced Stage Lung Cancer

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Background: Patients with advanced stage lung cancer suffer high rates of distress and impaired social and role function, yet few interventions have been designed for these patients. Previous research shows patient "hope," a positive psychological construct reflecting goal-directed determination and planning, is associated with decreased distress and better function among patients with advanced stage lung cancer, making it a promising intervention target. The aim of this research was to test the feasibility and acceptability of "Pathways," a brief, hope-based intervention delivered during routine lung cancer care.

Methods: Between September 2020 and July 2022, patients with advanced stage lung cancer were recruited from an academic cancer center to participate in a single-arm pilot of Pathways. Pathways consists of five sessions (two in-person; three phone sessions) in which patients discuss personal values, goals, and goal pathways with a nurse or occupational therapist. Patients completed measures pre- and post-intervention. A priori we defined feasibility as ≥60% of eligible patients enrolling, ≥70% of patients completing three or more sessions, ≥70% completing post-assessments, and mean acceptability ratings ≥7 out of 10 on intervention relevance, helpfulness, and convenience.

Results: Of 98 eligible patients, 53 enrolled (54%); 52 completed baseline (98%); 48 started the intervention (91% initiation); 37 of the 48 who started the intervention completed at least three intervention sessions (77% adherence); and 40 completed post-assessments (77% retention). Adherence and retention rates were higher after excluding non-completion due to death or hospitalization (82% adherence; 83% retention). Participants were on average 61 years old (SD = 10.7); 40% were male and 52% had a high school degree or less. Participants completed an average of 4.2 (SD = 1.36) sessions; 69% completed all 5 sessions. Participants rated Pathways highly in terms of convenience (M = 8.79, SD = 1.17), helpfulness (M = 8.54, SD = 1.55), relevance (M = 8.46, SD = 1.67). Pre-post changes adjusted for declines in physical function suggested moderate to large effects on patients' hope (d = .50) and goal interference (d = -.70) and small, but clinically meaningful effects on potential outcomes of anxiety (d = -.21), depression (d = -.26), demoralization (d = -.32), and social/role function (d = .15).

Conclusion: Pathways, a brief hope-enhancing intervention designed to overcome patient access barriers through delivery at the point of lung cancer care by a range of healthcare providers, is feasible and acceptable. A phase II efficacy trial is needed test the effects of pathways on advanced stage lung cancer patient distress and function.

Abstract 67

Translational

Isolation of Exosomal Immune Checkpoints from Pancreatic Cancer Patient-Derived Organoids

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Introduction: Exosomes are extracellular vesicles produced by tumor cells which promote cancer growth, metastasis, and immune evasion. Our previous studies have shown that innate immune checkpoint PD1 signaling activated oncogenic MET pathways in pancreatic ductal adenocarcinoma (PDAC) cell lines. This work was the foundation for our ongoing phase II investigator-initiated trial treating metastatic PDAC patients with anti-MET (cabozantinib) and anti-PD1 (pembrolizumab) therapy with concurrent drug sensitivity testing in patient-derived organoids (PDOs). We sought to isolate and quantify peritumoral exosomal PD1/PDL1, MET, and related pathway proteins using patient-derived organoids.

Methods: Patient-derived organoids (PDOs) were created from PDAC tumor samples originating from two different clinical trial patients (hPT27 and hPT50), passaged to confluence, and utilized for exosome isolation. Exosome isolation from media was performed using precipitation, followed by size-exclusion chromatography. Reverse-phase protein array (RPPA), a high-throughput state of the art proteomics platform, was used to characterize the exo-PDL1 and associated pathway proteins. Immunohistochemistry (IHC) was performed on hPT27 PDO showing both MET and PD-1 expression. In vitro cell viability assays were performed after 48 h treatment with pembrolizumab (PEM) and cabozantinib (CABO).

Results: PDOs were successfully created from two patient samples using previously validated methods, passaged to confluence for media exosome isolation and drug testing. RPPA of exosomes isolated from the two PDO lines revealed variable levels of exosomal protein between PDO lines. Exo-PDL1 was identified in exosomes from both PDO lines. Analysis of the two PDO lines showed expression of exosomal tumor promoting proteins including PD1, MET, PDL1, CXCR4, EPCAM, and HER2 (Figure 1A). IHC of PDOs showed both PD-1 and MET expression (1B). In vitro cell viability assays for this patient (hPT27), CABO alone had the greatest effect (1C).

Conclusions: This is the first known report of isolation of exosomes from PDOs and detection of peritumoral exo-PDL1 expression. Exo-PDL1 is required for potent immune suppression, so our results suggest a PD1/MET pathway in PDAC cells that may regulate this mechanism. Further studies are needed to explore therapeutic opportunities, some of which will ultimately be assessed in the ongoing investigator-initiated trial.

Abstract 68

Basic Science

Portable Optical Spectroscopic Assay for Non-Destructive Metabolic Characterization in In Vitro Cancer Cell Model

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In vitro immortal cancer cell models and organoids are widely used to study the role of tumor metabolism reprogramming in tumor growth and survival under therapeutics stresses. Although conducting longitudinal metabolic measurements on the same tumor sample during a course of therapy is critical for therapeutic studies, there are surprisingly few techniques that can provide a systems-level view of tumor metabolism on in vitro cells or organoids non-destructively. Several tools, such as Seahorse Assay and Metabolomics, provide standardized metabolic measurements but often require destructive sample preparation. Relying on the non-invasive nature of the optical technique, we aimed to fill the technology gap by providing an optical spectroscopic assay to enable non-destructive metabolic measurements on in vitro cancer models.

We built a portable optical fluorescence spectroscopy platform for non-destructive metabolic measurements. The system was built based on a cost-effective Solis™ white LED (SOLIS-3C, Thorlabs), a compact spectrometer (FLAME-T-VIS-NIR, Ocean Optics), and proper excitation and emission filters. A low-cost fiber probe (BF19Y2HS02, Thorlabs) was used for light delivery and collection. To demonstrate the proof-of-concept of our assay, we conducted optical metabolic characterization on human breast cancer cell lines (MDA-MB-231 vs MCF-7) with different radio-sensitivities. Fluorescent probes including 2-NBDG (glucose uptake) and TMRE (mitochondrial membrane potential) were used to quantify the tumor cell metabolism.

Our optical metabolic imaging using a standard microscope showed that MDA-MB-231 (Warburg) has a higher rate of glucose consumption but lower mitochondrial activities in comparison to MCF-7 (oxidative), which is consistent with published data. Our optical spectroscopic assay captured the same results of the two cell lines with high sensitivity and signal-to-noise ratio. Our optical assay may complement the existing metabolic tools (Seahorse Assay and Metabolomics) in cancer research laboratories, but with the added capability of being used for repeatable non-destructive metabolic characterization on different tumor models.

We demonstrated an optical spectroscopic assay that enables non-destructive metabolic characterizations on in vitro cells, which may provide a new way to enable longitudinal therapeutic studies through the lens of tumor energetics.

Abstract 69

Basic Science

Production of EVs Containing Mitochondria is a Selective Survival Mechanism for Prostate Cancer Upon RT

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Around 50% of prostate cancer (PCa) patients receive radiation treatment (RT) over the course of their treatment. The recurrence of tumors after radiation remains one of the significant challenges in the treatment and eradication of PCa. In addition to induction of DNA damage, ROS production, and cell death, we recently found that RT promotes EV production in PCa cells, at least in part, through H2O2 activation (Miller CE et al., Antioxidants 2022, PMID 36358489). Since we observed EV production upon RT, EVs and their contents could play role(s) in cancer survival after RT. By using Cytoflex flow cytometry coupled with Mitotracker Green for particle counting, we found that 40-50% of RT-derived EVs from prostate cancer cell line PC3 carried mitochondrial components. These RT-derived EVs contained an increased amount of mitochondrial antioxidants, such as Peroxiredoxin 3, Glutathione peroxidase 4, and Manganese superoxide dismutase as well as a mitochondrial transcription factor, TFAM. Significantly, this phenomenon is specific for PC3 cells since we did not observe a significant increase in EV number and mitochondrial contents in EVs of normal prostate epithelial cells PZ and PrEC post 6 Gy RT. The selective release of EVs containing mitochondria upon RT could act as a survival mechanism for PCa. Next, we sorted EVs-containing mitochondria, pre-treated PC3 with the sorted EVs, then radiated the cells. Our preliminary data shows that the uptake of EVs containing mitochondrial contents promotes PC3 survival post RT. Given that TFAM is essential for cell survival and significant levels of TFAM were found in RT-derived EVs, we plan to use PC3 shTFAM cells (TFAM KD) to determine if TFAM is responsible for EV production and its contribution to the survival of recipient cells post-RT.

Abstract 70

Population-Based/Behavioral

Acceptability of Patient Components of mPal, a Multilevel Intervention to Improve Palliative Care Integration into Advanced Lung Cancer Treatment

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Background: Despite guidelines and robust evidence for the benefits of early co-management between oncology and specialty palliative care, most patients with advanced lung cancer do not see a palliative care clinician in ambulatory care, even in academic medical centers with well-established palliative care programs. Whether and how patients discuss unmet palliative care needs (e.g., symptom concerns, illness stressors) with their oncologist is a major driver of palliative care utilization. However, efficacious interventions to improve patient-oncologist discussions of unmet palliative care needs are lacking. To address this problem, we developed mPal, a multilevel intervention (i.e., addresses patient, provider, and system-level factors) to improve palliative care integration into ambulatory lung cancer care. The aim of this research was to test the acceptability of mPal's patient component, a web-based patient application designed to improve patients' understanding of palliative care, identify their palliative care needs, and facilitate discussion of those needs with their oncology team.
Methods: Between April 2021 and July 2021, patients undergoing treatment for advanced stage lung cancer were recruited from Markey Cancer Center. Patients completed a baseline survey of their palliative care knowledge and attitudes, interacted with the mPal application, and then completed repeated measures of palliative care knowledge and attitudes and rated mPal's acceptability, appropriateness, and feasibility. The mPal application consisted of: brief education about palliative care delivered via text and video, a 17-item assessment of their palliative care needs (e.g., pain, worry about the future) and whether they wanted help for those needs, and a 4-item assessment of their interest in discussing palliative care needs and a palliative care referral with their oncology team. mPal was delivered via a tablet provided by study staff.
Results: Twenty patients participated. Participants were on average 60 years old (SD = 9.8); 40% were male; 65% had a high school degree or less; 70% had limited or marginal health literacy. Patients rated the mPal application highly with respect to acceptability (M = 4.48, SD = 0.55), appropriateness (M = 4.37, SD = 0.62), and feasibility (M = 4.43, SD = 0.59). A total of 75% of patients reported that after interacting with mPal, they were interested in using palliative care; 80% reported palliative care seemed less scary and 80% reported they were more motivated to talk to their oncologist about symptoms and concerns. Data revealed 100% of those who held misperceptions about palliative care prior to interacting with mPal (e.g., "palliative care is the same thing as hospice") no longer held the misperception after using mPal.
Conclusion: The mPal application, a brief tool designed to educate patients about palliative care and assess their unmet palliative care needs, is acceptable to patients with advanced lung cancer. It also appears to have high potential for addressing common misperceptions about palliative care and motivating patients to discuss palliative care with their oncologist. An efficacy trial is warranted to test the effects of mPal on advanced stage lung cancer patient palliative care knowledge and utilization. We are currently piloting mPal's clinician, system, and patient components at Markey Cancer Center in preparation for an efficacy trial.

Abstract 71

Translational

PLK1-Mediated PDCD4 Phosphorylation Confers Resistance to Enzalutamide in Prostate Cancer

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Enzalutamide is a second-generation androgen receptor inhibitor used to treat metastatic castration-resistant prostate cancer (mCRPC) patients. However, acquired resistance to enzalutamide remains a significant clinical challenge, implying an urgent need for additional approaches to overcome enzalutamide resistance. Previous studies have indicated that Polo-like kinase 1 (PLK1) negatively associates with programmed cell death protein 4 (PDCD4) in types of cancers. In particular, both PLK1 and PDCD4 were found tightly involved in enzalutamide resistance. Here we reported that PLK1 mediates the phosphorylation of PDCD4 at serine 239 (S239), leading to the degradation of PDCD4, which subsequently promotes enzalutamide resistance both in vitro and in vivo. Mechanistically, phosphorylation of PDCD4 at S239 upregulates the expression level of UDP-glucuronosyltransferase 2B15 (UGT2B15) via activating c-MYC-Hedgehog axis, which bypasses the androgen receptor pathway and de-sensitizes the cells to enzalutamide treatment. Inhibition of UGT2B15 increases enzalutamide-induced cell apoptosis and growth arrest in a PDCD4-S239 phosphorylation-dependent manner. Our work provides a novel perspective to understand the roles of PLK1-meidated PDCD4 phosphorylation in enzalutamide resistance and determines a potential therapeutic strategy to overcome enzalutamide resistance in prostate cancer.

Abstract 72

Population-Based/Behavioral

Lung Cancer Screening Programs: The Impact of SARS-CoV-2/COVID-19

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Introduction: The COVID-19 pandemic has had a persistently negative impact on healthcare delivery, and substantial barriers have caused delays and omissions of cancer risk reduction and early detection activities. Lung cancer screening (LCS) was particularly vulnerable to the adverse impacts associated with the pandemic because its widespread implementation is relatively recent, it serves a community that disproportionally experiences socioeconomic hardships, and it requires healthcare expertise required to manage the pulmonary effects of COVID-19 infection. While substantial research has monitored cancer screening rates over the course of the pandemic, relatively less research has explored decision making among LCS programs and other concerns raised regarding implementation of screening during pandemic restrictions. This cross-sectional mixed methods study utilized brief surveys and interviews conducted between Sept. 2020 and Jan. 2021, to understand the operational decisions made in response to the COVID-19 pandemic by LCS programs in Kentucky. **Methods:** The study sample consisted of LCS staff representing 18 screening programs across Kentucky. Participants were recruited through email invitation and dissemination of study flyers through cancer control organizations. Study participants were initially tasked with completing an online REDCap questionnaire consisting of nine item sets. These addressed decision-making, recent changes, and future directions of their LCS programs. Participants then engaged in semi-structured interviews to expand on a variety of domains pertaining to the initial and ongoing impact of COVID-19 on their programs and the decision-making process resulting from that impact. Data was analyzed using descriptive statistics, including means and standard deviations for continuous responses and frequencies for nominal/ordinal responses. Correlational analyses were used to explore patterns in responses with information regarding program history and characteristics. Qualitative data was analyzed using directed content analysis to identify prominent themes from the interview data regarding challenges and opportunities. **Results:** Participants included 1 LCS program medical director, 11 program coordinators/navigators, 2 administrators, 3 radiology directors, and 1 radiology technician, which represented 18 LCS programs operating in Kentucky in 2020. The initial impact of COVID-19 on LCS programs caused 78% (n=14) of programs to temporarily close or pause screening services from March to June 2020 and 72% (n=13) to experience reductions in caseloads within their programs. While most programs reported that their relationships with program participants (50%, n=9), referring clinicians (61%, n=11), and the community (55.6%, n=10) had not been harmed as a result of the COVID-19 pandemic, there were reports of delayed treatment which put these relationships at risk. Overall, programs were forced to make programmatic changes to adapt to the COVID-19 pandemic, including the implementation of telehealth (47%, n=8), the adoption of increased safety protocols (100%, n=18), and the expansion of communication efforts to maintain relationships with patients and referral sources. Although many of the programs came to similar decisions on what changes were necessary, the programs varied drastically in how those decisions were discussed among internal teams and how those decisions were ultimately implemented among LCS programs. **Discussion:** Despite the devastating impact of the COVID-19 pandemic on cancer screening behaviors, LCS programs in Kentucky closed relatively briefly and, through varied decision-making processes, engaged in several programmatic changes to ensure the continued provision of screening opportunities for Kentuckians.

