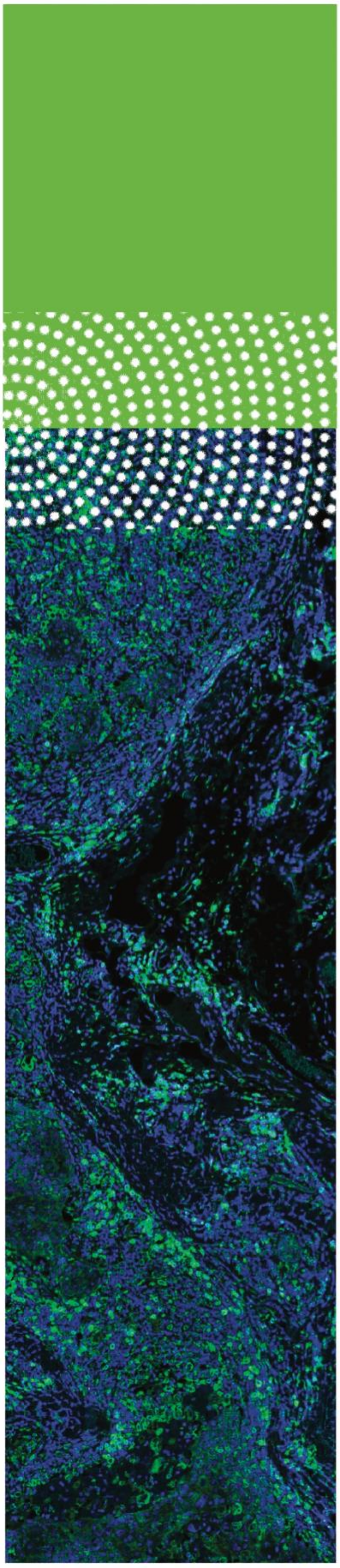


Markey Cancer Center
An NCI Comprehensive Cancer Center

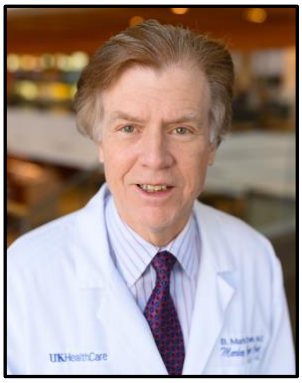
MARKEY CANCER CENTER
RESEARCH DAY

PRESENTED BY THE MARKEY CANCER FOUNDATION

Discover the Latest Advances in Cancer Research

May 10, 2024 | 8 a.m. – 5 p.m.
UK Gatton Student Center

Markey Cancer Center
An NCI Comprehensive Cancer Center



May 10, 2024

Dear Colleagues and Friends,

What a historic year it has been for the Markey Cancer Center. Since we gathered last May, we successfully renewed our NCI Cancer Center Support Grant and achieved the designation as an NCI Comprehensive Cancer Center; we broke ground on the new Cancer and Advanced Ambulatory building; our research community grew by more than 30 new cancer researchers; and for a seventh year in a row Markey was recognized as a top 50 cancer hospital by U.S. News and World Report.

All these milestones are underpinned by the extraordinary work you do and it is transformative for Kentucky. From researchers and clinicians to trainees and staff, our team moved the needle closer to our mission for a *cancer-free tomorrow*. And what better way to showcase some of your notable work than with our fourteenth-annual Markey Cancer Center Research Day. As I said last year, this event is one that I look forward to each year. No other event spotlights the depth and breadth of the Markey Cancer Center. This year we return to the UK Gatton Student Center to showcase the impactful work of researchers from numerous disciplines and colleges across the University of Kentucky, each highlighting the latest advancements in cancer research. This year, more than 100 posters will be on display representing all aspects of cancer research, prevention and control, treatment and clinical care.

The Faculty Oral Presentations will feature two of Markey's rising scholars—Yasminka Jakubek Swartzlander, PhD, and Samuel Awuah, PhD—and the Student Oral Presentations will offer four graduate students the opportunity to share their work.

This year we are fortunate to welcome two exceptional keynote speakers: Wei Zheng, MD, PhD, MPH, Professor and Director of Division of Epidemiology at Vanderbilt University School of Medicine and the Associate Director for Population Sciences at Vanderbilt-Ingram Cancer Center, will present the Gilbert H. Friedell, MD, Memorial Lecture; and Raymond N. Dubois, MD, PhD, Director and Associate Provost of Medical University of South Carolina Hollings Cancer Center, will present the Susan B. Lester Memorial Lecture.

I must thank our exhibitors, advertisers, and the UK Markey Cancer Foundation for their continued support in making this day a success, and for their help in furthering our educational mission throughout the University of Kentucky campus. Today, please take the opportunity to interact with our poster presenters, exhibitors, and Markey community. I'm eager to hear your comments and look forward to your suggestions for future Markey Cancer Center events.

Sincerely

B. Mark Evers

B. Mark Evers, MD

MARKEY CANCER CENTER
RESEARCH DAY
Program Contents

Markey Cancer Center
An NCI Comprehensive Cancer Center

Markey Cancer Center Research Day 2024
Agenda

Morning

8:00 – 8:30	Registration and Breakfast
8:30 – 9:30	Student Oral Presentations
	Mariah Geisen: Targeting Lipid Metabolism to Improve Efficacy of BRAF-Targeted Therapy in Colorectal Cancer
	Jessica Amezcua: HPV Vaccine Uptake and Completion among Hispanic Adults in Kentucky
	Murong Ma: Lipid Metabolism Reprograming Triggered by Spermine Synthase Inhibition Creates a Therapeutic Vulnerability for Targeting Ferroptosis in Colorectal Cancer
	Namrata Anand: Identification of Target Protein Molecules Responsible for M2 to M1 Macrophage Repolarization
9:30 – 10:30	Faculty Oral Presentations
	Samuel Awuah, PhD: Discovery of a Small Molecule Inhibitor of ARID4B
	Yasminka Jakubek Swartzlander, PhD: Chromosomal Alterations across the Cancer Continuum
10:30 – 11:45	Poster Presentation Session #1
11:30 – 1:00	Lunch Break

Afternoon

12:15 – 1:30	Poster Presentation Session #2
1:30 – 2:30	**Wei Zheng, PhD**: Gilbert H. Friedell, MD, Memorial Lecture: Epidemiologic and Genomic Research in Diverse Populations to Address Health Disparities and Gain New Insights into Cancer Etiology and Biology
2:30 – 2:40	Break
2:40 – 2:45	**Markey Women Strong Awards**: Presented by the Markey Cancer Foundation
2:45 – 3:15	**B. Mark Evers, MD**, Director of the Markey Cancer Center: State of the Cancer Center Address
3:15 – 4:15	**Raymond N. DuBois, MD, PhD**: Susan B. Lester Memorial Lecture: Cancer Prevention: Links to the Immune Response
4:15 – 4:25	Poster and Mentor Awards Presentation
4:20	Reception

Student Oral Presenters

Mariah Geisen

Targeting Lipid Metabolism to Improve Efficacy of BRAF-Targeted Therapy in Colorectal Cancer

Mariah Geisen is a 4th year Ph.D. candidate in Dr. Kate Zaytseva's laboratory in the Department of Toxicology and Cancer Biology in the College of Medicine at the University of Kentucky. Her project focuses on development of novel therapeutic strategies for BRAF mutant colorectal cancer. More specifically, her project focuses on understanding the mechanisms of resistance to a second-generation BRAF inhibitor, plixorafenib (PLX8394). The main goal in this project is to understand the mechanisms of resistance to plixorafenib to increase the efficacy of this drug and improve clinical outcomes for patients with BRAF mutant colorectal cancer. Mariah's studies show that upregulation of lipid metabolism contributes to plixorafenib resistance and inhibition of fatty acid synthase, a key enzyme of de novo lipid synthesis, increases efficacy and postpones development of resistant to plixorafenib. Mariah obtained her undergraduate degree in biology and a minor in biochemistry at Bellarmine University in Louisville, Kentucky. As a graduate student at the Department of Toxicology and Cancer Biology, she has presented her work at multiple scientific meetings including an invited presentation at the Dean's Distinguished Lecture Series.

Jessica Amezcua

HPV Vaccine Uptake and Completion among Hispanic Adults in Kentucky

Jessica is a Markey STRONG post-baccalaureate fellow. She has a background in public health and earned her bachelor's degree at Eastern Kentucky University. Previously, she worked with Dr. Canedo to address social inequalities affecting Hispanic/Latino access to cancer care through community-engaged research. Currently, she is engaged in research with Dr. Moore, examining health disparities resulting from the interplay of social identity, place, and genetics on cancer health outcomes. Jessica is committed to advancing health equity in cancer care, striving to ensure that all individuals have fair and equal opportunities to lead healthy, cancer-free lives.

Murong Ma

Lipid Metabolism Reprograming Triggered by Spermine Synthase Inhibition Creates a Therapeutic Vulnerability for Targeting Ferroptosis in Colorectal Cancer

Murong Ma is a Ph.D. candidate under the guidance of Dr. Qing-Bai She in the Department of Pharmacology and Nutritional Sciences in the College of Medicine, University of Kentucky. She earned her MS in Nutritional Sciences at UK. Her current project focuses on elucidating the mechanism underling lipid metabolism reprogramming upon spermine synthase depletion in colorectal cancer cells. Her overarching objective is to identify an innovative therapeutic strategy tailored to combat spermine synthase overexpressing colorectal cancer.

Namrata Anand

Identification of Target Protein Molecules Responsible for M2 to M1 Macrophage Repolarization

Dr. Namrata Anand is a Scientist I in the College of Pharmacy at the University of Kentucky. Dr. Anand obtained her Ph.D. from the Postgraduate Institute of Medical Education and Research, Chandigarh, India, where she worked on therapeutic applications of lactoferrin protein nanoformulation on parasitic diseases. Her postdoc at the University of Kentucky had an emphasis on parasite and cancer immunology (November 2019 to March 2024). She has been invited twice to an international nanomedicine conference in Switzerland to present her work. She has extensive experience in parasitology and cancer immunology. She has authored twelve peer-reviewed publications of which she was first author on seven.

Faculty Oral Presenters

Yasminka (Sasha) Jakubek Swartzlander, PhD

Assistant Professor, Biomedical Informatics
University of Kentucky College of Medicine
Cancer Prevention and Control Research Program
Markey Cancer Center

Chromosomal Alterations across the Cancer Continuum

Dr. Jakubek Swartzlander's research is focused on mutations that humans acquire with age. The goal of her work and others in the field is to understand which mutations are associated with higher risk for chronic disease such as cancer. She investigates factors that cause these mutations such as environmental exposures and inherited mutations. Through this work, she wants to improve how to identify people at high risk for disease and tailor approaches for disease prevention.

Samuel Awuah, PhD

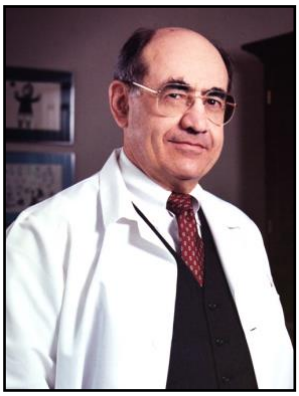
Associate Professor, Chemistry (College of Arts & Sciences)
Joint Appointment, Pharmaceutical Sciences (College of Pharmacy)
Translational Oncology Research Program
Markey Cancer Center

Discovery of a Small Molecule Inhibitor of ARID4B

Dr. Awuah's research program explores the chemical synthesis and biological impact of organic and transition metal complexes as a basis for chemical probe and therapeutic lead discovery. Recent contributions in his lab towards nano-based drug delivery continues to grow. Dr. Awuah's early studies contributed significantly to the development of photodynamic therapy agents that absorb in the near-IR region for deeper tissue penetration of solid tumors (Org. Lett. 2011; J. Med. Chem. 2013; JACS 2014). His attention quickly shifted to understanding and developing transition metal compounds for cancer therapy. Notable contributions in this area include unraveling the role of high mobility group box protein 4 (HMGB4) in sensitizing testicular germ cell tumors to the FDA approved platinum drug, cisplatin (PNAS 2017). Other discoveries included platinum, rhenium, osmium transition-metal compounds as anticancer agents (JACS 2015; ACS Nano 2016; JACS 2016).

In Dr. Awuah's independent laboratory at the University of Kentucky, he has been intrigued by the central role of the mitochondria and mitochondrial metabolism in physiological and pathophysiological conditions including cancer, neurodegenerative disorders, infectious diseases, and inflammatory bowel diseases. To this end, his laboratory has taken advantage of expanding the structural diversity of gold-based compounds to modulate distinct mitochondria processes, including mitochondrial morphology and biogenesis (Chem. Sci. 2021; JACS Au 2021; Chem. Sci. 2020). Dr. Awuah's work has reignited enthusiasm to investigate the effects of gold and other metal/metalloid compounds in perturbing cellular metabolism, chemical biology, and their application in biomedicine including anticancer and antimicrobial therapies (J Med. Chem 2019; Inorg. Chem 2019; Nature Sci. Rep. 2019, Inorg. Chim. Acta 2020). Opportunities to leverage synthetic chemistry to resolve long-standing biomedical problems is his passion. They have developed an innovative drug discovery campaign that deploys artificial intelligence, molecular docking, synthetic chemistry, in vitro assay prioritization, and relevant animal models to identify hit-to-lead compounds, serving as probes and therapeutic agents for c-MYC, ARID4B, KRAS mutants and SHP-2 protein targets. Dr. Awuah envisions the development of their therapeutic compounds as safe and potent agents for the cure of diseases in humans including lung cancer.

Gilbert H. Friedell, MD Memorial 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 PI 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.

Wei Zheng, MD, PhD, MPH

Associate Director for Population Science Research
Anne Potter Wilson Professor of Medicine
Director, Vanderbilt Epidemiology Center
Chief, Division of Epidemiology
Vanderbilt-Ingram Cancer Center

Dr. Wei Zheng is Professor and Director of Division of Epidemiology at Vanderbilt University School of Medicine and the Associate Director for Population Sciences at Vanderbilt-Ingram Cancer Center. He has published more than 1,250 research papers and served as the principal investigator for more than 35 NIH-funded large epidemiologic and genetic studies. His research focuses mainly on the nutrition, molecular and genetic epidemiology of cancer. Dr. Zheng has been the primary mentor for more than 80 graduate students, postdoc fellows and junior investigators.

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.

Raymond N. DuBois, MD, PhD, FAAAS, FAGA, FAACR, FRCP (London)

Director, MUSC Hollings Cancer Center
Associate Provost for Cancer Programs at MUSC
MUSC Distinguished University Professor
Charles W. Coker Endowed Chair in GI Cancer
Medical University of South Carolina, Charleston, SC

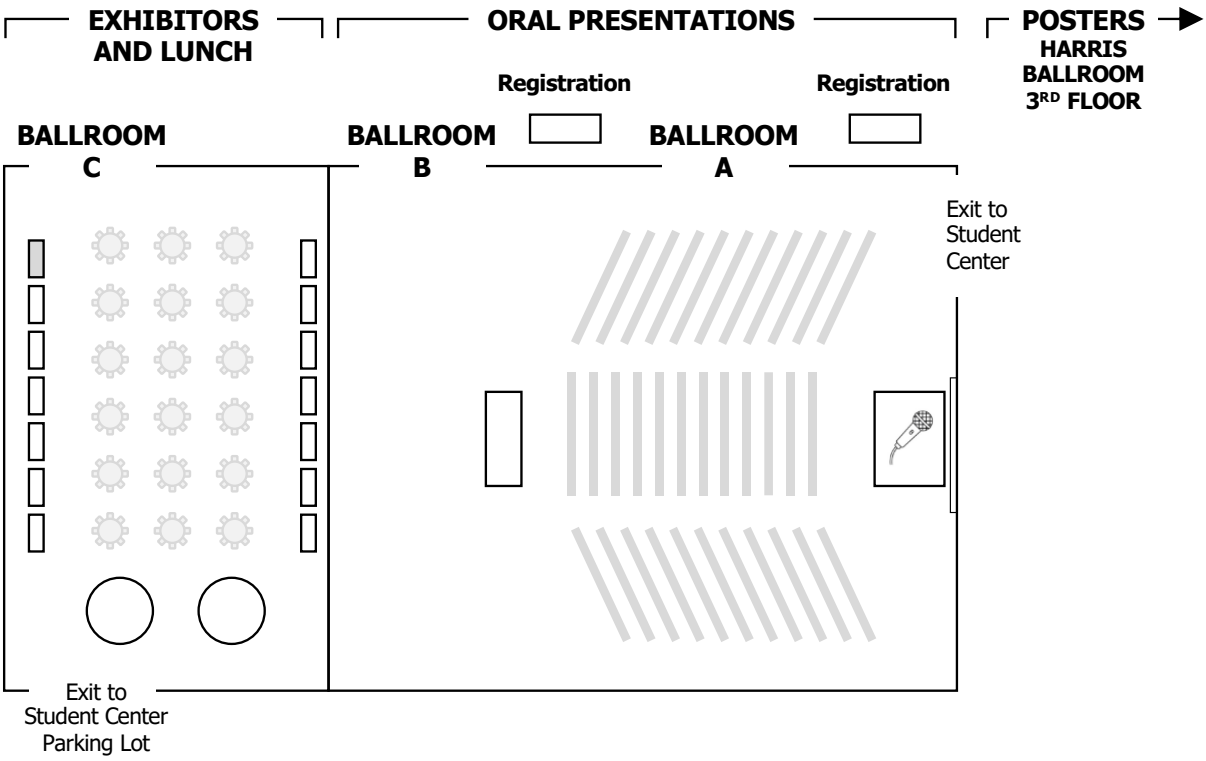
Dr. Raymond N. DuBois is a renowned physician-scientist and cancer researcher who has made significant contributions to understanding the role of inflammation and inflammatory mediators in the progression of colon cancer and other gastrointestinal malignancies. His work has led to a better understanding of the molecular basis for anti-inflammatory agents, such as aspirin, in reducing cancer risk. It has also led to clinical trials showing how drugs that inhibit this pathway could prevent or intercept the cancerization process.

Dr. DuBois has over 160 peer-reviewed research articles out of 274 publications, more than 72 review articles, 25 book chapters, and three books during his career, and his work has been cited over 70,000 times with an H-index of 120. He is also a co-inventor of a method to identify and target cellular genes needed for viral growth and cellular genes that function as tumor suppressors in mammals, which led to the creation of a biotech company that utilized this technology to discover new drug targets for the treatment of cancer and viral infections.

DuBois has been recognized with numerous awards for his cancer research, including the Richard and Hinda Rosenthal Research Award, the Dorothy P. Landon-AACR Cancer Prize, the Margaret Foti Award for Leadership and Extraordinary Achievements in Cancer Research, the AACR Distinguished Service Award, and the Anthony Dipple Carcinogenesis Award from Oxford University Press. He was inducted as a National Academy of Medicine member in 2019. He is a Fellow of the American Association for the Advancement of Science, the Academy of the American Association for Cancer Research, the American Gastroenterology Association, and the Royal College of Physicians (London). In addition, he currently holds the position of Executive Chair of the Mark Foundation for Cancer Research and was previously Vice Chair of the Stand Up to Cancer Foundation as well as President and Chair of the AACR Foundation Board.

Dr. DuBois earned a bachelor's degree in Biochemistry with honors from Texas A&M University, a Ph.D. in Biochemistry from The University of Texas Southwestern Medical Center, and a medical degree from The University of Texas School of Medicine in San Antonio. In addition, he completed his internship/residency in medicine and a gastroenterology fellowship at Johns Hopkins Hospital, where he studied under Nobel Laureate Daniel Nathans as a Howard Hughes Research Associate.

Site Map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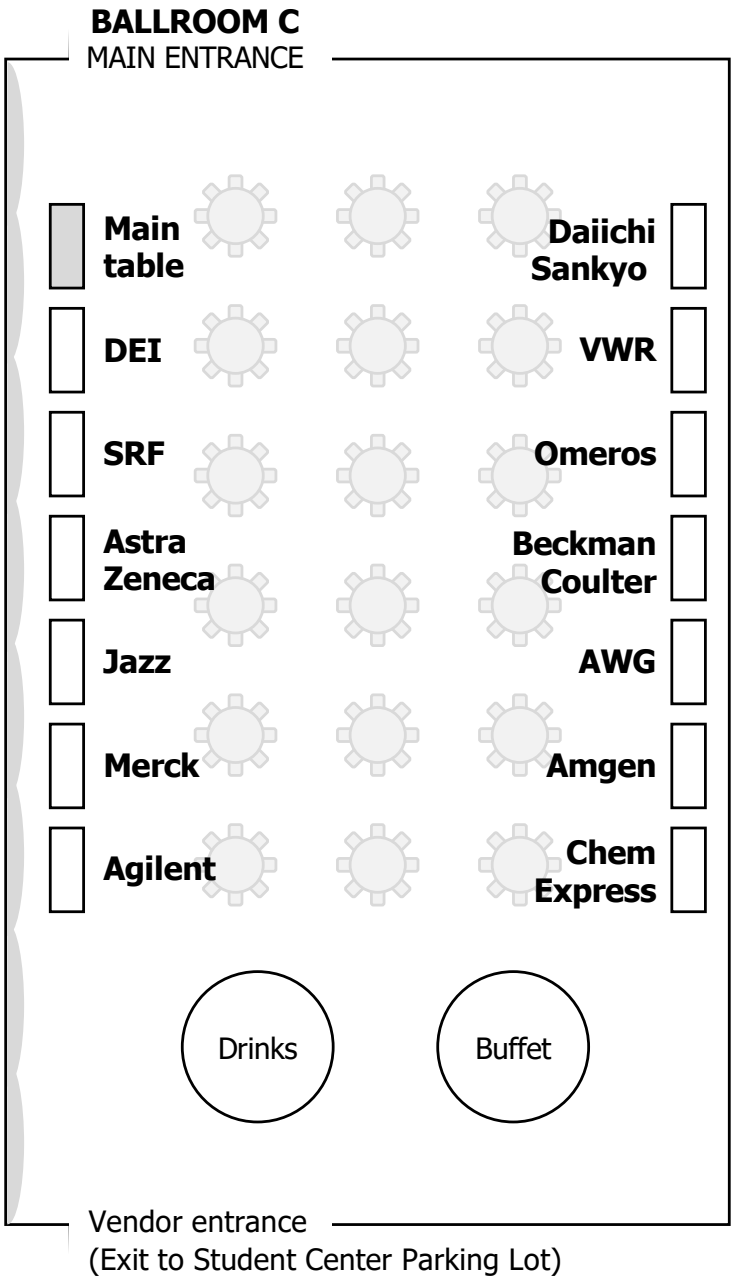

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Abstract 1

A Phase 1 Dose Escalation Study of Lapatinib and Paclitaxel in Recurrent Ovarian Cancer

Connie D. Cao[1, 2], J. Robert McCorkle, Donglin Yan, Rani Jayswal, Rachel W. Miller, Charles S. Dietrich, Frederick R. Ueland, Jill M. Kolesar

[1]UK Markey Cancer Center; [2]UK College of Medicine, Department of Obstetrics and Gynecology

Objectives: Paclitaxel is a commonly used chemotherapy agent for women with recurrent ovarian cancer; however, its efficacy is limited by the development of ABCB1-mediated drug resistance. Lapatinib is an EGFR and HER2 inhibitor approved for treating breast cancer but without single agent activity in ovarian cancer. Lapatinib is also an ABCB1 inhibitor, and we hypothesize that lapatinib could prevent the development of ABCB1-mediated paclitaxel resistance.

Methods: This phase 1 dose escalation study used a Bayesian optimal interval (BOIN) design to determine the recommended phase 2 dose of the combination of paclitaxel and lapatinib. Secondary endpoints were to assess adverse effects, determine the objective response rate, and assess pharmacokinetics. Eligible patients had ovarian, peritoneal, or fallopian tube cancer and had recurred within 12 months of platinum-based chemotherapy. Patients received three weekly paclitaxel doses of 80 mg/m2 in 28-day cycles. Patients were pretreated with escalating doses of lapatinib twice daily in the 48 hours preceding paclitaxel for up to 3 cycles starting with the second paclitaxel dose of cycle 1. Three lapatinib doses were evaluated, escalating from 750 to 2000 mg orally twice daily.

Results: Fifteen of nineteen patients were eligible and evaluable for efficacy and toxicity. All were female, the median age was 66, and the median number of prior therapies was 3. Three patients were treated at dose level 1, six at dose level 2, and seven at dose level 3. One patient treated at dose level 3 was not evaluable for DLT. There was one dose-limiting toxicity (DLT) in dose level 2 (diarrhea) and another in dose level 3 (elevated liver function tests), with a posterior DLT estimate of 0.17, 95% Credible Interval of (0.01, 0.53) for dose level 3. The most common grade 1-2 adverse effects were diarrhea (75%) and neutropenia (43.8%). Among the 15 evaluable patients, there were two partial responses and one stable disease for an objective response rate of 13% and a disease control rate of 20% in this heavily pretreated population.

Conclusion: The combination of paclitaxel and lapatinib is safe and with an efficacy signal. The recommended phase 2 dose of the combination is 80 mg/m2 of weekly paclitaxel combined with 2000 mg bid of lapatinib two days before the paclitaxel dose.

Abstract 2

ABL Tyrosine Kinases Stabilize The Transcription Factor ZEB1 to Enhance Melanoma Resistance to MAPK Inhibitors

Bhuvanesh Sukhlal Kalal[1], Rakshamani Tripathi[1], Anastasia Lyon[1], Daheng He[2, 3], Chi Wang[2, 3], Rina Plattner[1]

[1]UK College of Medicine, Department of Pharmacology and Nutritional Sciences; [2]UK College of Medicine, Department of Internal Medicine, Division of Cancer Biostatistics; [3]UK Markey Cancer Center

Melanoma is the fifth most common cancer among adults globally, and is projected to increase by 50% by 2040, with a 68% rise in associated deaths. Constitutive activation of the MAPK pathway which occurs primarily via hyperactive mutations in BRAF (50% of cases) or NRAS (15-25% of cases), drives the development of metastatic melanoma. Targeted therapy using BRAF/MEK inhibitors increases median overall survival for BRAF-mutant metastatic melanoma patients (from 9 to 14 months); however, resistance inevitably develops. For patients with NRAS-mutant melanomas, no effective targeted therapy exists as BRAFi paradoxically increase melanoma growth, and MEKi provide little survival benefit due to intrinsic and acquired resistance.

Melanomas dynamically shift between different transcriptional states. Downregulation of epithelial-mesenchymal transition (EMT) transcription factors (TF), SNAI2 and ZEB2, and upregulation of TWIST1 and ZEB1, drives an aggressive invasive, metastatic phenotype. Our lab previously showed that ABL kinases, (ABL1/2) play critical roles in tumor progression, drug resistance, and metastasis, and their kinase activities and/or expression are increased in resistant melanoma cell lines and patient samples. Here, we show that increased expression of ZEB1 and N-cadherin (NCAD) and reduced expression of SNAI2, ZEB2, MITF, and in some cases E-cadherin, correlate with resistance to MAPK inhibitors (MAPKi). Moreover, silencing or inhibiting ABL1/2 with nilotinib or the highly specific allosteric inhibitor (GNF-5) reduces ZEB1 and NCAD expression in resistant cells and, in some cases, coordinately induces SNAI2 and E-cadherin. Conversely, expression of constitutively active forms of ABL1/2 into parental melanoma cells increases ZEB1 and NCAD expression and promotes MAPKi resistance. RT-qPCR assays demonstrate that these effects are not mediated by changes in ZEB1/NCAD mRNAs. In contrast, cycloheximide assays show that ABL1/2 promote ZEB1 and NCAD protein stability, and MG-132 (proteasome inhibitor) treatment rescues nilotinib-mediated degradation of ZEB1 and NCAD. ABL1/2 regulate protein stability via a number of different mechanisms in other cell contexts. We found that ZEB1 and ABL1/2 are in the same complex, but ABL1/2 do not stabilize ZEB1 by impacting previously identified ZEB1 E3 ligases, such as SIAH1. We currently are focused on identifying the mechanism by which ABL1/2 stabilize ZEB1/NCAD, and are using siRNA screens to identify ZEB1/NCAD E3 ligase(s) regulated by ABL1/2.