Abstract 73

Basic Science

NRP1 Exon 4-Skipping Variant Promotes Colorectal Cancer Metastasis by Secreted Exosomes and Activated Endosomal Signals

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UK Markey Cancer Center

Neuropilin-1 (NRP1) is a transmembrane glycoprotein that acts as a critical co-receptor for tyrosine kinase receptors in growth factor signaling in both physiological and pathological context. The characterization of the NRP1 variants generated by alternative splicing mechanisms has provided profound insights into our understanding of the regulation of NRP1 function in cancer growth, angiogenesis, invasion, and metastasis as well as for the modulation of therapeutic outcomes. Here, we identified a novel human NRP1 splice variant resulting from the skipping of exon 4 (NRP1-ΔE4) in colorectal cancer (CRC). We found that NRP1-ΔE4 was largely expressed in CRC and significantly correlated with CRC progression. Furthermore, NRP1-ΔE4 exhibited increased endocytosis/recycling activity via secreted exosomes and decreased levels of degradation, leading to accumulation on endosomes. In addition, NRP1-ΔE4 enhanced interactions with the Met and β1-integrin receptors, resulting in Met/β1-integrin co-internalization and co-accumulation on endosomes. This provided persistent endosomal signals to activate the FAK/p130Cas pathway, thereby promoting CRC cell migration, invasion, and metastasis. Blocking exosome secretion, endocytosis or endosomal Met/β1-integrin/FAK signaling using genetic and pharmacological approaches profoundly inhibited the oncogenic effects of NRP1-ΔE4. Taken together, these findings reveal a unique function of NRP1-ΔE4 in the regulation of endocytic trafficking for CRC cell dissemination.

Abstract 74

Basic Science

A Portable Multiparametric Intravital Microscopy Platform for Metabolic Imaging on Biological Tissues

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Cellular metabolism is a complex and dynamic process that is strongly influenced by the local vascular microenvironment. In order to acquire a comprehensive understanding of the metabolism of tissues and the vasculature in vivo, a systems-level approach is of utmost importance. This becomes especially crucial in areas such as cancer biology, neuroscience, cardiovascular biology, and diabetes, where an understanding of tissue metabolism and vasculature is important in advancing significant biomedical research.

Currently available tools to measure tissue metabolism and vasculature are limited by practical and scientific constraints, making it challenging to obtain a complete picture of these metabolic and vasculature parameters simultaneously in vivo, in real-time, and with ease of access. Core facilities that require transporting samples or animals to their site, excessive costs (often in the hundreds of dollars per service), and time-consuming sample preparation (often multiple days) and data processing are just some of the challenges cancer researchers are currently facing, and all of these aforementioned challenges limit the access to high frequency measurements.

To overcome these limitations and enable more frequent and cost-effective quantification of tissue metabolism and vasculature, it is essential to develop new multi-modal metabolic tools with low-cost and point-of-care footprints. These tools will enable easy quantification of tissue metabolic and vascular endpoints together in vivo, in near real-time, and without the need for complex sample preparation or data processing.

In this study, we introduce a novel portable multiparametric intravital microscope for head and neck cancer tumor models for in vivo analysis is small animal models. The 2-NBDG has been used in cancer cells to report glucose uptake, like the clinically available FDG-PET. TMRE has been utilized to quantify cell mitochondrial membrane potential (MMP) to study OXPHOS. By measuring SO2 along with MMP, one can discern between OXPHOS vs. non-metabolic proton gradient changes. The system combines key metabolic probes with fluorescence microscopy techniques to simultaneously image glucose uptake, mitochondrial membrane potential, and oxygen saturation in small tumors in vivo. Previously, we have characterized the system using tissue mimicking phantom studies. In our recent in vivo studies, we have been successful in capturing key metabolic and vasculature endpoints from small mice tongue. We believe that this technology will provide a comprehensive view of cancer biology, facilitating the understanding of critical grey areas such as tumor radiation resistance, chemotherapy, and potential cancer therapeutics research.

Abstract 75

Clinical

Demographics and Outcomes in Hospitalized Patients with Acute Leukemia and Central Nervous System Involvement at the University of Kentucky

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Context: Acute leukemia is a heterogenous condition with a wide spectrum of disease states. In the adult population, myeloid is more common that lymphoid. Central nervous system involvement is largely considered an ominous finding, however its true incidence and effect on prognosis is unknown due to discordant findings in previous studies and lack of cytogenetic risk information.
Objective: To determine the incidence of CNS involvement in adult acute leukemia patients at the University of Kentucky and the effects on leukemia outcomes.
Design: A retrospective chart review was conducted and identified all adults (18 and older) hospitalized with an ICD-10 billing diagnosis of acute leukemia at the University of Kentucky inpatient leukemia service from 2016 to 2022. Subjects were filtered to identify those presenting at index hospitalization.
Setting: Single-center academic institution, inpatient leukemia service.
Patients: A total of 99 adult patients with acute leukemia were identified: 80 patients had AML, 4 T-ALL, 11 B-ALL, 2 APL, and 2 acute leukemia-not otherwise specified. Average age of population was 53.8 years; 35 females and 64 males. Patients were divided into cohorts based on presence or absence of CNS leukemic involvement. CNS involvement by acute leukemia was defined as presence of leukemic cells on lumbar puncture or the presence of imaging findings consistent with leptomeningeal involvement. Eleven acute leukemia patients had CNS positive disease: 9 with AML and 2 with ALL. No patients with APL were CNS positive.
Interventions: Patients received therapy based off their specific subtype of leukemia. AML patients received either 7+3 (cytarabine and daunorubicin) induction (with or without FLT3 inhibitor), hypomethylating agent and venetoclax, or hypomethylating agent alone. ALL patients received some form of Hyper CVAD. Intrathecal therapy was utilized in all cases.
Outcome measures: Length of stay, number of red blood cell/platelet transfusions, remission status at discharge, and survival at three and six months were collected and compared across each cohort (CNS involvement and control).
Results: Patients with CNS involvement averaged 7.9 RBC transfusions and 12.2 platelet transfusions for an average length of stay of 40.2 days. Patients without CNS involvement averaged 5.8 RBC, 8.1 platelet transfusions and average length of stay of 34 days. Survival for the CNS cohort at 3 and 6 months were 81.8% and 72.7%, respectively. Induction remission rate of 63.6%. One patient died during induction treatment. Survival for the cohort without CNS involvement at 3 and 6 months was 92.0% and 80.5%, respectively, with a remission rate of 57.5% at discharge. Of the AML patients with CNS positive disease, 2 of 9 patients (22%) were FLT-3 positive, whereas 3 (27.2%) were NPM1 mutated. One patient was t(8;21) and another patient demonstrated TP53 mutated status.
Conclusions: CNS involvement at time of index hospitalization for adult patients with acute leukemia appears to confer both increased morbidity and mortality. Patients with CNS involvement experienced greater transfusion requirements, longer length of stay, and increased mortality. With a CNS positivity rate of just 11.1%, a larger population will need to be evaluated in order to demonstrate any significance of genetic mutation or treatment approaches. Early survival and remission rates for CNS positive patients are favorable and should not preclude aggressive treatments for leukemia patients.

Abstract 76

Clinical

Getting to the Heart of the Issue: Algorithm Proposal for Treatment of Primary Intimal Cardiac Sarcoma

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Purpose: Although rare, cardiac sarcomas remain the most common malignant primary cardiac tumor in adults (Patel et al., 2014). Intimal cardiac sarcoma is the rarest subtype with a paucity of literature on management. No universally accepted consensus guidelines for its treatment algorithm exist (Kobayashi et al., 2022). Thus, we report a case of primary cardiac sarcoma managed with adjuvant chemoradiation after surgery to supplement the extremely sparse radiation management literature.

Methodology: This is a case description of an adult male patient with primary cardiac sarcoma managed post-surgically with adjuvant chemoradiation.

Results: A 35-year-old male initially presented with persistent concussive symptoms one week after a low impact motor vehicle accident. He denied any cardiac, pulmonary, or other symptoms at the time. Work-up revealed an incidental parieto-occipital brain mass, a large hypo-enhancing left atrial mass with protrusion into the left ventricle, and a 5mm nonspecific right upper lobe pulmonary nodule. Patient had sternotomy for left atrial mass resection with reconstruction of the left atrium followed by a left occipital craniotomy. Pathology confirmed high grade intimal sarcoma with left occipital metastasis and a focally positive atrial mass margin. Patient then received adjuvant chemoradiation (4000 cGy in 20 fractions followed by 1400 cGy in 7 fractions, using VMAT with 6X-FFF with concurrent Ifosfamide daily on days 1–5 every 28 days and Mesna). Cardio-targeted radiation was followed by additional six months of adjuvant chemotherapy (AIM). Patient underwent Gamma Knife Radiosurgery (GKRS) for his left occipital lobe resection cavity and five additional brain metastases. At 14 months following diagnosis, patient has excellent local control from cardio-targeted radiation, stable performance status, and mixed response intracranially from GKRS.

Conclusion: Managing intimal sarcoma includes surgery, chemotherapy, and radiation treatment either alone or in combination (Filho et al., 2002). Given positive margin at primary tumor site, limited extra-cardiac burden of disease, patient underwent chemoradiation with additional adjuvant chemotherapy. The use of image fusion, intensity modulated radiotherapy, and daily image guided RT were a proactive strategy to limit radiation to the normal heart and maximize tumor control.

Abstract 77

Clinical

Noninvasive Noncontact Optical Imaging of Tumor Blood Flow Contrasts in Human Breasts

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Introduction: X-ray mammography and ultrasound are the most commonly used imaging modalities for breast cancer diagnosis; however, they provide primarily tumor morphologic changes, which may not distinguish breast cancers from benign lesions. Eventually, patients undergo invasive biopsies as the gold standard for final accurate diagnosis. However, about four out of every five biopsies are negative for breast cancer, leading to unnecessary harm to patients. An innovative portable speckle contrasts diffuse correlation tomography (scDCT) technology has been recently developed in our laboratory, which enables noninvasive, noncontact, and high-density optical imaging of blood flow contrasts in deep tissues. This project aims to optimize and test the scDCT for noninvasive imaging of blood flow contrasts in breast tumors, with the ultimate goal of distinguishing aggressive cancers from benign lesions.

Methods: In scDCT, a galvo mirror remotely delivers coherent point near-infrared light (at 785 nm) to multiple source positions. A scientific CMOS camera measures spatial diffuse speckle contrasts on the tissue boundary with a typical exposure time of 2 ms. These boundary blood flow index (BFI) data from multiple sources and detectors are input into a customized parallel computation program for 2-D or 3-D reconstruction of blood flow distributions. To assess depth sensitivity of scDCT, solid phantoms with empty channels of the University of Kentucky (UK) logo and different top layer thicknesses were fabricated by 3D printing technology. The empty UK logos was filled in an Intralipid liquid solution to generate Brownian particle motions (i.e., particle flow). A total of 30 × 30 scanning sources was used to scan regions of interest of 50 × 50 mm ^2 and 70 × 70 ^2 for imaging UK logo phantoms and 12 patients with breast tumors, respectively. Patients scheduled for ultrasound guided biopsy as standard practice for a lesion of more than 15 mm and within 15 mm from the skin were enrolled. Contralateral normal breasts were included as healthy control tissue. Optical imaging with target lesions was obtained before the biopsy.

Results: Phantom measurement results demonstrate the ability of scDCT for high-density 2D mapping of flow contrasts with depth sensitivity. 2D and 3D imaging results from the patients show large variations in blood flow contrasts across different regions of breast tissues.

Discussion and Conclusions: The innovative scDCT enables noninvasive, high-density, depth-sensitive, 2D and 3D imaging of breast blood flow contrasts in the clinic. We are currently optimizing the system to reduce the time for data acquisition and imaging reconstruction. We are also adding more wavelengths to image both blood flow and oxygenation contrasts. With more subjects recruited, functional changes in breast tumors are expected to distinguish aggressive cancers from benign lesions.

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Abstract 78

Translational

Conferred Radioresistance via Treatment Trained Extracellular Vesicles in Pediatric H3K27M-driven Diffuse Midline Glioma

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Introduction: H3K27M-driven Diffuse Midline Gliomas (DMG) are a subset of uncurable malignant pediatric gliomas. In all cases, patient tumors evolve to be resistant to radiation therapy, the current standard of care. DMGs are multi-clonal, and each subclone has distinct genetic and transcriptional profiles. Data suggest that activation of oncogenic pathways through sub-clonal communication, specifically extracellular signaling, impacts how tumors respond to therapy and evolve. Secreted extracellular vesicles (EVs) are a mode of intratumor communication. However, EVs in H3K27M-DMG have not been extensively characterized, and their role in multi-clonal tumor evolution and development of radioresistance is unknown.

Methods: We isolated small EVs (<1000um) from a panel of primary and relapsed patient-derived H3K27M-DMG cells and individual sub-clones from a primary patient-derived cell line. We utilized Next-Gen sequencing and mass spectrometry to profile miRNA, proteins, and metabolites in H3K27M-DMG EVs. Additionally, we identified H3K27M-DMG EV membrane signatures using surface-enhanced Raman spectroscopy. We are using DMG cells that express a genetically encoded death indicator (GEDI) and a cell cycle indicator (FUCCI) for longitudinal imaging to assess the functional impact of EVs on radiation-induced cell death and cell senescence, two dynamic processes.

Results: EVs isolated from radioresistant H3K27M-DMG conferred radioprotective effects to radio-sensitive DMG cells given 8 Gy ionizing radiation, preventing apoptosis. These EVs also induce enhanced glycolysis in radiosensitive H3K27M-DMG, compared to treatment with autologous EVs.

Conclusion: Pediatric DMG-derived EVs induce functional changes in radiation-naive DMG cells and confer radioresistant phenotypes.

Abstract 79

Basic Science

Metformin Enhances the Efficacy of Enzalutamide Treatment in Castration-Resistant Prostate Cancer

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Prostate cancer continues to be the most commonly diagnosed cancer among men in the United States with approximately 268,500 new cases a year. One of the first treatment options for men with prostate cancer is androgen deprivation therapy (ADT), however, recurrence and metastases after ADT remain an issue and are categorized as castration-resistant prostate cancer (CRPC). Enzalutamide, an FDA approved drug currently prescribed to patients with CRPC, inhibits androgen receptor (AR) nuclear translocation preventing the transcriptional activity of AR. This therapy typically only extends the survival of patients with CRPC and can lead to enzalutamide resistance over time, accenting the importance of new therapy targets. Metformin, a commonly used FDA approved therapy for type 2 diabetes, has been recently investigated as potentially having anti-tumor effects in many cancer types. In this study, we use enzalutamide and metformin in combination to explore possible re-sensitization of enzalutamide-resistant CRPC to enzalutamide. Recently, we have tested the effects of this combination treatment on cell viability, drug synergy, and cell proliferation in enzalutamide-resistant CRPC. After treatment, we see a decrease in cell proliferation and viability as well as a synergistic effect of both enzalutamide and Metformin in enzalutamide-resistant CRPC. Following these results, we sought to explore how this combination treatment affected key signaling markers for EMT, WNT signaling, and TGF signaling as well as key signaling markers for prostate cancer such as AR and prostate specific antigen (PSA). Since Metformin inhibits Complex I of oxidative phosphorylation, we also tested this combinational treatment using Seahorse analysis to determine the mitochondrial respiration. After initial testing, we will continue to move forward in testing the effects of this combination treatment in enzalutamide-resistant CRPC as well as further elucidate the underlying mechanisms contributing to this phenotype.

Abstract 80

Clinical

Use of Novel 3D-Conformal MLC-Based Spatially Fractionated Radiation Therapy (SFRT) Treatment on "Same Day" via Conebeam-CT Guidance for Management of Bulky Tumors

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Purpose/Objectives: Large and bulky nonlymphoid, unresectable tumors (≥ 8.0 cm) have very poor prognosis, frequently progress despite systemic therapy, and are often managed palliatively. Recent publications have indicated that SFRT is a viable treatment option to manage these large tumors. We now report the clinical use of a novel MLC-based SFRT on "same day" as CT simulation, a safe and efficacious treatment for bulky disease that confers rapid reduction in disease burden and pain relief with minimal normal tissue toxicity.

Materials/Methods: Patients with bulky tumors received 15 Gy in 1 fraction via 3D-conformal MLC-based SFRT to the gross tumor volume (GTV-GRID) derived from CT scans with 10%-15% hotspot into the GTV. Dose was calculated using Acuros-based engine, and treatments were delivered using pre-treatment Conebeam CT imaging for set up corrections. Patients underwent post-treatment CT imaging in 3-month intervals to evaluate for changes in tumor size and post-radiation sequelae. Outcomes reported include decreases in tumor shrinkage, pain control, and treatment-related toxicity.

Results: Twenty-six patients with large and bulky tumors of various histologies (non-lymphomatous) in various treatment sites who underwent MLC-based SFRT were evaluated. These patients received consolidated radiation therapy treatment after receiving SFRT. Mean GTV-GRID volume was 514.3 ± 315.6 cc (range, 131.1–1251.0 cc). This novel MLC fitting algorithm provided excellent dose parameters with mean GTV(V7.5Gy) and mean GTV dose of 54.2% and 7.9 Gy respectively for 15 Gy plans. Average peak-to-valley-dose-ratio was 3.4. Mean beam-on time was 3.32 minutes. Overall treatment time including patient setup, conebeam CT imaging, and patient re-positioning to beam-off was within 15 minutes. Average 3-dimensional couch correction from original skin-markers was < 1.1 cm. The 3D MLC-based SFRT plans enhanced target dose for bulky masses including deep-seated large tumors while protecting skin and adjacent critical organs. Median follow-up interval from treatment delivery day was 3 months (range, 0–21 months). All patients tolerated the MLC-based SFRT treatment well. Eighteen of 26 (69.2%) patients received post-treatment CT imaging at 3-month interval. Tumor shrinkage was observed in 12/18 (67%) patients who underwent post-therapy evaluation. Improved pain relief was reported in 14/19 (73.6%) patients. Thirteen (50%) patients were confirmed as deceased. Four (15.4%) patients passed away before the 3-month follow-up, and three (11.5%) patients passed away after the 3-month follow-up, none of whom exhibited acute toxicity. Amongst the 22 patients evaluated in total for post-radiation toxicity; acute toxicity included grade 1 skin erythema (n = 4) and grade 1 odynophagia (n = 1), and chronic toxicity included grade 3 wound complication (n = 1) and grade 4 necrotizing fasciitis of the neck (n = 1). Otherwise, 16/22 (72.7%) of clinically evaluated patients reported no post-radiation toxicities, and no grade 5 toxicities were observed.

Conclusion: The novel 3D-conformal MLC-based SFRT to large and bulky unresectable tumors is a fast, safe, and effective treatment that confers decreased tumor burden, improved pain control, and generally low morbidity rates. This simple MLC-based SFRT method can provide effective palliation for a wide range of tumor pathologies, improving patient comfort and compliance as well as better clinic workflow, as a "same day" treatment option avoiding the need of longer contouring, treatment planning and physics quality assurance times.

Abstract 81

Translational

Identification and Characterization of Iminoquione-1 as PD-L1 Inhibitor

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UK College of Medicine

Cancer immunotherapies through immune checkpoint blockade by therapeutic antibodies have achieved remarkable tumor regression in limited cancer patients. Immune checkpoint ligand PD-L1 has been validated as a viable target in different types of cancers. However, poor bioavailability and immune-related adverse effects of monoclonal therapeutic antibodies limit their applications. In contrast, small-molecule inhibitors of the PD-L1 pathways have shown promising immunotherapeutic effects but none have been approved by the FDA to date. The identification of additional small-molecule PD-L1 modulators warrants further effort. We present here the discovery of iminoquione-1 as a novel PD-L1 inhibitor. Through screening our in-house compounds, we identified iminoquinone-1 that selectively induces premature PD-L1 expression in cancer cells in a dose-dependent manner. Mechanism of action studies by RNA-seq analysis revealed that iminoquinone-1 activated unfolded protein response.

Abstract 82

Clinical

Clinical Outcomes of Synchronous Lung Stereotactic Body Radiation Therapy Lesions via Single-Isocenter/Multi-Lesion Treatments

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Purpose/Objectives: Lung cancer patients with synchronous primary or oligometastatic (< 5 lesions) with associated co-morbidities may not retain their treatment position for the extended stereotactic body radiotherapy (SBRT) treatment times with individual isocenter plans for each lesion due to discomfort or shortness of breath. SBRT using a single-isocenter/multi-lesion (SIML) volumetric arc therapy (VMAT) plan with flattening filter free (FFF) beam could significantly shorten overall treatment time, improve patient comfort and compliance and clinic efficiency. We report clinical results of treating multiple lung metastases or synchronous primary lung cancers with SIML lung SBRT.

Materials/Methods: Eighty patients with synchronous primary lung cancers or oligometastatic lung tumors (two, n=61; three, n=10; four, n=4; five n=5; total treated lesions, n=193) were simulated with abdominal compression and/or 4D-CT MIP images and treated with a highly conformal SIML VMAT lung SBRT plans via 3-4 non-coplanar arcs. Common prescriptions were 50-55 Gy/5 fractions and 54 Gy/3 fractions prescribed 70-80% isodose line to the each PTV. Acuros dose calculation for 6MV-FFF beam was used for tissue heterogeneity corrections. RTOG-0618/0813 criteria was used to dose constraints to organs at risk (OAR) and target conformality. Treatment was delivered every other day or twice weekly with CBCT-guidance, adjustments made with 6DOF couch corrections on TrueBeam Linac, and treatment delivery time within 15 minutes. Local control rates and toxicity profile was evaluated using CTCAE v. 5.0 grading for pneumonitis, rib fracture, and chest wall pain.

Results: All plans met RTOG-0618/0813 requirements for each tumor coverage, dose to OAR including normal lung and ribs. Mean follow up after last fraction of treatment was 16.9 months (range, 1.0–54.2 months). PTV volume ranged from 2.17 to 167.8 cc with mean volume of 16.1 cc. Of the 80 patients treated, 71 had adequate post-treatment thoracic CT imaging to assess local control. Local control was achieved in 167/175 (95.4%) of treated and followed lesions. CTCAE grade 1 asymptomatic pneumonitis was noted on thoracic CT scans in 42/71 (59.2%) of patients and occurred, on average 4.8 months after SBRT. Symptomatic pneumonitis and rib fracture did not occur in any patient. CTCAE grade 2 chest wall pain occurred in 4/80 (5.0%) treated patients and was managed conservatively with over-the-counter NSAIDS or acetaminophen.

Conclusion: SIML lung SBRT for synchronous primary lung cancers or multiple lung metastases can be used as a variant to traditional multiple isocenter SBRT or chronologically separate treatment courses, and has excellent local control rates and low toxicity profile in our patient population. It can help improve comfort and compliance of the patients who have difficulty lying still for an extended treatment course, and significantly reduces treatment time via isocenter shifts/repeated CBCTs for image guidance, thus improving clinic efficiency.

Abstract 83

Basic Science

Diffuse Optical Spectroscopy for Rapid and Accurate Monitoring of Glucose Uptake in Biological Models

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Increased glucose uptake in tumors regardless of oxygen levels has been routinely used as one of the effective clinical biomarkers for human cancer diagnosis. Glucose uptake monitoring in small animals in vivo during a course of tumor therapeutics study has been explored as an effective way to understand the role of tumor metabolism reprogramming in tumor therapeutics. Currently, the available device for in vivo glucose uptake imaging is clinical PET-CT scanning, which has low user access due to the expensive equipment and user fees. To meet the unmet need for glucose uptake monitoring in small animals for pharmacological studies, optical technologies have been explored as an effective way to quantify glucose uptake in biological tissues. Specifically, fluorescence imaging or spectroscopy of 2-NBDG has been used in cancer cells or tumors to report glucose uptake, similar to the clinically available FDG-PET. The drawbacks of the fluorescence technique include: (1) fluorescence signal suffers from tissue absorption and scattering distortions; (2) it requires high-power light to excite the fluorescent probes. Both the drawbacks increase the optical device cost and user complicity. To further minimize the device cost and maximize the ease of the technique, we report a novel diffuse reflectance spectroscopy tool and a novel algorithm for accurate monitoring of glucose uptake in biological tissues. Rather than looking at fluorescence, our proposed approach focuses on the absorption properties of 2-NBDG from which the 2-NBDG concentrations can be accurately and rapidly estimated by examining the absorption-caused diffuse reflectance changes. We performed tissue-mimicking phantom studies and our data showed that our technique can accurately quantify 2-NBDG concentrations from tissue-mimicking phantoms. We will further test our technique in small animals to monitor the 2-NBDG uptake in vivo. We expect the developed technology could be a useful tool for cancer diagnosis or pharmacological research.

Abstract 84

Clinical

Single Center Review of the Characteristics and Outcomes of Patients with Acute Leukemia Admitted to the Intensive Care Unit During Initial Hospital Stay

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Context: Acute leukemia patients may present with critical illness at time of diagnosis or develop during initial treatment. Some leukemia patients present with leukostasis, an indication for leukophereses. Other patients present with DIC prompting aggressive transfusions and supportive care. Many patients will develop multi-organ impairment that requires ICU level of support. One study demonstrated 22.5% of acute myeloid leukemia patients will require ICU care with most cases involving the initial treatment. Cardiovascular disease, COPD, renal insufficiency, and cytopenia are associated with ICU admissions in leukemia patients. Among these populations, 36.7% required mechanical ventilation and in-hospital mortality was reported up to 31%. The outcomes and characteristics of acute leukemia patients admitted to the University of Kentucky who require ICU level of care has not been investigated.
Objective: The intention of this study is to review acute leukemia patients treated at the University of Kentucky and compare the demographic, pathologic, and outcomes between those patients who require ICU level of care versus those who do not and determine if differences exist which might have predictive capabilities.
Design: A chart review was conducted and identified all adults hospitalized with an ICD-10 billing diagnosis of acute leukemia at the University of Kentucky from 2016 to 2022. Information about patient demographics and outcomes was collected through a retrospective chart review.
Patients: A total of 99 patients with acute leukemia were identified: 80 patients had AML, 15 with ALL, 2 APL, and 2 acute leukemia-not otherwise specified. Average age was 53.8 years. Patients were divided into cohorts based on whether they required ICU level of care at any point during the induction hospitalization: 11 patients required ICU care (9 AML; 2 ALL). Comorbidity included 1 CAD, 3 diabetics, 1 cervical cancer, 1 MPN, and 1 Crohn's disease. All patients had preserved cardiac function at diagnosis. No patients had COPD. BMI and age were comparable between the cohorts.
Interventions: Patients received ICU care: 4 due to respiratory failure, 4 sepsis, 2 leukopheresis, and 1 hemorrhage. ICU care ranged from 1-15 days.
Outcomes: Patient demographic information: length of stay, number of blood/platelet transfusions, remission status at discharge, and survival at six months were collected and compared across each cohort (ICU requirements and control).
Results: ICU leukemia patients required more transfusions of RBC (8.36 vs 5.76) and platelets (13.9 vs 7.9) during the hospitalization. Overall LOS for ICU leukemia patients averaged 42 days versus 34 days for control. All ICU patients went on to receive induction chemotherapy. One died during hospitalization and a second soon after discharge to hospice. Nine patients survived more than six months following diagnosis. Despite the ICU requirements, eight patients were able to achieve a morphologic remission.
Conclusions: In this single center population of adult patients with acute leukemia, ICU level of care during initial therapy did not appear to confer significant risk in morbidity or mortality. Patients with ICU care did require longer LOS and increased transfusion requirements. With an ICU admission rate of just 11.1%, a larger population will need to be evaluated in order to demonstrate any significance of comorbidity or treatment approaches. Early survival and remission rates for ICU leukemia patients are favorable and should not preclude aggressive treatment.

Abstract 85

Clinical

Health Gaming Intervention as Neurostimulation Therapy for Cancer Patients Suffering from Acute Cognitive Impairment (Chemo Brain): An Ongoing Clinical Study to Improve Synaptic Plasticity

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Millions of patients each year suffer from acute cognitive impairment caused by traumatic brain injury, stroke, Parkinson's disease, and post-intensive care syndrome (PICS). One cause of cognitive dysfunction gaining increasing attention affects cancer patients with similar cognitive challenges after chemotherapy, often referred to as "chemo brain." While patient survival from cancer drugs extend life, there are considerable adverse cytotoxic effects that extend to the central nervous system. Such effects, which outlast the period of chemotherapy, result in chronic dysfunction in sensitive areas of mental performance. Studies refer to this condition as chemotherapy-related cognitive impairment (CRCI), affecting concentration and executive function, e.g., working memory, cognitive flexibility, and inhibitory control. CRCI is the manifestation of central nervous system toxicity which contributes to the decline of quality of life. Besides the persistent cognitive effects of brain fog, these patients often find it difficult to execute simple tasks without extra concentration, while others cannot return to work or hold a job. CRCI patient care with physical/occupational therapy or neuropsychology have shown limited success in activating the necessary neural pathways due to neurotoxicity.