Resistance to targeted therapy is often accompanied by the EMT-TF switch, which drives a more invasive and metastatic phenotype. ABL1/2 promotes invasion and metastasis of resistant cells; however, unexpectedly, ABL1/2 don't promote invasion via ZEB1 as silencing ZEB1 paradoxically potentiates the ability of constitutive active ABL1/2 to increase invasion. Thus, current experiments are focused on identifying the biological role of ZEB1 and NCAD upregulation in resistant cells. Importantly, the pathway we identified is clinically relevant as single-sample Gene Set Enrichment Analysis demonstrates a robust positive correlation between ABL1/2 kinase activity and ZEB1 transcriptional activity (assessed using downstream target gene sets sourced from the IPA Ingenuity Knowledge Base) in samples from melanoma patients. In summary, our work identifies a new role for ABL1/2 in regulating the stability of transcription factors with critical roles not only in invasion and metastasis but also resistance to therapy.

Abstract 3

ABL1/2 Promotes a Pro-Tumorigenic Microenvironment during BRAFi/MEKi and MEKi Resistance in BRAF- and NRAS-Mutant Melanomas

Rakshamani Tripathi[1], Daheng He[2, 3], Jinpeng Liu[2, 3], Chi Wang[2, 4], Bhuvanesh Sukhlal Kala[1], Anastasia Lyon[1], Christina Meeks[1], Siva Gandhapudi[5], Shulin Zhang[4], Rina Plattner[1]

[1]UK College of Medicine, Department of Pharmacology and Nutritional Sciences; [2]UK College of Medicine, Department of Internal Medicine, Division of Cancer Biostatistics; [3]UK Markey Cancer Center; [4]UK College of Medicine, Department of Internal Medicine; [5]UK College of Medicine, Flow Cytometry and Immune Monitoring Core Facility

Mutually exclusive, oncogenic BRAF and NRAS mutations are critical drivers of metastatic melanoma. Despite the development of newer therapeutic agents, metastatic melanoma remains an incurable disease for many patients (5-year survival rate; 20% in 2023). While immunotherapy is curative for some patients, others (50%) are resistant to its effects or cannot tolerate the therapy. Targeted BRAF/MEK inhibitor (BRAFi/MEKi) combination therapy is effective in reducing metastatic burden for most patients with BRAF-mutant melanoma; however, for the vast majority, resistance develops, which results in rapid disease progression. For patients with NRAS-mutant melanomas, BRAFi are not utilized as they paradoxically increase melanoma growth, and MEKi only provide a modest improvement in survival, due to intrinsic and acquired resistance. Thus, new drug combinations are urgently needed for patients that cannot tolerate or develop resistance to the above therapies. ABL family non-receptor tyrosine kinases (ABL1, ABL2) are most known for their involvement in human leukemia. Recently, we showed that ABL1/2 are required for acquired resistance to BRAFi/MEKi in BRAF-mutant melanoma, and drive intrinsic and acquired MEKi resistance in NRAS-mutant melanomas by inducing reactivation of the MEK/ERK signaling pathway, and promoting proliferation and survival. Importantly, targeting ABL1/2 with the FDA-approved 2nd generation inhibitor, nilotinib, dramatically prevents and reverses BRAFi/MEKi (BRAF-mutant) and MEKi (NRAS-mutant) resistance in xenograft models.

Here, we demonstrate that nilotinib also significantly delays/prevents and reverses BRAFi/MEKi and MEKi resistance in immune-proficient, syngeneic and genetically engineered mouse models. Moreover, addition of nilotinib to BRAFi/MEKi or MEKi not only shrinks and prevents melanoma growth, in vivo, but also impacts the immune microenvironment. Using multiparameter flow cytometry and single-cell RNA sequencing, we show that nilotinib treatment (in the presence BRAFi/MEKi or MEKi) increases infiltration of anti-tumorigenic, cytotoxic immune cells (e.g. CD8+ T cells, NK cells) and reduces pro-tumorigenic, immunosuppressive myeloid cells (e.g. myeloid-derived suppressor cells-MDSCs, tumor-associated macrophages-TAMs). Moreover, in vivo depletion of CD8+ T cells blocks the ability of nilotinib to prevent BRAFi/MEKi resistance. Thus, nilotinib reverses/prevents resistance not only by inhibiting melanoma proliferation/survival but also by increasing cytotoxic immune cell infiltration. Furthermore, using bulk RNA sequencing and membrane-based cytokine arrays, we show that cytokines known to drive infiltration of MDSCs (CXCL1, 2, 8, IL6), which blunt the effects of cytotoxic immune cells, are upregulated in resistant melanoma cells compared with their parental counterparts, and silencing or inhibiting ABL1/2 (in the presence of BRAFi/MEKi or MEKi) dramatically blocks cytokine mRNA upregulation and secretion. Experiments are now focused on testing whether ABL1/2 drive MDSC infiltration by promoting melanoma secretion of CXCL1, 2, 8, and IL6. Finally, our data have clinical relevance as ABL1/2 activities (assessed by single-sample Gene Set Enrichment analysis-ssGSEA using downstream target gene sets sourced from the IPA Ingenuity Knowledge Base) are positively correlated with CXCL1, 2, 8 and IL6 mRNAs in melanoma patient samples. In summary, ABL1/2 drive resistance not only by impacting the melanoma cells themselves (intrinsic) but also by affecting the melanoma microenvironment (extrinsic), indicating that targeting ABL1/2 is likely to be a highly effective treatment for reversing and preventing targeted therapy resistance in both BRAF- and NRAS-mutant melanoma subtypes.

Abstract 4

African American (AA) and African Born-Black (ABB) Women's Perspectives and Experiences with a Cervical Health Education and HPV-Self Sampling Intervention

Shania A. Jones[1, 2], Princess Agbozo[3], Samuela Musasa[3], Adebola Adegboyega[3]

[1]UK College of Medicine; [2]Markey Cancer Center; [3]UK College of Nursing

Introduction: About 99% of cervical cancer cases are linked to Human Papilloma Virus (HPV) a sexually transmitted infection. Approximately 95% of cervical cancers could be prevented by regular screening and HPV vaccination. Despite cervical cancer being preventable, systemized racial and ethnic disparities continue to exist in cervical cancer screening rates among AA and ABB women. A cervical health intervention was developed to promote cervical screening, HPV knowledge, and screening efficacy. These abstract reports the experiences of women who took part in a cervical health education and received HPV-self-sampling Kit.

Methods: Twenty AA/ABB women aged 30–65 years who participated in a 1-hour online cervical health education and were provided with HPV self-sampling kits were invited for a one-on-one interview via Zoom. Interviews were guided with a semi-structured guide and took 20-30 minutes. Each interview session was recorded, transcribed, and content analyzed.

Results: Reported data was organized into two major categories: Intervention facilitators and opportunities. Participants reported having positive experiences with the flexibility of the online education session and HPV self-sampling process. Factors facilitating participation included spousal support, length of session, desire for cervical education, and ease of HPV self-screening. Participants suggested more language options, shorter surveys, and hybrid presentation formats to promote engagement.

Conclusion: Overall, interventions such as education and provision of HPV self-sampling kits, can be viable strategies to empower AA and ABB for preventative cervical cancer screening and or follow-up care. These findings will be used to further refine the intervention for future implementation.

Abstract 5

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

Kristen S. Hill[1, 2], Erin E. Schuler[3], Sally R. Ellingson[2, 4], Jill M. Kolesar[2, 5]

[1]UK College of Pharmacy; [2]Markey Cancer Center; [3]UK College of Medicine, Department of Pathology and Laboratory Medicine; [4]UK College of Medicine, Department of Internal Medicine, Division of Biomedical Informatics, Cancer Research Informatics Shared Resource Facility; [5]UK College of Pharmacy, Department of Pharmacy Practice and Research

Introduction: 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 a multi-disciplinary approach combining computational modeling and UV-visible spectroscopy to identify potential direct targets of artesunate from a list of mitochondrial associated heme bound proteins. An identified target, cytochrome c, was confirmed in pediatric AML cell lines using drug combination studies and phenotypic assays including assessing caspase 3/7 activity and mitochondrial membrane potential by florescent imaging, and cytochrome c localization by ELISA.

Results: Computational modeling determined the predicted binding score of artesunate with heme-bound mitochondrial proteins and identified cytochrome c as a potential artesunate target. UV-visible spectroscopy showed changes in the absorbance spectrum, and thus protein structure, when cytochrome c was incubated with artesunate. Artesunate induces apoptosis, disrupts mitochondrial membrane potential, and is antagonized by methazolamide in pediatric AML cells indicating a probable mechanism of action involving cytochrome c. Additionally, artesunate treatment induced increase cytoplasmic cytochrome c, consistent with artesunate inducing the release of cytochrome c from the mitochondria.

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 6

Biological Impact of True-to-Life Nanoplastics, Derived from PET Water Bottles, on the Polarization of Alveolar Macrophages

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The increasing presence of secondary micro/nanoplastics (MNPLs) in the environment requires knowing if they represent a real health concern. To such end, an important point is to test representative MNPLs such as the denominated true-to-life MNPLs resulting from the degradation of plastic goods in lab conditions. In this study, we have used polyethylene terephthalate (PET) NPLs resulting from the degradation of PET water bottles. Since inhalation is an important exposure route to MNPLS, we have used mouse alveolar macrophages (MH-S) as a target cell, and a set of biomarkers including intracellular reactive oxygen species (ROS) levels, variations on the mitochondrial membrane potential values, and the macrophage polarization to M1 (pro-inflammatory response) and M2 (anti-proinflammatory response). After exposures lasting for 3 and 24 h to a range of concentrations (0, 25, 50, and 100 µg/mL) the results indicate that no toxicity was induced despite the 100% internalization observed at the highest concentration. Significant intracellular levels of ROS were observed, mainly at exposures lasting for 24 h, in an indirect concentration relationship. Interestingly, a reduction in the mitochondrial membrane potential was observed, but only at the exposure lasting for 24 h, but without a clear concentration-effect relationship. Finally, PETNPL exposure shows a significant polarization from M0 to M1 and M2 subtypes. Polarization to M1 (pro-inflammatory stage) was more marked and occurred at both exposure times. Polarization to M2 (anti-inflammatory stage) was only observed after exposures lasting for 24 h. Due to the relevance of the described biomarkers, our results underscore the need for further research, to better understand the health implications associated with MNPL exposure.

Abstract 7

Biostatistics and Bioinformatics Shared Resource Facility (BB SRF)

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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. 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.

Abstract 8

Biospecimen Procurement and Translational Pathology Shared Resource Facility (BPTP SRF)

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The Biospecimen Procurement and Translational Pathology Shared Resource Facility (BPTP SRF) is a cancer center-managed resource providing biospecimen services that are cost-effective and comprehensive. BPTP SRF aims to: 1) Provide high-quality biospecimens, including basic specimen histology and blood processing, to support Markey Cancer Center (MCC) investigators and collaborators; 2) Provide advanced tissue-based services, including quantitative image analysis artificial intelligence (AI), multiplexed assays and digital spatial profiling with access to pathologist expertise, to support MCC investigators and collaborators; and 3) Work efficiently and economically with other resource centers, including other MCC SRFs and other University of Kentucky (UK) research facilities and clinical departments, to optimally tailor project support for MCC investigators and collaborators. BPTP's unlimited access to UK HealthCare and MCC clinical programs provides a strong, well-organized system that enables researchers to request prospectively collected specimens from patients of interest throughout the course of treatment. This streamlined centralization enables investigators to engage in clinical research in a quality-, time- and resource-efficient manner. BPTP support for MCC clinical trials occurs at any time from trial inception to completion with hand-off to other SRFs or direct shipment. In addition to procuring, processing, and providing high-quality biospecimens, BPTP offers translational pathology services including in situ proteomic and transcriptomic analyses (Nanostring, Visium), AI image analysis (Halo AI), multiplex immunohistochemistry, immunofluorescence, in situ hybridization (Ventana, RNAScope) and complex blood processing for clinical trials (extracellular vesicle isolation). Tissue microarrays designed and constructed by BPTP using an automated platform, 3D Histech, facilitate high-throughput biomarker discovery.

Abstract 9

Blocking p38δ-ADAM17 Axis in Neutrophils to Inhibit Triple-Negative Breast Cancer Progression and Metastasis

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Neutrophils, a type of white blood cells, have been regarded as first line of defense in the innate immune system. Multiple studies have found the elevated neutrophil to lymphocyte ratio (NLR) is associated with adverse breast cancer (BCa) prognosis and survival. Triple-negative breast cancer (TNBC) is a particular breast cancer subtype which lacks effective targeted therapies and often displays early metastasis, with a poor prognosis and short survival. Growing evidence has proved that neutrophils are heterogeneous population. Identifying which neutrophil subpopulation is associated with TNBC progression and metastasis and understanding how they are generated are critical for providing a therapeutic strategy. In several TNBC mice models, we surprisingly observed "emergency" granulopoiesis (EG), concomitant with an aberrant accumulation of a previously unrecognized immature CD62Lneg circulating neutrophil (cNeu) population with immunosuppressive function. Importantly, high cNeu counts and CD62Lneg cNeu levels were also detected in metastatic cancer patients and associated with worse outcome. We further found that high G-CSF (granulocyte colony-stimulating factor) expression in TNBC cells is prerequisite for eliciting EG and immature CD62Lneg cNeu accumulation. Moreover, we identified that p38δ-ADAM17 axis is highly activated in neutrophils from mice bearing G-CSF high- but not low-expressing TNBC tumors. Strikingly, inhibition of p38δ-ADAM17 axis reduced G-CSF-induced EG, immature CD62Lneg cNeu accumulation, tumor growth and metastasis. Taken together, blocking p38δ-ADAM17 axis in neutrophils could be a potential and novel therapeutic strategy for metastatic TNBC.

Abstract 10

Cancer Research Informatics (CRI) Shared Resource Facility (SRF)

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The Cancer Research Informatics (CRI) Shared Resource Facility (SRF) is a cancer center resource that provides comprehensive informatics support for basic, clinical, translational, and population-based research at the Markey Cancer Center (MCC). Important advances in precision medicine and molecular-based research led to the development of a Cancer Research Data Commons that integrates molecular and omics data with Surveillance, Epidemiology and End Results (SEER) data from the Kentucky Cancer Registry. CRI provides easy access to the MCC data ecosystem and assists investigators with unique patient datasets specific to their research aims. CRI has supported over 130 molecular data requests and 2,124 case reviews by the Molecular Tumor Board. CRI has also expanded its expertise and use of artificial intelligence and high throughput computational methods. CRI is deeply engaged in the development of leading-edge natural language processing methods that assist MCC investigators with rapid case ascertainment and access to tissues. In addition, CRI has developed natural language processing methods for social media data mining used by the Cancer Prevention and Control Research Program. Machine learning methods to derive pathomics features from digital whole slide images are driving new research. New methods have also been developed for in silico protein and drug interaction modeling, used in Translational Oncology Program research. CRI maintains MCC's strategic computational infrastructure, software, and databases (including mobile app development for use in clinics) and facilitates MCC data sharing. CRI resources and expertise are broadly categorized into three Specific Aims: 1) Provide the cancer data ecosystem that empowers MCC investigators with integrated datasets from diverse sources ranging from molecular biomarkers to population-based SEER patient data; 2) Develop artificial intelligence and high throughput computational methods that elevate MCC Research Program science; 3) Provide secure computational resources, biomedical informatics applications and expertise to facilitate interoperability among MCC SRFs and Research Programs. CRI SRF coordinates services with other MCC SRFs to ensure complementary and efficient service delivery.

Abstract 11

Characterization of p53 T253I as a Pathogenic Mutation Underlying Li-Fraumeni Syndrome in an Infant with Adrenal Cortical Carcinoma

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An 8-month-old patient with signs of precocious puberty was found to have an adrenal cortical carcinoma (ACC) characterized by a T253I mutation in the TP53 tumor suppressor gene. p53 protein levels were overexpressed in the patient's ACC, as determined by immunohistochemistry, and molecular profiling of the tumor suggested suppression of the remaining wt TP53 allele. As this type of malignancy has been reported to occur in Li-Fraumeni Syndrome (LFS) patients with germline inheritance of loss-of-function TP53 mutations, we evaluated the patient's TP53 gene in non-tumor tissue and found evidence of a constitutional heterozygous germline TP53 T253I mutation. The p53 T253I mutation is characterized as a variant of uncertain significance (VUS) on ClinVar, an NIH repository for genomic variations and their relationship with human health. Since the mutation has not yet been linked to LFS, we sought to characterize p53 T253I mutation to determine its impact on cell growth and damage responses. We acquired TP53 CRISPR deleted HEK293 cells and stably transduced them with GFP-tagged wild type (T253) or T253I mutant TP53. We compared growth kinetics of p53-null, p53-wild type (wt) and TP53-T253I HEK293 cells in log growth phase and documented similar cell doubling times, comparable growth rates, and no differences in cell cycle stages between conditions. Whereas HEK293 p53 -/- cells complemented with p53 wild-type exhibited canonical damage-induced activation of p21, p53 -/- cells complemented with T253I-p53 exhibited attenuated responses comparable to uncomplemented p53 -/- cells, suggesting that T253I represents a loss-of-function variant of TP53. In complemented HEK293 cells, we observed that levels of T253I p53 were higher as compared to wt p53, suggesting a loss of MDM2-mediated clearance of T253I p53. Accordingly, MDM2 levels were lower in T253I p53 complemented cells as compared to wild type p53 complemented cells, suggesting that the high expression of T253I-p53 may be explained by reduced p53-mediated MDM2 transactivation and subsequent diminished negative feedback. These data, coupled with the clinical observation of an LFS-associated tumor in a child with germline T253I TP53 mutation, lead us to conclude that p53 T253I represents a pathologic variant that predisposes to cancer.

Abstract 12

Characterizing and Targeting the Oncogenic Mechanism of Phosphatase of Regenerating Liver 3

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The Phosphatase of Regenerating Liver 3 (PRL-3) is one of the most oncogenic phosphatases characterized to date. High PRL-3 expression levels correlate with increased metastatic potential and a poor patient prognosis in many cancer types. Despite its prominent implications in metastasis, the protein substrates of PRL-3's oncogenic phosphatase activity have not been clearly defined. Recently, PRL-3 has been found to use its phosphatase catalytic site to tightly bind to the CBS-pair Domain Divalent Metal Cation Transport Mediators (CNNM) family of proteins. This interaction promotes the accumulation of intracellular magnesium, though the collective signaling implications of this for cancer progression are unclear.

To determine whether PRL-3's phosphatase or CNNM-binding activity is responsible for PRL-3's oncogenic function, we utilized a set of PRL-3 mutants deficient in one activity or the other and expressed them in a transgenic zebrafish model of Rhabdomyosarcoma (RMS). Tumors expressing wild-type PRL-3 or PRL-3 capable of binding CNNM but lacking phosphatase activity were approximately two-fold larger ($p \leq 0.0001$) and displayed highly invasive phenotypes. Interestingly, the PRL-3 mutant that had phosphatase activity but lacked CNNM binding did not significantly enhance tumor progression compared to control.

When these same mutant constructs were expressed in human cancer cells, we found that PRL-3's CNNM binding properties could enhance cancer cells' survival in vitro when exposed to various stress conditions reminiscent of the tumor microenvironment (such as hypoxia and nutrient starvation), while phosphatase-only PRL-3 did not enhance survival ($p \leq 0.0039$ and ≥ 0.7915 respectively). Taken together, our results suggest that PRL-3 exerts its oncogenic activity by binding CNNM proteins and regulating intracellular magnesium rather than dephosphorylating cellular substrates.

These data have implications for PRL-3 drug discovery, which, to date, has been focused on the inhibition of PRL-3's phosphatase activity. This may explain the failure of these drugs in targeting PRL-3's oncogenic activity in vivo, as our data suggests CNNM binding may be PRL-3's true oncogenic function.

To address this issue, we developed a novel FRET-based assay to rapidly identify inhibitors of the PRL:CNNM interaction. We paired this methodology with thermal stability and enzymatic assays to develop a high-throughput, comprehensive pipeline to identify PRL-3 inhibitors. We screened 96 compounds identified as top hits from an in silico screen as well as a library of 440 drug fragments. We identified two compounds that could inhibit the PRL:CNNM interaction and validated this inhibition through co-immunoprecipitation (Co-IP) which revealed a 72-88% decrease in the amount of PRL-3 pulled-down with CNNM ($p \leq 0.0012$). Using these same methods, we further demonstrate that the current PRL-3 "inhibitors" are not capable of inhibiting the CNNM interaction.

We are further characterizing the PRL:CNNM interaction to determine how it promotes cancer progression and survival and to examine its usefulness as a drug target to combat metastatic disease.

Abstract 13

Characterizing the Small Cell Lung Cancer Tumor Immune Microenvironment for Precision Immunotherapy: A Pilot Study

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Background: Small Cell Lung Carcinoma (SCLC) comprises only 15% of lung cancer yet is the sixth leading cause of death from all cancers. Survival beyond three years occurs in 5-10% of patients. This exceptional survival may be attributable to an enhanced anti-tumoral immune response, although small cell carcinoma is generally described as an "immune desert" or as immersed in an immunosuppressive tumor immune microenvironment (TIME). Immunotherapy is thus far effective in only a subset of patients. We posited that specific, measurable features of the TIME in primary and matched metastatic SCLC significantly affect survival. Furthermore, TIME features could inform optimal immunotherapy selection, tailored to an individual's specific immune microenvironmental conditions.

Design: We identified SCLC patients from our SEER registry. Two matched cohorts were created: one with 12 patients of expected survival (<14 months) and another with 12 exceptional survivors (>36 months). Using GeoMX (NanoString Inc.) and Comet platform (Lunaphore), we assessed 78 immuno-oncology markers via digital spatial profiling and immunofluorescence on tumor cell block sections. These markers included lineage antigens, cytokines, and hypoxia-related proteins. Also, four cytopathologists blindly reviewed sections for independent prediction of exceptional survival.

Results: Exceptional survivors exhibited statistically significant differential gene expression in four immune response-related proteins (CCND1, CD27, CD274, CD74) relative to expected survivors (principal component analysis $P = 4.135 \times 10^{-3}$) on GeoMX spatial profiling. Multiplex IF staining correlated with tumor cells and immune cells quantified by the spatial profiling platform. Notably, diminished CD27 expression correlated with shorter survival ($P = 0.003163$). Morphologic groupings of cases devised independently by cytopathologists showed a significant correlation with survival but in only one of four personally devised tumor stratification systems ($P = 0.014$).

Conclusion: Pilot findings from 12 expected and 12 exceptional SCLC survivors underscore the potential substantial impact of the SCLC TIME on survival. The pattern of expression of panels of immune markers including level of CD27 expression could infer variable responsiveness to specific immunotherapy regimens. Partially successful correlation of survival with morphologic features suggests potential for development of a prognostic and immunotherapy predictive AI algorithm based on microscopic review of hematoxylin and eosin-stained cell block sections.

Abstract 14

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

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Non-small cell lung cancer (NSCLC) is a problem across much of the country with many thousands expected to develop and pass on from this disease. There are many different subtypes of NSCLC, and adenocarcinomas are the most common. EGFR activating mutations are the second most frequently identified driver mutation in lung adenocarcinoma. Treating EGFR mutant NSCLC usually is done with the EGFR inhibitor osimertinib. This therapeutic binds to EGFR covalently and blocks ATP from accessing the kinase domain. But resistance is an issue with this therapy and many patients develop resistance and progress. Epigenetics is an emerging hallmark of cancer showing that epigenetic change can influence cancer outcomes and treatments. Polycomb Repressive Complex 2 (PRC2) is an epigenetic modifier that catalyzes trimethylation of histone 3 at the 27th lysine residue which induces gene silencing. 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 tazemetostat as a combination treatment for EGFR mutated NSCLC, specifically in the EGFR mutated cell lines HCC4006, PC9 and PC9GR4. We found a strong synergy between osimertinib and tazemetostat in each of these parental cell lines. To test efficacy after osimertinib resistance, we generated osimertinib resistant cell lines for each of these parental cell lines. We have found the combination treatment can induce apoptosis in osimertinib resistant cells in vitro. When injected in vivo, we are able to shrink both the HCC6006 OsiR and the PC9GR4 OsiR tumors more when compared to either monotherapy. We are now exploring modulation of MYC as a mechanism through which PRC2 inhibition causes re-sensitization to osimertinib. The work suggests that combining PRC2 inhibition with osimertinib will be an effective way to overcome EGFR TKI resistance in the clinic. This work is funded by NCI-CA237643, the Markey Women Strong Grant, and many SRFs (P30-CA177558).

Abstract 15

Comprehensive Connected Cancer Care (C4) Program

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Program Purpose and Goals: To refine, implement, and evaluate the Comprehensive Connected Cancer Care (C4) Program at Markey Cancer Center and three community-based oncology programs in the Markey Cancer Center Research Network (MCCRN) in Kentucky. The overall goal of the C4 Program is to advance health equity in cancer care by improving timely access to community-focused, patient-centered, and high-quality care. To achieve this goal, the C4 Program provides digital tools to screen for and address social determinants of health that pose barriers to initiating and completing cancer treatments. These digital tools enhance the ability of oncology programs to successfully implement two Commission on Cancer (CoC) standards: 5.2 Psychosocial Distress Screening and 8.1 Addressing Barriers to Care.

Program Target Population: The C4 program is intended to facilitate assistance and support for social needs and mental health needs of patients with cancer, in particular patients from vulnerable and underserved groups, including rural, Appalachian, low-income, Medicaid-enrolled, and racial/ethnic minority populations.

Program Components: The components of the C4 Program include a mobile application for cancer patients and caregivers. The mobile app will provide an on-demand distress assessment tool (NCCN Distress Thermometer) for patients to report unpleasant experience(s) of a mental, physical, social, or spiritual nature. The app will deliver personalized recommendations for external community resources to address identified practical needs, a methodology to provide updated statuses on those referrals to patients, and relevant patient education information based on cancer type. A navigation dashboard will provide status of distress assessments reported via the mobile app and referral information for social workers and non-clinical patient navigators. Provider/staff education modules support this technology with topics including patient navigation, patient-centered care, and diverse populations, and addressing barriers to cancer care.

Navigation Capacity-Building Initiative: Supplemental to the C4 program is the American Cancer Society Navigation Capacity-Building Initiative project which aims to enhance the patient navigation services provided through Markey Cancer Center's Psych-Oncology Program by 1) adding a non-clinical patient navigator position to complement existing clinical social workers, 2) utilizing continuous quality improvement, and 3) augmenting the program with innovative digital tools. This project will improve health equity by increasing Markey Cancer Center's capacity to address social determinants of health that pose barriers to timely access to care. Through this project, Markey Cancer Center will also enhance its tools for tracking and reporting metrics for continuous improvement in oncology patient navigation and addressing the social needs of patients with cancer.

Abstract 16

Core Fucosylation is a Novel Metabolic Vulnerability in MYCN-Amplified Neuroblastomas

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Introduction: MYCN-amplification (MYCN-amp) is a hallmark of nearly half of all high-risk neuroblastomas (NBs). N-MYC is an oncogenic transcription factor that metabolically reprograms cells in the transformation process. We have previously reported enrichment of core fucosylated glycans in MYCN-amplified human NB tissues. Herein, we hypothesized that aberrant core fucosylation is a critical metabolic vulnerability in NB tumor progression.