Early studies have demonstrated that video games have improved cognition and attention/working memory for older adults and patients suffering from stroke and dementia. We posit that we can augment/enhance neuroplasticity through a newly developed digital substrate, which we identify as a form of neurostimulation therapy. By means of an interactive gaming environment that includes cityscapes, persons and objects, the patient moves through a 3D space as a (first-person) avatar of her/himself. Importantly, in addition to the 3D experience, the game is designed to increase cognitive and sensory engagement through (embedded) selective attention (SA) exercises. Studies have shown that non-pharmacological interventions, such as SA testing, contributes to success in addressing cognitive impairment. Our gaming environment manages SA stimuli by directing the patient to focus and filter visual and auditory information. Each challenging SA exercise acts like a spotlight on a particular task within the patient's visual and auditory field. By neurologically recruiting executive function and processing speed, this non-pharmacological intervention promotes the ability of neurons to alter the functional properties of the brain by stimulating plastic changes.

The game targets the strengthening of brain synapses, i.e., the connection at the end of neurons. As the patient-player learns and adapts to each new cityscape environment and SA exercise, electrical signals assist in the release of neurotransmitters into the synapse. That is, the game is intended to support the brain's ability to strengthen synaptic plasticity. The game includes three levels (environments), with 84 exercises within 14 modules. To mitigate CRCI and restore patient pre-cancer cognitive function and quality of life, we are currently usability testing the game (referred to as Hometown Bound), followed by clinical study experiments with existing cancer and ICU PICS patients by late spring/summer 2023. Currently, the game intervention is played using PC/laptop and mouse/pad. The game's backend tracks patient performance measures in all movements and exercise completion/error. If clinical study patient outcomes prove positive, we intend to convert the game to a virtual reality environment using a VR headset for a full-patient immersive experience, which we posit will further maximize synaptic plasticity and cognitive restoration.

Abstract 86

Clinical

Benefit of Immune Checkpoint Inhibitors Across the Age Spectrum in Patients with Advanced Squamous Cell Lung Cancer: An NCDB Analysis

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Introduction: Non-small cell lung cancer remains the leading cause of cancer death worldwide, especially for patients over 75 years old. However, because elderly patients are often underrepresented in clinical trials, insight is limited into the benefits of cancer therapeutics including immunotherapy, which revolutionized treatment of advanced lung cancer. There are conflicting data on outcomes of elderly patients treated with immunotherapy, with immunosenescence and increasing tumor mutational burden as possible competing factors for potential response.

Methods: A retrospective analysis using National Cancer Database (NCDB) was conducted in patients diagnosed with squamous cell lung carcinoma between 2016 and 2019. Squamous cell carcinoma was chosen to reduce the confounder of molecular targeted therapy. Patients were separated into treatment groups of those having received cytotoxic chemotherapy only, chemotherapy + immunotherapy, immunotherapy only, and no chemotherapy or immunotherapy. Patients were stratified by age groups of the young (19-49), the average (50-79), and the elderly (80 or older). Age 50-59, 60-69 and 70-79 traced each other for survival hence grouped together in analysis. The primary outcome analyzed was overall survival. Secondary analyses evaluated differences amongst treatment institution type, and patient specific socioeconomic factors including sex, race, insurance, etc. Kaplan-Meier (KM) plots were used to examine survival outcomes and descriptive analysis was performed to examine the characteristics of clinical variables by the different treatment modalities. Cox regression analysis was used to identify factors associated with OS.

Results: Of the 24,611 patients evaluated, 11,063 (45%) were 70 years old or older. Data showed 9,261 patients received chemotherapy only, 3,037 immunotherapy only, 4,889 chemotherapy + immunotherapy, and 7,024 had neither chemotherapy nor immunotherapy. There were 400 patients for whom treatment data was unknown. Compared to treatment with chemo only, treatment with immunotherapy only was associated better outcomes (HR 0.84), with chemotherapy plus immunotherapy the best outcome (HR 0.73), whereas the no treatment group had the worst outcome (HR 2.90). One-year and two-year survival in these groups are chemo and immune (52.98%; 18.10%); Immunotherapy (46.85%; 16.34%); chemotherapy (40.11%; 10.73%) and no treatment (11.13%; 3.81%), respectively, all statistically significant. In multivariate analysis, male sex, uninsured status are associated with slightly worse outcome whereas location and education were not. Interestingly, while the survival outcome is worse in the elderly (age 80 or older), different age groups benefited similarly to all treatments (HR 1.02, Range 0.98-1.06, P=0.397 in age 50-79; and HR 1.01; range 0.91-1.11; P=0.862), suggesting that differences in survival would be related to factors other than cancer treatment.

Conclusion: Chemotherapy and immunotherapy in combination are associated with increased survival, including in elderly patients. Immunotherapy alone is also associated with a survival benefit compared to chemotherapy. Elderly patients should be considered for immunotherapy, in combination with chemotherapy or alone, in treatment of advanced squamous cell carcinoma of the lung. Future studies should evaluate for correlation of tumor mutational burden with immunotherapy outcomes in the elderly population.

Abstract 87

Translational

Artesunate Increases Enzalutamide Efficacy in Advanced Prostate Cancer

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UK College of Medicine, Toxicology and Cancer Biology

Prostate cancer (PCa) is one of the most commonly diagnosed cancers worldwide. In advanced prostate cancer, Bromodomain-containing protein 4 (Brd4) has been found to play a critical role in promoting tumor growth and survival by transcribing c-MYC (MYC), which is involved in cell proliferation and survival pathways. Furthermore, Brd4 has been implicated in the development of resistance to androgen deprivation therapy, which is a standard treatment for prostate cancer. Although second-generation anti-androgen drugs plus androgen deprivation therapy (ADT) remain the first-line treatment for advanced prostate cancer patients, approximately one-third of patients will relapse within a short period. MYC overexpression has been shown to contribute to not only tumor progression but also prostate cancer recurrence, making targeting MYC a promising therapeutic strategy for advanced prostate cancer. However, direct targeting of MYC can lead to high toxicity, making it crucial to identify novel therapeutic strategies. One potential approach is to use Artesunate (ART), a semi-synthetic ingredient derived from Artemisia annua, which is commonly used to treat malaria globally and has recently been discovered to have anticancer properties. However, the efficacy of ART in treating advanced prostate cancer and its direct target have not been thoroughly investigated. In this study, we examined the efficacy of combining enzalutamide (Enza) and ART in advanced prostate cancer cell lines. We also conducted an unbiased bioinformatics analysis using RNA-seq results in C4-2R cells and 22RV1 cells to investigate the cell response to ART treatment. Our findings revealed that ART can downregulate the MYC signaling pathway, highlighting the potential of targeting the Brd4-MYC axis through ART treatment as a promising and valuable therapeutic strategy in the clinic.

Abstract 88

Translational

Disparities in Small Cell Lung Cancer Care: Addressing the Unique Challenges Faced by Oncology Care Providers in Appalachia

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Introduction: Patients with small cell lung cancer (SCLC), especially those located in more rural communities face substantial challenges in their health care. Low socioeconomic status, barriers to healthcare access, and geographic isolation are contributors to disparate lung cancer care in the Appalachian region. Until recently, improving treatment-related outcomes remained an unmet medical need for patients with extensive-stage small cell lung cancer (ES-SCLC). To understand the factors that can inhibit the provision of accessible, evidence-based care for SCLC patients living in Appalachia, education was delivered regarding evolving treatment standards of care and e-brief resources were disseminated to oncologists and support care teams with the goal to improve the care of patients with ES-SCLC.

Methodology: The initiative follows the care of patients from seven regional sites in Kentucky and Ohio in the Appalachia region. Current clinical practice patterns were evaluated through an audit of the electronic records of patients with newly diagnosed SCLC three months prior to baseline data abstraction across 10 quality measures that provided information on smoking status/cessation, ECOG performance, first-line therapy options, and dedicated palliative care among others. A baseline practice assessment survey was also provided to oncologists and other clinicians managing patients with SCLC. An intent to change survey was distributed following an educational virtual workshop that spotlighted treatment standards of care within SCLC, proposed three case studies for participant feedback, and barriers/challenges regarding access to treatment were identified. Three e-briefs were also distributed with topics including tobacco cessation, talking with patients about ES-SCLC treatment, and managing immune-related adverse events. Following this distribution, a second practice assessment survey was collected, and the post-initiative EHR quality measures were abstracted for final review.

Results: A final summary report was provided to each of the sites that included aggregate data from the EHR quality measures and the two practice assessment surveys that examined factors influencing treatment selection including clinical evidence, prior experience, and confidence in managing patients with ES-SCLC. At final review there was an increase in the percent of patients who had documentation of ECOG performance status (44% baseline (BL) to 61% final), and tobacco cessation being offered (41% BL to 64% final). In terms of self-reported confidence in managing patients with ES-SCLC on the practice assessment survey, nurses reported an increase in being extremely or moderately confident post-initiative in discussing SCLC development and growth (15% BL to 35% final), providing patients with educational materials and resources (30% BL to 65% final), and counseling patients about financial resources (15% BL to 53% final).

Conclusions: In order to improve the management for patients with ES-SCLC the following improvements were proposed to the participants: 1) providers should encourage treatment to start as soon as possible after the ES-SCLC diagnosis is made. If waiting on results (e.g., biopsy) calling the pathologist (instead of waiting for the biopsy results to post in the electronic record) will reduce the time to start treatment. Providers should also encourage and refer ES-SCLC patients undergoing treatment to palliative care services as many patients have symptoms that could benefit from these services.

Acknowledgement: This initiative was funded by an educational grant from Genentech. The funder had no role in the development, execution, or analysis of this work.

Abstract 89

Core Resources (Informational and not judged)

Comparison of Pre-Approval and Post-Approval Study Benefits of Oncology Drugs Approved through the U.S. Food and Drug Administration Accelerated Approval Program

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Pharmacy

Background/Rationale: The Food and Drug Administration (FDA) accelerated approval (AA) program was created in 1992 to expedite the approval of drugs that treat serious and life-threatening conditions to fill an unmet medical need based on a surrogate endpoint.[1,2] The surrogate endpoint is a marker, such as a laboratory measurement, radiographic image, physical sign or other measure that is reasonably likely to predict clinical benefit. Approximately 85% of medications receiving approval through the AA process over the past decade have oncology indications.[3]

The FDA requires drug companies to conduct post marketing trials to confirm clinical benefit and attain traditional approval using clinical endpoints such as overall survival. Due to recent criticism, the FDA Oncology Center of Excellence performed an analysis of the current AA process. Based on the analysis, they suggest granting AA based on an interim analysis of a surrogate endpoint from a single randomized trial. This trial would then serve as the post marketing trial for traditional approval.[1] Currently, there is no published data evaluating the variation between the surrogate endpoint in the AA approval trials and the confirmatory trials.

Objective(s): The purpose of this study is to explore a relationship between surrogate endpoints in AA studies with post marketing confirmatory trials.

Methods: Oncology drugs that were granted accelerated approval from Jan. 1, 2010 to Dec. 31, 2019 were identified using publicly available FDA databases. Follow-up ended on Jan. 1, 2023. FDA approval letters, product label updates, and drug sponsor press releases were used to determine the clinical trials on which accelerated and traditional approval were based. Data extracted from trials included drug name, indication, approval date, line of therapy, trial location, study design, participation enrollment, and primary and secondary endpoints.

Results: From Jan. 1, 2010 to Dec. 31, 2019, 51 drugs received AA for 82 oncology indications. As of Jan. 1, 2023, 14 indications were withdrawn from the market, 48 indications were converted to traditional approval, and 20 indications have yet not confirmed clinical benefit. The median time to traditional approval was 2.5 years (range 0.5 years to 8.9 years). Further results to be presented at HOPA 2023.

Conclusions/Discussion: Final conclusions will be presented at HOPA 2023.

References

[1] Fashoyin-Aje LA, Mehta GU, Beaver JA , Pazdur R. The on- and off-ramps of oncology accelerated approval. N Engl J Med. 2022;387(16):1439-1442
[2] Guidance for industry-Food and Drug Administration. https://www.fda.gov/media/86377/download. Published May 2014.
[3] Beaver JA, Pazdur R. "Dangling" accelerated approvals in oncology. N Engl J Med. 2021; 384(18):e68.

Abstract 90

Population-Based/Behavioral

Adherence to Follow-Up Care after Positive HPV Test among Hispanic Women

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Background: The human papillomavirus (HPV) causes 91% of cervical cancers. HPV testing plays an important role in cervical cancer screening in addition to traditional Pap tests. Women with a positive HPV infection have a higher risk of developing cervical cancer, even if their Pap test was normal. A positive HPV result determines the follow-up care and procedures women need to prevent the infection from progressing to cervical cancer. Hispanic women have higher cervical cancer incidence but lower cervical cancer screening rates than non-Hispanic white women. Hispanic women are also less likely to adhere to the recommended follow-up care after cervical cancer screening. This study aims to examine barriers to follow-up care after a positive HPV test among Hispanic women.

Methods: Using an observational, cross-sectional design, we collected individual in-depth interviews and demographic questionnaires with Hispanic women and their partners.

Study Population: Interviews and analysis are ongoing. To date, interviews have been conducted with six self-identified Hispanic women aged 30-65 who received a positive HPV test result in the past three years, as well as two romantic partners of these women.

Results: Over two-thirds (67%) of interviewed women were from Mexico, one-third (33%) were from South American countries, and none spoke English. Half of the participants (50%) did not graduate from high school, and one-third (33%) were unemployed. Over two-thirds (67%) of participants had a monthly household income of less than $2,000. All participants were unmarried and lived with a romantic partner. None of them had a family history of cancer. Lack of HPV knowledge was a common barrier. Most women and their partners knew that HPV is sexually transmitted, but they did not know there was a test to detect HPV infection. The women and partners were not familiar with what HPV was and that it causes cervical cancer. All women sought health information online and on social media. The two interviewed romantic partners said they supported their partners to adhere to their recommended follow-up care. The other women did not want to tell their partners about the HPV infection because they feared that their partners would accuse them of being unfaithful. Additional barriers to follow-up care included transportation, language, lack of health insurance, and undocumented immigration status.

Conclusion: Hispanic women face several barriers to adhering to follow-up care after a positive HPV test that includes traditional healthcare access barriers, as well as cultural gender roles and stigma around sexually transmitted infections. To address cervical cancer health disparities affecting Hispanic women, these findings will be used to develop an intervention to increase adherence to recommended follow-up care in this population.