Methods: Kaplan-Meier analysis was performed to determine whether key fucosylation genes GMDS, FX, and FUT8 expression correlated with overall survival using the R2 Platform. GDP-mannose 4, 6- dehydratase (GMDS) genetic knockdown (GMDS-KO) was performed. 2-fluorofucose (2-FF) was a small molecule inhibitor of fucosylation. Core fucosylated glycan abundance was measured by western blotting, ELISA, and flow cytometry using Aleuria aurantia lectin (AAL). Cellular adhesion and migration were measured in vitro using a Vybrant blue adhesion assay and wound healing assays. Cyclin D1 and PARP cleavage were quantified by western blotting for markers of proliferation and apoptosis induction. Subcutaneous tumor formation using MYCN-amp BE(2)-C cells was our measure of in vivo tumorigenesis. For small molecule trials, mice with established BE(2)-C tumors were randomized to receive water-supplemented with 2-FF or vehicle control.

Results: High levels of de novo fucose synthesis genes (GMDS and FX) and core fucosyltransferases (FUT 8) were associated with advanced stage disease and poor overall patient survival. Genetic inhibition of GMDS blocks core fucosylated glycan abundance and secretion in human MYCN-amplified cell lines. GMDS-KO impedes NB cell adhesion and migration in vitro and blocks subcutaneous tumor formation in vivo. 2-FF blocks NB core fucosylation, and NB cell growth and adherence in vitro. 2-FF induces PARP cleavage and cell cycle arrest. Importantly, GMDS-KO blocks NB tumor formation ($p<0.01$). 2-FF water supplementation blocks core fucosylation in vivo and impedes established tumor progression (<0.05) via the induction of cancer cell death.

Conclusions: Blocking de novo fucose synthesis impairs NB adherence and migration in vitro and impairs tumor growth in vivo. Small molecule inhibition with 2-FF blocks established tumor growth via the induction of cancer cell death in vivo. These critical findings identify core fucosylation as a novel metabolic vulnerability that may be exploited in designing novel treatment paradigms for high-risk NBs.

Abstract 17

Delineating Contributions of Genotype and Lineage to Lung Cancer Therapy Response

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Non-small cell lung cancer (NSCLC) heterogeneity is a major challenge for determining effective treatment strategies. Adenocarcinomas (ADCs) and squamous cell carcinomas (SCCs) are histologically and epigenetically distinct subtypes of NSCLC. Patients with ADCs harboring mutations in both KRAS and LKB1 (aka STK11) have lower survival rates and respond poorly to immunotherapy. However, these data are limited to ADCs, and it is unclear if SCCs with this genotype are also resistant to immunotherapy. Our mouse model of Krasmut/Lkb1mut produces aggressive ADCs, and a proportion of tumors transition to SCC. This lineage switch is driven by a reduction in Polycomb Repressive Complex 2 (PRC2) activity, which can be targeted directly with EZH2 inhibitors (EZH2i). We hypothesize that EZH2i will be more effective at de-repression of genes involved in immune recognition in SCCs than ADCs. To test this hypothesis, we developed tumoroid models that are ADC and SCC in phenotype and have mutant Kras with either WT or mutant Lkb1. When treated with a combination of EZH2i and IFN-gamma, PD-L1, MHCI, and MHCII expression were increased in the SCC but not the ADC tumoroids. These data suggest that combining PRC2 inhibition with immunotherapy may be very efficacious in KRAS/LKB1 tumors with SCC characteristics, but not those with ADC characteristics. However, there may be other ways to improve treatment of Krasmut/Lkb1mut ADCs. For example, PRC2 requires S-adenosyl methionine (SAM) to methylate histones, and SAM production requires exogenous methionine or methionine recycling. Therefore, we predict that methionine restriction (MR) will enhance treatment efficacy in KRAS/LKB1 mutant NSCLCs via lowering PRC2 activity. We placed cohorts of Krasmut/Lkb1mut mice on control methionine and MR diets during tumor progression and carboplatin treatment. We observed that Krasmut/Lkb1mut mice on MR diets had reduced tumor burden in both the prevention and chemotherapy studies. Furthermore, MR increased the number of tumor-infiltrating macrophages and decreased tumor-infiltrating neutrophils, and this change could indicate that MR will alter TIME and change immunotherapy responses. To investigate potential mechanisms of MR sensitizing cells to carboplatin, we used human lung cancer cell lines. Growth in high methionine protected cells, while low methionine conditions sensitized cells to carboplatin. LKB1 rescue increased carboplatin survival in regular and low methionine conditions, and reduced carboplatin-induced apoptosis and cell cycle arrest in high methionine conditions. We observed that LKB1 rescue increased the mRNA and protein levels of the methionine pathway protein cystathionine-β-synthase (CBS). CBS condenses homocysteine and serine for downstream glutathione production and may therefore reduce methionine recycling and modulate PRC2 activity. Knock-down of CBS in LKB1 mutant and rescued cells could sensitize cells to carboplatin treatment. Together these data suggest an important role for PRC2, methionine restriction, and LKB1-mutational status in NSCLC lineage fate and therapy response. This also demonstrates the importance of considering not only the genotype of NSCLCs, but also the phenotype when selecting the best course of treatment for each individual patient.

Abstract 18

Demonstration of Feasibility and Safety of Multiple Courses of Gamma Knife Radiosurgery Treatment to Recurrent Trigeminal Neuralgia: A Single-Institutional Clinical Outcomes

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Purpose/Objective(s): Leksell Perfexion Gamma Knife Radiosurgery (GKRS) has been utilized to provide a treatment alternative for treatment resistant Trigeminal Neuralgia (TN) patients with a refractory or contraindication to surgery. If a minimally invasive, same day frame based GKRS can effectively provide pain relief, then multiple retreatments via highly precise GKRS unit can be a patient's best alternative to polypharmacy. We analyzed the effectiveness, complication rate, and time intervals between GKRS retreatments at our historic GKRS center.

Materials/Methods: This study focuses on the cohort of patients with a history of multiple treatment episodes spanning from 2009 to 2023. A comprehensive examination of patient records, encompassing notes, and imaging reports was conducted. A single 4 mm shot was used; first treatment was at prepontine cistern area. To avoid the shot overlap, subsequent retreatment shot(s) were placed at the posterior aspect of Meckel's cave while respecting the temporal lobe and brainstem (keeping maximum dose <18 Gy). The initial prescription was 45 Gy to 50% isodose line (IDL) with a maximum dose of 90 Gy at the middle of the trigeminal nerve. Re-treatment dosage ranged between 30–40 Gy to 50% IDL (maximum 60–80 Gy). Outcomes were assessed with the Barrow Neurological Institute (BNI) pain scoring system (Class I–V). Retreatments were considered ineffective if BNI class did not improve after retreatment or if symptoms recurred, requiring additional intervention within 6 months post-retreatment.

Results: Thirty-one patients were identified that received two or more courses of GKRS. Average patient age was 65.8 years (yrs) (range, 37-89 yrs) at the first treatment. 20 of the 31 patients (65%) had atypical TN, including all 3 patients that received three or more treatments. One atypical patient received a total of five courses of GKRS over 11 yrs intervals without complications. 3 (9.6%) atypical TN patients failed GKRS retreatment. Of these one underwent surgery within 6 months due to pain and 2 others failed to improve BNI scoring, however they didn't receive additional interventions. Average and median intervals between first and second treatment were 2.9 yrs and 1.4 yrs, respectively. Following the second course of GKRS, 3 patients experienced post-retreatment facial numbness, which was managed with medication. No patients demonstrated treatment-related intracranial toxicity.

Conclusion: Most patients (>90%) undergoing multiple courses of GKRS experienced improved pain control based on a decreased BNI score with none of them experiencing radiation induced toxicity concerns. This suggests that multiple GKRS treatments, with adequate recovery time and shot placement strategy, are a safe and effective treatment option for recurrent or refractory patients of TN including atypical cases. Further dedicated prospective studies are needed to validate the potential of dose escalation.

Abstract 19

DNA-PK Inhibition to Enhance DNA-Damaging Therapies in Neuroblastoma

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Background: Neuroblastoma (NB) is a pediatric cancer, representing with variable clinical and genetic features. This heterogeneity is closely linked to resistance to treatment continuous relapses, and often leading to fatal outcomes. It has been demonstrated that aberrant function of DNA damage response in High-risk (HR) NB patients can lead to tumor progression and therapy resistance. Numerous therapeutic approaches that are applied in NB treatment, including chemo and radio therapy, target cancer cells by inducing DNA damage. However, in HR-NBs aberrant function of DNA repair pathways can lead to tumor progression and therapy resistance. Here, we have demonstrated that high DNA-PKcs expression, a pivotal upstream component of the non-homologous end joining (NHEJ) DNA repair pathway, is associated with poor overall survival in NB patients. DNA-PKcs is highly activated in NB cell lines and inhibition of DNA-PKcs enhances therapeutic effect from DNA damaging therapies.

Methods: Patient data were obtained from the Kocak dataset, and survival curves were generated using the Kaplan-Meier method. Gene expression and protein levels were analyzed using qPCR and Western blotting (WB), respectively. DNA strand double break (DSB) was induced with topoisomerase II inhibitor etoposide. DNA-PKcs activity was inhibited with peposertib (M3814) or DNA-PKCs siRNA. Clonogenic and proliferation assays were used to evaluate efficacy of M3814 in combination with etoposide in BE-2-C and SK-N-DZ NB cell lines. DSB-induced DNA repair was visualized with confocal laser scanning microscopy using γ-H2AX immunofluorescence (IF). Apoptosis was measured by using cell death ELISA and WB analysis to examine fragmented DNA and cleaved PARP expression in different treatment groups, respectively.

Result: qPCR and WB analysis showed high expression of DNA-PKcs in six NB cell lines. Patient data analysis revealed that the elevated expression of DNA-Pkcs is associated with poor overall survival in NB patients. DNA-PKcs inhibition resulted in enhancement of etoposide cytotoxic therapy in both BE-2-C and SK-N-DZ cell lines. IF analysis detected γH2AX foci increase in etoposide and M3814 combination group. DNA-PKcs inhibition in combination with etoposide treatment increased PARP cleavage in BE-2-C and SK-N-DZ cell lines. DNA-PKcs knockdown by siRNA alone increased PARP cleavage to the levels comparable to etoposide treatment. The combination of etoposide and DNA-PKcs knockdown enhanced PARP cleavage further in BE(2)-C cells.

Conclusion: High DNA repair activity is commonly linked to tumor drug resistance. This study emphasizes the crucial role of elevated DNA-PKcs in HR-NB and suggests its involvement in tumor progression in HR patients. Our data indicated that combining DNA-Pkcs inhibition with etoposide resulted in enhanced apoptosis in NB cells, suggesting the potential of this approach to enhance the anti-tumor effects of DNA damaging therapy. These findings point out the necessity of further investigating DNA-PKcs inhibitors in clinical practice. However, extensive in vivo studies are required to validate their efficacy in combination with etoposide.

Abstract 20

DNA-PK Inhibition to Enhance **DNA-Damaging Therapies in Neuroendocrine Cancer**

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Introduction. Gastroenteropancreatic (GEP) neuroendocrine tumors (NETs) are neoplasms originating from the gastrointestinal track and pancreas. Unfortunately, most patients at the time of diagnosis have extensive metastatic disease and are not candidates for curative surgical resection of metastatic tumors. In such cases, chemotherapy can slow the progression of metastatic disease, but eventually tumors develop resistance to genotoxic agents through DNA repair pathways activation. Therefore, targeting DNA repair pathway can increase the tumor sensitivity to cancer therapies. In this study, we examined whether DNA-PK inhibition could sensitize pancreatic NETs to DNA damaging chemotherapy.

Methods. DNA double-strand break damage was induced with topoisomerase II inhibitors etoposide or doxorubicin. DNA-PK activity was inhibited with peposertib. Clonogenic and proliferation assays were used to evaluate the efficacy of peposertib in combination with etoposide or doxorubicin in BON and QGP-1 neuroendocrine cell lines. DSB-induced DNA repair was visualized with confocal laser scanning microscopy using γ-H2AX immunofluorescence. Induction of apoptosis in chemotherapy combination groups was visualized with confocal laser scanning microscopy using cleaved caspase-3 immunofluorescence.

Results. DNA-PK inhibition resulted in enhancement of etoposide cytotoxic therapy (BON IC50 ET = 11.4 μM, ET+Pep = 7.24 μM; QGP IC50 ET = 7.2 μM, IC50 ET+Pep = 2.5 μM) and enhancement of doxorubicin cytotoxic therapy (BON IC50 DOX = 608.6 nM, DOX+Pep = 83.6 nM; QGP IC50 DOX = 269.2 nM, DOX+Pepo = 105.5 nM) in both BON and QGP-1 cell lines. Immunofluorescence analysis detected γH2AX foci increase in the etoposide/doxorubicin and peposertib combination groups. DNA-PK inhibition in combination with etoposide or doxorubicin treatment significantly increased caspase-3 cleavage in BON and QGP-1 cell lines.

Conclusions. High DNA repair activity is commonly associated with cancer drug resistance. The combination of DNA-PK inhibition with DNA-damaging therapies stalled DNA damage response and enhanced neuroendocrine cancer cells apoptosis.

These findings demonstrate that selective DNA-PK inhibition provides a potent therapeutic strategy for disruption of non-homologous end joining DNA double-strand break repair and may offer a novel therapeutic approach in advanced NET chemotherapy.

Abstract 21

Effectiveness of Remote Recruitment Strategies to Enroll Youth and Young Adults

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Introduction/Background: Youth and young adults disproportionately use flavored tobacco products due to their appeal compared to that of non-flavored products. They are establishing adult tobacco use patterns. However, engaging youth and young adults in online studies has challenges. Young adults often start college or work and move to different residences. Recruiting remotely in various cities across the US necessitated a variety of outreach strategies to reach 15 to 24-year-old potentially eligible participants. Recruitment challenges were heightened due to the disruptions of the COVID-19 pandemic. The present study analyzes the effectiveness of various recruitment strategies, including paid media and social media platforms, community partners, and participant registries.

Methods: We used multiple recruitment approaches and tracked them through online survey links and self-reported methods of hearing about the study. We analyzed screener data from n=1572 potential respondents to understand which recruitment approaches were most effective in producing respondents who had potentially eligible responses and ultimately enrolled in the study.

Results: When examining sources for links clicked to complete the recruitment screener, we found that most individuals who completed the screeners heard about the study from community partners, followed by paid Facebook/Instagram and then Reddit. However, participant registry (Research Match) (78%) and community partners (68%) resulted in the highest yield of eligible participants. Actual study enrollment was highest for Listserv/email contacts that were likely sent out by community partners or referrals from some other person.

Conclusions: We conclude when recruiting remotely it is crucial to have community partners in the site location to help spread the word as these resulted in the most eligible and enrolled participants. Paid social media ads are a valuable alternative when community partners cannot be identified; however, identifying eligible participants may take more effort.

Abstract 22

Elucidating the Region of SHP2-DDX3X Interaction that Contributes to Translational Control of CD274 mRNA in KRAS-Active Non-Small Cell Lung Cancer

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Immune checkpoint inhibitors (ICIs) significantly shifted the landscape in management of non-small cell lung cancer (NSCLC) and improved treatment outcomes for these patients. Clinical benefit of ICIs is correlated with tumoral expression of PDL1. However, many patients develop resistance to ICIs often linked to expression levels of PD-L1. To improve patient treatment outcomes, identification of other therapeutic targets that could overcome inherent and acquired ICI resistance is needed. Downstream of KRAS lies SHP2, a tyrosine phosphatase, that can negatively regulate PDL1 expression in KRAS-active NSCLC, and we sought to understand the mechanism. Using immunoprecipitated of ectopically-expressed SHP2 in H460 cells, interacting proteins were identified using mass spectrometry. DDX3, an RNA helicase, and eukaryotic translation elongation factor 1a were identified as potential partners of SHP2. We hypothesize that SHP2 interacts with DDX3x on newly transcribed CD274 mRNA to control translation of the PDL1 message.

Materials and Methods: Mass Spectrometry Analysis: WT-SHP2 was cloned into a pIRES2 (Addgene) and transfected into H460 cells. SHP2 was immunoprecipitated with an anti-SHP2 monoclonal antibody (Cell Signaling). Immune complexes were captured by Protein A magnetic beads (Cell Signaling) and separated by SDS-PAGE. Excised gel slices were treated with dithiothreitol and iodoacetamide, digested with trypsin, and subjected to liquid chromatography-mass spectrometry (LC-MS/MS) analysis. MS data sets were searched in MASCOT software against a database from UniProt to determine interacting proteins. In vitro assessment of Protein-Protein interactions: DDX3X and SHP2wt (and mutants) were cloned into pET28a and expressed in BL-21 cells. His-tagged proteins were Ni-column-purified (Thermo Fisher). Magnetic Ni-NTA beads (Cell Signaling) were used to immobilize DDX3X and SHP2 ('bait'). The bait was incubated with extracts from A549 and H460 cells as 'prey'. Western blot analysis was performed to visualize the interactions using SHP2, DDX3X, and GAPDH (control) antibodies (Cell Signaling Technologies) and detected using chemiluminescence (Promega). Cloning of mutant SHP2 proteins: A series of SHP2 deletion mutants were constructed in pET28a-SHP2 generated by PCR amplification of SHP2 gene with a specific primer sets for each mutant. SHP2 point mutations were generated with the Quik change-direct mutagenesis kit (Agilent). The vectors were transformed into DH5a cells and confirmed by Sanger sequencing. In vivo assessment of Protein-Protein interactions: SHP2 and DDX3 proteins were immunoprecipitated from A549 and H460 extracts with an anti-SHP2 monoclonal (Cell Signaling) or an anti-DDX3 monoclonal (Abcam) antibody. Immune complexes were captured with Protein A magnetic beads (Cell Signaling), separated by SDS-PAGE, and transferred to nitrocellulose for Western blot analysis. The blots were incubated with indicated antibodies and detected using chemiluminescence (Promega). Assessment of SHP2 phosphatase activity: Synthetic phosphor-DDX3x peptide (AAPPTec) was incubated with SHP2 protein with a fluorescent substrate of SHP2, DiFUMP (BPS Bioscience). The catalytic activity of SHP2 was monitored using a fluorescent signal at the absorbance of 620nm.

Results and Conclusions: Mass spectrometry analysis revealed that DDX3x, with other components of the translation machinery, interacts with SHP2. This interaction between DDX3x and SHP2 has been confirmed both in vivo and in vitro. Mutagenesis will allow confirmation of the binding region. It has been observed that phosphorylated peptides derived from DDX3 are incapable of activating the phosphatase activity of SHP2, suggesting DDX3 is not a substrate for SHP2.

Abstract 23

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

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Introduction: Osteosarcoma (OS) is a primary bone malignancy that affects children and young adults. In 2024, it is anticipated that 3, 970 new cases will be diagnosed, with 2050 deaths in the USA. Treatment includes cisplatin-based chemotherapy, but dose escalation and improved efficacy are limited by toxicity. Therefore, a treatment strategy that could deliver chemotherapy specifically to cancer cells has the potential to both enhance efficacy and reduce toxicity.

Methods: Human blood macrophages were stimulated to the M1 phenotype and used to prepare empty and cisplatin-loaded vesicles (E-MEVs and C-MEVs) using N2 cavitation for in vitro studies. MEVs were characterized by electron microscopy, and in vivo localization was evaluated using DiR dye-labeled vesicles. HOS and 143B cells were treated with free cisplatin and C-MEVs at their respective IC50s to assess cell viability and double stranded DNA breaks using the Y-H2AX antibody. OS mouse xenografts were generated by injecting luciferase-labeled 143B cells intratibially. RAW 264.7 M1 macrophages were cultured to prepare C-MEVs for animal studies. Weekly treatments of C-MEVs, free cisplatin, and vehicle (1xPBS) were given. Tumor volumes were assessed weekly by measuring the bioluminescence intensity (BLI) of tumor cells for 12 weeks and followed for survival. Treatment-induced toxicities were examined by body weight, blood chemistry, and pathophysiological changes in the liver and kidney of mice.

Results: MEVs had a size range of 100-150nm and localized to the liver and tumor of the mice. C-MEVs had significantly lower IC50s than free cisplatin. When dosed at IC50 values, double-stranded DNA damage was equivalent between free cisplatin and C-MEVs despite >32% lower dose administered is C-MEVs. (Table 1). Bioluminescence relative ratio to baseline at 12 weeks was significantly lower in C-MEV treated mice compared to the free cisplatin and vehicle treated mice (332 fold change (C-MEV) versus 662 fold change (free cisplatin) versus 1276 fold change (control); $p<0.05$). Body weight at 12 weeks was significantly lower in free cisplatin treated mice compared to the C-MEV and control mice (24.91 grams (g) (free cisplatin) versus 30.56 g (C-MEVs) and 30.36 g (control); $p<0.05$). Albumin/globulin ratio (normal range 1-2), a measure of nephrotoxicity, at sacrifice was significantly higher in the free cisplatin-treated mice compared to the C-MEV and control mice (3.07 (free cisplatin) versus 1.78 (C-MEVs) and 1.66 (control); $p<0.05$).

Discussion: Cisplatin loaded MEVs (C-MEVs) are more effective and less toxic than free cisplatin in an osteosarcoma mouse model and warrant further development as a novel nano delivery system.

Abstract 24

Enhancing Cancer Care through Patient Education Material Analysis and Community Resource Validation

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Introduction: Cancer patients in Kentucky face diverse challenges, necessitating a comprehensive approach to address their needs. Our study builds upon the Comprehensive Connected Cancer Care (C4) project, focusing on assessing and validating patient education materials (PEM) and community resources. The aim is to support the development of a smartphone-based cancer patient navigation system, enhancing cancer care delivery and patient engagement in Kentucky.

Methods: We compiled and coded 397 patient education materials (PEM) from the Markey Cancer Center Psych Oncology team, reducing them to 234 validated PEM. Additionally, 401 Lexington community resources were collected, validated, and reduced to 356 resources. Each resource underwent tagging and categorization based on the 43 concerns listed in the National Comprehensive Cancer Network (NCCN) Problem List.

Results: The majority of PEM and community resources primarily addressed practical concerns. However, both lacked resources addressing spiritual or religious concerns and other NCCN concerns. PEM focused on personal care practical concerns, while community resources prioritized basic needs practical concerns such as finances, housing, and food sufficiency.

Conclusions: Our study, in collaboration with the C4 project, highlights the importance of assessing and validating patient education materials and community resources for the development of a smartphone-based cancer patient navigation system. Limitations include the current focus on practical concerns, indicating the need for ongoing refinement and usability testing. Nonetheless, our findings contribute valuable insights to the C4 program, emphasizing the significance of addressing practical concerns and empowering patients with accessible information and resources. Integrating validated resources into a smartphone-based navigation system represents a significant advancement in enhancing cancer care delivery and patient engagement in Kentucky.

Abstract 25

Enhancing Immunotherapy by Modulating MLH1 Phosphorylation with ABL Kinase Inhibitors

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The DNA mismatch repair (MMR) pathway identifies and corrects misincorporations that arise during DNA replication. Mutations within the MMR pathway, both germline or somatic, lead to development of cancer. Microsatellite instability (MSI) is a hallmark of MMR deficient tumors. Microsatellites are short, repeated sequences prone to slippage during DNA replication. MSI is routinely measured clinically in multiple tumor types and used to determine treatment. MSI-high/MMR defective tumors are ideal candidates for immunotherapy as they have increased mutational load and a higher number of neoantigens. Our work shows that ABL1 kinase directly phosphorylates critical MMR protein, MLH1. In the absence of ABL1 activity, MLH1 binds to chaperone HSP70 and undergoes lysosomal degradation. We propose that treatment with FDA-approved ABL kinase inhibitor, Nilotinib, will decrease MLH1 protein over time and convert an MSI-low tumor to an MSI-high tumor that can be targeted with immunotherapy checkpoint inhibitors. We show that ABL1 phosphorylates MLH1 at tyrosine 646. Mutation of this residue leads to loss of MMR and reduced MLH1 stability that can be partially reversed with HSP70 inhibition. Chronic treatment with Nilotinib over three months was tolerated by cancer cells, resulted in the loss of MLH1 protein, and began to convert the cells to an MSI-high phenotype as measured by digital droplet PCR. Understanding MLH1 regulation by ABL1 will allow us to predict the effects of long-term tyrosine kinase inhibitor use on genomic stability both in immunotherapy and in chronic myelogenous leukemia patients on long-term ABL kinase treatment therapy.

Abstract 26

Equitable Implementation of Lung Cancer Screening in Kentucky: A Learning Collaborative

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Kentucky has the highest rates of lung cancer incidence and mortality in the nation. However, collaborative statewide efforts from 2015-2019 led by the Kentucky Cancer Consortium's Lung Cancer Network and the Kentucky LEADS (Lung Cancer Education Awareness Detection Survivorship) Collaborative™, have helped Kentucky experience some of the greatest reductions in late-stage lung cancer incidence in the country – a two times faster decline in Kentucky than the U.S. and a three times faster decline in Appalachian Kentucky than the U.S.

To continue the momentum of late-stage incidence reduction and achieve equity in lung cancer screening, the Kentucky LEADS Collaborative™/QUILSTM Group (Quality Implementation of Lung Cancer Screening) implemented a six-session virtual learning collaborative from January – June 2023. Evaluation of this effort included pre- and post-intervention surveys-test registration and a post-test end of the session survey. There were 55 participants in the first session, participation increased over the six sessions to a high of 66, and the 142 unique participants overall all sessions combined represented 34 of the 120 Kentucky counties with nine extended areas represented outside of the state. Nineteen lung cancer screening programs were represented across the six sessions with twelve navigators participating it at least one session. Most participants consisted of those involved with lung cancer screening programs, community-based organizations, clinical settings, non-profit organizations, networks/ collaboratives, and state/ government agencies.

At the conclusion of the collaborative, (N=52, 76%) of respondents in the post-test intervention agreed that they had the sufficient skills and knowledge to perform equitable lung cancer screening in their program role and (N=13, 20%) indicated their overall knowledge of equity regarding lung cancer screening as excellent. There was also strong agreement reported on shared decision making (N=52, 80%), patient engagement and retention methods (N=63, 97%), and responsible marketing and outreach (N=58, 89%) strategies as all being important for achieving equitable lung cancer screening.

Participants' primary interest areas included community outreach/assessment/education, implementation of health equity practices, and improved general knowledge of LCS data. Attendees indicated that they plan to apply the material from the collaborative to address health equity/social drivers of health as well as implement and educate others on best practices. As a result of the collaborative, participants gained a better understanding of the role of geography as a social driver of health, the economic burden of health disparities and the complex factors that are associated and demonstrated continued interest in future lung cancer-related learning collaboratives.

Abstract 27

Evaluating the Intensity of Inpatient and Outpatient Resource Utilization Following Diagnosis of Acute Leukemia

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Introduction: Acute leukemia is a life-changing diagnosis. Aside from the initial direct clinical care including hospitalization and induction chemotherapy, there are also indirect demands on resources required in order to effectively manage these patients. These indirect resources include transportation to treatment facility, outpatient appointments, central line care, laboratory tests, blood transfusions, pharmacy expenses and unplanned admissions. Multiple studies have examined the healthcare costs and resources associated with treatment of AML. One study examined the financial cost and required resources of AML patients compared the costs generated from chemotherapy alone versus patients receiving allogeneic hematopoietic cell transplant [Preussler, 2017]. Use of resources was determined by the number of inpatient hospitalizations, number of inpatient days, and number of outpatient visits per patient for a year post-AML diagnosis. Not surprisingly, patients with allogeneic hematopoietic cell transplant had higher resource utilization and healthcare costs than those patients receiving chemotherapy alone ($579, 649; 88.0 inpatient days versus $296, 529; 57.1 inpatient days). Another retrospective study assessed costs and resource utilization of patients with relapsed or refractory AML based on physician office visits, emergency department visits, length of stay, number of hospital days, and use of pharmacy services [Pandya, 2019]. Both studies showcase the immense financial and logistical toll that AML treatment can have on patients and healthcare as a whole. The objective of this study is to determine inpatient and outpatient resource utilization by acute leukemia patients during and subsequent to induction chemotherapy at the UK Markey Cancer Center (MCC).

Methods: A retrospective chart review in which information was obtained from patient EMR. Population was identified using all adult hospitalized patients with an ICD-10 billing diagnosis of acute leukemia at the UK from 2016-2022. Data extracted included: diagnosis, age at diagnosis, gender, location of residence (to calculate distance from Markey Cancer Center), date of hospital admission, date of hospital discharge, number of trips to healthcare facility following diagnosis (outside hospital, inpatient visit, clinic visit, infusion center appointment), number of blood product transfusions, and number of infections.

Results: 97 patients were identified with acute leukemia for which initial hospitalization information and post hospitalization information could be compiled. This included 79 patients with AML, 4 T-ALL, 11 B-ALL, 1 APL, and 2 Leukemia – NOS. Average age was 53.8 years. 34 females and 63 males. Patients received initial therapy based upon their subtype of leukemia. Medical records were reviewed to determine resource utilization for the first 12 months following diagnosis. Number of initial inpatient hospital days averaged 34.9 days. Number of inpatient days within the first 12 months, exclusive of the initial hospital stay averaged 39.4 days. Outpatient visits including infusion center, local or UK clinics, ER visits averaged 50.8 days. Remaining days were assigned as free from active medical care and averaged 239.8 days over the year. Patients required on average 34.9 units of blood products during the assigned period.

Discussion: The results of our analysis demonstrated the significant resources required during and after initial therapy for newly diagnoses acute leukemia patients. This includes the frequency of outpatient appointments, readmissions, blood product transfusions, infusion appointments, distances travelled, and provides some indication as to the expenses incurred. This information will be useful when discussing expectations of leukemia therapy with patients and their families.

Abstract 28

Examining the Impact of Financial Toxicity on Healthcare Transitions of Adolescent and Young Adult Cancer Survivors: Kentucky Cancer Registry

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Adolescent and young adult (AYA) cancer survivors, particularly those diagnosed between 15 and 39 years of age, are susceptible to financial toxicity (FT) due to their developmental life stages, healthcare transitions (HCTs) from pediatric to adult or primary care, and the enduring effects of cancer. Despite these challenges, limited research has delved into the consequences of FT on AYA HCTs. This study aimed to assess the impact of FT on HCTs and health-related quality of life (QOL) among AYA cancer survivors in Kentucky. A cross-sectional survey design recruited 260 survivors from the Kentucky Cancer Registry, analyzing FT (COST-FACIT), HCT outcomes (transition readiness, healthcare utilization, long-term health impact), and health-related QOL (PROMIS Global Health, anxiety, depression) through descriptive statistics and bivariate analysis. Results showed a predominantly white, female, employed, and educated sample with moderate FT scores, yet significant financial challenges were reported. Participants demonstrated high transition readiness and QOL, often relying on oncologists for healthcare. Notably, there were no significant associations between FT and HCT or health-related QOL outcomes, contrasting existing literature. These findings highlight the need for further research to comprehend FT's intricate effects on HCTs among specific AYA subgroups.

Abstract 29

Exploring the Heterogeneity of Infiltrating Immune Cells in Non-Small Cell Lung Cancer

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Non-small cell lung cancer (NSCLC) is current the leading cause of cancer related death in the United States. NSCLC can be divided into two major subsets, squamous cell carcinomas (SCC) and adenocarcinomas (ADC). ADCs are more treatable, given they possess many targetable mutations that SCCs do not. These two lung cancer subtypes also differ in the composition of the immune microenvironments, and SCCs are often heavily infiltrated with neutrophils. In general, in cancer biology, neutrophils can be classified into two major groups, either tumor promoting or tumor eliminating. Our lab has shown that the epigenetic inhibitor, tazemetostat, in combination with immunotherapy, not only decreased lung SCC tumor volume significantly, but also altered neutrophils. Tazemetostat inhibits the Polycomb Repressive Complex 2 (PRC2), which tri-methylates lysine 27 on histone 3 to silence gene expression through its catalytic subunit, EZH2. Prior research suggests that neutrophils in an immature state are more tumor promoting, and often large increases in immature neutrophil numbers are observed as tumors grow. We observed that EZH2 inhibition shifted populations towards more IFN-responsive neutrophils in the tumor, and more mature neutrophils in the bone marrow. However, the functionality of varied immune cells depleted for EZH2 is unclear. Our lab employs the use of a mouse model on SCC involving the deletion of Lkb1 and Pten (LP) via the inhalation of Adeno-cre virus. To complement the pharmacological approaches, we also have mice in which we can conditionally delete EZH2 with the addition of doxycycline water to understand how neutrophils and T cells utilize EZH2. Lastly, we have optimized use of a carcinogen, called NTCU, that can be painted on the backs of mice and drive spontaneous development of SCC within the lungs. We have generated NTCU-driven tumors in mice with the EZH2 conditional alleles, and established tumoroids from these mice. These models will allow us to delete EZH2 in the tumor only, in the immune cells, or both, depending on the transplant strategies we use. Our tumoroid models are 3D cultures that recapitulate the liquid air interface seen within the lung. Prior work within our lab has shown that 3D cultures better predict in vivo treatment results. We have already performed several "multi-culture" experiments where we combined the lung tumoroids with varied immune cells to replicate what is seen within the lung. Our data has shown that addition of total bone marrow to the cultures helps the tumors proliferate significantly more than those that did not receive bone marrow. This proliferation is then reduced via the addition of tazemetostat and anti-PD1 antibody. This work was funded by: R01 CA237643 and P30 CA177558 for MCC shared resources.

Abstract 30

Extending the Impact of Cancer Research Through Communication Dissemination

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Purpose: An essential component of bidirectional community engagement is the dissemination of research findings back to the community. Many researchers do not prioritize community research dissemination because they do not perceive it as important and have low skills in plain language communication. However, its importance is gaining momentum nationwide as many grant funding mechanisms are now requiring communication dissemination plans and lay community abstracts as part of funding requirements. UK Markey Cancer Center (MCC) Community Impact Office (CIO) developed a Community Research Dissemination Toolkit which can be utilized by a myriad of groups, including researchers, community outreach and engagement teams, marketing, communications, and public relations teams. The Toolkit can be used to guide the development and dissemination of materials about cancer research to the lay public.

Methods: The Toolkit offers recommendations for researchers to communicate with non-scientific audiences following plain language principles. Components include a sample dissemination plan for grant proposals, dissemination strategy options, tips for visual presentations and writing, substitutions for frequently used cancer and research terms, and templates for project summary paragraphs and infographics. CIO's Community Impact Ambassadors from each MCC Research Program contributed to the development and refinement of the Toolkit. CIO created sample summary paragraphs and infographics about Ambassadors' research projects. MCC Community Advisory Board provided feedback on the sample materials' overall appearance, relevance of the graphics used to convey information, and comprehensiveness of the information.

Results: From February 2022 – April 2024, 30 Toolkits were provided to MCC researchers through CIO Consultation Requests. Seventeen MCC researchers utilized the Toolkit to create plain language abstracts and dissemination plans for 15 internal and 5 external grant proposals. Thirteen MCC researchers utilized the Toolkit to develop lay audience presentations. One of the lay summary infographics was shared via MCC social media and reached 1,776 people. Another infographic was used to educate a state lawmaker and informed a biomarker testing bill. From October 2023 to March 2024, we have disseminated the Toolkit externally to 41 cancer centers who have requested the Toolkit for their bidirectional community engagement efforts.

Conclusion: MCC CIO has received positive feedback from researchers and other cancer centers on the usefulness of the Toolkit. Utilization of the Toolkit can further extend the reach, philanthropy, and impact among cancer centers nationwide, and ensure that the community is prioritized as we work to become a cancer-free catchment area.

Abstract 31

Extracellular Vesicles Derived from Glioblastoma After Radiation Promote Microglia-Mediated Neurotoxicity

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Little is known about the underlying mechanisms of glioblastoma (GBM) and/or therapy-derived cognitive impairment (CICI). Our data indicates that GBM patients exhibit higher numbers of extracellular vesicles (EVs) compared to non-cancer patients and levels of EVs release are increased after radiation therapy (RT). Importantly, these radiation derived EVs (named Redox EVs), contain high levels of highly reactive α, β-unsaturated aldehyde 4-hydroxynonenal (HNE), which participates in multiple pathological processes. Given that EVs can function as messengers between cells, we seek to elucidate if GBM-derived Redox EVs trigger molecular mechanisms within glial cells that induce neurotoxicity. As a first step, we evaluated if microglia cells (HMC3) would uptake Redox EVs. EVs were collected from LN18-RFP, a GBM cell line transfected to express RFP in the plasma membrane, specifically in phosphatidylserine. After adding the EVs to microglia cells, confocal images showed that EVs are taken up within minutes of exposure and they spread evenly throughout the cells. To determine if Redox EVs cause ROS release from microglia cells, we treated HMC3 cells with Redox EVs and monitored H2O2 levels in the medium. Results showed that Redox EVs from LN18, GBM-PDX cells (G44 and G84), and GBM patients; caused a significant increase in H2O2 production as early as 3h and continued to increase at 24h. These data suggest that Redox EVs activate microglial cells that in turn release ROS. To probe whether H2O2 is toxic to neuronal cells, Redox EVs were added to co-culturing chambers containing HMC3 cells and neuron cells (HCN2) for 48h. Cell viability of HCN2 cells was significantly reduced after co-culturing with Redox EVs-activated HMC3 cells. More importantly, the viability of HCN2 cells was rescued by pre-treating them with catalase. Next, we tested if altering the microglial redox state using BMX-001 (an MnSOD mimetic, currently in clinical trials for high-grade gliomas), could mitigate glial cells activation. Adding BMX-001 in combination with RT increased the levels of 4HNE-adducted proteins in GBM cells but decreased in microglial cells. Cytokines were measured as markers of microglia activation and inflammatory response. Overall data suggest that H2O2 released from microglia could be a key for Redox EV-mediated neuronal injury and that BMX-001 could reduce GBM damage to non-cancer cells such as microglia and neurons.

Abstract 32

Fatty Acid Synthase Enhances Stem-Like Properties of Colorectal Cancer Cells via an Increase in Notum Expression and Secretion

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Introduction: Overexpression of lipogenic enzymes has been associated with a poor clinical outcome in colorectal cancer (CRC). Fatty acid synthase (FASN) synthesizes palmitate which can be utilized for post-translational modifications of various proteins. The hyperactivation of the canonical Wnt/β-catenin signaling pathway greatly contributes to the initiating event in CRC via the inactivating mutation of a vital tumor suppressor, APC. Notum, a palmitoleoyl-protein, inhibits downstream activation of Wnt signaling by de-palmitoylating Wnt ligands. Interestingly, a recent study found that Apc mutant intestinal stem cells utilize Notum to outcompete the wild type cells by promoting differentiation. Furthermore, Notum is also a CRC stem cell marker, and its expression correlates with poor prognosis in CRC. We found that upregulation of FASN is associated with an increase in Notum expression in mouse adenoma tissues. Furthermore, we found that FASN positively correlates with Notum expression in human CRC. Therefore, the purpose of this study is to elucidate the mechanisms and contribution of FASN/Notum axis in CRC.

Methods: Adenoma and normal intestinal organoids, established from ApcMin/VillinCre-ERT2 and Apc/VillinCre-ERT2 mouse models with hetero- and homozygous deletion of FASN. 4-hydroxytamoxifen (4-OHT) and TVB-3664 (FASN inhibitor) treatments were used to assess the effect of FASN deletion/inhibition on organoid growth and viability. LIVE/DEAD™ Viability/Cytotoxicity and Cell Titer-Glo® 3D Cell Viability Assays were used for quantitative analysis of growth. Human NOTUM/Protein notum homolog ELISA Kit was used to analyze Notum secretion. CRC cells, NTC and FASN shRNA, and control and FASN overexpression, were used for Notum analysis. TCF/LEF reporter luciferase assay was used to assess active β-catenin activity. MSAB (β-catenin inhibitor) treatments were conducted to assess the effect of β-catenin inhibition on spheroid/colony formations.

Results: The TCGA data analysis shows that Notum is significantly upregulated in CRC and its expression correlates with expression of FASN in tumor tissue. Utilizing qRT-PCR and Western blot analyses, we show that downregulation of FASN leads to a decrease in expressions of active β-catenin, Notum, and other stem cell markers in transgenic mouse models. Moreover, 4-OHT-mediated inhibition of FASN results in decrease viability and size in ApcMin/Fasn/VillinCre-ERT2 and ApcMin organoids. Consistently, shRNA-mediated deletion of FASN decreases expression of Notum and active β-catenin activity in HT29 cells. In contrast, overexpression of FASN increases the expression levels of active and total β-catenin, Notum, and other stem cell markers and restores β-catenin activity in SW480 cells. Furthermore, pharmacological inhibition of β-catenin decreases Notum expression and spheroid/colony formation in CRC cells; however, FASN expression remains constant.

Conclusion: In summary, downregulation of FASN leads to a decrease in expression of Notum and β-catenin activity and is associated with significant decrease in viability and growth in organoid models. Conversely, FASN overexpression upregulates β-catenin activity and Notum expression suggesting a potential cross talk between de novo lipid synthesis and Notum. Delineating the role of FASN regulation of stem-like properties via upregulation of β-catenin signaling and expression of Notum and other stem cell markers will provide the rationale for targeting the FASN/Notum axis in CRC.

Abstract 33

Flow Cytometry and Immune Monitoring Shared Resource Facility (FCIM SRF)

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The Flow Cytometry and Immune Monitoring Shared Resource Facility (FCIM SRF) facilitates high-impact pre-clinical and clinical cancer research within the Markey Cancer Center (MCC). It is jointly managed by MCC and the University of Kentucky (UK). The FCIM SRF provides state-of-the-art flow cytometry and immune monitoring services supporting all Research Programs. Flow cytometry services include cell immunophenotyping for surface and intracellular biomarkers, DNA content, cell cycle, apoptosis, cytokine/growth factor synthesis, signal transduction and cell activation. Immune monitoring services include cryopreservation/banking of blood and tumor tissue cells, immunophenotyping for biomarkers and/or cytokines/growth factor analysis by enzyme-linked immunosorbent assay, enzyme-linked immunosorbent spot and Luminex multiplexing. Additional assays include analysis of antigen-specific T cells, cytotoxicity, antibody synthesis as well as generation of dendritic cells and macrophages from blood, and T cell expansion from blood and/or tumor tissue. FCIM offers high dimensional data analysis for multi-parameter flow cytometry and Luminex multianalyte profiling for complex cell populations and rare cell subsets. FCIM has three Specific Aims: 1) Provide cost effective state-of-the-art flow cytometry and cell sorting services for basic, pre-clinical and clinical researchers; 2) Provide cost effective state-of-the-art immune monitoring services for basic, pre-clinical and clinical researchers; and 3) Provide educational training and mentoring to MCC members and/or laboratory staff in proper use of self-service instrumentation and appropriate experimental design and data analysis. The comprehensive flow cytometry, immune monitoring and immune assay design services offered by FCIM provide MCC with exceptional, cost-effective, and in-depth analysis of the immune system for pre-clinical and clinical cancer research.

Abstract 34

Follow-Up Care among Rural and Urban Cancer Survivors: Parents' Perceptions of Access and Acceptability

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Introduction: Childhood cancer survivors require ongoing follow-up services to monitor for cancer recurrence, manage treatment side effects, and address long-term health concerns. Rural survivors face unique challenges accessing follow-up care due to limited availability of survivorship clinics and primary care providers experienced in cancer care. This study explores rural and urban childhood cancer survivors' access to follow-up care and their perceptions of its accessibility.

Method: A cross-sectional, quantitative survey was conducted among parents/guardians of childhood cancer survivors (aged 2-17 and at least 2 years since cancer diagnosis) identified through the Kentucky Cancer Registry. Of 698 eligible parents/guardians, 371 granted permission for study staff to contact them and 238 (116 rural and 122 urban) completed a survey. The survey assessed 1) past 6-month receipt and sources of follow-up care, 2) perceptions of local access to follow-up care, and 3) ratings of the acceptability of various sources of follow-up care (university-based cancer center, university-affiliated cancer center, primary care providers, and primary care providers working with a cancer treatment center).

Results: Rural survivors were significantly more likely to be Non-Hispanic White, Male, slightly older at primary cancer diagnosis, and have greater number of symptoms than urban survivors. Regarding insurance coverage, rural children were less likely to have private coverage and more likely to receive Medicaid coverage. Regarding follow-up care in the past six months, rural parents reported that their children had greater receipt of counseling or psychological services (26.5% rural, 12.4% urban; P<.006). Lower percentages of rural than urban parents rated local access to follow-up care as "good" (34.2% rural, 62.0% urban; P<.0001) and "effective" (36.75% rural, 63.6% urban; P<.0001). There were no rural/urban differences in the acceptability of follow-up care sources by location or type.

Conclusions: Counseling and psychological services need to be expanded for childhood cancer survivors, especially those residing in urban areas. Rural parents' ratings of local follow-up care services suggest that the accessibility and quality of local services would need to be improved to encourage the use of local follow-up care.

Abstract 35

Free Nicotine Replacement Therapy as a Strategy to Promote Tobacco Treatment Equity at Cancer Center Cessation Initiative Sites: A Mixed-Methods Study

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Significance: While clinical practice guidelines recommend integration of tobacco treatment into cancer care, this is not yet realized in most settings. Nicotine replacement therapy (NRT) is highly effective for cancer patients trying to quit smoking but coverage, especially with public insurance for older and low-income adults, varies widely. Offering free NRT to cancer patients has potential to increase tobacco treatment engagement and reduce disparities around tobacco-related disease burden. This study aims to describe NCI-designated Cancer Center Cessation Initiative (C3I) sites that have free NRT programs and present their strengths and obstacles.

Methods: Tobacco treatment engagement and medication use rates at a sample of n=8 C3I sites with free NRT programs were gathered via standardized survey. Semi-structured interviews based on the Consolidated Framework for Implementation Research were then conducted with n=9 C3I sites in 2023. Descriptive statistics were used for survey data and directed content analysis for interview data.

Results: On average, 37.4% of patients who smoke engaged in a C3I tobacco treatment program and 48.4% of those were prescribed NRT. Based on interviews, barriers to widely disseminating free NRT included unclear or inconsistent eligibility determinations, with some sites requiring proof of financial hardship; healthcare systems requiring prescription orders for over-the-counter NRT formulations; and pick-up limited to a single location. Also, most sites operated under institutional policies barring advertisement of free NRT plus funding uncertainty that jeopardized program sustainability. Strengths across free NRT programs included integration of tobacco treatment programs into electronic health records and robust availability of evidence-based cessation counseling paired with combination NRT.

Conclusion: Results show significant heterogeneity in how some cancer centers provide free NRT to patients, an initiative that is largely driven by the desire to improve equitable access to tobacco treatment. Unfortunately, barriers related to prescription and distribution of NRT, eligibility, advertisement, and sustainability mean that providing accessible, gold standard treatment to all cancer patients who smoke is not yet a reality. Future research should investigate how to improve access and delivery of free cessation medications for all patients with cancer who smoke.

Abstract 36

Functional ROLE of Shoc2 SCAFFOLD in LYMPHANGIOGENESIS

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The fundamental extracellular signal-regulated kinases 1 and 2 (ERK1/2) signaling cascade is essential for the morphogenesis and maintenance of most tissues. Mutations within the genes of the ERK1/2 pathway cause debilitating congenital abnormalities or cancer. To offset the catastrophic events of deregulated ERK1/2, signals are tightly controlled and modulated at different levels, including assembling the scaffold signaling complexes. We study how scaffold protein Shoc2 guides the ERK1/2 signals in normal physiology to understand the pathogenesis of the disease caused by mutations in the Shoc2 gene- Noonan Syndrome with Loose anagen hair (NSLH).

Our lab developed a zebrafish CRISPR/Cas9 model for Shoc2 deficiency and established that loss of Shoc2 has a systemic effect on early development recapitulating congenital malformations observed in patients with Shoc2 mutations. Our recent discoveries showed that Shoc2 loss in zebrafish results in striking loss of lymphatic vasculature. Notably, lymphatic anomalies of patients with genetic disorders of the ERK1/2 pathway include intestinal lymphangiectasia, thoracic duct dysfunction, dilated lymphatic channels, and lymphatic vessel dysmotility.

Using the comparative RNAseq analysis of the human lymphatic endothelial cells (LEC), we uncovered remarkable induction of Interferon (IFN) response genes and activation of the JAK/STAT pathway. We also found that Shoc2 regulates JAK/STAT pathway activation in an ERK1/2-independent manner. Importantly, Shoc2 loss triggered the expression of genes of the innate immunity sensors RIG-1/MDA5 pathway. Furthermore, the expression of IFN response genes in our CRISPR/Cas9 Shoc2 zebrafish mutants was also elevated. Hence, these studies identified the unknown functions of Shoc2 in lymphangiogenesis and regulating LEC.

We will next determine if Shoc2-mediated IFN response is followed by apoptosis or autophagy-mediated cell death. Future studies will also examine whether IFN-induced response contributes to lymphatic abnormalities in NSLH and determine Shoc2's function in lymphangiogenesis

Abstract 37

ABSTRACT WITHDRAWN

Abstract 38

HPV Testing for Cervical Cancer: Barriers to Follow-Up Care among Hispanic Women

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Background: Of all cervical cancers, 91% are caused by the human papillomavirus or HPV. Therefore, HPV testing plays an important role in cervical cancer screening in addition to traditional Pap tests. A positive HPV test result determines the type of follow-up testing and procedures women need to prevent the infection from progressing to cervical cancer. Hispanic women in the United States (U.S.) have lower cervical cancer screening rates and higher cervical cancer incidence than non-Hispanic White Women. In addition, they are less likely to adhere to the recommended follow-up care after a positive HPV test, increasing their risk of developing cervical cancer. Using the Intervention Mapping Framework (IMF), the objective of this research is to increase understanding of barriers and facilitators that influence adherence to recommended follow-up care after positive HPV test results among Hispanic women.

Methods: The study population consisted of self-identified Hispanic women aged 30-65 who received a positive HPV test in the last three years. Using an observational, cross-sectional design, we conducted individual in-depth qualitative interviews and a demographic questionnaire. We also performed thematic analysis of the interview transcripts.

Results: Thirty-three individual interviews were conducted in Spanish. All participants were born outside the U.S., with a mean age of 38.8. Most participants (87.9%) had no health insurance, and less than half (42.2%) reported having a gynecologist. More than half (60.6%) had less than a high school degree, over three-quarters (78.8%) had a monthly income of less than $3,000, and only 18.2% were employed full-time. A common barrier was the lack of knowledge about HPV and HPV testing. Most women knew that HPV is sexually transmitted, but they did not know there was a test to detect HPV infection. Another barrier was patriarchal cultural gender roles. Many women did not want to tell their partners they tested positive for HPV because they feared their partners would accuse them of being unfaithful. Additional barriers to follow-up care included new requirements to qualify for financial assistance, lack of transportation, low literacy levels, limited English proficiency, undocumented immigration status, and lack of health insurance. Facilitators included access to safety net community clinics, receipt of financial assistance from safety net clinics, and transportation vouchers issued by safety net clinics.

Conclusions: Hispanic women face several barriers to adhering to follow-up care after a positive HPV test, including traditional healthcare access barriers, cultural gender roles, and stigma around sexually transmitted infections. The assistance provided by safety net clinics—without stringent requirements—is crucial to help low-income, uninsured Hispanic women overcome healthcare access barriers. Culturally and linguistically tailored, low-literacy educational materials about HPV and HPV testing should be created in English and Spanish for Hispanic women and their romantic partners to improve knowledge and address the cultural stigma about sexually transmitted infections in this population.

Abstract 39

HPV Vaccine Uptake and Completion among Hispanic Adults in Kentucky

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Purpose: The incidence rates of two HPV-related cancers, cervical and penile cancers, are higher for Hispanics versus non-Hispanics in the U.S., but little data exists on HPV vaccination among Hispanic adults. This study examined self-reported uptake and completion of the HPV vaccine among Hispanic adults in Kentucky (KY) and potential associations with age, gender, country of birth, English proficiency, educational attainment, income, and health insurance status.

Methods: An observational cross-sectional survey was conducted with a convenience sample of 297 self-identified Hispanic men and women (ages 18+) living in KY in 2023 and 2024, as part of a Hispanic cancer needs assessment (275 in Spanish and 22 in English).

Results: HPV vaccine uptake (at least one dose) among Hispanic adults was 30.2% for 18-26 year-olds and 20.6% for 27-45 year-olds, though not significantly different. Completion of all recommended doses of the HPV vaccine was lower than uptake and was higher in the younger group (15.7% 18-26 years vs. 4.8% 27-45 years, P =.009). Women had higher uptake than men (27.0% vs. 14.7%, P=.038), but completion did not have a significant gender difference. Uptake differed by country of birth (45.5% born in U.S., 25.3% born in Mexico, 16.7% born in other countries, P =.010) and was positively associated with English proficiency (43.2% speak English very well, 25.9% well, 20.0% not well vs. 13.6% not at all, P=.007). Further, English proficiency was positively associated with completing the recommended HPV vaccine doses (21.2% speak English very well, 7.7% well, 4.0% not well vs. 5.1% not at all, P=.010). Uptake was higher among those with health insurance than those without (34.5% vs. 19.0%, P=.018). Completion of the recommended HPV vaccine doses was positively associated with health insurance (14.0% vs. 5.4%, P=.041) and those with a high school degree or higher education compared to those with no high school degree (32.8% vs. 11.5%, P =.001). Income was not significantly associated with HPV vaccine uptake. The same patterns of association were observed for education and income for HPV vaccine completion.

Conclusion: Hispanic adults ages 18-26 in KY had slightly lower HPV vaccination rates than recent national estimates for adults of this age (39.9% uptake, 21.5% completion, CDC 2018). National estimates are not available for ages 27-45. The findings provide insights into the social determinants of health contributing to low HPV vaccine uptake and completion among adult Hispanics in Kentucky.

Abstract 40

Identification of Target Protein Molecules Responsible for M2 to M1 Macrophage 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 M1Ø, M2Ø, and tumor-associated macrophages (TAMs). M1Ø can shift the phenotype of M2Ø or TAMs to M1Ø and inhibit cancer progression. Previous studies from our group have suggested the role of M1Ø engineered vesicles (MEVs) and their repolarization efficacy against lung and ovarian cancer models. Therefore, this study aims to identify the proteins and protein-protein interactions responsible for this repolarization effect.

Materials and Methods: Human blood monocytes were stimulated to M1Ø & M2Ø. These M1Ø were dissociated using N2 cavitation using a pressurized chamber and further ultracentrifuged to collect MEVs. The M2Ø were treated with these MEVs for 24 hours and M1Ø was kept as positive control. The mRNA expression for TNF-α (M1) and CD206 (M2) markers were analyzed and plotted in fold change ratio to gapdh levels. The protein lysates of these samples were subjected to proteomics analysis by LC-MS to identify the proteins responsible for repolarization. Proteomic results were analyzed using Proteome Discoverer 2.4 and were searched using the human protein database from UniProt, and were further filtered based on at least 2 peptides and 3 protein spectra matched (PSM). These proteins were quantified based on the abundance in each sample and protein complexes were identified using the online string database.