Abstract 91

Population-Based/Behavioral

Kentucky Lung Cancer Screening Learning Collaborative: Achieving Equity

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Background: Kentucky is #2 in the country in eligible participants being screened for lung cancer, and experienced a 10% reduction in late-stage diagnosis from 2015 to 2018 as a result of collaborative efforts including leadership from the Kentucky LEADS Collaborative. Despite this, the burden of lung cancer remains significant. Kentucky has the worst overall lung cancer incidence (54% higher than national average) and mortality (82% higher than national average) with significant geographic, socioeconomic, and racial disparities in outcomes. Due to these disparities, achieving equity in lung cancer screening programs is critical.
Objective: As leaders of the Kentucky LEADS Collaborative QUILS™ (Quality Implementation of Lung Cancer Screening) team and the UK Markey Cancer Center, we are delivering a virtual collaborative learning environment for all LCS programs in Kentucky. The purpose is to provide education and mentoring in a bidirectional, virtual environment addressing disparities in lung screening and increase the impact on all regions, especially Appalachian Kentucky, where the need is greatest.
Methods: The Learning Collaborative hosted a launch event in November 2022, promoted among contacts for the Kentucky LEADS Collaborative, Kentucky Cancer Consortium, Markey Cancer Center Research Network, and Markey Cancer Center Affiliate Network. A brief research description and connection with content experts was provided prior to the launch. A landing page and website was established for the Learning Collaborative. Recruitment began in January 2023, collecting LCS program information including organization, main county of practice, and specific programmatic role. When participants enroll in the collaborative, a pre-survey is sent to collect data on attendees including knowledge, skills, and attitudes on LCS. More specific survey questions are targeted to program navigators on their LC specific operations. Specific content, plan, and roles among the team is established, and recruitment is on an individual session basis with promotion of the event and speakers highlighted for potential participants. The Learning Collaborative series is hosted from January 2023 to June 2023 with the following session topics monthly: 1. Launch Event and Introduction; 2. QUILS and Equitable Lung Cancer Screening; 3. Equity & Multidisciplinary Components; 4. Equity and Lung Cancer Risk Reduction; 5. Equity and Candidate/Participant Engagement; 6. Equity and Community Engagement; 7. Learning Collaborative Conclusion and Next Steps. Each session includes an opening section, presentation, case presentation, discussion, summary, and open time for questions. By utilizing the Zoom application, we host interactive polling questions to collate information on disparities and where future efforts and resources may best be allocated to achieve equity in LCS. Following the six learning collaborative sessions, a post-survey will be distributed to participants collecting data from the pre-survey as well as a robust evaluation for the navigator population. Tracking reach is another critical component of this series, tracking program and attendee participation for each session to identify relevant trends and identify where resources can be aligned to contribute to achieving equity within the Commonwealth in this specific patient population. Following data collection and analysis, the comprehensive report will be sent to UK Healthcare for further steps and to identify where resources should be aligned for future projects and collaborations across the state.
Results: The Learning Collaborative will be ready for analysis in June 2023 when the series concludes.

Abstract 92

Clinical

Use of HyperArc Stereotactic Radiotherapy (SRT) for Large Recurrent Head and Neck Cancers

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Purpose/Objectives: Conventional radiotherapy treatment for recurrent large head and neck tumors poses significant technical and dosimetric challenges due to treatment-related toxicity. Recent studies have shown stereotactic radiotherapy (SRT) is a viable treatment option to manage these difficult patients. Here, we present a novel HyperArc volumetric modulated arc therapy (VMAT) SRT option for fast, safe, and effective treatment that allow for dose escalation to the tumor center while protecting adjacent organs at risk (OAR).

Materials/Methods: Patients with large recurrent head and neck cancers underwent 30–40 Gy in 5 fractions HyperArc SRT to the planning target volume (PTV) of 2-3 mm margin around the standard gross tumor volume (GTV) derived from MRI or CT images with 15-20% hotspot into the GTV. Highly conformal SRT plans were generated using a fully automated HyperArc VMAT module on TrueBeam with 6MV-FFF beam (1400MU/min), Encompass support, Q-fix mask, and advanced Acuros-XB dose calculation algorithm for tissues heterogeneity corrections. Treatments were delivered every other day via pre-treatment conebeam CT guided procedure and perfect pitch couch corrections. Patients underwent post-treatment computed tomography in 3-month intervals to evaluate for locally recurrent disease and distant metastases. Outcomes reported include tumor local control (LC), distant failure (DF), and treatment related head and neck toxicity following Common Terminology Criteria for Adverse Events (CTCAE, version 5.0) criteria.

Results: Twenty-three head and neck cancers patients who either underwent retreatment (n = 17) or boost to primary tumor (n = 6) were evaluated. Median follow-up interval was 8 months (0 to 19 months). Mean GTV and PTV volume was 23.9 cc (range, 5.5–53.1 cc) and 50.3 cc (range, 16.9 ± 91.6 cc). HyperArc VMAT SRT plans provided highly conformal target coverage, steep dose gradient, and low doses to the immediately adjacent critical organs including maximal spinal cord was kept below 5 Gy. Average perfect pitch couch correction was < 1.7 mm and 2.2o in each direction. Average beam-on-time was approximately 3.53 min. Overall mean treatment time including pre-treatment CBCT imaging, patient repositioning, and dry-run was within 15 min. All patients tolerated the HyperArc treatment. Tumor LC was observed in 17/20 (85%) evaluable patients with follow-up CT imaging and physical exam. Six (25%) patients died prior to three-month follow-up; two (8.3%) before post-treatment imaging could be obtained. Three (12.5%) patients experienced local tumor progression. No acute toxicities were reported in this cohort. Eight (33.3%) patients had distant progression, including metastases to the neck and hilar region (n = 1), occipital scalp (n = 1), carotid encasement (n = 1), pulmonary metastases to left upper lobe (n = 1), dermal metastasis (n = 1), and leptomeninges (n = 1) spread. No patients developed grade 2 or higher toxicity in the Head Neck area.

Conclusion: The novel HyperArc SRT treatment to large recurrent head and neck cancers is fast, safe, and effective treatment with promising LC rates and no adverse treatment related toxicity due to limitation of normal tissue exposure on account of the steep dose gradient of HyperArc. HyperArc confers effective local control with decreased toxicity to normal tissues.

Abstract 93

Clinical

Comparison of the Clinical and Genomic Profiles of Endometrial Cancers in Appalachian and non-Appalachian Patients

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In 2018, Kentucky had the highest incidence and mortality rate of gynecologic cancer in the United States; furthermore, there is increased incidence of endometrial cancer (EC) in the Kentucky Appalachian population than non-Appalachian population. The Kentucky population has been found to have more frequent DACH1 mutations than the general population. Further investigation revealed that DACH1 mutations are associated with a higher tumor mutation burden and several genome destabilizing mutations, which was present in the Kentucky population but also generalized using ORIEN data. To our knowledge, this is the largest study with complete molecular profiling of EC with clinicopathologic and outcomes data. Our study aims to 1) compare the molecular profile of the EC in the Appalachian and non-Appalachian populations; 2) assess molecular profile of subgroups including the TCGA groups, multiclassifiers, rare histologies and, 3) associate molecular profiles with demographic, clinicopathologic data, and outcomes.

This is a descriptive study in which the Total Cancer Center (TCC) enrolled, ORIEN Avatar subset was used to extract whole-exome tumor sequencing, RNA-sequencing, germline sequencing, and lifetime follow up. Zip code was used to define Appalachian status. Descriptive statistics were used to summarize molecular profiles, demographics, clinicopathologic characteristics, treatment outcomes, and survival. EdgeR will be used to compare gene expression profiles between groups of patients. Fisher's exact tests will be used to compare gene mutation frequencies between groups. Logrank tests and proportional hazards models will be used to assess associations between gene expression/mutation features and time-to-event outcomes. In all analyses, multiple comparisons adjustment will be performed by using the Benjamini-Hochberg procedure.

This is a brief preliminary summary of Appalachian versus non-Appalachian patient demographics and molecular profile. Complete WES, RNA sequencing, germline sequencing, and outcomes are pending. Appalachian status was incomplete and pending in 250 patients. The Appalachian population had a higher rate of serous adenocarcinoma and lower endometrioid adenocarcinoma than the non-Appalachian population (Table 1). TCC enrolled, Avatar subset resembles TCGA data regarding the four molecular subtypes of endometrial cancer.

Preliminary analysis suggests a different histology between Appalachian population, however all four molecular subtypes are represented (Figure 1a-c).

Abstract 94

Basic Science

Phosphorylation of AHR by PLK1 Promotes Metastasis of LUAD via DIO2-TH Signaling

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Metastasis of lung adenocarcinoma (LUAD) is a major cause of death in patients. Aryl hydrocarbon receptor (AHR) is an important transcription factor involved in the initiation and progression of lung cancer. Polo-like kinase 1 (PLK1), a serine/threonine kinase, is an oncogene that promotes the malignancy of multiple cancer types. Nonetheless, the interaction between these two factors and its significance in lung cancer remains to be determined. Here, we demonstrate that PLK1 phosphorylates AHR at S489 in LUAD, which leads to epithelial-mesenchymal transition (EMT) and metastatic events. RNA-seq analyses show that type 2 deiodinase (DIO2) is responsible for EMT and enhanced metastatic potential. DIO2 converts tetraiodothyronine (T4) to triiodothyronine (T3), which then activates thyroid hormone signaling. In vitro and in vivo experiments demonstrate that treatment with T3 or T4 promotes the metastasis of LUAD, whereas depletion of DIO2 or deiodinase inhibitor disrupts this property. Taken together, our results identify the phosphorylation of AHR by PLK1 as a mechanism leading to the progression of LUAD and provide possible therapeutic interventions for this event.

Abstract 95

Basic Science

Rad5 and its Human Homologs, HLTF and SHPRH, are Novel Interactors of Mismatch Repair

Anna Kristin Miller[1], Guogen Mao[1], Breanna Knicely[1], Hannah Daniels[1], Christine Rahal, Christopher Putnam, Eva Goellner[1]

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DNA mismatch repair (MMR) is the DNA repair mechanism that repairs base-base mispairs and small insertions or deletions remaining after replication. MMR is also required for apoptosis after certain types of exogenous DNA damage that result in damage-associated mispairs. The basic MMR mechanism is well understood; however, proteins associated with MMR continue to be identified, and the roles of these proteins in MMR are largely unknown. We have identified the yeast protein Rad5 as a novel interactor with the critical MMR proteins, Msh2 and Mlh1. Rad5 is a DNA helicase and E3 ubiquitin ligase involved in post-replicative repair. However, to date, Rad5 has no known role in MMR despite interacting with both two MMR factors. We show that deletion of yeast RAD5 does not have the mutation rate or mutation spectrum associated with defective canonical MMR. Rad5's interactions with MMR are conserved throughout evolution and split between its two human homologs, HLTF and SHPRH, with human MSH2 interacting with HLTF and human MLH1 interacting with SHPRH. Loss of HLTF, SHPRH, or both does not affect canonical MMR. SHPRH knockdown with siRNA or knockout with CRISPR/cas9 induces moderate resistance to MMR-mediated apoptosis. We recently confirmed that our HLTF and SHPRH knockout cells affect survival after exposure to DNA damage that is a substrate for post-replicative repair versus MMR and are currently investigating whether the interactions between HLTF, SHPRH, and the MMR proteins influence post-replicative repair pathways or pathway choice. This study defines a novel accessory factor that binds with MMR proteins and is conserved throughout evolution. Additionally, this study provides a deeper understanding of how accessory proteins factors may provide a mechanistic distinction between canonical and non-canonical MMR and how MMR influences post replicative repair pathways. HLTF and SHPRH are often disrupted in cancer, especially in endometrial tumors. Endometrial cancers often have MMR defects that are either sporadic or due to the hereditary cancer predisposition syndrome, Lynch syndrome. Understanding the interplay between the MMR pathway and other repair pathways has important implications in cancer development and treatment.

Abstract 96

Clinical

Addressing the Mental Health Disparities of Families (Caregivers/Next-of-Kin) of Cancer Patients from Rural Kentucky: A Clinical Study on the Efficacy to Reduce Mental Trauma Using the Mobile App Intervention FamCare+

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Families, caregivers, or next-of-kin (NOK) with a family-member receiving cancer treatment require medical updates, communication from staff, and proximity to their loved one. When the needs of family members are met, there are desirable consequences for both the cancer patient and NOK. Unfortunately, these needs are often challenged in the overall care plan. One study found that 54% of families had poor comprehension of the patient's diagnosis and treatment due to the lack of communication. Such a situation has a direct impact on the mental health of family members. Decades of compelling research have demonstrated that patient NOK are at higher risk for developing mental trauma such as post-traumatic stress disorder (PTSD), anxiety, depression, and disruptions to family relationships. For example, during the COVID-19 pandemic hospital visitation privileges were limited/suspended. The results were catastrophic for families, resulting in a severity of mental health conditions, even three months after patient discharge. Isolation due to the pandemic especially exacerbated the mental health of rural cancer patient NOK. Although a majority of rural communities use smartphones, racial and socioeconomic disparities still exist due (in part) to their geographic location. Added to the weight of socioeconomic inequalities, cancer patient NOK must travel long distances to stay connected to the bedside, where comfort can be found in knowing what is happening with their loved one. This is especially true for older Kentuckians, who are both limited financially and in their knowledge of the use of technology.

In exploring ways to support access to cancer patient health updates for NOK, we have developed a new mobile health (mHealth) intervention (FamCare+) that enhances communication and coordination between (remote) families and healthcare staff at point-of-care. The purpose of this mobile app service platform is to provide NOK a supplemental link to the bedside. Apart from its direct impact on reducing psychological trauma, maintaining communication, and the flow of bedside information, FamCare+ is intended to create informed families, with lower anxiety and depression, while building trust and satisfaction with healthcare services. Our recent UK usability and user experience study of the FamCare+ service provided extremely positive findings for all quantitative/qualitative data. Based on the positive response of these participants, we are currently forming a planning committee of community stakeholders, referred to as MERCCI (mHealth Equity Research for Community Connected-Health Impact). MERCCI is collaborating on identifying a strategy to study the efficacy of FamCare+ on cancer patient NOK mental health throughout rural Kentucky, particularly those with socioeconomic disparities. FamCare+ functionality provides families outside the clinical setting (in a remote location) with vitals/wellness updates, video conferencing, chat/texting, and counseling social/mental health services. Clinicians at the bedside input real-time information in qualitative measures, along with chat/video if needed. Clinical studies using FamCare+ with families at two Markey Cancer Center Research Network locations is currently in progress, with findings anticipated for late spring/summer 2023. Post study work will include co-design (feedback) studios for multi-stakeholder and family/community participation. MERCCI long-term goals and partnering opportunities are intended to produce inclusive research approaches, leading to meaningful future collaborations/studies that benefit community mental health of cancer patient families in impactful ways throughout Kentucky.