Results: M2Ø macrophages treated with MEVs had significantly increased mRNA expression of TNF-α compared to M2Ø (0.20±0.01 vs 0.03±0.01) (p=0.01). Whereas, CD206 had lower levels in MEVs treated M2Ø vs M2Ø (1.90±0.77 vs 4.14±0.83) (p=0.07). More than 4500 proteins were identified from M1Ø, M2Ø, and MEVs treated M2Ø. A total of 12 and 16 proteins were identified only in M1Ø and M2Ø, respectively. The 12 proteins in M1Ø were associated with the Interferon gamma signaling complex. The 16 proteins from M2Ø belonged to the hematopoietic cell lineage and Ras-related protein complex. A total of 7 proteins were found only in MEVs treated M2Ø, belonging to heat shock proteins (HSP) and tubulin complexes. A total of 1206 & 794 proteins were found to be more abundant in M1Ø vs M2Ø and more in M2Ø vs M1Ø respectively. A total of 764 proteins were highly expressed in MEVs treated M2Ø vs M2Ø belonging to complexes of histone proteins, antigen presentation, and tubulin proteins. Perilipin, Histone2B, hsp70, sialoadhesin, and importer subunit-α4 proteins, proteins known to negatively regulate T cells, were highly expressed in MEVs treated M2Ø and were also elevated in M1Ø.

Discussion: TME consists of both M2Ø and TAM which support cancer growth and progression and make many cancers resistant to immunotherapy. Repolarizing M2Ø to M1Ø with MEVs is a potential therapeutic strategy for suppressing tumor growth and metastasis as well as sensitizing cancers to immunotherapy. Using a proteomic approach, several potential, novel, mediators of M2Ø re-polarization by MEVs have been identified for further study.

Abstract 41

Immunological Profiling of Adult Human Peripheral Nerve as Framework for Understanding the Cellular and Noncellular Constitution of Nerve-Related Cancers

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In this study, we developed fluorescent and chromogenic immunostaining methods, myelin-selective fluorophores, and standard histological stains to define cellular and noncellular constituents of the intact adult human nerve and their representation in a neuroma biospecimen. We combined Schwann cell (SC)-specific markers, such as S100β, NGFR, Sox10 and myelin protein zero (MPZ), together with axonal, extracellular matrix (collagen, fibronectin, laminin and CSPG) and fibroblast markers to assess the SC's relationship to myelin, axons, other cell types, and the acellular environment. Whereas S100β and Sox10 were sufficient to reveal mature SCs in the absence of other stains, discriminating myelinating and non-myelinating SCs required immunodetection of NGFR along with axonal and myelin markers. Surprisingly, our analysis of NGFR+ profiles uncovered the existence of at least 3 different novel populations of NGFR+ nonglial cells in the stroma and perivascular areas of the endo-, peri- and epineurium of normal nerve and neuroma. Indeed, an important proportion of the nerve's cellular content, including circa 30% of cells in the endoneurium, consisted of S100β- cells that were not associated to axons and expressed markers such as CD34, SMA, Thy1 and Glut1, a perineurial cell marker. To conclude, we provide a generic immunochemical roadmap to reveal the complex arrangement of peripheral nerve-resident cells in normal and pathological human tissues.

Abstract 42

Improving the Docetaxel-Based Chemotherapy in Therapy Resistance Prostate Cancer

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Prostate cancer is known to have a relatively good prognosis, but long-term hormone therapy can lead to castration-resistant prostate cancer (CRPC). Docetaxel, a third-generation chemotherapy drug based on inhibiting depolymerization of microtubules, provide excellent initial response in patients for the CRPC treatment. Despite the improved survival duration and reduction of tumor size observed in some patients, many have no response to the drugs or develop resistance over time. In this study, to elucidate the molecular basis of docetaxel resistance, we established docetaxel resistance prostate cancer PC3-TR, C4-2-TR and DU-145-TR cell lines, which exhibited higher LD50 and lower drug uptake against docetaxel than parental PC3, C4-2 and DU-145 cell lines. We employed quantitative high throughput screen with a collection of 2500 FDA approved drugs to identify drugs that can enhance the docetaxel sensitivity of docetaxel resistance cell lines. ML385 has been identified as a potent NF-E2 related factor-2 (Nrf2) synthetic inhibitor, and its treatment of docetaxel resistance cells significantly suppressed the Nrf2 signaling by decreasing the cytosolic and nuclear Nrf2 levels. In addition, docetaxel resistance cell lines showed marked increases in nuclear accumulation of Nrf2 compared to parental cell lines, suggesting that Nrf2 signaling is homeostatically activated in docetaxel resistance cell lines. Here we identified that silencing of Nrf2, docetaxel sensitivity of resistance cell lines was increased, and that of parental cell lines were reduced by activation of Nrf2. Combination treatment of ML385 with docetaxel induced cell death in docetaxel resistance cell lines. Together, these results provide new insight in the understanding of docetaxel resistance and direct a therapeutic approach to overcome docetaxel resistance in CRPC treatment.

Abstract 43

Incorporating Surgery into Multimodal Therapy Leads to Improved Survival for Stage IIIa Non-Small Cell Lung Cancer: Evidence from an Inverse Probability Treatment-Weighting Study

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Introduction: The treatment for Stage IIIa non-small cell lung cancers (NSCLC) typically involve a surgical based multimodality approach or a definitive chemoradiation therapy plus durvalumab consolidation. However, it remains unclear whether surgery-based multimodality therapy has any survival advantage over definitive chemoradiation plus immunotherapy consolidation.

Method: The National Cancer Database (NCDB) provided data for NSCLC patients at stage IIIa (AJCC8, T3N1/T4N0-1 or T1N2/T2N2) treated either with a surgery-based multimodality approach or definitive chemoradiation plus durvalumab. Survival comparisons were conducted using inverse probability of treatment weighting (IPTW)-adjusted Kaplan-Meier curves and Cox proportional hazards regression. Results were independently confirmed by Landmark Inverse and Clone Censor Weight analyses to address immortal time bias.

Results: Between 2017 and 2019, 24, 170 patients were identified with potentially resectable stage IIIa NSCLC. Of these, 2, 664 (11.2%) underwent surgery-based multimodality therapy, and 2, 985 (12.4%) were treated with definitive chemoradiation plus durvalumab. The surgery-based approach showed a survival benefit compared to definitive chemoradiation plus durvalumab (HR 0.75; 95% CI 0.70-0.80, P<0.001), with the median overall survival not yet reached in the surgery group and 48.59 months in the chemoradiation plus durvalumab group. This advantage was consistent across both N2 negative and positive tumors. Neoadjuvant chemotherapy was just as effective as adjuvant chemotherapy and delay of immunotherapy consolidation to 6 weeks after completion of chemoradiation did not negatively affect survival outcome.

Conclusion: In patients with stage IIIa NSCLC, surgery-based multimodality treatment outperformed chemoradiation plus durvalumab in survival. Future strategies incorporating neoadjuvant and perioperative chemoimmunotherapy hold the potential for further improving survival.

Abstract 44

Induction of Epithelial-to-Mesenchymal-Like Reprogramming in Human Bronchial Epithelial Cells by Acute Cigarette Smoke Exposure

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Cigarette smoke is a significant risk factor for lung cancer, a leading cause of cancer-related deaths in the United States. Despite containing numerous harmful and carcinogenic chemicals, the broader effects of tobacco smoke on lung physiology remain underexplored. Our study aims to investigate the impact of tobacco constituents on human bronchial epithelial cells (HBEC), using HBEC2, HBEC3KT, and HBEC14 cell lines as models for lung diseases. We exposed these cells to cigarette smoke condensate (CSC) from the 1R6F reference cigarette, observing changes in cell morphology over a short timeframe. Higher CSC concentrations induced cell elongation within 24 hours, while lower concentrations produced more pronounced effects after 48 hours, highlighting both time and dose dependencies. Notably, CSC concentrations exceeding 40 ug/ml exhibited cytotoxicity, while doses below 5 ug/ml failed to induce noticeable changes. Intermediate doses (5-20 ug/ml) led to varying degrees of elongation, simulating chronic smoke exposure. Morphological alterations were evident across all HBECs, with HBEC3KT displaying heightened sensitivity to CSC. Furthermore, CSC hindered cell proliferation over time, indicating its inhibitory effect. These morphological changes potentially signify an epithelial-to-mesenchymal transition (EMT), crucial in cancer progression and lung tissue remodeling. Elevated levels of vimentin and alpha smooth muscle actin (α-SMA), observed two days post-CSC exposure across all HBECs, correlated directly with CSC dose, supporting the hypothesis of CSC-induced cellular reprogramming akin to EMT. Our findings underscore the importance of short-term bronchial epithelial cell exposure to CSC and cigarette smoke in initiating cellular reprogramming pathways relevant to cancer and lung diseases. Ongoing investigations aim to delve deeper into underlying mechanisms and ascertain their association with EMT.

Abstract 45

Inhibition of FASN Postpones Development of Resistance to BRAF Inhibitors in Colorectal Cancer

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Introduction: Mutations in proto-oncogene BRAF occur in about 10-15% of CRC patients and BRAFV600E is the most common. For patients who undergo BRAF-targeted therapy, resistance develops 4-6 months after treatment initiation and results in more aggressive disease. We found that development of resistance to BRAF inhibitors (BRAFi) is associated with an increase in lipid metabolism and expression of fatty acid synthase (FASN). FASN is a key enzyme of lipid synthesis overexpressed in CRC. Therefore, our hypothesis is that inhibition lipid metabolism via FASN will postpone development of resistance to BRAFi.

Methods: We established CRC cells resistant to PLX8394, a novel BRAFi. To evaluate differences in parental and resistance cells, CellTiterGlo 2.0, PrestoBlue, CytoSelectTM Invasion Assay, Triglyceride Assay, Seahorse XF, and confocal microscopy were used. Combination of PLX8394 and TVB3664 or C75 (FASN inhibitors) was tested on cell viability, colony formation, and synergy studies in parental and BRAFi resistant cells.

Results: The development of resistance to BRAFi promotes cellular proliferation and increases cyclin D and survivin expression in vitro and in vivo. BRAFi resistance is also associated with an increase in invasive properties and loss of E-cadherin expression. Metabolic changes include an increase in lipid metabolism, oxidative phosphorylation, and triglycerides storage.

RNAseq and western blot analysis show significant upregulation of FASN in BRAFi resistant cells. Using cell viability and soft agar colony formation assays, we show that combined PLX8394 and TVB3664 treatment leads to a significantly higher decrease in cell viability and colony formation as compared to each drug alone in parental cells but not in BRAFi resistant cells. The calculation of a Bliss synergy score confirms that combination treatment with C75 and PLX8394 has synergetic effect in BRAFV600E cells. To further confirm that FASN contributes to resistance to BRAFi, we show that HT29 FASN shRNA cells are more susceptible to PLX8394 treatment as compared to control cells. Importantly, our data show that the long-term treatment with PLX8394 in combination with TVB3664 postpones development of resistance to BRAFi as compared to cells treated with PLX8394 alone.

Conclusion: Our study demonstrates that resistance to BRAFi is associated with a significant increase in proliferation, metastasis, and lipid metabolism. We demonstrate that combination of FASN inhibitors and BRAFi postpones development of resistance in BRAFV600E cells. However, FASN inhibition does not sensitize cells to BRAFi in already resistance cells, suggesting that this approach cannot be used to overcome acquired resistance to BRAFi. In summary our data suggest that an addition of TVB3664 at the beginning of BRAF treatment regimen could be an efficacious treatment strategy for BRAFV600E CRC patients.

Abstract 46

Integration of Gene and Lipid Profiles in Metabolically Driven Human Hepatocellular Carcinoma Tumor and Adjacent Nontumor Tissue

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Background: Hepatocellular carcinoma (HCC) is the most common form of liver cancer worldwide. Metabolic dysfunction-associated steatohepatitis (MASH) is the fastest growing etiology of HCC, which is largely attributed to the parallel rise in obesity and diabetes mellitus. Thus, it is imperative we understand how fatty acid metabolism contributes to the development of HCC for translatable disease prevention and therapeutic development.

Methods: Human HCC tumor (n=8) and adjacent non-tumor samples (n=9) were obtained from the Biospecimen Procurement and Translational Pathology Shared Resource Facility at the UK Markey Cancer Center. All individuals were negative for viral hepatitis while 87.5% (7/8) had confirmed hypertension, diabetes, or were hyperlipidemic. RNA and protein were isolated and used for bulk RNA-sequencing and immunoblotting, respectively. Lipids were extracted using a methyl-tert-butyl ether extraction method for high-throughput lipidomics. Data were analyzed using paired nonparametric analyses via a Wilcoxon or Mann-Whitney test, where appropriate.

Results: Lipid profiling of human tumors revealed significant increases in long chain nonesterified monounsaturated fatty acids (MUFAs; C16:1, C18:1, C19:1, and C20:1) and MUFA-enriched phospholipids (PC30:1, PC32:1, PE32:1, PC34:1, PC36:1) relative to nontumor tissue. Further, there was trend to increase in total triglycerides (P=0.0645) while total cholesterol levels were reduced in tumor tissue. Consistent with lipid profiles, the expression of genes regulating fatty acid oxidation (CPT1A, CPT2, ACADL, ACADM, and HADHA) were significantly lower in tumor versus nontumor tissue.

Conclusions: These results suggest HCC tumors exhibit reduced fatty acid oxidation resulting in an accumulation of MUFAs and triglycerides, as compared to adjacent non-tumor tissue.

Abstract 47

Integrin α6β4 Associates with Overall Survival in Triple Negative Breast Cancer and Sensitizes Cells to Adriamycin/Cytoxan Treatment

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Integrin α6β4 is expressed in approximately 80% of triple negative breast cancer (TNBC) cases, and it associates with a transition to more aggressive phenotypes. Currently, Adriamycin (doxorubicin) and Cytoxan (cyclophosphamide) are employed as first-line chemotherapeutics in TNBC care plans, with platinum agents generally reserved for metastatic cases. Previous investigations from our lab demonstrated that TNBC cells bearing integrin α6β4 had greater sensitivity to platinum based chemotherapeutic agents. However, the impact of integrin α6β4 on the current standard of care protocols used in TNBC treatment remains untested. A breast cancer tissue microarray was used to confirm that integrin α6β4 is highly expressed in a majority of TNBC cases and that integrin α6β4 expression associates with greater overall survival. To investigate this association, we treated BT549 (EV vs. β4) and MDA-MB-231 (Cont. vs β4 knockout) cells with different doses of doxorubicin and mafosfamide (an analog of the active species produced by the prodrug Cytoxan), or with a combination of doxorubicin and mafosfamide. MTT assays were performed to assess cell viability, followed by data analysis using SynergyFinder software to calculate ZIP and Bliss synergy scores. We found that integrin α6β4 expression leads to significantly increased levels of synergistic sensitivity to doxorubicin and mafosfamide dual treatment in BT549 (EV vs β4, p=0.00017) and MDA-MB-231 cells (Cont. vs β4 knockout, p=0.000073). Clonogenic survival assays using 4T1 cells (Cont. vs β4 knockout) further supported the result that integrin α6β4 promotes synergistic effects following a combination treatment with doxorubicin and mafosfamide. Immunoblot assay analysis demonstrated that integrin α6β4 expression in these cells enhanced the activation of critical proteins in the DNA damage response (DDR) pathway including DNA-PK, p53, 53BP1, and γH2X in response to a combination treatment. Immunocytochemical staining was used to qualitatively confirm the activation levels of p53 and γH2X in MDA-MB-231 cells (Cont. vs knockout) in response to single and dual drug treatments. We observed that phospho-p53 on serine 15 and γH2X were detected in greater abundance following dual drug treatments in the integrin α6β4 bearing cells. Finally, by analyzing the Cancer Genome Atlas (TCGA) Pan-Cancer database for patient outcomes with basal-like breast cancers treated with cyclophosphamide, we found that patients with integrin α6β4 levels above median expression had superior overall survival rates compared to patients with levels below the median. In summary, these results provide evidence that integrin α6β4 promotes the efficacy of dual administration of Adriamycin and Cytoxan, the current first-line therapy for TNBC. We further suggest that integrin α6β4 could be used as a potential biomarker in determining whether or not TNBC patients will be responsive to the standard of care employed in their treatment plans.

Abstract 48

Integrin α6β4 Regulates Tryptophan Metabolism through the Kynurenine Pathway in Triple Negative Breast Cancer

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The laminin receptor integrin α6β4 is overexpressed in more than 80% of triple negative breast cancer (TNBC) cases and contributes to the aggressive nature of TNBC. The kynurenine pathway, which degrades tryptophan to NAD+, is often upregulated in TNBC and is associated with poorer outcomes. While the role of the kynurenine pathway has been well described in the context of immunosuppression in the tumor microenvironment, the significance of de novo NAD+ generation in TNBC has yet to be elucidated. In this study, we seek to identify how the kynurenine pathway is regulated by integrin α6β4 and its impact on tumor growth in TNBC. To investigate these events, we performed RNA sequencing on BT549 cells that stably expressed integrin β4 and empty vector (control). We found that compared to control cells, integrin β4-expressing cells have significant upregulation of key kynurenine pathway enzymes including IDO1, KYNU, and QPRT, which were further confirmed via qPCR and Western blot. These data suggest that integrin α6β4 drives tryptophan metabolism through the kynurenine pathway toward de novo NAD+ synthesis. To further investigate this relationship, we knocked-down integrin β4 in other TNBC cell lines and found that integrin β4 knock-down decreases expression of IDO1, the rate limiting enzyme of tryptophan degradation, thus suggesting that IDO1 expression is regulated in an integrin α6β4-dependent manner. The positive correlation of integrin β4 and IDO1 expression in TNBC cells is further supported by analysis of the TCGA Breast Invasive Carcinoma dataset. To test if integrin β4 upregulation of IDO1 impacts tumor growth in a physiologically relevant condition, we treated cells in 3-dimensional (3D) culture with Epacadostat, an IDO1 inhibitor. Upon treatment with Epacadostat, we demonstrate no basal differences in cell viability between our integrin α6β4 positive and negative lines; however, treatment with Epacadostat in our integrin α6β4-expressing model exhibits a decrease in invasive growth in 3D culture. Metabolite assessment shows that integrin α6β4 knock-in results in a decrease in the NAD+/NADH ratio, suggesting that consumption of NAD+ is potentially higher in the model expressing integrin α6β4. In summary, our data suggests that integrin α6β4 promotes the kynurenine pathway through transcriptional regulation of IDO1 to facilitate tumor growth. Moving forward, this alteration of tryptophan metabolism towards the kynurenine pathway has the potential to increase de novo NAD+ synthesis. Physiologically, some of the largest consumers of NAD+ are PARPs, which utilize NAD+ to recruit base excision repair machinery to the site of damage. Given that the current standards of care for TNBC are DNA-damaging chemotherapeutics and the majority of TNBC tumors express integrin α6β4, the upregulation of the kynurenine pathway by integrin a6b4 has significant implications with regards to disease progression and treatment efficacy.

Abstract 49

Investigating the Role of HOXB13 Phosphorylation by Plk1 in Castration Resistant Prostate Cancer (CRPC) Progression

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Prostate cancer is a prevalent malignancy in men worldwide. Besides conventional therapies such as chemotherapy, because androgen and its receptor (AR) has a crucial role in prostate cancer progression, initially it could be treated with androgen deprivation therapy (ADT) as a standard therapy, but it frequently progresses to develop castration-resistant prostate cancer (CRPC) with poor outcomes.

HOXB13 is a critical transcription factor belonging to the homeobox gene family, that has a vital role in developing prostate tissue during embryonic development and tissue differentiation, however, its persistent low-level expression in adult prostate tissue contributes to prostate cancer development. HOXB13 modulates androgen receptor (AR) function and plays a multifaceted role in prostate cancer and is linked in both promoting and inhibiting prostate cancer growth and metastasis for example, mutations like G84E in the HOXB13 gene have been associated with an increased risk of more aggressive form of PC.

Recent research highlights two distinct facets of HOXB13 in prostate cancer. Phosphorylation of HOXB13 by mTOR leads to HOXB13 degradation due to association with E3 ligase SKP2 that enhances the HOXB13 oncogenic properties, while recruitment of HDAC3 by HOXB13 leads to lipid metabolism suppression, by repressing the expression level of lipogenesis regulators such as FASN. HOXB13 loss mediates lipid accumulation, tumorigenesis, invasion and metastasis of prostate cancer.

Polo-like kinase 1 (Plk1) is a serine/threonine kinase crucial in mitosis, often overexpressed in cancers, including prostate cancer, leading to tumorigenesis and progression of prostate cancer with poor prognosis. It has a very vital role in facilitating mitotic cell division, which particularly increased in cancer cells.

Investigating the relationship between Plk1 and HOXB13, our findings demonstrate that Plk1 directly phosphorylates HOXB13 at S56 and S147 sites. Understanding this interaction between phosphorylated HOXB13 by Plk1 and CRPC progression could unveil critical insights into developing potential therapeutic interventions for CRPC.

Abstract 50

Iron Oxide Nanozymes Enhanced by Ascorbic Acid for Macrophage-Based Cancer Therapy

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Introduction: Pharmacological ascorbic acid has emerged as a promising therapeutic approach in cancer treatment, owing to its capacity to induce extracellular hydrogen peroxide (H2O2) production in solid tumors. The H2O2 is converted into cytotoxic hydroxyl free radicals (HO·) by redox-active Fe2+ inside cells. However, the high dosage of ascorbic acid required for efficacy is hampered by adverse effects such as kidney stone formation. In a recent study, we demonstrated the efficient catalytic conversion of H2O2 to HO· by wüstite (Fe1-xO) nanoparticles through a heterogenous Fenton reaction. Here, we explore whether iron oxide nanoparticles (IONPs) can enhance the therapeutic potential of ascorbic acid, thus mitigating its dose-related limitations.

Materials and Methods: Magnetite and wüstite nanocrystals were synthesized by thermal decomposition. The size and composition of nanocrystals were analyzed by TEM and XRD. Hydroxyl free radical and hydrogen peroxide were measured by TMB assay and Amplex red assay. The cytotoxicity of nanoparticles was measured by MTT assay. Polarization of RAW 264.7 cells toward pro- or anti-inflammatory phenotypes was analyzed using RT-qPCR. The cytotoxicity effect and targeting efficiency were evaluated using a transwell and a flow channel system.

Results: We have discovered that wüstite nanoparticles exhibit markedly superior catalytic activity compared to magnetite and maghemite nanoparticles, including ferumoxytol. Besides, wüstite nanoparticles exhibit the capability to either impede or enhance the cytotoxic effect of ascorbic acid, depending on the spatial segregation of the two reagents by cellular compartments. Macrophages treated with magnetite nanoparticles possess tumor targeting via magnetic guidance. Importantly, treatment with ascorbic acid promotes the polarization of IONPs-loaded macrophages toward a pro-inflammatory M1 phenotype, significantly suppressing the growth of 4T1 breast cancer cells.

Conclusions: Our investigation illuminates the distinct pH dependency of two promising reagents in cancer therapy. While IONPs exhibit efficacy as nanozymes, effectively curtailing tumour growth through the generation of free radicals, their synergistic utilization with other chemotherapeutic agents necessitates meticulous coordination in accordance with their respective mechanisms of action. Moreover, our study underscores the potential of macrophages engineered with IONPs as potent cellular adjuncts to ascorbic acid in cancer treatment. Here, wüstite nanoparticles and magnetite nanoparticles play pivotal roles in catalysis and magnetic targeting, respectively. This discovery prompts further exploration utilizing native macrophages and in vivo models, charting a path toward an innovative combination therapy.

Abstract 51

L858R/L718Q and L858R/L792H Mutations of EGFR Inducing Resistance Against Osimertinib by Forming More Hydrogen Bonds: A Molecular Simulation Study

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A deeper understanding of the resistance mechanisms associated with epidermal growth factor receptor (EGFR) mutations could expedite the development of innovative therapeutic drugs to overcome this acquired resistance. This work utilizes all-atom molecular dynamics simulations to investigate the conformational variation of two reported EGFR mutants (L858R/L718Q and L858R/L792H) that cause resistance of the covalent inhibitor Osimertinib. The wild-type EGFR kinase domain and the one with L858R mutation are used as the reference. The analysis of the simulation trajectories revealed that both the L718Q and L792 secondary mutations would induce additional hydrogen bonds between the residues in the active pocket and the residues with the water molecules. Those additional hydrogen bonds are expected to reduce the exposure area of C797, the covalent binding target of Osimertinib. The additional hydrogen bonds are also expected to influence the binding affinity of the EGFR kinase domain by alternating the secondary structure and flexibility of the amino acid residues in the domain. Our work highlights how the two reported mutations may alter the protein-protein and protein-solvent hydrogen bonds and affect the protein properties, which could be helpful to drug discovery.

Abstract 52

Lattice Therapy on Halcyon Linac: A Novel, Automated Spherical Lattice Placement and Treatment Planning Method for Spatially Fractionated Radiation Therapy to Large and Bulky Unresectable Tumors

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Background: The Varian Halcyon ring-mounted linear accelerator (Halcyon Linac) is a relatively new radiation oncology treatment delivery system that was originally designed with underserved/low resource communities in mind. Certain advanced cancers have a predilection to present as large and bulky, unresectable tumors (≥ 8 cm) at a disproportionate rate in underserved communities. Due to advances in technology, spatially fractionated radiation therapy (SFRT) has seen an increase in interest for effective management of these large tumors in recent years. In addition to direct DNA double-strand breaks, SFRT enhances indirect cell-killing mechanisms via bystander signaling, intratumor immune response, and intratumor microvasculature damage through the delivery of highly heterogeneous radiation dose distributions. Due to fundamental differences in design from conventional C-arm Linacs, traditional methods of SFRT treatment planning and delivery using standard multileaf-collimator-based 3D-crossfire techniques cannot be readily utilized by the Halcyon Linac. The Halcyon Linac was designed for the fast delivery of inversely optimized volumetric modulated arc therapy (VMAT) treatments, which require the use of complicated objective function(s). This, compounded with the need to draw numerous optimization and control structures to generate the desired lattice pattern(s), renders SFRT treatment planning on a Halcyon Linac time consuming and difficult, particularly for clinics with minimal physics support. This study aims to provide a rapid lattice structure generating tool and treatment planning method that makes lattice SFRT accessible to clinics regardless of equipment/resources, and eliminates the need for patient data import/export to third party software.

Methods: Automated lattice structure placement was implemented within a Varian Eclipse plugin script that establishes a bounding box surrounding the target structure, systematically places a lattice within, and crops the lattice to the target. Ten previously treated site-specific SFRT patients (15 Gy in one fraction) with cylindrical dose distributions using an MLC-based 3D-crossfire method on a Varian C-arm Linac were replanned using the proposed lattice contouring tool and inverse planning for Halcyon VMAT. Key SFRT metrics for tumor dose and maximum dose to adjacent critical organs were compared between methods. Shapiro-Wilk and paired t-tests were used to test for normality and differences in methods, respectively.

Results: The automated script allows for cylindrical or spherical lattice structure placement and specification of lattice diameter, separation, and orientation; runtime lasts 1-5 minutes depending on tumor volume. Dose heterogeneity is quantified using peak-to-valley-dose ratios (PVDRs), defined as the ratio maximum dose delivered to 90% of the tumor volume to the maximum dose delivered to 10% of the tumor volume. Spherical-lattice plans obtained a near-unit increase in PVDR compared to the clinical 3D-crossfire method (ΔPVDR = 0.9; $p<0.001$) and maintain similar maximum doses to nearby critical organs (ΔDmax = 6.7 cGy; $p = 0.79$). Spherical-lattice plans exhibited increased total monitor units and beam-on time (ΔMU = 5876 and Δt = 6.4 minutes; $p<0.001$) but can be delivered within the standard patient treatment time slot.

Conclusions: Our study provides proof-of-concept that lattice therapy via Halcyon VMAT is achievable. Spherical lattice structures help to introduce steeper dose gradients (providing higher PVDRs), and potentially increasing bystander signaling effect. Neoadjuvant radiotherapy via lattice SFRT opens avenues for future dose escalation, protection of nearby critical organs, and better/faster tumor response with longitudinal mass reduction using combination immunotherapy treatment or providing rapid pain relief. Identifying optimal lattice spacing and orientations, generating additional optimization structures, and developing formal treatment planning guidelines to streamline SFRT planning on Halcyon is ongoing.

Abstract 53

Leveraging Whole Slide Images for Predicting Mutations in Lung Cancer Patients: A Preliminary Study

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Next-generation sequencing (NGS), tumor microarray, and pathology images have significantly advanced disease diagnosis, drug development, prognosis monitoring, and treatment guidance. Despite NGS's potential in cutting-edge research and translational medicine, its high cost limits its accessibility, leaving many patients unable to benefit from NGS-based tests. Particularly in preventative medicine, early identification of genetically predisposed mutations in cancers can greatly benefit cancer patients' families. However, this is only feasible if NGS testing is affordable for every cancer patient's family. This study explores whether whole slide images can directly identify certain mutations necessary for cancer diagnosis and staging.

Using clinical data, whole slide images, and Reverse-phase protein array (RPPA) data from The Cancer Genome Atlas (TCGA) and The Human Protein Atlas, this study focuses on site-specific protein expression in lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC). The Random Forest Machine Learning algorithm is employed as the main classifier, and 13 site-specifically expressed proteins are identified using the Chi-Square test, with p-values adjusted using the Benjamin Hochberg method. The corresponding class label of the whole slide image is annotated based on the expression of these genes in RPPA results.

Results demonstrate that using clinical features extracted from whole slide images achieves an accuracy ranging from 43% to 96%. Incorporating simple whole-slide image features significantly enhances protein expression prediction accuracy. This suggests that certain mutations and proteins can be accurately predicted using deep learning-based enriched feature extraction tools or embedded as an AI model learning directly from multimodal data combining clinical and whole slide images.

Moreover, this preliminary study highlights the possibility of identifying germline mutations or high-risk cancer patients within families, enabling early enrollment in cancer surveillance programs. It also proposes retrospectively screening all cancer patients diagnosed in Kentucky's population with tissue blocks or whole slide images to identify germline test candidates and suggest germline screening, thereby facilitating early enrollment of high-risk families in cancer surveillance programs.

Abstract 54

Lipid Metabolism Reprograming Triggered by Spermine Synthase Inhibition Creates Therapeutic Vulnerability for Targeting Ferroptosis in Colorectal Cancer

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Dysregulation of polyamine metabolism has been linked to colorectal cancer (CRC) development. We recently reported that spermine synthase (SMS), a polyamine biosynthetic enzyme converting spermidine to spermine, is overexpressed in CRC (Nat Commun 11:3243, 2020). The overexpression of SMS is necessary to balance cellular spermidine levels, facilitating CRC tumorigenesis. Our findings suggest SMS as an attractive therapeutic target in CRC. However, genetic depletion of SMS expression only shows a modest antitumor effect. Through unbiased metabolomics and transcriptomics analyses, we identified reprograming of lipid metabolism as among the most impacted metabolic changes by SMS depletion in CRC cells. Specifically, SMS inhibition significantly promotes phospholipid hydrolysis by upregulating PLA2G2A expression, leading to a marked increase in the levels of free long-chain fatty acids, especially polyunsaturated fatty acids (PUFAs), and their acylcarnitines to sustain fatty acid oxidation and mitochondrial respiration. Furthermore, we found that the FOXO3a transcriptional factor was activated by SMS depletion via spermidine-targeted inhibition of EP300-mediated acetylation of FOXO3a, leading to the transcriptional upregulation of PLA2G2A expression. Ferroptosis occurs due to the accumulation of oxidized PUFAs on membrane phospholipids and is triggered by failure of the glutathione-dependent lipid peroxide scavenging pathway (system xc-/GPX4). While given the established role of PUFAs in promoting ferroptosis, SMS inhibition-induced elevation of PUFAs only slightly increases lipid peroxidation in CRC cells. However, genetic depletion or pharmacological inhibition of SMS in combination with pharmacological inhibition of GPX4 or its upstream regulator system xc- synergistically induces lipid peroxidation, leading to ferroptosis induction and marked suppression of CRC cell growth in vitro and xenograft tumor growth in vivo. Notably, silencing of either FOXO3a or PLA2G2A expression in SMS-depleted CRC cells profoundly attenuates lipid peroxidation induced by the blockade of the lipid peroxide scavenging pathway. Collectively, these results reveal that the FOXO3a/PLA2G2A signaling axis mediates SMS inhibition-induced lipid metabolism reprograming to sustain mitochondrial respiration and cell survival, thereby maintaining CRC tumorigenesis while creating vulnerability that can be therapeutically targeted by induction of ferroptosis. Thus, co-targeting SMS and system xc-/GPX4 is a potentially promising therapeutic strategy for treating SMS-overexpressing CRC.

Abstract 55

Long-Term PFOS Exposure Promotes Colon Cancer Progression

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Background: Perfluorooctanesulfonic acid (PFOS), a subtype of PFAS that is classified as a possible human carcinogen, has high bioaccumulation potential with a long elimination half-life. According to scientific literature, PFAS exposures promote intestinal inflammation and gut barrier dysfunction. However, how a long-term PFOS exposure affects colorectal cancer (CRC) progression is not known. Therefore, the purpose of this study is to delineate the effect of PFOS exposure on CRC cell proliferation and test the potential mitigation strategy for the harmful effects of PFOS.

Methods: SW480 and HCT116 cells have been treated with 1 ug/mL PFOS and proliferation was measured at 1, 2, and 3 months using the Presto Blue Cell Viability Reagent fluorescence assay. Proliferation markers were assessed by qPCR and Western blot. Control and PFOS exposed cells were treated with sulforaphane (SFN), a nuclear factor erythroid 2-related factor 2 (Nrf-2) inducer, at concentration 5, 10 and 20 uM for 72 hours.

Results: We show that chronic, low-dose PFOS exposure promotes proliferation of SW480 and HCT116 cell lines starting at 3 months. The increase in proliferation is associated with upregulation of Cyclin D, pAkt and FASN. We also show that sulforaphane has a higher efficacy in PFOS exposed CRC cells. The current studies show that SFN has no effect on normal colon epithelium cells.

Conclusion: Our studies suggest that chronic PFOS exposure promotes proliferation of CRC cells by increasing pro-carcinogenic gene expression and signaling. Furthermore, our findings suggest that supplementation of diet sulforaphane can potentially mitigate the harmful PFOS effects.

Abstract 56

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

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Background: Advanced ovarian cancer continues to be a leading cause of cancer-related female deaths in the United States, so novel therapies are needed. Extracellular vesicles are cell membrane-derived structures either endogenously released by cells or engineered from various cell types. Cell-derived vesicles exhibit cell-targeting capabilities and are easily loaded with cargo such as cisplatin. M1 macrophage-derived engineered vesicles (MEVs) target macrophages and cancer cells, allowing for targeted drug delivery.

Methods: We generated ovarian cancer xenografts with CAOV-3 luciferase-expressing ovarian cancer cells that were injected intraperitoneally into female BALB/c scid mice. Mice were randomized into four groups: control, cisplatin, empty MEVs (E-MEVs), and cisplatin-loaded MEVs (C-MEVs). We tracked the progression of mouse xenograft tumors with bioluminescence imaging. We made M1 MEVs from cultured RAW 264.7 cells, a mouse macrophage cell line, and stimulated the cells with IFN-Ɣ and LPS to the M1 phenotype. M1 MEVs were generated by nitrogen cavitation in the presence and absence of cisplatin to generate C-MEVs and E-MEVs, respectively. The NanoSight NS300 assessed MEV size and quantity. MEV quantity was matched between C-MEVs and E-MEVs. We quantified the cisplatin concentration in C-MEVs with mass spectrometry and matched the dose in the cisplatin group. Mice received treatments via intraperitoneal injection. The mean dose of cisplatin administered via C-MEVs and free cisplatin was 6 mg/kg per mouse. Thirty-four mice received ten weekly treatments and were followed for 120 days post-treatment initiation for a planned survival endpoint. A second cohort of 24 mice received five treatments prior to the collection of mouse serum and tumor tissue to study biomarkers. We performed statistical tests with GraphPad Prism and R.

Results: In the cohort of 34 mice, the mice treated with cisplatin received eight weekly doses before meeting toxicity endpoints. Control, E-MEV, and C-MEV groups received ten weekly treatments. Last measured luminescence values were used to assess tumor burden. Mean radiance for the four treatment groups from highest to lowest were: control mice 3.53x1010 (SEM 1.55x1010), E-MEVs 1.04x1010 (SEM 2.56x109), and C-MEVs 2.13x109 (SEM 6.97x108, and free cisplatin 1.56x109 (SEM 7.19x108). A pairwise comparison of luminescence between treatment groups demonstrated radiance was significantly different between vehicle control vs. free cisplatin ($p=0.0010$), control vs. E-MEVs ($p=0.0141$), and control vs C-MEVs ($p=0.0010$). Tumor size was not significantly different between cisplatin and C-MEV mice ($p=0.9989$). We followed the mice for 120 days post-treatment initiation, and on survival analysis, a Log-rank test demonstrates a difference in survival between groups ($p<0.0001$). In the cohort of 24 mice, the C-MEV group had a serum cisplatin half-life of ~40 hours. In C-MEV mice, the mean tissue cisplatin concentration at 65 hours post-treatment is 1.21 µg/g tumor tissue.

Conclusion: M1 MEVs are a novel and exciting therapeutic modality and drug delivery vehicle for the treatment of ovarian cancer. We were able to generate M1 MEVs in consistent numbers, sizes, and concentrations. C-MEVs at matched doses have equal efficacy as free cisplatin with less toxicity in vivo in an ovarian cancer mouse xenograft model. E-MEVs are also well tolerated and have more significant anticancer activity than control.

Abstract 57

Markey Cancer Center Research Network Coordinating Center

Timothy W. Mullett[1], Joseph Alexander[2], Marta Wood[2], Kris Damron[2], Melissa Shuey[2], Jane Noble[2], Yvonne Beatty-Warner[2], Lora Maynard[2], Ranjani Balasuriya[2], Rebecca Ingledue[2]

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Background: 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 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, 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 1,017 patients to studies in 63/120 counties (53% of the state), with 13 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 58

MCC Research Communications Office

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

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, and facilitate opportunities for continuing education. We also maintain Markey Connect, Markey's employee website and produce marketing materials for cancer-related projects.

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 UK—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, 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: CC415, CC416, and CC418 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

—Helping Markey researchers with editing, graphics, grants and more

Abstract 59

Molecular Insights into the Impact of APOE Gene Variants and Mutations on Cancer Immunotherapy: A Molecular Dynamics Study

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Apolipoprotein E (APOE) gene variants affect tumor development and progression by influencing processes such as cell growth, blood vessel formation, and spread. Excessive production of APOE is linked to aggressive tumor behavior and worse prognosis. While immunotherapy is a key cancer treatment, some tumors resist it, still APOE's impact on cancer prognosis remains uncertain. To better understand the immune modulation of the APOE gene on melanoma, there is a need to study its behavior at the molecular level. This study uses molecular dynamics simulations to examine how variation in conformation and dynamics among the three APOE variants (ε2, ε3, ε4) and three known mutants (APOE2-V236E, APOE3-R154S, APOE4-R269G) affects the lipidation and oligomerization functions of the protein using APOE3 as a reference. The residue-residue and residue-water hydrogen bond analysis is used to further understand how these changes impact the functions of the protein. From our findings, APOE2 and APOE2-V236E C-terminals are the most flexible among the three variants and mutants, respectively. The mutations did not significantly improve the solvent accessible surface area (SASA) for the six proteins, but their hydrophilic residues presented more SASA when compared to the hydrophobics in both the lipid and oligomerization binding domains. For both functional domains, a higher number of hydrogen bonds were observed between the proteins' residues and solvent, while the least number was with themselves. This suggests that the mutations in the APOE2 and APOE2-V236E C-terminals may have altered the protein's interaction with the surrounding solvent molecules, potentially affecting its overall structural stability and functionality. Additionally, the increased flexibility observed in these variants could indicate a higher degree of conformational adaptability, which might influence their biological activities and interactions with other molecules in cellular processes.

Abstract 60

Molecular Signature for Treatment-Responsive and Recurrent Wilms Tumors

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Wilms tumor is the most common solid renal malignancy in children. To better understand the biology of Wilms tumors and develop drugs especially against tumors that relapse, we sought to identify mutations in the exome, differential gene expression patterns, and tumor suppressor expression status of Wilms tumors in Kentucky. The Kentucky Cancer Registry (KCR) Virtual Tissue Repository identified 29 cases of Wilms tumor diagnosed between 2015 and 2021 at hospitals across the Commonwealth of Kentucky. The Markey Cancer Center Biospecimen Procurement and Translational Pathology Facility prepared tumor microarrays (TMAs) using Wilms tumor and matching normal tissue cores for immunohistochemistry (IHC) analysis. RNA-Seq analysis performed at the Markey Cancer Center Oncogenomics Facility identified a gene signature that was further validated by reverse-transcription quantitative-PCR (RT-qPCR) and IHC. Outcome data provided by KCR indicated that increased expression of an anti-apoptotic protein Bcl-2 in the tumor counter-intuitively provided better prognosis and outcome to treatment, indicating that the treatments either directly or indirectly targeted this protein. Loss of function of the tumor suppressor proteins Par-4/PAWR, WT1 and p53 did not explain the higher expression of Bcl-2. Thus, our multidisciplinary team approach involving basic science/translational researchers, population scientists, biomedical informaticists, and clinicians has resulted in well-characterized, Wilms tumor tissue microarrays, and our studies identified a unique gene signature in the Wilms tumor cases.

Abstract 61

Neurotensin Expression and Microbial Diversity in Colorectal Cancer Patients

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Neurotensin (NT), a neuropeptide implicated in gastrointestinal function and tumorigenesis, shows elevated expression in gastrointestinal cancers, indicating its potential role in tumor development. Recent research suggests an association between NT, intestinal tumors, and its interaction with lipid metabolism and the gut microbiota, highlighting its significance in tumorigenesis.

In our previous study, we investigated NT's role in specific intestinal tumor development and progression. We observed significantly higher NT expression levels in tumor tissues compared to adjacent healthy tissues, implying a potential association between NT and tumorigenesis. Furthermore, heightened NT expression correlated with increased absorption and metabolism of fats in tumor cells, suggesting NT's involvement in regulating lipid metabolism within the tumor microenvironment.

Considering the established link between the gut microbiota and tumorigenesis, we hypothesized that NT expression might influence microbial diversity within tumors. To explore this, we collected tumor samples from patients alongside adjacent healthy tissue samples and conducted 16S rRNA gene sequencing to assess microbial diversity. Additionally, we quantified NT and its receptor expression levels in these tissues.

Our analysis revealed a significant positive correlation between NT expression and the abundance of specific microbial genera, including Sellimonas, Lachnospira, Helicobacter_D, and Alistipes_A_871404 ($P < 0.05$). Moreover, certain microbial genera, such as Bifidobacterium_388775 and Chlamydia, exhibited elevated expression levels in tumor tissues compared to healthy tissues. Additionally, utilizing Picrust2 analysis, we found that alterations in microbial diversity were associated with increased expression of metabolic pathways, including biotin biosynthesis II and pyrimidine deoxyribonucleotides de novo biosynthesis II, within tumor tissues.

In summary, our findings suggest a potential link between NT expression, microbial diversity, and metabolic alterations within the tumor microenvironment, shedding light on novel mechanisms underlying colorectal tumorigenesis."

Abstract 62

Novel Approaches to Reduce the Risk of Colorectal Cancer Caused by Campylobacter Infections in Humans

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Campylobacter jejuni is the most common cause of self-limiting gastroenteritis worldwide. C. jejuni infection has been associated with complications such as inflammatory bowel disease (IBD), a known risk factor for colorectal cancer in humans. Campylobacter infections can be transmitted to humans by the consumption of contaminated poultry and poultry products. C. jejuni infections are normally treated with antibiotics. However, the emergence of antibiotic resistant C. jejuni has made treatment challenging. If the infection is left untreated, chronic inflammation can supervene which increases the risk of developing cancer. In this study, we aim to develop next-generation probiotics (NGPs) as antibiotic alternatives to control C. jejuni infections in humans. Primarily, we screened 38 different probiotic strains for their effect on the growth of C. jejuni using an agar-well diffusion assay. All the probiotics demonstrated growth inhibition of C. jejuni. However, we selected the top 7 probiotics strains demonstrating the highest growth inhibition of C. jejuni. Interestingly, all 7 candidates significantly inhibited C. jejuni's growth when co-cultured in broth media. They also inhibited the growth of other strains of Campylobacter such as C. fetus, C. lari, C. hyointestinalis, and C. coli. Similarly, the cell-free supernatants of all 7 candidates demonstrated up to 100% inhibition of biofilm formation and pre-formed biofilms of C. jejuni. The inflammation of the intestine usually initiates when the bacteria invade the intestinal epithelial cells. Here, the pre-treatment of human colorectal adenocarcinoma cell lines with the selected candidates significantly (p<0.05) inhibited the adhesion, invasion, and intra-cellular survival of C. jejuni in the cells. All the selected candidates significantly downregulated the expression of genes associated with virulence factors, motility, and biofilm formation. In the future, our studies will focus on understanding how the selected probiotics modulate their action in the human colorectal adenocarcinoma cells. Our results will facilitate the development of NGPs as alternatives to antibiotics for controlling C. jejuni infections and reduce the risk of colorectal cancer in humans.

Abstract 63

Novel Quorum Sensing Inhibitors as Potential Therapeutics for the Control of Non-Typhoidal Salmonella Causing Colon Cancer

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Background: Non-typhoidal Salmonella is a significant foodborne pathogen. Untreated Salmonella infections have been linked to variety of chronic health issue such as colon cancers. Release of effector proteins by T3SS mediates the uptake of Salmonella by colon epithelial cells resulting in the activation of oncogenic pathways. QS is a cell-to-cell communication which allows the bacteria to sense its population density and regulate its virulence including cell invasion. This communication is conducted by signaling molecules called autoinducers 2 (AI-2). This study is aimed to identify QS inhibitors and evaluate their effect on virulence and biofilm formation of Salmonella in vitro.

Methodology: We tested 1,900 small molecules (SMs) to assess their impact on QS/AI-2 production of Salmonella. Bacterial cultures (100µL; OD=0.05) were treated with 1µL of each small molecule (SM; 10 µM - 0.7µM) in 96 well plates and incubated for 6 hours at 30 ºC to assess their effect on bacterial growth. SMs demonstrating no significant impact on bacterial growth were subsequently screened via a bioluminescence assay. Cell-free supernatants of treated bacteria were incubated with Vibrio harveyi BB170 to evaluate their effect on AI-2 production. SMs exhibiting the highest inhibitory activity for AI-2 were then selected for their effect on biofilm formation and the expression of virulence, biofilm, and quorum sensing-associated genes using RT-PCR.

Results: Ten SMs with more than 95% inhibition of AI-2 activity without affecting bacterial growth were selected for further evaluation. These compounds possessed inhibition (95-100%) of biofilm formation. Furthermore, all 10 compounds downregulated the expression of genes associated with quorum sensing, virulence, biofilm development, and motility.

Conclusions: Quorum sensing inhibitors offer a promising new strategy to combat Salmonella infections mitigating the invasion of Salmonella in colon epithelial cells which reduces the risk of cancer. To accelerate the in vitro development of these SMs as antibiotic alternatives, the cytotoxic potential will be assessed on human intestinal and chicken macrophage cells.

Abstract 64

Oncogenomics Shared Resource Facility (OG SRF)

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The Oncogenomics Shared Resource Facility (OG SRF) plays a vital role in supporting the genomic investigations of the three Markey Cancer Center (MCC) Research Programs. The OG SRF's mission is to provide comprehensive genomic services to facilitate basic and translational research, clinical trials and precision medicine initiatives at MCC. Aligned with its mission, the service scope includes: 1) Project consultation; 2) State-of-the-art genomic platforms; 3) Genomic data curation and interpretation; 4) Liaising between investigators and commercial NGS vendors to ensure service and data quality. The OG SRF's main platforms include one HiSeq 2500, two NextSeq 2000, one NovaSeq 6000, an automatic liquid handling system, a NanoString nCounter Sprint Profiler and a 10x Genomics Chromium X. The services include whole genome sequencing, whole exome sequencing, cancer panel sequencing, RNAseq, single cell-related sequencing and digital spatial profiling. In addition to wet bench services, OG SRF assists in choosing the proper platform(s) for genomic investigation and participates in integrated data discussion with MCC members. The service expertise of OG SRF has greatly facilitated the genomic, transcriptomic and epigenomic research of the MCC Research Programs. OG SRF has the following Specific Aims: 1) Provide comprehensive and high-quality genomic services; 2) Provide genomic consultation services; 3) Enhance coordinated operations with project-relevant SRFs to support the team science research model of MCC. With genomic service expertise and the newly obtained NGS platforms, OG SRF will continue to provide comprehensive and cost-effective genomic services to MCC members. In addition, the cutting-edge sequencing capacity will enable OG SRF to catalyze spatial biology and multi-omics research at MCC. The integrated service workflow between OG SRF and other SRFs and the streamlined process of data review and discussion between OG SRF and researchers will continue to contribute to the team science model of the MCC.

Abstract 65

Orally Bioavailable DCN+ Inhibitor NAcM-OPT Disrupts the Trafficking of EGFR through Endomembrane System

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Background: Defective in cullin neddylation 1 (DCN1) is a component of enzymatic machinery, which transfers the NEDD8 tag to the target proteins. This enzyme-based machinery includes the E1, E2, and E3 enzymes, which subsequently append the NEDD8 tag to the protein of interest. DCN1 is a co-E3. NAcM-OPT is a selective inhibitor of neddylation requiring DCN1 as co-E3. By binding selectively to the DCN1 protein, NACM-OPT inhibits its binding to UBC12. UBC12 is an E2 and inhibition of the binding between UBC12 and DCN1 blocks the transfer of NEDD8 to the target protein. Selectively blocking neddylation showed accumulation of vesicles and generation of abnormal vesicular phenotype. The purpose of this study was to investigate this abnormal vesicular phenotype.

Methods: Immunocytochemistry for colocalization analysis and western blot.

Results: EGF after binding to its receptor EGFR is cycled through the endomembrane system before being degraded in the lysosomes. Early endosome cargo (EGFR) fuses with LAMP vesicles leading to the development of a lysosome which attenuates EGFR signaling and degrade EGFR. NAcM-OPT treatment significantly reduces the colocalization between the EGFR and LAMP vesicles. NAcM-OPT treatment increased particle size of EGF. KLHL22 is a cul3 adaptors whose expression changes NAcM-OPT treatment. NAcM-OPT treatment causes increased colocalization between KLHL22 and EGFR vesicles and decreases colocalization between KLHL22 and LAMP1 vesicles. A corresponding increase in the particle size of KLHL22 is observed after NAcM-OPT treatment.

Conclusion: Inhibition of cullin neddylation by NAcM-OPT disrupts endomembrane system producing the characteristic abnormal vesicular phenotype. NAcM-OPT treatment prevents the fusion of early endosomes and late endosomes and prevents their maturation into lysosomes.

Abstract 66

Overall Survival and Mutational Landscape Comparison between Appalachian and Non-Appalachian Patients with Ovarian Cancer—An Oncology Research Information Network (ORIEN) Analysis

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Objectives: Few studies have examined the mutational landscape in ovarian cancers, and none have focused on the Appalachian region which has a higher incidence of ovarian cancer, higher rates of poverty, and limited access to care compared to non-Appalachian region. Women with ovarian cancer enrolled in the Total Cancer Care prospective cohort study were evaluated to compare overall survival, mutational landscape, and immune cell composition between subjects in Appalachian and Non-Appalachian regions.

Methods: Clinical and genomic data are available for 788 ovarian cancer patients, including 71 from Appalachian Kentucky. MutSigCV was used to identify significantly mutated genes. CIBERSORTx was used to estimate the immune cell composition based on RNA sequencing data. Fisher's exact test was used to compare clinical and genomic characteristics, Cox regression analysis was used to compare survival, and Wilcoxon rank-sum was used to compare immune cell composition.

Results: Most participants were white (90.4%). Mean age at diagnosis was 58.6 years, and mean BMI was 27.5 kg/m2. Appalachian women with ovarian cancer were more likely to be non-Hispanic (100% vs. 94.5%, p=0.04), older at time of diagnosis (63 years vs. 58 years, p= 0.0006), have a higher BMI (29.1 vs. 27 kg/m2, p= 0.048), and be diagnosed at later stage (III/IV, 82.5% vs 66.2%, p=0.0115) than non-Appalachian residents. In univariate analysis, women in Appalachia had poorer survival compared to non-Appalachia (HR 2.16, p<0.001, 95% CI 1.44-3.25). In multivariate analysis including Appalachian status, age at diagnosis, BMI and stage, Appalachian residence trended towards increased risk for worse survival, but was not significant (HR 1.63, p=0.054, 95% CI 0.37-1.01), while older age at diagnosis (HR 1.02, p=0.005, 95% CI 1.01-1.04) and advanced stage (stage III vs. I HR 2.49, p=0.008, 95% CI 1.27-4.89; stage IV vs I HR 3.13 p= 0.002 CI 1.52-6.43) were significantly associated with poorer survival. After correcting for multiple comparisons, significantly mutated genes in the Appalachian residents were TP53 (70%) and RPL38 (2%), while TP53 (61%), PIK3CA (14%) and ARID1A (13%) were the most frequently mutated among non-Appalachian residents. The frequency of BRCA1/BRCA2 mutation in Appalachia was 7.8%, compared to 14.7% in the non-Appalachia cohort (p=0.18). Immune cell composition analysis demonstrated multiple significant differences, with non-Appalachian residents having increased proportions of naïve B cells, T regs, gamma-delta T cells, resting NK cells and M2 macrophages. However, Appalachian residents had increased proportion of activated dendritic cells.

Conclusion: Women with ovarian cancer residing in Appalachia are older at time of diagnosis, have a higher BMI and are diagnosed at later stages compared to non-Appalachian residents. While the current Appalachian population size did not reach statistical significance, the hazard ratio is likely due to later stage and age at diagnosis. Along with our other findings, this suggests other factors like variable mutation frequencies and immune activation, may contribute to excess mortality in the region and should be further explored.

Abstract 67

Overcoming Immune Checkpoint Blockade Resistance in Prostate Cancer by Suppressing PLK1-Mediated MSH6 Phosphorylation

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Despite the remarkable success of immune checkpoint blockade (ICB) in treating various malignant tumors, its efficacy in prostate cancer (PCa) remains constrained by the inherently cold tumor microenvironment characteristic. Studies have identified a small subset of PCa patients exhibiting high microsatellite instability (MSI-H) and deficient mismatch repair (dMMR) who demonstrate atypical responses to ICB treatment, potentially linked to dMMR-induced activation of the cGAS-STING pathway. This underscores a significant challenge: MMR-mediated cGAS-STING inactivation renders PCa patients resistant to ICB treatment, compounded by limited data regarding the regulation of MMR. To address this gap, the present study aims to potentially enhance the efficacy of ICB treatment in PCa through studying the critical role of PLK1-mediated MSH6 phosphorylation in regulation of cGAS-STING-IFN pathway activation. Our preliminary data showed that PLK1 directly phosphorylates MSH6, which is responsible for DNA mismatch-recognition and -binding, at T986 site in vitro. The T986 phosphorylation site resides within the clamp domain, which makes extensive interactions with the DNA near mismatch. This finding about MSH6 phosphorylation by PLK1 appears to enhance the DNA mismatch-binding ability of MSH6, potentially influencing the downstream activation of the cGAS-STING pathway. Building upon this discovery, the central hypothesis of this study is that PLK1-induced phosphorylation of MSH6 at T986 augments DNA mismatch-binding, reduces cytosolic DNA, silences of cGAS-STING pathway, and ultimately curtails immunotherapy response. This hypothesis will be tested with three specific aims: Aim 1 will help to investigate whether PLK1-mediated MSH6 phosphorylation at T986 is essential for the functions of MSH6 in DNA mismatch repair. Aim 2 will determine the mechanism by which inhibition of PLK1 changes immunologically "cold" PCa tumor environment to immunologically "hot". Aim 3 will elucidate the role of PLK1-mediated MSH6 phosphorylation in anti-tumor immunity in vivo. Successful completion of these three aims will provide a comprehensive understanding of the role played by PLK1-mediated MSH6 phosphorylation in modulating anti-tumor immunity via the cGAS-STING pathway. Moreover, the expected results will hold a potential to unveil a novel ICB combinational cancer therapy whereby PLK1 inhibition enhances the efficacy of ICB treatment in PCa, as evidenced by scientific studies in mice.