Abstract 97

Core Resources (Informational and not judged)

Markey Cancer Center Research Network Coordinating Center

Timothy Mullett[1,2], Joseph Alexander III[2], Marta Wood[2], Kris Damron[2], Melissa Shuey[2], Jane Noble[2], Yvonne Beatty[2], Lora Maynard[2], Ranjani Balasuriya[2]

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Established in 2016, the Markey Cancer Center Research Network (MCCRN) is an alliance of physicians conducting clinical research studies in the prevention, early detection, and treatment of cancers. The MCCRN conducts studies initiated by our own physicians and scientists, selected industry studies, as well as national studies available through the Markey Cancer Center's membership in the National Cancer Institute's National Clinical Trials Network. The MCCRN serves as a liaison between the Markey Cancer Center and investigators throughout Markey's catchment area. The network provides innovative research studies, support and education for our network research centers, and thorough quality assurance so our studies meet the highest ethical standards. By allowing patients across Kentucky and beyond to participate in clinical trials close to home, the MCCRN supports Markey Cancer Center's mission of reducing the cancer burden with a focus on Kentucky and its most vulnerable populations through research, prevention, treatment, education, and community engagement.

Research Collaborations & Development Opportunities: Achieving our mission requires collaboration and leadership among our members. We assist physicians and research programs in initiating or expanding their research portfolios, and selecting studies appropriate for their patient populations. The network is guided by the input of multidisciplinary healthcare professionals, including medical oncologists, radiation oncologists, radiologists, and surgeons involved in developing innovative approaches to cancer care. MCCRN provides expertise and guidance for MCC/MCCRN investigators and research teams. MCCRN offers education and training, study monitoring, budget and billing expertise, and regulatory support.

Markey Investigator-Initiated Trials: Because of our collaborative relationships, our investigator-initiated studies are developed with a unique insight to operations of community-based sites in mind, while maintaining compliance and integrity of the project. Research studies are targeted to focus both on the areas with the highest rate of disease and the types of cancers that most affect these regions.

MCCRN Coordinating Center: Services include network membership and onboarding, needs assessment and program development, research education and training, monitoring and research oversight, data management, protocol development and site selection, project management, contract and budget negotiation, and centralized processing of site and subject payments. We also facilitate interaction with other Markey programs including the Data and Safety Monitoring Committee, the Markey Quality Assurance Program, and the Molecular Tumor Board. Site Research Teams are provided with a variety of resources: CRA mentoring, screening support, recruitment materials, audit support, and assistance with IRB submissions.

MCCRN Members: Site membership requirements include regulatory review, a site assessment, research training, and submission of qualifying documents. A contractual relationship is established, and an onboarding process completed. Our members are dedicated clinicians qualified by relevant expertise and training, assuring quality conduct of clinical trials.

Research at Home: MCCRN allows patients throughout the state of Kentucky and neighboring Appalachia to participate in clinical trials while remaining at home under the direct care of their trusted local physicians.

Achievements: MCCRN sites have enrolled 837 patients to studies in 50/120 counties (40% of state), with 12 additional counties outside the state in OH, WV, and IN. The MCCRN program contributes to removing barriers to research participation and reducing the burden of cancer care by bringing important research opportunities into the communities we serve.

Abstract 98

Basic Science

PLK1-Dependent Phosphorylation of EZH2 Contributes to Its Oncogenic Activity in Castration-Resistant Prostate Cancer

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Enhancer of zeste homologue 2 (EZH2), the catalytic subunit of Polycomb-repressive complex 2 (PRC2), plays a critical role in repressing gene expression by tri-methylation of histone 3 at lysine 27 (H3K27me3). Emerging data have demonstrated that there is a link between EZH2 and oncogenesis as EZH2-mediated methylation acts as an important factor in epigenetic silencing of tumor suppressor genes in cancer. Expression of EZH2 is often upregulated in castration-resistant prostate cancer (CRPC), thus EZH2 has been proposed as a target for CRPC. Importantly, it has been demonstrated that EZH2 becomes hyperphosphorylated in CPRC cells. Further, it has been shown that the oncogenic function of EZH2 is regulated by these post-translational modifications. Polo-like kinase 1 (PLK1), a regulator of various stages of mitosis, has been shown high activity in CRPC. However, whether PLK1 is involved in EZH2 phosphorylation is not known. Herein, we show that Plk1 physically interacts with EZH2 and negatively regulates H3K27 trimethylation (H3K27me3). Furthermore, Plk1 can phosphorylate EZH2 at T144, and Plk1-mediated phosphorylation of EZH2 is involved in inhibiting EZH2 activity toward H3K27me3. More importantly, EZH2 phosphorylation by Plk1 is inhibitory for PRC2-mediated gene repression but required for gene activation toward oncogenesis. Finally, by combination with Plk1 inhibitor BI2536, we show a robust sensitization of EZH2 inhibitors in CRPC cell lines, as well as in CRPC xenograft tumors. Our findings provide a new mechanism to define the oncogenic activity of EZH2 and suggest that inhibition of Plk1-mediated EZH2 activity may provide a promising therapeutic approach for CRPC.

Abstract 99

Basic Science

Integrin a6b4 Upregulates Laminin Subunit Expression in Breast Cancer

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Prior research has demonstrated that integrin α6β4 is a major contributor of the aggressive properties of select carcinomas through its ability to alter the transcriptome. To function, integrin α6β4 must bind one of its ligands, which includes multiple isoforms of the extracellular matrix protein laminin. We have evidence that integrin α6β4 can alter the transcription of its ligands. The objective of this study is to determine how integrin α6β4 regulates laminin expression in breast cancer cells by assessing the change in expression of the individual subunits that make up the various laminin isoforms when expression of integrin α6β4 is knocked out through the deletion of the β4 subunit. Our hypothesis is that integrin α6β4 upregulates the expression of select laminin subunits that lead to an increase of at least one isoform of laminin. This study was conducted utilizing several techniques. First, RNA was extracted from MDA-MB-231 cells with and without integrin α6β4 expression. From the RNA, cDNA reverse transcriptase reactions were used to make cDNA for each sample that were subsequently used for q-PCR. For the q-PCR assays, expression of laminin subunit genes was compared against internal control gene, r18S. Results of this study showed the expression of laminin subunits LAMA3, LAMA4, and LAMC2 decreased significantly when integrin β4 subunit was knocked out in MDA-MB-231 cells. Western blotting confirmed the expression of LAMC2 at the protein level. Other laminin subunits did not showcase definitive results. To supplement these data, we utilized the cancer genomic database, cBioPortal. Analysis from cBioPortal of the TCGA breast cancer database showed a correlation between integrin β4 (ITGB4) expression and laminin subunits LAMA1, LAMB3, LAMC2, and LAMC3. In conclusion, these data show that cells that express integrin α6β4 upregulates the expression of its primary ligand, laminin 5 which contains laminin subunits α3, β3, γ2. This observation suggests that integrin α6β4 controls expression of its primary ligand, and therefore does not need externally supplied laminin to activate it. With further investigation, uncovering more intrinsic properties of integrin α6β4, in terms of epigenetics and the transcriptome, will be valuable to the field.

Abstract 100

Basic Science

A Point-of-Care Multi-Parametric Functional Optical Spectroscopy Platform for Longitudinal Monitoring of Tumor Metabolism and Vasculature

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Tumor metabolism reprogramming plays a crucial role in tumor survival and recurrence following therapy. Several tools with a variety of practical and scientific limitations are currently used to report on different endpoints to piece together a narrative on cell metabolism or vasculature. Unfortunately, none of them can simultaneously quantify the major metabolic and vascular parameters in vivo in a systems level view. Moreover, most of these devices are expensive and not user friendly, which make them have low user access. Therefore, there is a pressing need for easy-to-access metabolic tools to overcome these limitations. To address this challenge, we developed a highly portable multi-parametric functional optical spectroscopy platform with real-time data processing algorithms for longitudinal monitoring of tissue metabolism (glucose uptake and mitochondrial membrane potential) and vasculature (oxygen saturation (SO2) and total hemoglobin concentration) in small animals. Our novel optical device is intended for pharmacological studies in biomedical research laboratories, similar to the Seahorse Extracellular Flux Analyzer, but with the added capability of non-invasive in vivo investigations. To achieve this goal, we first built a point-of-care functional spectroscopy system for real-time diffuse reflectance and fluorescence measurement on small animals, using white LED light source, band-pass filters, and a custom-designed fiber probe. Secondly, we introduced ratio-metric techniques for real-time oxygenation quantification and fluorescence correction. This method allows rapid extraction of intrinsic 2-NBDG and TMRE fluorescence, enabling accurate measurements of metabolic parameters—glucose uptake and mitochondrial membrane potential (MMP) in solid tumor model. To validate our point-of-care spectroscopy and ratio-metric method as an effective tool to quantify vascular and metabolic features of tumors, we did tissue-mimicking phantoms study and are currently doing in vivo animal study in murine flank tumors models. This innovative platform is expected to have a broad impact on biomedical research, drug discovery, and clinical translation of therapeutic interventions.

Abstract 101

Translational

Emergency Granulopoiesis in Breast Cancer is Regulated by the Inflammation-Associated Receptors Toll-like Receptor 4 and Interleukin-1 Beta Receptor

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In many patients with breast cancer, increased circulating neutrophils were reported. This has been linked to an enhanced risk of metastasis and poor survival. Increased differentiation of granulocyte progenitors through a process called "emergency granulopoiesis" (EG) is possibly regulated by tumor cells. However, the molecular mechanism underlying tumor-mediated EG is not well established. This study aimed to shed light on the cross-talk between tumor cells and granulocyte progenitors in mouse models of triple-negative breast cancer. The proteomics and ELISA analyses of bone marrow supernatant of 4T1-bearing Balb/c mice (TB) showed that S100A8/A9 and IL-1β proteins were significantly increased compared to tumor-free mice. In vitro supplementation of S100A8/9 or IL-1β increased IL-1β and S100A8/9 secretion from neutrophils, respectively. IL-1βR was also upregulated in TB granulocyte/ macrophage progenitors (GMPs). In addition, both IL-1β and S100A8/A9 independently and corporately were able to promote colony-forming unit-granulocyte (CFU-G) formation and GMP proliferation. Moreover, tumor-secreted G-CSF directly increased S100A8/A9 and IL-1β release from neutrophils. Inhibition of S100A8/9 binding to TLR4 reduced the absolute number of GMPs in the bone marrow of TB mice. Inhibition of IRAK1/4, the common downstream kinase of TLR4 and IL-1βR signaling pathways, reduced GMP proliferation by decreasing NF-κB phosphorylation. Thus, our data support a new role of inflammation-associated receptors IL1-βR and TLR4 and their downstream signaling pathways in driving cancer-induced EG.

Abstract 102

Informatics/IT

Deep Learning Model for Breast Cancer Lumpectomy Specimen Margin Prediction on Radiography

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Background: Complete removal of breast cancer tumor with a negative specimen margin during lumpectomy is essential to minimize cancer recurrence. However, 2D radiography, the current method used by surgeons and radiologists to assess margin status in the operating room, has a margin sensitivity of only 36-58%, resulting in the need for subsequent surgeries in approximately one in four patients. This leads to additional healthcare costs, worse cosmetic outcomes, and significant patient stress.

Purpose: The objective of this study is to develop a deep learning model that improves the detection of positive margins in intraoperative lumpectomy specimens on radiographs.

Methods: In this HIPAA-compliant retrospective study, we collected data from 100 localized lumpectomy intraoperative specimen radiographs performed between 2020-2022 at the University of Kentucky and Markey Cancer Center Comprehensive Breast Care Center. We annotated the lumpectomy radiograph images with masking that denoted regions of known malignancy, non-malignant tissue, and the area of pathology-confirmed positive margin. We preliminarily trained the model with annotated radiographs of 22 lumpectomy specimens (9 positive margin; 13 negative margin) and performed preliminary positive vs. negative margin detection on an image level with pre-trained ImageNet ResNet-18. We plan to perform end-to-end automated segmentation on this dataset with state-of-the-art deep learning segmentation models to get pixel locations for positive, negative, and positive margins. We plan to evaluate our segmentation performance with dice and intersection-over-union metrics, allowing us to effectively detect pixel locations of our target margin.

Results: We are currently in the process of performing segmentation with deep learning. Our preliminary classification from this ongoing study has achieved 85.71%, 87.50%, 87.50%, 85.42% accuracy, f1, precision, recall, and ROC-AUC, respectively on the test set. We anticipate that all data collection and analysis will be completed before the symposium. We aim to develop a model that can identify subtle differences between non-malignant tissue and tissue containing lower densities of malignant cells consistent with positive margins identified by pathology. Identifying visually imperceptible indicators of positive lumpectomy margins could significantly impact the prevention of cancer recurrence and re-excision rates of breast cancer patients.

Conclusion: The purpose of this study is to develop a deep learning model that improves the detection of positive margins in intraoperative lumpectomy specimen radiographs. The findings of this research could potentially reduce the rate of lumpectomy re-excision and improve patient outcomes.

Abstract 103

Basic Science

Short-Term Exposure to Cigarette Smoke Activates an Epithelial-to-Mesenchymal Like Reprogramming in Human Bronchial Epithelial Cells

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Cigarette smoke is one of the main risk factors for lung cancer, which comes at the top of the list of cancer-related deaths in the United States in 2021. Cigarette smoke contains more than 7,000 chemicals, of which 250 have been identified as harmful and 70 as carcinogenic. While some of these compounds cause DNA damage and mutations that contribute to carcinogenesis, other tobacco smoke exposure effects on lung physiology are not as well understood and need to be investigated in more depth. The broad objective of our study is to examine the individual and combined effects of tobacco constituents on cellular and molecular endpoints observed in non-transformed human bronchial epithelial cells (HBEC). These HBEC cell lines (HBEC2, HBEC3KT, and HBEC14) were used as excellent precursor models for lung cancer and perhaps other human lung diseases. Cigarette smoke condensate (CSC) was derived from the new-generation reference cigarette 1R6F from the Kentucky Tobacco Research and Development Center in the College of Agriculture.

HBECs were exposed to different CSC concentrations over a short time frame, and a change in cell morphology was observed. Using high concentrations of CSC, the cells started to elongate after 24 h, while with low concentrations, more pronounced elongation was observed after 48 h. The effect of CSC is not only time-dependent but also dose-dependent. With a single treatment, doses > 40 ug/ml became cytotoxic, while doses < 5 ug/ml were too low to induce an observable change in cell appearance. At intermediate doses between 5-20 ug/ml of CSC, cells showed varying degrees of conversion into elongation in relation to the dose. Repetitive treatment with low CSC concentrations showed a more uniform effect after 72 h, which makes it more physiologically relevant because it mimics the chronic exposure of smokers to cigarette smoke. The morphological change was shown in all HBECs, but with different ratios; however, HBEC3KT was more sensitive to CSC and demonstrated a more uniform phenotype in more than 90% of the treated cells. CSC can also affect cell proliferation in all HBECs, where the number of treated cells gets reduced over time compared with the untreated ones, suggesting CSC's inhibitory effect on cell proliferation. The CSC-induced morphological change may reflect a reprogramming process called epithelial-to-mesenchymal transition (EMT). During EMT, epithelial cells convert from a cubic shape to a more elongated fibroblast-like shape, acquiring invasive and metastatic capacity. EMT is not only relevant to cancer progression but also to the remodeling of lung tissue related to a variety of pulmonary diseases. During EMT, several mesenchymal markers get elevated, while epithelial markers get reduced. Two days after exposure to CSC, all three HBECs showed an elevation in vimentin, one of the primary markers driving the EMT process. Vimentin elevation was directly proportional with the dose of CSC, where 20 ug/ml showed high expression while low expression with 5 ug/ml. We hypothesize that short-term exposure of bronchial epithelial cells to CSC and cigarette smoke, in general, can trigger a cellular reprogramming resembling EMT. This may be important in developing and progressing cancers and other lung diseases. Ongoing studies are underway to examine further the mechanism underlying it and whether it is EMT.