Abstract 68

Patient-Oriented and Population Sciences Shared Resource Facility (POP SRF)

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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. 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 69

Perspectives of Facilitators and Barriers to Cancer Clinical Trial Participation: A Mixed-Methods Study

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Background: Inclusive clinical trials (CT) are integral to ensure that all patients benefit from advancements in cancer treatment. Having a diverse makeup of CT participants is imperative. Yet nationally, only 5% of trial participants are racial/ethnic minorities despite persistent disparities in cancer incidence and mortality. Determining the barriers and facilitators to CT participation will inform strategies to advance CT diversity, and cancer care equity.

Methods: We conducted a mixed methods study exploring perceived barriers and facilitators to CT participation. We asked CT staff and Black adult cancer patients who enrolled in a CT to engage in semi-structured focus groups or interviews via Zoom. Based on emergent themes and data from extant literature we developed and deployed to CT staff a 24 item 5-point Likert style (strongly disagree – strongly agree) survey regarding clinician, institution, and patient-level factors impacting CT participation; one item ranked overall CT participation barriers.

Results: Ten patients and thirteen CT staff participated in the focus groups and interviews. Table 1 presents major themes that emerged. CT survey respondents (N=30) included clinicians (40%) and research associates (43%) and roughly half (55%) were White, non-Hispanic. The majority (80%) responded that personal skills and confidence with enrolling diverse patients were high. The top ranked barriers to CT enrollment were travel distance (77%) and added financial burden (60%). Facilitators were altruism (70%) and clinician awareness of available trials (73%). Most (60%) endorsed perceptions that enrolling racial/ethnic patients was more challenging than White and that patient attributes (e.g., higher education, timeliness to appointments increased adherence to CTs protocols.

Conclusions: Complex, multi-level factors influence CT enrollment and diversity with patient level factors of most concern. Multi-level approaches both internally and community-focused are necessary for CT participation and diversity. Efforts are necessary to mitigate implicit bias among CT staff, particularly as it relates to perceptions of patient attributes. To advance cancer care, CT participation equity must remain a priority.

Abstract 70

PFOS Exposure Downregulates HMGCS2 Protein Levels and Upregulates Pro-Carcinogenic Signaling in Intestinal Cells

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Introduction: Long-term exposures to per- and polyfluoroalkyl substances (PFAS) have been increasingly linked to negative health outcomes including metabolic disorders, reduced immune responses, and increased risk of cancer. PFOS, one the most frequently detected PFAS in drinking water, is readily absorbed in the GI tract and distributes predominantly to the plasma and liver. Even though recent studies demonstrate that PFOS can cause an imbalance of cecal gut microbiota, alter microbiota diversity, and promote inflammation and gut barrier dysfunction, scientific literature on PFOS exposure in the GI is limited and the association between this environmental pollutant and GI-associated diseases remains to be determined.

Methods: The effect of PFOS on gene expression profiles was assessed through RNAseq analysis conducted on the intestinal tissue of C57BL/6 mice. Normal primary human colon cell line (NPHC) was treated with 1µg/mL of PFOS for 6 months. ShRNA-mediated knockdown of HMGCS2 was used to analyze the functional role of HMGCS2. To access the mechanisms involved in PFOS-induced intestinal alterations, we performed qRT-PCR, western blot, immunofluorescence, and tissue microarray analysis.

Results: Through RNAseq analysis, we identified the top pathways upregulated by PFOS as cancer, lipid metabolism and immune system. The KEGG analysis further highlighted significant gene enrichment in pro-oncogenic signaling pathways, including NOTCH, WNT/β-catenin and TGF-β. The RNAseq, q-RT-PCR and western blot analyses of normal intestinal tissues revealed that PFOS exposure leads to downregulation of 3-hydroxy-3-methylglutaryl-Coa synthase 2 (HMGCS2), a key enzyme in the synthesis of β-hydroxybutyrate (βHB), a critical ketogenic molecule. Additionally, PFOS exposure upregulated the levels of fatty acid synthase (FASN), a crucial enzyme of de novo lipogenesis, and pro-oncogenic proteins such as β-catenin and MYC. shRNA-mediated knockdown of HMGCS2 in NPHC cells resulted in upregulation of CD36 expression, a fatty acid transporter, and an increase in lipid accumulation, as evidenced by BODIPY staining. To further support our findings, we also showed that HMGCS2 mRNA and proteins levels are downregulated in colorectal cancer.

Conclusion: In summary, our data suggests that PFOS may induce GI pathological changes that can increase the risk of CRC development. We identified that downregulation of ketogenesis and upregulation of lipid metabolism are the major effects of PFOS exposure in intestinal epithelium. Further studies are warranted to determine the functional significance of PFOS-mediated downregulation of HMGCS2 expression and upregulation of lipid metabolism in intestinal cells. Delineating the effects of PFOS on intestinal epithelium may contribute to development of interventional strategies to eliminate harmful effects of these environmental pollutants.

Abstract 71

PGD as an AMPK-Independent Target of AICAR for Its Anti-Leukemic Effect

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Acadesine inhibits the viability of many different types of leukemic cells and reverses drug resistance. To further improve the anti-leukemic effect, a better understanding at the molecular level is needed. However, the anti-leukemic effect is independent of AMPK, the best-known target of AICAR, which is the active form of acadesine. The molecular target relevant to the anti-leukemic effect is not yet clear. Here we report that 6-phosphogluconate dehydrogenase (PGD) is a molecular target of AICAR relevant to the anti-leukemic effect. AICAR inhibits PGD in vitro, while AMP does not. Expression of an AICAR-insensitive PGD variant protects K562 chronic myelogenous leukemia cells from acadesine. PGD inhibitor RES reproduced acadesine's anti-leukemic effect and reversed the imatinib resistance of K562 cells. This result indicates that PGD is a molecular target of AICAR relevant to the anti-leukemic effect and might be a potential target to develop the next generation of anti-leukemic compounds.

Abstract 72

Phase I Clinical Trial Designs in Oncology: A Systematic Literature Review from 2020 to 2022

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Background: Phase I clinical trials aim to find the highest dose of a novel drug that may be administrated safely without having serious adverse effects. Model-based designs have recently become popular in dose-finding procedures. Our objective is to provide an overview of phase I clinical trial in oncology.

Methods: A retrospective analysis on phase I clinical trials in oncology was performed by using PubMed database between 1 January 2020 and 31 December 2022. We extracted all papers with inclusion of trials in oncology and kept only those in which dose-escalation or/ and dose-expansion were conducted. We also compared the study parameters, design parameters, and patient parameters between industry sponsored study and academia sponsored study.

Result: Among the 1450 papers retrieved, 256 trials described phase I clinical trials in oncology. Overall, 71.1% trials were done with single study cohort, 50% trials collected a group of 11-30 study volunteers, 55.1% were sponsored by industry, and 99.2% of trials had less than 10 patients who experienced DLTs.

The traditional 3+3 (73.85%) was still the most prevailing method for dose escalation approach. More than 50% of the trails did not reach MTDs. Industry sponsored study enrolled more patients in dose escalation trials with benefits of continental cooperation. Compared to previous findings, the usage of model-based design increased to about 10%, and percentage of traditional 3+3 design decreased to 74%.

Conclusions: Phase I traditional 3+3 designs perform well, but there is still room for development in novel model-based dose-escalation designs in clinical practice.

Abstract 73

Plk1 Attenuates Colon Inflammation and Tumorigenesis

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Inflammation-associated colon cancer, linked with long-standing colon inflammation, is often seen in patients with inflammatory bowel diseases (IBD), such as Crohn's disease and ulcerative colitis. Typically, these patients are diagnosed at a younger age compared to those with sporadic colon cancer. Despite some advancements, the detailed molecular mechanisms driving cancer development in IBD remain unclear. Polo-like kinase 1 (Plk1), a serine/threonine kinase and cell cycle regulator, is recognized as an oncogene in various cancers. Intriguingly, higher Plk1 expression correlates with better survival rates in colon cancer patients. Besides, Plk1 also elevates IBD cases, suggesting its significant role in both colitis and colon cancer. Our study explores Plk1's role in IBD-associated colon cancer using the azoxymethane (AOM)/dextran sulfate sodium (DSS) mouse model, revealing Plk1's suppressive impact on both colitis and related colon cancer.

Abstract 74

PLK1 Phosphorylates CHAF1A Sensitizing PCa to Olaparib Treatment

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Prostate cancer (PCa) ranks as the second most prevalent malignant neoplasm worldwide and stands as the fifth leading contributor to cancer-related mortality among men. Polo-like kinase 1 (PLK1), a highly conserved serine/threonine kinase, assumes a pivotal role in orchestrating cell mitosis and facilitating DNA damage repair (DDR). Emerging evidence underscores its involvement in promoting malignancy and conferring drug resistance in a spectrum of cancer types, including PCa. Chromatin assembly factor 1 subunit A (CHAF1A), the largest constituent of the Chromatin assembly factor 1 (CAF-1) complex, serves as a scaffold that furnishes binding sites for various substrates, notably the H3-H4 dimer, thus attesting to its indispensable role in DDR. Preliminary investigations have suggested that CHAF1A may serve as a plausible substrate for PLK1. However, the precise nature of the interaction and phosphorylation events between these two factors remains enigmatic, as does their biological significance in the context of DDR in PCa. In this study, we have identified threonine 591 (T591) on CHAF1A as a site susceptible to phosphorylation by PLK1. By introducing exogenous CHAF1A variants with a phosphorylation-mimicking mutation (CHAF1A-T591D) and a phosphorylation-deficient mutation (CHAF1A-T591A) into C4-2 cells, we have illuminated the functional consequences of this phosphorylation event. Our findings reveal that phosphorylation sensitizes C4-2 cells to the PARP inhibitor olaparib, primarily by expediting the activation of cell cycle checkpoints and enhancing the affinity of the H3-H4 dimer for facilitating chromatin reassembly before DDR reaches completion. In summary, our current results underscore the potential therapeutic benefit of olaparib in PCa cases characterized by heightened PLK1 expression.

Abstract 75

PLK1 Phosphorylation of BRN2 Regulates Neuro Endocrine Prostate Cancer Progression

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Prostate cancer is a common and deadly cancer in North American men. While it is initially managed with hormonal therapies, the cancer often recurs and becomes resistant to treatment, known as castration-resistant prostate cancer. Currently, drugs like Enzalutamide, which are inhibitors of the androgen receptor pathway, can extend survival, but the cancer eventually becomes resistant. Recent studies show that BRN2, a protein encoded by the gene pou3f2, plays a key role in the neuroendocrine phenotype in prostate cancer. It is also discovered that the expression of BRN2 is suppressed by androgen receptor and that decreasing BRN2 levels can reduce the neuroendocrine marker expression and slow down the growth of neuroendocrine prostate cancer both in vitro and in vivo. On the other hand, PLK1, a widely recognized regulator of cell division, is also considered a potential treatment target for cancer. Publications from our lab have always emphasized the negligible role of Plk1 in prostate cancer and our preliminary data shows that Plk1 can phosphorylate BRN2. We also found that inhibition of this phosphorylation can significantly reduce NEPC formation. This interaction between Plk1 and BRN2 may contribute to the resistance of prostate cancer to treatment, leading us to suggest blocking the Plk1/BRN2 pathway as a potential new treatment method for prostate cancer.

Abstract 76

PLK1 Phosphorylation of NANOG Promotes Prostate Cancer Lineage Plasticity in Response to Androgen Deprivation Therapy

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Developing resistance to enzalutamide as a second-generation androgen receptor inhibitor is a challenge for treatment-induced castration-resistant prostate cancer. By continuing usage of androgen receptor inhibitors, tumors can reprogram to a different lineage, such as neuroendocrine that no longer rely on AR signaling and belongs to the most aggressive kinds of prostate cancer. Cancer cells undergo extensive transcription rewiring during this lineage plasticity process and gain stem cell phenotype by increasing stemness. Studies have shown that prostate cancer (PCa) cells acquire stem-like properties through the expression of NANOG, particularly in stable and accumulated conditions. The post-translational modification of NANOG at specific sites is essential to maintain NANOG stability, thereby enhancing tumorigenic properties. RNA-seq data analysis confirmed that in androgen-independent LNCaP cells that overexpressed NANOG, Plk1, and other important cell cycle-related genes also increased. Plk1 functions as a serine/threonine kinase, playing a crucial role as a key regulator in cell cycle progression. Notably, Plk1 has been extensively identified as an oncogene, with its overexpression linked to genomic instability. This heightened expression promotes cell transformation, and its correlation with a poor prognosis in tumor patients has been consistently reported. Correlation analysis validated a positive association between Plk1 expression and NE or stemness genes, while revealing a negative correlation with Androgen Receptor (AR) signaling, however in adenocarcinoma or non-NE groups, this correlation is not that significant, even opposite. Our central hypothesis is that Plk1 phosphorylates NANOG, and this phosphorylation makes NANOG more stable. Accumulation of NANOG in stable conditions is essential for prostate cancer cells to acquire stem-like properties. After patients with CRPC develop resistance to androgen receptor pathway inhibitors (ARPI), a subgroup may present with AR-indifferent disease. This condition is distinguished by low levels of PSA and may display neuroendocrine differentiation, indicating a form of lineage plasticity. We hypothesized that the phosphorylated and stable form of NANOG drives this lineage shift from CRPC to NEPC. To figure out whether phosphorylation of NANOG by Plk1 promotes this lineage plasticity and progression of CRPC to NEPC, we will address three aims to test this hypothesis. The first specific aim of the proposed study is to determine if Plk1 phosphorylates NANOG and map the phosphorylation site. The second aim of the proposed study is to investigate the functional significance of NANOG phosphorylation by Plk1. By using the LNCaP/shP53-RB cell line as a working model to overexpress NANOG WT/A and D mutations in the Plk1 site we will test this hypothesis that when NANOG is phosphorylated by Plk1, the stability of NANOG increases and this acts as a driver for lineage plasticity. Lastly, for the third specific aim of the study, we will elucidate whether the phosphorylation of NANOG by Plk1 regulates lineage plasticity with NANOG A mutation mouse and TRAMP mouse. This project for the first time will elucidate the mechanism by which Plk1 regulates NANOG and how Plk1 plays a pivotal role in prostate cancer progression through NANOG regulation. We will establish a novel pathway through Plk1-NANOG crosstalk which will provide a foundation for further research in the future, opening new doors for therapeutics in the field of cancer biology. This study will also provide insight into new targets for novel therapeutic options regarding NANOG and Plk1 co-targeting to improve the efficacy of treatments.

Abstract 77

Plk1 Phosphorylation of PHGDH to Regulate Serine Metabolism

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Polo-like kinase 1 (Plk1) has been reported to be highly expressed in most tumors, especially in advanced tumors, while how Plk1 elevation benefits tumor growth remains elusive. Metabolic reprogramming is one of the hallmarks of cancer, but how the tumors fully take advantage of deregulated metabolism is an enigma. Here, we found that Plk1 could divert serine metabolism from de novo synthesis to exogenous uptake by regulating PHGDH (phosphoglycerate dehydrogenase), the first rate-limiting enzyme of de novo serine biosynthesis. We show that PHGDH is phosphorylated by Plk1 at a cluster site (S512, S513, S517) and that Plk1-associated phosphorylation of PHGDH results in its protein degradation, thus reduced de novo serine biosynthesis. As a compensatory response, cells with an elevated level of Plk1 significantly increase the uptake of serine to produce more sphingosines, which are pivotal metabolites for the growth of cancer cells. Our finding reveals how cancer cells orchestrate macro biomolecules like glucose, amino acids, lipids to benefit cancer growth and may provide guidance on how to target de novo biosynthesis of serine, serine uptake or sphingosine metabolism to treat advanced prostate cancer.

Abstract 78

PLK1-Dependent Phosphorylation of PRMT5 Promotes DNA Damage Response in Prostate Cancer

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Prostate cancer stands out as the most frequently diagnosed cancer in men and is projected to be the second leading cause of cancer-related deaths among men in the US. Consequently, there is an urgent need for studies focusing on innovative therapeutic approaches. DNA Damage Response (DDR) is critical for cell survival, as it promotes genomic stability and reduces the risk of inheriting damage. DDR also promotes cancer cells' survival, making it a therapeutic target in cancer. PRMT5, an enzyme in the methyltransferase family, is frequently activated and overexpressed in various cancers, including prostate cancer. Furthermore, research has revealed that PRMT5 has been implicated in DDR in prostate cancer, and the regulation of PRMT5-dependent DDR is influenced by its phosphorylation. Polo-like kinase 1 (PLK1) is a serine/threonine kinase also reportedly involved in DDR in prostate cancer. Our preliminary study found that PRMT5 is positively correlated with PLK1 in prostate cancer based on the TCGA database. Nonetheless, the mechanism through which PRMT5 is regulated needs to be clarified. Here, we found that PLK1 phosphorylates PRMT5 at the S470 site, and this specific phosphorylation is required for maintaining the enzymatic activity of PRMT5. Moreover, Plk1-associated phosphorylation of PRMT5 subsequently promotes DNA damage repair, indicated by the decreasing level of γH2AX after DNA double-strand breaks (DSB). Our RNA-seq analyses of prostate cancer cells with wild-type or mutant S470 sites indicate that Plk1-associated phosphorylation on the S470 site exerts an influence on DNA replication and DDR pathways. These findings shed light on a novel perspective regarding the roles of PLK1-mediated PRMT5 phosphorylation in DDR, offering potential therapeutic strategies in prostate cancer.

Abstract 79

PLK1-Mediated SOX2-S159 Phosphorylation Enhances Cell Plasticity in Neuroendocrine Prostate Cancer Progression

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Prostate cancer research is of paramount importance, particularly in understanding its multifaceted nature. Neuroendocrine prostate cancer (NEPC), an aggressive variant, presents unique challenges due to its resistance to standard treatments. The pathogenesis of NEPC is intricate, characterized by the transformation of adenocarcinomas into neuroendocrine phenotypes, a process intricately linked to cell plasticity. Our study delves into the cell plasticity hypothesis in NEPC progression, with a specific focus on the pivotal role of the SOX2 gene, recognized as an essential player in cell plasticity. Importantly, we have unveiled a critical link between SOX2 and PLK1 (Polo-like kinase 1), a key driver of NEPC. Through in vitro kinase assays, we have demonstrated that PLK1 phosphorylates SOX2 at the S159 site. Luciferase assays have further revealed that the SOX2-S159A mutant, which resists PLK1 phosphorylation, exhibits reduced transcriptional activity, underscoring the significance of this modification. Additionally, overexpression of wild-type (WT) SOX2 amplifies the cell plasticity potential of LNCaP cells, while the S159A (phosphorylation-resistant) mutant loses this ability. In conclusion, our investigation establishes a compelling link between PLK1-mediated SOX2-S159 phosphorylation and cell plasticity in NEPC progression. This novel insight opens avenues for innovative therapeutic strategies targeting this critical molecular pathway, offering promise for improved NEPC management and patient outcomes.

Abstract 80

Preliminary Results of a Phase II Randomized Comparison of Flat vs Weight based Mitomycin C Intra-peritoneal Chemotherapy for Patients Undergoing Cytoreductive Surgery and Heated Intraperitoneal Chemotherapy

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Introduction: Cytoreductive surgery (CRS) ± heated intraperitoneal chemotherapy (HIPEC) has demonstrated increased survival in patients with peritoneal carcinomatosis (PC) from advanced gastrointestinal (GI) cancer. Mitomycin C (MMC) is the historic most common drug utilized worldwide with highly variable dosing and is associated with neutropenia and increased morbidity. Two dominant dosing strategies exist: flat dose (FD) and weight-based (WB), however, neither has been compared and lack of consensus in dosing prevents standardization of HIPEC technique.

Methods: A single institution investigator-initiated Phase II trial comparing FD and WB MMC in patients undergoing CRS+HIPEC for advanced GI cancer. Eligible patients were randomized to MMC FD 40 mg vs WB 12.5 mg/m2 utilizing 1:1 allocation ratio. Plasma (PLA) and peritoneal (PER) fluid samples were collected at (0, 15, 30, 60, 90 min) in addition to PLA postoperatively (2, 4, 12 hour) for pharmacokinetic (PK) analysis including Cmax (ng/mL) and area under the curve (AUC) (hour x ng/mL). Clinicopathologic data including age, sex BSA, dose, and rate of neutropenia between the two arms were compared. Recurrence data was evaluated for all patients with ≥ 1 year follow-up, excluding those with LAMN and completeness of cytoreduction (CC) score = 2.

Results: Of 45 patients enrolled, 31 have undergone CRS + HIPEC, with mature PK data. Clinicopathologic characteristics were comparable between arms (Table 1). Dose (mg/m2) was significantly higher in the FD group (21.2 vs 12.2, P < 0.00001). PER Cmax and AUC were significantly higher in FD compared to WB patients, as was PLA Cmax and AUC, though not significantly. Neutropenia requiring filgrastim administration occurred in 3/14 (21.4%, P = 0.04) patients in the FD arm and 0/17 patients in the WB arm. Recurrence data existed for 19 patients, which showed no significant difference.

Conclusions: Lack of consensus MMC dosing in HIPEC leads to surgical variation and further complicates efforts to standardized practice. Early results of a randomized comparison of FD vs. WB MMC dosing during HIPEC demonstrate a higher PER (and PLA) Cmax and AUC in FD with 21.4% of these patients required filgrastim support. These preliminary data suggest higher dosing in FD arm is reflected in PK data and may lead to higher incidence of neutropenia, with no difference in recurrence rate.

Abstract 81

Prevalence of Mental Health Disorders in Medicaid Beneficiaries Before and After Childhood Cancer Diagnosis

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Background: Recent years have seen a heightened focus on the mental health of childhood cancer patients. However, there's limited understanding of the prevalence of mental health disorders before and after a childhood cancer diagnosis, particularly among the relatively underserved Medicaid beneficiaries.

Objective: This study aims to investigate the prevalence of mental health disorders before and after childhood cancer diagnoses among Medicaid beneficiaries in Kentucky.

Methods: We used data from the Kentucky Cancer Registry to identify individuals aged 19 or younger diagnosed with their first primary childhood cancer between 2001 and 2017. Linking KCR data with Medicaid claims, we included patients with continuous Medicaid enrollment 12 months before and after their cancer diagnosis. Mental health disorders were determined using the International Classification of Diseases (ICD)-9 and ICD-10 codes. A descriptive analysis was conducted.

Results: Among 898 patients, the median age was 9 years (Interquartile Range: 4-14 years), 54% were male, and 39% were from Appalachian counties. The most common cancers included leukemias (n=217), central nervous system cancers (n=193), and lymphomas (n=141). Within 12 months before their cancer diagnosis, 32% (282 patients) had been diagnosed with a mental health disorder, which rose to 55% (489 patients) within 12 months after diagnosis. The leading mental health disorders identified were mood disorders (before n=108; after n=282) and neuropsychiatric/developmental disorders (before n=210; after n=250). The prevalence of mood disorders was higher in older patients and those with certain types of cancer, both before and after diagnosis. Specifically, mood disorder diagnoses rose from 10% to 40% post-diagnosis for cancers diagnosed between 2014 and 2017, from 10% to 38% in lymphoma cases, and from 15% to 60% in bone cancer cases. Diagnoses of neuropsychiatric/developmental disorders were more common in males and those living in non-Appalachian areas, both before and after the cancer diagnosis.

Conclusion: The study shows that over half of the Medicaid-enrolled childhood cancer patients in Kentucky were with mental health disorder within one year of their cancer diagnosis, with a notable increase from before their diagnosis. This increased prevalence post-diagnosis may result from the identification of pre-existing mental health conditions during cancer treatment, or the emergence of new mental health issues as a consequence of the cancer diagnosis and treatment. Further research is critical to fully comprehend these trends and their wider implications.

Abstract 82

PTPRF Negatively Regulates c-MET Signaling to Inhibit Cell Migration in Colon Cancer

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The spatiotemporal 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 c-MET signaling in colon cancer. Downregulation of PTPRF in colon cancer cells activates c-MET signaling at the level of GAB1 scaffolding protein downstream of HGF-induced receptor activation. This resulted in prolonged phosphorylation of GAB1 in both 293T cells and colon cancer cells. Co-immunoprecipitation experiments indicated that PTPRF interacts with GAB1. Interestingly, PTPRF downregulation promotes signaling propagation through the SHP2/MEK/ERK axis but not PI3K/Akt. Functionally, PTPRF-loss markedly increases cell migration towards HGF as a chemoattractant. Taken together, our findings identify GAB1 as a novel substrate of PTPRF. Downregulation of PTPRF may promote tumor progression by enhancing c-MET-mediated oncogenic signaling.

Abstract 83

Quality Implementation of Lung Cancer Screening using the QUILS™ System: Baseline Data from Ten Programs in Kentucky

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Introduction: Lung cancer screening (LCS) using low-dose computed tomography (LDCT) has emerged as an evidence-based approach to detect lung cancer early and reduce lung cancer mortality when conducted among individuals at high risk. Despite robust evidence supporting LCS implementation and a favorable insurance coverage climate due to established guidelines and recommendations, national LCS implementation has encountered a host of multilevel barriers, including incomplete LCS awareness among clinicians, limited engagement among eligible individuals, suboptimal adherence to the screening algorithm, and lack of integration into healthcare system operations. To address barriers and optimize LCS delivery, the Kentucky LEADS Collaborative developed a comprehensive system (QUILSTM System 1.0: Quality Implementation of Lung Cancer Screening) to evaluate LCS delivery, with a focus on supporting rural and low-resource LCS programs. The purpose of this study was to implement the QUILSTM System 1.0 in ten LCS programs to evaluate quality of LCS delivery in diverse settings.

Methods: A multidisciplinary development team created the QUILSTM Index 1.0, an evaluation system to assess quality LCS program operations across six domains: (1) Screening Eligibility, (2) Clinical Radiology Operations, (3) Interdisciplinary Team Operations, (4) Lung Cancer Prevention Efforts, (5) Patient Education, Counseling, and Support, and (6) Community Outreach. Each domain included multiple quality elements, creating 16 scored elements and a total score ranging from 0 to 75. The score was normalized to a range of 0 to 100% quality points. Ten LCS programs at academic, private, and rural community hospitals across Kentucky were evaluated using the QUILSTM Index 1.0. Three data sources contributed to calculating the QUILSTM Index 1.0, including: surveys and interviews conducted with LCS program navigators and medical directors and site-level LCS implementation data.

Results: Overall quality scores ranged from 55% to 87%, with an average score of 69% (SD=9%) across the ten sites evaluated. LCS Programs consistently received high scores on elements involving (1) Screening Eligibility Policy, (2) Screening Frequency & Duration Policy, (3) LDCT Performance, (4) Lung Nodule Identification, and (5) Provider Outreach with most programs receiving optimal scores. LCS Programs scored consistently low on several other elements, including (1) Team Review of Radiology Results, (2) Tobacco Treatment Interventions, (3) Tobacco Treatment Targets, and (4) Shared Decision Making.