Abstract 104

Clinical

Examining Outcomes of Adult Patients Diagnosed with Acute Leukemia Based on Distance from Treatment Center

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Context: Studies have examined how various factors such as socioeconomic status or geography impact outcomes in acute leukemia patients. A retrospective study to determine whether living in an urban region or rural region affected overall outcome in patients with acute myeloid leukemia showed no difference in outcome. Another retrospective review examined distance living from leukemia center and survival in younger patients demonstrated distance of over 50 miles conferred worse outcomes for ALL patients, but not for AML. These studies highlight how geography could represent a metric to consider when assessing survival outcomes in acute leukemia patients. Distance to Markey Cancer Center effects on outcomes in leukemia treatment has not been investigated.
Objective: The objective of this study is to determine whether distance from MCC affects outcomes of patients being treated for acute leukemia and if there are significant demographic or cytogenetic differences between the populations.
Design: Using ICD-10 billing diagnosis, a retrospective chart review was conducted and identified all adults hospitalized with an of acute leukemia at the University of Kentucky from 2016 to 2022. Data pertaining to demographics and outcomes was compiled.
Patients: A total of 99 patients with acute leukemia were identified: 80 patients had AML, 15 ALL, 2 APL, and 2 acute leukemia-NOS. Average age of population was 53.8 years. M/F was 64/35. Patients divided into cohorts based on residing within 70 miles (54 patients) or more (45 patients) from MCC. (Range was 0-257 miles). In the local cohort, 45 AML patients included 11 FLT-3 positive (25%) and two TP53 mutated (4.4%). In the distant cohort, 36 AML patients with 14 FLT-3 positive (38.9%) and 7 TP53 mutated (19.4%).
Interventions: Patients received therapy based on subtype of leukemia. AML patients received either cytarabine and daunorubicin induction (with or without FLT3 inhibitor) or hypomethylating agent (with or without Venetoclax). ALL patients received some version of HyperCVAD. APL patients had ATRA with ATO or Idarubicin.
Outcomes: Patient demographic information: length of stay, number of blood/platelet transfusions, remission status at discharge, and survival at three and six months were collected and compared across each cohort.
Results: There was no significant difference in comorbidity between the two cohorts in respect to age, BMI, COPD, or diabetes. Patients in the local cohort had higher prior malignancy (35.1% versus 17.7%) and less cardiac disease (22.2% versus 35.5%). Both cohorts had similar length of hospital stay 35.3 d (local) vs 34.7 d (distant) and transfusion products: RBCs 6.3 vs 5.7, platelets 8.0 vs 9.2. Data showed 9.3% of local patients required ICU level of care versus 13.3% (distant). Remission rate for leukemia patients was 55.6% (local) opposed to 75.5% (distant). Three-month and six-month survival was 83% and 74% (local) versus 95.6% and 84.4% (distant).
Conclusions: In this single center population of adult patients with acute leukemia, there did not appear to be a significant difference in outcomes or transfusion requirements regardless of proximity to the leukemia center. There was a propensity towards higher incidence of other malignancies in the local cohort whereas there was an increase in cardiac disease in the distant cohort. Higher adverse cytogenetics were seen in the distant cohort. Early survival and remission rates for leukemia patients living far from leukemia center are favorable and should not preclude aggressive treatment.

Abstract 105

Translational

Effect of Oxygen in the Promotion of GBM-Derived EVs: An Insight into GBM Survival After Radiation Treatment

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Despite the multifaceted treatment approach including radiation therapy (RT), glioblastoma multiforme (GBM) often reoccurs, developing resistance to current therapies. To investigate the mechanisms that impart radioresistance in GBM, our laboratory has developed radiation-resistant glioblastoma cells that can survive clinically relevant doses of radiation using the GBM cell line LN18. Based on Trypan blue and Prestoblue cell viability assays, radiation-resistant cells LN18-RR-60Gy grew faster than the parental LN18 cells. Colony survival assay and 3D spheroid formation confirm that the LN18-RR-60Gy cell survives RT at a greater proportion than LN18 cells. Seeing as GBM is often characterized by regions of hypoxic conditions, we elected to investigate the effect of this atmospheric state on LN18-RR-60Gy survival. As expected, under 1% O2, RT decreases the 3-dimensional spheroid formation of LN18 cells but not of LN18-RR-60Gy cells. Given that extracellular vesicles (EVs), cell-derived membrane vesicles, and reactive oxygen species (ROS) are being released upon RT, we further examined the association of EVs and their redox-related protein cargo with the survival of radiation-resistant cells under hypoxic conditions. Following RT, the parental LN18 cells and LN18-RR-60Gy cells demonstrated an increase in EV number under 21% O2 as a coping mechanism to manage unneeded proteins and promote communication. In contrast, only parental LN18 cells demonstrated a decrease in EV number, but not LN18-RR-60Gy, under 1% O2. Intriguingly, upon RT, the levels of antioxidant proteins cargo such as manganese superoxide dismutase (MnSOD) in LN18-RR-60Gy-derived EVs are significantly higher at 21% O2 but lower at 1% O2, compared to non-RT EVs. The differences in EVs number and their cargo upon RT suggest that LN18-RR-60Gy survive RT during hypoxia, potentially by maintaining the number of EVs but altering cargo contents such as essential redox-related proteins MnSOD to maintain redox hemostasis balance.

Abstract 106

Translational

KDM3A Inhibitors Block Wnt Signaling and Repress Tumor Growth in Mouse Models

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Aberrant activation of Wnt signaling triggered by mutations in either Adenomatous Polyposis Coli (APC) or CTNNB1 (beta-catenin) is a hallmark of colorectal cancers (CRC). Wnt signaling also plays important roles in other types of cancers, including liver cancer and prostate cancer. As part of a program to develop epigenetic regulators for cancer therapy, we developed carboxamide-substituted benzhydryl amines (CBAs) bearing either aryl or heteroaryl groups that selectively targeted histone lysine demethylases (KDM) and functioned as inhibitors of the Wnt pathway. A biotinylated variant of N-(5-chloro-8-hydroxyquinolin-7-yl)(4-(diethylamino)phenyl)-methyl)butyramide (CBA-1) identified KDM3A as a binding partner. KDM3A is a Jumonji (JmjC) domain-containing demethylase that regulates the demethylation of histone H3's lysine 9 (H3K9Me2), a repressive marker that is significantly upregulated in CRC. Inhibiting KDM3A increased H3K9Me2 levels, repressed Wnt target genes, and curtailed in vitro CRC cell proliferation. CBAs also exhibited in vivo inhibition of Wnt signaling in a Zebrafish model and repressed tumor xenografts models of both colon cancer cells and prostate cancer cells in SCID mice.

Abstract 107

Translational

Targeting Energy Metabolism for Colon Cancer Treatment

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[1]UK Markey Cancer Center; [2]College of Medicine, Molecular and Cellular Biochemistry

Cancer cells undergo significant "metabolic remodeling" to provide sufficient ATP to maintain cell survival and to promote rapid growth. In colorectal cancer (CRC) cells, ATP is produced by mitchondrial oxidative phosphorylation (OXPHOS) and by substantially elevated cytoplasmic glucose fermentation (i.e., the Warburg effect). Glucose transporter 1 (GLUT1) expression is significantly increased in CRC cells, and GLUT1 inhibitors block glucose uptake and hence glycolysis crucial for cancer cell growth. In addition to ATP, these metabolic pathways also provide macromolecule building blocks and signaling molecules required for tumor growth. In this study, we identify a diaminobutoxy-substituted isoflavonoid (DBI-1) that inhibits mitochondrial complex I and deprives rapidly growing cancer cells of energy needed for growth. DBI-1 and the GLUT1 inhibitor, BAY-876, synergistically inhibit CRC cell growth in vitro and in vivo. We performed metabolomic study and RNA-seq study and found that DBI-1 regulates CRC cell metabolism at multiple levels.

Abstract 108

Translational

The Immune Microenvironment of Pancreatic Ductal Adenocarcinoma and Patient Derived Organoids

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Introduction: Pancreatic ductal adenocarcinoma (PDAC) is an aggressive cancer with an extremely poor five-year survival rate of 11%, and is on track to become the second leading cause of cancer death by the end of the decade. PDAC's poor prognosis is due in part to fact that a large percentage of patients have distant metastases at the time of diagnosis. There have been only slight changes in chemotherapeutic regimens since the introduction of FOLFIRINOX in 2011. Currently there is evidence to show that the tumor microenvironment of PDAC promotes resistance to chemotherapy, and given this insight, investigations into the composition of the PDAC TME are needed. We hypothesize that the PDAC tumor microenvironment (TME) contains significant populations of immune infiltrates and stromal cells, and these populations can be recapitulated in a 3D model.

Methods: Human surgical specimens were granted to us with the approval of the institutional review board for research use. The surgical specimens were mechanically and chemically digested for the creation of patient derived organoids and flow cytometry assays. Pieces of tissue were also paraffin embedded and used for immunohistochemistry. Digested tumor and daughter PDOs were stained with immune markers anti CD45, CD3, CD4, CD8, CD56, CD19, CD14, HLA-DR, and anti fibroblast activation protein (FAP) antibodies, and subsequently analyzed using a BD FACSymphony machine. Data was processed using FlowJo. On human tissue sections and daughter whole mount PDOs immunofluorescent staining was performed using antibodies against the immune markers CD45, CD3, CD4, CD8, CD19, CD56, CD14, interleukin receptor 2-α (IL-2Rα), and forkhead box protein 3 (FOXP3), and against the cancer associated fibroblast markers alpha smooth muscle actin (α-SMA) and FAP. Sections were additionally stained with CK19 to identify PDAC cells.

Results: Significant amounts of immune and stromal infiltrates were identified in the PDAC tissue specimens. Daughter organoids reflected the tumor microenvironment findings in the parental tumor specimens. Flow cytometry revealed large populations of T-killers (CD45+, CD3+, CD4-, CD8+), T-helpers (CD45+, CD3+, CD4+, CD8-), as well as populations of NK cells (CD45+, CD3-, CD56+), B-cells (CD45+, CD3-, CD19+), myeloid cells (CD45+, CD14+) and tumor promoting cancer associated fibroblasts (CAFs) (CD45-, FAP+) in both PDAC tissue and early passage organoids. Immunohistochemistry of the tissue showed populations of T-killers (CD45+, CD3+, CD8+), T-helpers (CD45+, CD3+, CD4), NK cells (CD45+, CD3-, CD56+), and the aggressively pro-tumor FAP+, α-SMA+ CAFs called myofibroblasts, as well as single positive CAFs. It was observed that the majority of the tumor space was occupied by CK19-, α-SMA+ cells. The presence of IL-2Rα+, FOXP3+ Tregs was also noted. Immunofluorescent staining of whole mount PDOs reflected the findings of the parental tissue with positive identification of CD45+, CD3+, CD8+ T-killers, CD45+, CD3+, CD4+ T-helpers, NK cells (CD45+, CD3-, CD56+), and FAP, α-SMA dual positive CAFs.

Discussion: Our findings show that the TME PDAC tissue is rich in diversity of immune infiltrates, which can significantly affect the tumor itself, and that this diversity is represented in their daughter PDOs. We will continue development of our 3D model and improve this model for the in vitro testing of cytotoxic therapy and immunotherapy in order to provide a personalized medicine approach based on the unique immunophenotype of each patient.

Abstract 109

Informatics/IT

An Efficient Solution for Gathering and Visualizing Catchment Area Geospatial Data

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Purpose: To provide an efficient way of gathering and visualizing publicly available data at various geographic levels for any cancer center catchment area.

Methods: We constructed programs in Python to access data from various publicly available sources through application programming interfaces, automated data downloads, and web scraping. This data was then manipulated into datasets at different geographic levels and exported as an organized collection of files. Two pathways for turning this data into interactive mapping applications were then constructed: one using ArcGIS Online and one using R Shiny. All code was structured to allow for automation of updates and generalized for easy adaptation to any cancer center catchment area structured as a set of U.S. counties.

Results: This process resulted in a comprehensive software solution licensed under the name of Cancer InFocus. Cancer InFocus creates a quick, efficient, and automatable mechanism for gathering much of the data necessary to characterize the cancer burden in any U.S. geographic area of interest and translating it into simple applications for either internal or external distribution. Cancer InFocus is available through a no-cost licensing agreement with the University of Kentucky. The functionality of Cancer InFocus is maintained and expanded upon by the online community of users who have chosen to adopt this platform.

Conclusions: Gathering and visualizing publicly available data on the cancer burden for a given cancer center catchment area at the county and census tract levels can be performed using modern computer programming techniques. This makes doing an initial assessment of the cancer burden more efficient, allowing greater time to be spent on developing strategic priorities and operationalizing insights. The use of open source tools to perform this task allows for its free dissemination to other institutions looking for a ready-made solution to characterize the quantitative data of their catchment area. This also demonstrates the ability to develop efficient solutions for gathering and visualizing geospatial data relevant to other disease fields.

Abstract 110

Translational

Effectiveness and Synergistic Effects Among Select PARP1/2 and ATR Inhibitors in Small Cell Lung Cancer Cell Lines

Samuel Clark[1], Tanvi Sawardekar[2], Amrita Machwe[1], Zhonglin Hao[3], David Orren[1]

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Respiratory cancers are the leading cause of cancer mortality in the U.S. and Kentucky has the highest lung cancer incidence, with small cell lung cancer (SCLC) as one of the most concerning. In this project, we have examined SCLC tumor cell lines for viability when treated with combinations of ATR and PARP inhibitors that are known or being investigated as relevant chemotherapeutic agents in order to observe potential synergistic effects. More specifically, the experiments used cultures of H196, H446, H524, H889, and DMS114 human tumor cell lines to test the ATR inhibitors elimusertib and berzosertib and PARP1/2 inhibitors olaparib, talazoparib, and niraparib. Olaparib and berzosertib are established chemotherapy treatments, while the other agents are in different stages of investigation. We have found that the degree of effect on viability varies considerably by cell line with H196 being less effected by treatments. Cell lines were generally more sensitive to elimusertib than berzosertib, and more sensitive to talazoparib and olaparib than niraparib, with all ATR inhibitors showing a decrease in cell viability when treating in combination with talazoparib. In general, the most sensitive combination of inhibitors was talazoparib/elimusertib with effects on cell viability with as little as 0.005 and 0.004uM respectively.

Abstract 111

Translational

Invasion, Metastasis, and Therapeutic Resistance in Colorectal Cancer

Tanisha Garg, Rakshamani Tripathi, Anastasia Lyon, Rina Plattner

UK College of Medicine, Pharmacology and Nutritional Sciences

Colorectal cancer (CRC) is one of the leading causes of morbidity and mortality. Activating the BRAF and MEK proteins in the MEK-ERK-MYC pathway leads to the ERK protein being activated, which is essential for CRC cell proliferation. Inhibiting these proteins using BRAF and MEK inhibitors has proven successful in the past, as it prevents the activation of the ERK protein. However, other cancers, such as melanoma, developed resistance and found another path to reach the ERK protein through a protein called ABL. The purpose of this project was to test whether ABL is present in the MEK-ERK-MYC pathway in BRAF-mutated CRC. To test its presence, cell proliferation, clonogenic, and western blot assays were used with different doses of Dabrafenib (a BRAF inhibitor), Trametinib (a MEK inhibitor), and Nilotinib (an ABL-kinase inhibitor). In cell viability and clonogenic assays, as Dabrafenib and Trametinib were added, cell survival and ATP production decreased. However, as Nilotinib was added, cell survival and ATP production significantly decreased. Similar results were found in western blots. Based on cell viability and clonogenic assays, it was shown that ABL-kinase inhibitors are involved in cell survival and proliferation while the western blots showed that they are involved in protein activation. Because cell proliferation, cell survival, and protein activation decreased after adding the ABL-kinase inhibitor, it can be said that ABL affects the MEK-ERK-MYC pathway. In the future, further tests can be performed to gather definite proof of ABL's presence.

Abstract 112

Translational

Understanding Lung Cancer Screening Resources and Needs in Appalachian Kentucky

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Appalachian Kentucky, an under-resourced rural region, has the highest rates of lung cancer incidence and mortality in the U.S. As lung cancer screening becomes increasingly available, in Appalachian Kentucky only 14% of those meeting guideline requirements are being screened. The aims of this study are to identify lung cancer screening health education material needs in the Appalachian Kentucky community and to uncover the social determinants of health (SDOH) affecting lung cancer screening for those inhabitants. We recruited five Appalachian KY residents who are eligible for lung cancer screening and not currently in a healthcare-based profession, to participate in 60-minute qualitative interviews. Here, we shared three lung cancer screening materials that are available online and asked the participants for their opinions. We then asked participants about specific SDOH in their community and how we can help members to access screening. The interviews were transcribed, and three independent coders analyzed the data to determine relevant themes. The insights gleaned from this study can help create better and culturally relevant infographics for this population. There are multiple SDOH at play for rural Appalachian Kentucky residents, which we have uncovered during our research, from accessing transportation to fear of discovering that they may have lung cancer. Future research can be conducted to determine how to improve access and understand the barriers to lung cancer screening. Overall, this study can help improve the disparate lung cancer incidence and mortality among individuals in this region through generating new, accessible health education materials on lung cancer screening.

Abstract 113

Translational

Leveraging Artificial Intelligence in the Characterization of Non-Small Cell Lung Cancer

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Despite decades of research, lung cancer remains one of the world's deadliest afflictions, with a five-year survival rate of only 28%. While recent advances in immunotherapy have offered hope to many, there is still a pressing need for additional treatment options. Epigenetic modulators, or drugs which alter the structure of chromatin in order to induce variations in gene expression, are one possible avenue for this advancement. In the case of non-small cell lung cancer (NSCLC), it has been reported that a key epigenetic regulator, the EZH2 subunit of the Polycomb Repressive Complex 2 (PRC2), is overactive. Typically, EZH2 operates by catalyzing the trimethylation (me3) of lysine 27 positions on histone 3 tails (H3K27), which then renders the associated genes unavailable for transcription. Unfortunately, many of the molecules necessary for immune self-surveillance are influenced by H3K27me3, causing EZH2 to take on a pathogenic role in tumor proliferation, though we do not fully understand the mechanisms involved. In this experiment we trained the HALO® artificial intelligence (AI) from Indica Labs to compare samples from 216 NSCLC patients. The tissue was stained for six immunohistochemical markers, EZH2, H3K27me3, B2M and HLA-DR,DQ,DP (representing the antigen presentation machinery MHC I and II, respectively), PD-L1 (programmed death ligand-1) and CBS (a determinant of methyl availability), with the goal of uncovering how epigenetic regulators impact immune signaling status. Interestingly, we found no significant correlation between H3K27me3 levels and EZH2 expression. We also discovered that while B2M and PD-L1 behaved in accordance with the standard model, CBS and HLA-DR,DQ,DP showed surprisingly positive associations with H3K27me3. This inconsistency may in part be explained by the individual characteristics of the major NSCLC phenotypes, with several outcomes suggesting that adenocarcinoma and squamous cell carcinoma have differing, or even opposing, epigenetic landscapes. Given the disconsonant nature of these results, it is clear that EZH2's role in tumorigenesis is not well understood and that additional investigation is required. To that end, next steps include supplemental training of the AI to refine its algorithm and an exploration of gene expression patterns in public databases to further validate the activity of these molecules.

Abstract 114

Translational

Combining Targeted and Epigenetic Therapies as a Treatment Approach for EGFR Mutated Non-Small Cell Lung Cancer

Christian M. Gosser, Xiulong Song, Christine Brainson

UK College of Medicine, Toxicology and Cancer Biology

Non-small cell lung cancer (NSCLC) is a growing problem across much of the population as it is frequently diagnosed and the leading cause of death from cancer. Within the histological subtypes of lung cancer, there are both squamous cell carcinomas and adenocarcinomas that can be driven by mutations in specific pathways. EGFR is a common driver of adeno-NSCLC, accounting for roughly 17% of cases of this subtype. Treatment of EGFR mutated NSCLC has evolved over years with several generations of EGFR inhibitors emerging throughout the past decade as first-line treatments in the clinic. However, resistance has emerged as a growing problem with EGFR, and more generally, tyrosine kinase inhibitors. EGFR-T790M is a second site mutation in response to first- and second-generation EGFR tyrosine kinase inhibitors. Osimertinib (AZD9291) was developed in response to the T790M mutation and has become first-line treatment for EGFR positive mutated lung cancer. Epigenetics, or the control of transcription through DNA organization, has emerged as a hallmark of cancer. The epigenetic Polycomb Repressive Complex 2 (PRC2) facilitates H3K27me3, an epigenetic silencing mark on histone 3 that represses transcription of genes on that region. The PRC2 inhibitor tazemetostat was recently FDA approved for other cancer types. The goal of this study is to establish if there is synergy between osimertinib and treatment tazemetostat as a possible combination for EGFR mutated NSCLC. We have found that osimertinib and tazemetostat synergize well in several EGFR mutated NSCLC cell lines and that this combination promotes cell death in the target cells. Our future work will test this drug combination in vivo and test potential mechanism of drug synergy. This work is funded by NCI CA 237643, NCI K22 career transition award, and many SRFs (P30 CA177558).

Abstract 115

Basic Science

Using EZH2 Inhibitor as a Tool for Targeting Lung Disease

Dave-Preston I. Esoe[1], Xiulong Song[1], Christine Brainson[1,2]

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Lung disease, including chronic obstructive pulmonary disease, fibrosis, and cancer are major health problems and together lead to the deaths of millions of individuals each year. Pulmonary fibrosis is a life-threatening respiratory disease caused by lung tissue inflammation, which leads to scarring and damage. People with fibrosis have more than a two-fold higher risk of lung cancer development than smokers without fibrosis, underscoring the importance of findings treatments to reverse fibrotic phenotypes. Anti-inflammatory and anti-fibrotic drugs have been shown to slow the progression of pulmonary fibrosis; however, there are no current methods to prevent or cure this disease. Various literature has identified Enhancer of zeste Homolog 2 (EZH2), which is responsible for mediating histone 3 lysine 27 trimethylation, as a key regulator of fibrosis in injured lungs. Commonly referred to as EPZ-6438, tazemetostat is an epigenetic drug that specifically targets and inhibits EZH2. FDA-approved in 2020, EPZ-6438 serves as an oral drug which provides a reliable and therapeutically tolerable treatment option for diseased patients. We believe that using EZH2 inhibitor to target cells that promote fibrosis could prevent or possibly reverse fibrosis. One of many pathways that have been identified in promoting fibrosis is the transition of Endothelial cells to Mesenchymal cells (ENDMT). Post-inflammation, pro-inflammatory cytokines such as TGFB, IL1B, and TNF-alpha cause the endothelial cells to lose their endothelial-specific markers and develop a more mesenchymal phenotype as well as express mesenchymal gene markers. For our current study, we use lung endothelial cells derived from c57BL/6 mice and treated them with cytokines in the presence of EZH2 inhibition. So far, our findings suggest that in mouse endothelial cells these cytokines promote endothelial differentiation, which is opposite of what is seen in human experiments. For further investigation, human endothelial lung samples will be used to investigate preventing ENDMT with use of EZH2 inhibition. We have also tested inhibiting the fibrotic response to bleomycin in mouse lungs with EPZ6438 and observed sting reduction in bleomycin response, which may be due in part to a reduction in inflammatory response. Further understanding the effects of EPZ6438 on each cell compartment in the lung will be essential for translating this drug for use in many lung diseases, including fibrosis and cancer.

Abstract 116

Translational

Defining the Role of EZH2 in the Tumor Microenvironment of Squamous Lung Cancer

Tanner J. DuCote[1], Xiulong Song[1], Xufeng Qu[2], Jinze Liu[2], Kwok-kin Wong[3], Christine Brainson[1]

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Immune checkpoint inhibitors (ICI) have become first line therapy for lung squamous cell carcinoma (LUSCC); however, ~80% of patients do not respond. To overcome the lack of response to ICI therapy, it is important to understand the fundamentals of resistance that occur. The work that our group has done demonstrates that the histone methyltransferase EZH2 controls expression of antigen presentation machinery and immunogenicity in LUSCC. Our central hypothesis is that EZH2 inhibition (EZH2i), when combined with an anti-PD1 ICI, will increase response and durability grounded on evidence from our group and others. In vitro studies from our laboratory show that combining IFNγ with EZH2i increases expression of MHC I and II expression in both 2D and 3D human and murine lines. MHC I and II are vital to activating the immune system by presenting antigens to T cells; therefore, this is evidence that this combination can encourage tumor elimination. Moreover, work from others has suggested that MHC I and II expression are vital for tumor elimination in different pre-clinical models of cancer. To study this further, we performed in vivo studies combining EZH2i with anti-PD1 ICI in two separate models of LUSCC. Through the ablation of Lkb1 and Pten we can recapitulate LUSCC in mice that resembles the human disease in an autochthonous manner as well as a syngeneic subcutaneous model. Furthermore, these models resemble the tumor microenvironment which include tumor-associated neutrophils (TANs) which can be immunosuppressive. This phenomenon is due to the ability for TANs to produce molecules such as cytokines, chemokines, and arginase which all lead to an immunosuppressive TME. We confirmed tumor burden through the utilization of MRI scans and caliper measurement and then placed mice on one of four treatment arms. The treatment modalities chosen were placebo and IgG isotype controls, as well as single agent EZH2i and anti-PD1 ICI, or a combination of both. After treatment we observed significant tumor control in the groups treated with EZH2i alone. Furthermore, we observed a significant decrease in tumor volume in the group treated with the combination therapy in the autochthonous cohort. This finding is incredibly exciting because of the issues of eligibility for, and tolerability to treatment of ICIs, as well as disease progression. We then did scRNAseq on dissociated tumors from the autochthonous model and observed five distinct TANs populations. What we discovered were significant decreases in populations of neutrophils which we believe are immunosuppressive. Additionally, we observed increases in TAN populations believed to be involved in tumor elimination. These data lead us to believe that combining EZH2i with anti-PD1 ICI and EZH2i alone are viable modalities for use in the clinic, which is currently in clinical trials of other disease types. This work is funded by R01 CA237643, T32 ES007266-30, and P30 CA177558 for Markey Cancer Center shared resources.

Abstract 117

Translational

Investigating the Roles of LKB1 in Non-Small Cell Lung Cancer Linage Fate

Kassandra J. Naughton, Xiulong Song, Christine Brainson

UK College of Medicine, Toxicology and Cancer Biology

The two major forms of non-small cell lung cancer (NSCLC), adenocarcinomas (LUAD) and squamous cell carcinomas (LUSC), are identified by distinct histology. These differences as well as variations in genetic mutations contribute to the divergent treatment modalities. A particular genetic subtype of NSCLC, with mutations in both KRAS and LKB1 (aka STK11), presents mostly as LUAD histology with lower survival rates compared to their KRAS-only counterparts. In addition, tumors with KRAS and LKB1 mutations have low levels of PD-L1 expression and respond poorly to immunotherapy. However, this data was limited to only LUAD in the clinic. We have shown that both LUSC as well as an adneosquamous histology, possessing characteristics of both LUAD and LUSC, develop in a Kras/Lkb1 mouse model. Evidence indicates that a transition from LUAD to LUSC in this mouse model is driven by a reduction in Polycomb Repressive Complex 2 (PRC2) activity with the deletion of LKB1. In addition, PRC2 is supplied with methyl groups to methylate DNA via the methionine pathway. Therefore, we predict that methionine restriction will enhance treatment efficacy in KRAS/LKB1 mutant NSCLCs via alterations to PRC2 activity. From this Kras/Lkb1 mouse model, we developed two tumoroid models: one LUAD (3690) and one LUSC (3650). Unsurprisingly, we saw high levels of expression of LUSC markers Sox2 and Krt5 in the LUSC organoids and higher levels of LUAD markers Ccsp and Spc in the LUAD organoids. In addition, we observed very divergent responses to a treatment with EZH2 (of PRC2) inhibitor tazemetostat (EPZ-6438) and IFN-gamma: LUSC organoids had significant increases in MHCI, MHCII, and PD-L1 while the LUAD organoids did not. This indicates an important role for the histology and PRC2 activity status in these NSCLCs on the response to immunotherapy. Furthermore, methionine metabolism and its role in PRC2 activity is being investigated. Thus far, we have rescued LKB1 and overexpressed (OE) cystathionine-beta-synthase (CBS), an important regulator of the methionine cycle, in A549 cells. We have found that rescue of LKB1 and OE of CBS decreases H3 lysine 27 trimethylation (H3K27me3, a PRC2-mediated histone modification). We have also employed altering concentrations of methionine in the growth media of A549 cells and normal lung epithelial cells with activated KRAS (BEAS-2B + KRAS) and observed that lowering methionine leads to increased carboplatin sensitivity and lowered soft agar colony formation. Lastly, the Kras/Lkb1 model was used to investigate the effect of methionine restriction on tumor progression and PRC2 activity. We found that mice on a diet of low methionine had decreases in tumor progression compared to those on a diet of regular levels of methionine. Currently, this same diet is also being used to investigate if methionine restriction can increase the efficacy of chemotherapy treatments. These investigations will provide a greater understanding of how methionine restriction could regulate PRC2 activity as well as determine which NSCLC histology may respond more positively to specific treatments.
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