Conclusion: Quality LCS implementation will be an essential component to achieving optimal individual and population health benefits promised by rigorous efficacy trials of LDCT. Across ten sites evaluated with the QUILSTM System 1.0, LCS programs performed well under the Screening Eligibility and Clinical Radiology Operations domains but demonstrated opportunities for improvement in the Interdisciplinary Team Operations and Lung Cancer Prevention Efforts domains. Despite the heterogeneity of sites, all ten LCS programs demonstrated areas for potential improvement. Next steps included delivering feedback to the sites using the QUILSTM Audit and Feedback process, and providing access to the QUILSTM Resource Portal and QUILSTM Coaching and Technical Assistance components to help LCS programs improve service quality. Longitudinal evaluations will also be conducted to assess change in service delivery and quality outcomes.

Abstract 84

Recurrence Detection of Stage IIB to IIID Cutaneous Melanoma: Is PET Superior to Other Imaging?

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Introduction: With the development of multiple effective systemic therapies for melanoma, timely detection of recurrent melanoma allows for earlier initiation of treatment when appropriate. NCCN guidelines for stage IIB through IIID cutaneous melanoma surveillance recommend consideration of imaging at least annually for 5 years. However, the type of surveillance imaging is at the discretion of the oncology provider and may include ultrasound, CT scan, PET scan, or brain MRI. Recent data suggests that routine surveillance with PET may lead to earlier detection of melanoma recurrence. We aimed to determine the impact of PET surveillance on recurrence detection rates at our institution.

Methods: We performed a retrospective review of the electronic medical record for all patients diagnosed with stage IIB-IIID cutaneous melanoma at the UK between July 2017 to January 2022. Demographic, clinicopathologic, surveillance, and melanoma recurrence data was collected and analyzed. P-value < 0.05 was considered significant.

Results: Out of 106 patients, 64 (60.4%) had surveillance imaging within one year of resection (26.6% stage IIB-IIC, 85.2% stage III). Reasons for no imaging within one year included lost to follow up, provider choice, death, or early recurrence. Eight patients had follow-ups outside our system and thus surveillance imaging is unknown. A total of 25 patients (23.6%) had a melanoma recurrence; 40% were detected by surveillance PET imaging, 24% by surveillance CT imaging, 28% by clinical exam, and 12% by patient history and subsequent diagnostic imaging (Table 1). One recurrence in the stage IIB-IIC group was detected by PET compared to 9 in the stage III group ($p = 0.01$). Average time to recurrence was 14.2 months with PET surveillance and 20.3 months without PET surveillance ($p = 0.23$).

Conclusions: PET scan detected the majority of melanoma recurrences at the UK. Additionally, patients who had regular surveillance with PET scan had earlier time to recurrence detection. Further multicenter studies are needed to evaluate if these trends are similar across institutions. Additional studies are also needed to determine if this earlier detection is associated with improved survival.

Abstract 85

Redox Metabolism Shared Resource Facility (RM SRF)

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The Redox Metabolism Shared Resource Facility (RM SRF) is a cancer center managed facility that provides critical services for Markey Cancer Center (MCC) Research Programs. Notably, the RM SRF is one of a small number of facilities that provides expertise and analyses in oxidative stress, cellular energetics, metabolomics and proteomics in cancer and cancer biology, a role underscored by increasing evidence that oxidative stress and consequent damage to cellular energetics, metabolism and protein alterations underlie both tumorigenesis and treatment resistance. RM SRF Specific Aims include its four major services: 1) Analysis of biomarkers of oxidative and nitrosative stress; 2) Measurement of redox state/redox signaling and mitochondrial functions; 3) Metabolomic services, i.e., profiling and stable isotope resolved metabolomics; and 4) Proteomic services. Key investments have included obtaining state-of-the-art Seahorse XFp, XFe96, and Biotek Cytation cell imaging and proteomic instrumentation. RM SRF has continued to exhibit its value-added impact through support for the renewal of the P20 Center of Biomedical Research Excellence (COBRE), several Molecular and Cellular Oncology (MCO) program R01 grants, and Translational Oncology (TO) program R01s and a project within the P20 COBRE for discovery/development of anticancer agents and mechanistic targets in models of cancer. RM SRF also collaborates with the Cancer Prevention and Control (CP) program to identify risk factors and interventional targets for cancer prevention and control in Kentucky's Appalachian catchment population. RM SRF leadership and staff actively participate in the development of new applications and methodologies for a better understanding of the roles of oxidative stress and metabolism in cancer and cancer chemotherapy and provide technical assistance with experimental design and interpretation of results for all MCC Research Programs. The RM SRF facilitates research with the other MCC SRFs to improve the analytical power of various cutting-edge technologies for understanding the enormous cancer burden in Kentucky.

Abstract 86

Regulation of Protein Tyrosine Phosphatase Type F by NEDD4L E3 Ligase

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The phosphorylation state of target proteins is tightly regulated by protein kinases and phosphatases. The vast majority of cellular signaling depends on this dynamic relationship for the propagation or termination of signaling cascades and thus maintenance of homeostasis. PTPRF belongs to a family of receptor type protein tyrosine phosphatases. Our previous studies have been shown that PTPRF acts as an oncogenic phosphatase through the upregulation of Wnt signaling in colon cancer. However, the regulation of PTPRF is not well studied. In this study, we show that NEDD4L, a HECT E3 ligase, regulates the stability of PTPRF protein. Specifically, overexpression of NEDD4L decreases the half-life of PTPRF whereas knockdown of NEDD4L has the opposite effect. In addition, wild-type NEDD4L, but not the E3 ligase activity deficient mutant, increases the ubiquitination of PTPRF, indicating that PTPRF is a substrate of NEDD4L. Interestingly, the protein stability of a catalytically inactive mutant PTPRF is higher compared to wild-type PTPRF, suggesting that the phosphatase activity promotes PTPRF degradation. Moreover, by utilizing modified ubiquitin, we demonstrate that NEDD4L preferentially utilizes K29 ubiquitin linkage to modify PTPRF. Functionally, NEDD4L-mediated degradation of PTPRF blocks the ability of PTPRF to enhance Wnt activation. Taken together, this study identifies a previously unidentified regulatory mechanism of PTPRF through NEDD4L mediated ubiquitination.

Abstract 87

Role of Cytoreductive Nephrectomy in Metastatic Renal Cell Carcinoma in the Era of Immunotherapy: An Analysis of the National Cancer Database (NCDB)

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Background: The landscape of metastatic renal cell carcinoma (mRCC) treatment has witnessed significant transformations with the emergence of immunotherapy-based combination regimens (IO-X), including IO-IO and IO-TKI. However, the role of cytoreductive nephrectomy (CN) in the era of IO-X remains controversial following CARMENA and SURTIME trials. We evaluated survival outcomes and timing of CN in patients treated with CN+ IO-X v/s IO-X using NCDB.

Methods: An NCDB analysis was conducted on 6,018 stage IV mRCC patients who underwent IO-based treatments between 2016 and 2022. Patients were stratified into three groups (grps): received IO-X alone (grp 1), underwent upfront CN followed by IO-X (grp 2) and received IO-X followed by delayed CN (grp 3). The life table method, Kaplan–Meier plot, and a multivariable Cox regression analysis were conducted to compare survival among grps, initiating follow-up 30 days post-recent treatment to mitigate bias.

Results: A total of 6,018 patients received IO-X: 4,155 pts (69%) had IO-X alone; 1,560 (25.9%) had upfront CN then IO-X; and 303 (5.0%) had IO-X followed by delayed CN. Most patients are male (71.3%), Caucasians (86.1%), and aged between 40 and 64 years (51.9%). Most patients had a comorbidity index of 0 (71.6%) and were treated in academic medical settings (58%). At the time of diagnosis, 42% had bone, 61.6% had lung, 19% had liver, and 11% had brain metastasis. Additionally, 31.3% received a combination of IO and TKI therapy. The distribution of histological types varied across the groups: 38.6% with clear cell histology in grp 1 compared to 68.9% grp 2 and 70.8% in grp 3.

Median overall survival was not reached in grp 3, 42.1 months in grp 2, and compared to 14.4 months in grp 1 ($p<0.0001$)- figure 1. The multivariate Cox regression analysis indicated improved survival in grp 2 (HR 0.63, 95% CI 0.57 to 0.70) and grp 3 (HR 0.41, 95% CI 0.33 to 0.50) compared to grp 1. Grp 3 demonstrated a 37% reduction in risk of death compared to grp 2 (HR 0.65, 95% CI 0.52 to 0.81)

Conclusion: In this study, we found that patients who underwent CN, particularly when delayed following initial systemic therapy with IO-based therapy, experienced notably enhanced survival outcomes compared to those treated with IO-based treatment alone. This study highlights the importance of the timing of surgical interventions in the era of evolving IO-based systemic therapies. Of note, given the varied characteristic distributions among the three groups, potential selection bias may influence the observed.

Abstract 88

RPS6KB1 is a Critical Target for Overcoming Tumor Lineage Plasticity and Therapy Resistance

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Most tumors initially respond to treatment, yet refractory clones subsequently develop owing to resistance mechanisms associated with cancer cell plasticity and heterogeneity. Moreover, tumor intrinsic features contribute to resistance to standard-of-care treatment. We used a chemical biology approach to identify protein targets in cancer cells exhibiting diverse driver mutations and representing models of tumor lineage plasticity, heterogeneity and therapy resistance. An unbiased screen of a drug library was performed against cancer cells followed by synthesis of chemical analogs of the most effective drug. The cancer subtype target range of the leading drug was determined by PRISM analysis of over 900 cancer cell lines at the Broad Institute, MA. RNA-sequencing and enrichment analysis of differentially expressed genes, as well as computational molecular modeling and pull-down with biotinylated small molecules were used to identify and validate RPS6KB1 (p70S6K or S6K1) as an essential target. Genetic restoration, as well as CRISPR/Cas knockout in cancer cells were used to test the functional role of RPS6KB1 in cell culture and xenograft models. We identified a novel derivative of the anti-histamine drug ebastine, designated Super-ebastine (Super-EBS), that inhibited the viability of cancer cells representing diverse KRAS and EGFR driver mutations and models of plasticity and treatment resistance. Interestingly, Super-EBS inhibited over 800 diverse cancer cell lines tested in the PRISM analysis and targeted the serine/threonine kinase RPS6KB, but not its upstream activator mTORC1. RPS6KB1 is upregulated in various cancers relative to counterpart normal/benign tissues and phosphorylated-RPS6KB1 predicts poor prognosis for cancer patients. Inhibition of RPS6KB1 phosphorylation was necessary for tumor cell growth inhibition, and restoration of phospho-RPS6KB1 above endogenous levels rendered tumor cells resistant to Super-EBS. Inhibition of RPS6KB1 phosphorylation by Super-EBS induced caspase-2 dependent apoptosis via inhibition of the Cdc42/Rac-1/p-PAK1 pathway that led to actin depolymerization and caspase-2 activation. The essential role of RPS6KB1 in the action of Super-EBS was recapitulated in xenografts, and knockout of RPS6KB1 abrogated tumor growth in mice. RPS6KB1 is a therapeutic vulnerability in tumors exhibiting intrinsic and/or acquired resistance to treatment.

Abstract 89

Simultaneous 1H NMR Quantification of Stable Isotope Enriched Metabolite in Multiplexed Labeling Stable Isotope Resolved Metabolomics

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Stable isotope-enriched precursors are frequently used to trace the metabolic fate of different atoms, including 13C, 15N and 2H. Simultaneous administration of different substrates enables tracking different metabolic pathways in a single experiment, which greatly increases the analytical power. For example, 2H7 glucose + [U-13C, 15N]-Glutamine enable pathway tracing through glycolysis, the Krebs cycle, nucleotide synthesis, the hexosamine pathway, as well as nucleobase synthesis, and can be applied to a wide range of models including cells in monoculture, 3D cultures, tissue slices, organisms, and even human subjects.

However, due to the natural low gyromagnetic ratios of these isotopes, direct detection via NMR is very time consuming, requiring long acquisition times with lengthy relaxation delays. In contrast, 1H NMR can capitalize on cryoprobe and intrinsic high sensitivity and use the introduced stable isotope for isotope editing (1). Furthermore, 1H NMR is routinely used in most metabolomics studies. Analysis of the proton peak patterns due to 1-3 bond scalar couplings and isotope shifts in different metabolites can identify and quantify the positional enrichments of the isotopically introduced metabolites.

We demonstrate the application of multiplexed labeling in cultured cells with Glutamine-derived 15N incorporation into purine nucleotides, glucose-derived 2H incorporation into lactate and acetate, as well as glutamine-derived 13C incorporation into the pyrimidine ring of uracil in UTP. Isotope shifts and Isotope distributions were determined using the isotope shifts (2H) and scalar coupling patterns quantified in 1D NMR, and validated by 2D NMR (HSQC and TOCSY). This information greatly aids determining the activity of different metabolic pathways in cells with a single multiplexed labeling experiment.

Abstract 90

Single-Fraction Stereotactic Body Radiotherapy (SBRT) to Early-Stage Primary Lung Tumors or Oligometastatic Lesions Confers Effective Local Control and Minimal Toxicity

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Purpose/Objectives: Single-fraction SBRT for early-stage NSCLC and oligometastatic pulmonary disease has been shown to be a safe and efficacious treatment modality. However, adoption of this regimen has been limited despite excellent local control rates, minimal rates of toxicity, and favorable survival outcomes. Here we present a prospective study of the use of singe-fraction SBRT in the definitive treatment of oligometastatic and primary NSCLC.

Materials/Methods: Fifty-six patients with early-stage (T1-2N0) non-small cell lung cancer (NSCLC) or solitary pulmonary metastases were treated using 4D-CT on TrueBeam, to deliver 30 Gy in a single fraction via SBRT. Treatments were delivered every other day using pre-treatment Conebeam CT-guided procedure and pitch-perfect couch corrections. Patients underwent post-treatment tomography in 3 to 6-month intervals per standard of care. Pulmonary and rib toxicities per CTCAE v4.0 were reported, as well as rates of local control, regional and distant failure, and survival.

Results: Fifty-six patients (51 early-stage NSCLC and 4 metastatic tumors) were evaluated, and thirty-nine (76.5%) of these patients underwent follow-up CT imaging. Median follow-up was 10.6 months (range 0 months—40 months). Local control was achieved in all patients (100%) evaluated radiographically, including 16 patients assessed at 1-year follow-up and 9 patients at 2-year follow-up. Regional or distant failures were found in 15/39 (38.5%), and 5/15 cases (33%) of these cases were confirmed as deaths from disease progression. Two of the fifteen patients with isolated recurrences underwent repeat SBRT to 30 Gy in 1 fraction, with effective local control and no acute toxicities. The toxicity profile amongst 47 clinically evaluated patients showed one (2%) episode of grade 1 acute rib toxicity. Thirty-eight of 39 (97%) radiographically evaluated patients demonstrated grade 1 radiation pneumonitis, but no chronic pulmonary toxicities were reported.

Conclusion: Use of single-fraction SBRT for early-stage NSCLC and oligometastatic pulmonary disease confers excellent local control and minimal rates of acute and chronic toxicities. We propose further investigation combining single-fraction SBRT with systemic therapy to improve recurrent and distant disease rates, as well as rates of survival. We recommend its extended use in other cancer centers, providing additional benefits of optimized clinic workflow, greater efficiency in treatment planning and physics quality assurance, and improved patient comfort, compliance, and access to care.

Abstract 91

Stereotactic Body Radiation Therapy (SBRT) Treatment of Synchronous Lung Tumors via Single-Isocenter/Multi-Target (SIMT) Method: Reporting Single-Institutional Clinical Outcomes

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Purpose: Oligometastatic (≤5 lesions) lung cancers or synchronous primary lung tumors with associated co-morbidities may not retain their treatment position during SBRT treatments when using multiple isocenters due to longer treatment times, back pain, or shortness of breath. Lung SBRT using SIMT VMAT plans with flattening filter free (FFF) beam can reduce treatment time, improve patient comfort, and improve clinic efficiency. Herein we present our clinical results for treating multiple primary or oligometastatic lung cancers using SIMT lung SBRT.

Materials/Methods: Sixty-two complex and difficult patients with synchronous primary lung cancers or oligometastatic lung lesions (two, n= 51; three, n= 6; four, n= 3; five n=2; total tumors = 142) were simulated with 4D-CT based MIP images and/or abdominal compression and treated with highly conformal SIMT SBRT plans using co/non-coplanar VMAT geometry. Common prescriptions were 50-55 Gy in 5 fractions (50 pts), and 54 Gy in 3 fractions (12 pts), prescribed to each PTV margin with the 70-80% isodose line. Advanced Acuros-XB dose engine for 6FFF beam was used for tissue heterogeneity corrections. NRG RTOG-0618/0813 protocols criteria were used for plan quality evaluation and dose constraints to organs-at-risk (OAR). CBCT-guided SIMT treatment was delivered every other day with 6DOF PerfectPitch couch corrections, and overall treatment time was within 15 minutes. Reported outcomes includes tumor local control (LC) rates and toxicities profiles using CTCAE v5 guidelines.

Results: All SIMT lung SBRT plans met RTOG-0618/0813 requirements for target coverage and dose to OAR. Average PTV volume was 16.2 cc (range, 2.17–167.8 cc). Mean follow up after last fraction dose was 20 months (range 0–67.2 months). Of the 62 patients treated, 54 had an adequate post-treatment chest CT scan to assess LC. Among patients with treated and followed up tumors, LC was achieved in 51/54 (94.4%), and for toxicities, 33/54 (61%) of patients developed CTCAE grade 1 asymptomatic pneumonitis on chest CT in on average, 6.2 months after SIMT lung SBRT. No symptomatic pneumonitis, esophagitis, or rib fracture occurred. CTCAE grade 2 chest wall pain occurred in 1 patient with pre-existing neuropathic rib pain which was managed with gabapentin. Thirty-six patients had distant metastasis and 22 patients died from distant failure or a competing comorbidity.

Conclusion: Highly conformal SIMT lung SBRT for synchronous primary or oligometastatic lung cancers was safe, fast, and an effective treatment option demonstrating excellent tumor LC rates (>94%) with low treatment related toxicity. SIMT improved patients' compliance and comfort for those who may not tolerate traditional extended treatment course/time and reduces isocenter shifts with repeated CBCT imaging for patient set up and verification. SIMT improves clinic workflow and potentially reduced intrafractional set up errors. Kaplan-Meier estimate with longer clinical follow up in a larger patient cohort of SIMT lung SBRT is ongoing.

Abstract 92

Targeting CDK1 Activity Suggests a Strategy for Ape1-Related Treatment in Cancer

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The exonuclease activity of Apurinic/apyrimidinic endonuclease 1 (APE1) plays a crucial role in processing matched/mismatched termini within various DNA repair pathways and in removing nucleoside analogs linked to drug resistance. In our study, we have identified APE1 as a novel substrate of Cyclin Dependent Kinase 1 (CDK1), a pivotal regulator essential for orchestrating cell cycle-related events, including G2/M and G1/S transitions, as well as G1 progression. Aberrations in CDK1 activity often drive uncontrolled cell proliferation, a hallmark of malignancy.

Our findings further demonstrate that CDK1 phosphorylates APE1, leading to the degradation of APE1. We aim to dissect the cellular consequences associated with CDK1-mediated APE1 degradation. Given APE1's classical role in DNA repair and transcriptional regulation of gene expression, our primary objective is to investigate whether CDK1 phosphorylation impacts APE1's exonuclease activity and its redox signaling in cellular processes in cancer development. Additionally, since APE1 is often overexpressed in cancer, and its expression and activity are correlated with processes such as proliferation, invasion, inflammation, angiogenesis, and resistance to cell death, our secondary objective is to search for and develop novel strategies for cancer treatment and therapy by targeting the CDK1-APE1 axis.

Abstract 93

The Effects of Cannabis on Cancer Burden: Protocol Description of Phase I Placebo-Controlled, Double-Blind, Randomized Trial

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Purpose: Several clinical trials have studied cannabinoids for cancer pain and symptom management; however, many lack necessary controls (e.g., controlled drug administration). Therefore, this trial aims to examine the effects of cannabis (via controlled dosing methods) on an array of symptoms that cancer patients experience (termed cancer burden), including problems with pain, sleep, appetite, everyday activities, quality of life. This study will also assess the highest tolerated dose in each arm to better inform cannabis dosing protocols and the overall risk/benefit profile of cannabis in advanced cancer patients.

Method: This study is a randomized, double-blind, placebo-controlled trial. All study participants will be randomized 1:1:1:1 into the 4 dosing groups: 1) 5 mg THC, 2) 15 mg THC, 3) 15 mg THC+15 mg CBD, 4) placebo. The primary objective is to evaluate the safety/tolerability of daily oral cannabis relative to placebo. Secondary outcomes include final tolerated dose, total cancer burden score; exploratory outcomes include quality of life, sleep quality, pain intensity, body weight. We anticipate screening approx. 250 participants to enroll n=80 total (n=20/arm). Each participant will be enrolled in the study for approx. 5.5 months (with 4 months of daily dosing).

Results: We have received approval from Markey Cancer Center and the UK Medical IRB. We anticipate launching enrollment in Spring 2024.

Conclusion: This study is one of the first controlled trials examining cannabis effects on cancer burden outcomes and will help develop dosing protocols for patients with cancer.

Abstract 94

The Functional Significance of Plk1 Phosphorylation of Nrf2 at S253 in Prostate Cancer Progression

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Prostate cancer is a complicated disease that primarily affects older men and is influenced by genetics, diet, environmental pollution, and inflammatory conditions. When there is an overproduction of reactive oxygen species (ROS), prostate cells shift towards a more oxidative state, promoting the initiation and progression of prostate cancer (PCa). To combat oxidative stress, PCa cells exhibit an adaptive mechanism by upregulating different antioxidant signaling pathways in response to elevated ROS levels. It is critical to comprehend the oxidative stress mechanism and antioxidant regulation in prostate cancer progression. In this project, to figure out the mechanism for enhancing the expression of several antioxidant genes in PCa, we established C4-2 stable cell lines expressing Nrf2 (WT, S253A, S253D) to study the Plk1/p-S253Nrf2/ARE axis. We performed the in vitro kinase assays to find out whether Nrf2 is indeed a substrate of Plk1. We observed that Nrf2 is being phosphorylated at the S253 site by Plk1 at the cellular level. We also examined that this phosphorylation increases the level of both cytosolic and nuclear Nrf2 levels. We found that the C4-2 expressing Nrf2 S253A cell line has the production of more unstable Nrf2 compared to WT, suggesting that this phosphorylation might contribute to Nrf2 stabilization. We found that this phosphorylation increases Nrf2 downstream target genes expression in both mRNA and protein levels. We observed that Nrf2 WT has more cellular ROS production compared to Nrf2 SA cell line, indicating that this phosphorylation increased oxidative stress and at the same time increased antioxidant genes expression to optimize PCa cell survival. We further designed experiments to elucidate whether this phosphorylation has any effect in cell proliferation and enzalutamide resistance. We identified that this phosphorylation increased the proliferation and colony formation capacity in with and without Enzalutamide treated groups. These results reveal novel ideas on how the Plk1/p-S253Nrf2/ARE axis contributes to prostate cancer progression.

Abstract 95

The Role and Regulation of OCT4 in Neuroendocrine Prostate Cancer Progression

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Prostate cancer, holding the dubious distinction of being the most prevalent malignancy and a leading cause of cancer-related mortality among men in the United States, faces an evolving landscape with the emergence of a distinct and formidable subtype known as neuroendocrine prostate cancer (NEPC). NEPC is characterized by its aggressive behavior, rapid progression, and notable resistance to conventional therapeutic interventions, including second-generation anti-androgen inhibitors. The clinical urgency in addressing NEPC is underscored by the substantial decline in patient survival rates associated with this challenging malignancy. In this context, the imperative to unravel the molecular intricacies of NEPC becomes paramount, necessitating the identification of novel therapeutic targets to circumvent its unique challenges. Polo-like Kinase 1 (PLK1), a key orchestrator of cell cycle progression and mitotic entry has emerged as a potential player in prostate cancer progression. With its phosphorylation capabilities, PLK1 exerts influence over genes and disrupts pathways, including those intricately tied to pluripotency maintenance. Concurrently, OCT4, a pluripotent transcription factor, holds significance not only for its impact on cancer cell proliferation but also as a marker indicative of cancer stem cell activation. This research endeavors to elucidate the intricate interplay between PLK1 and OCT4 within the context of NEPC. The central hypothesis posits that PLK1-mediated phosphorylation of OCT4 induces cancer stem cell pluripotency, thereby contributing substantively to the development and aggressiveness of NEPC. This multifaceted exploration is delineated into three specific aims: 1. To dissect OCT4 roles in regulating stemness in NEPC; 2. To understand how OCT4 can be regulated upon PLK1 phosphorylation; 3. To determine if inhibition of OCT4 phosphorylation reduces cell differentiation into NEPC in vivo. By successful completion of this project, the profound impact lies not only in advancing our fundamental understanding of the molecular underpinnings of NEPC but also in potentially unearthing actionable therapeutic targets. By unraveling the complex interplay between PLK1 and OCT4, this study has the potential to unveil critical nodes in the molecular network driving NEPC pathogenesis. The implications extend beyond basic science, offering a pathway toward precision medicine tailored to the unique molecular signatures of NEPC. Ultimately, this research aspires to contribute significantly to the development of innovative therapeutic strategies, offering renewed hope for improved outcomes and enhanced quality of life for individuals grappling with the challenges of neuroendocrine prostate cancer.

Abstract 96

The Role of PGK1 in Ferroptosis in Breast Cancer

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Breast cancer is a malignant disorder of the mammary tissue that is caused by the accumulation of mutations in neoplastic cells. It is the most commonly diagnosed cancer among women and one of the leading causes of cancer-related deaths in females. Breast cancer treatments have improved over the years due to the invention of highly sensitive diagnostic techniques, identification of druggable targets, and advancement in immunotherapies. Nevertheless, a fraction of the tumour cells escape therapeutic interventions, develop resistance and facilitate relapse and metastasis which eventually kills the patients. These residual cells are able to reprogram their metabolism to adapt to harsh conditions such as starvation, acidosis, hypoxia and escape cell deaths. Ferroptosis occurs when tumor cells die from a toxic build-up of lipid peroxides on their cell membranes. It is a cell death that is heavily dependent on transition metal iron, reactive oxygen species, phospholipids containing polysaturated fatty acids (PUFA-PLs), and can be repressed under glycolytic metabolism. Our kinase of interest is the Phosphoglycerate kinase 1 (PGK1), which in addition to its metabolic activity, has oncogenic properties and has been reported to be overly expressed in several human cancers. PGK1 modulates metabolic events in cancer cells such as glycolysis which represses ROS, coordinates the TCA cycle, and has been implicated in the promotion of tumorigenesis, chemoresistance, metastasis and survival of tumor cells. Our lab intends to target PGK1 to disrupt the metabolic equilibrium in breast cancer cells so as to further sensitize them to ferroptosis. Our preliminary data shows that silencing PGK1 in MCF7 and MDAMB231 breast carcinomas slowed down their tumor-promoting properties, as well as their cellular expression of GPX4 which is an anti-ferroptosis defense peroxidase. In view of this, the overall objective of this study is to better understand how PGK1 regulates ferroptosis in breast cancer cells. My central hypothesis is that PGK1 inhibition disrupts tumor metabolism which consequently inhibits ferroptosis resistance and sensitizes breast cancer to cell death. My hypothesis will be tested using a few approaches. Firstly, we evaluate how PGK1 regulates the phenotypes of breast cancer cells and how this facilitates tumor progression. Next, we will highlight the mechanisms by which PGK1 regulates ferroptosis in cancer cells using in vitro and in vivo models. Successful execution of this project will not only validate PGK1 inhibition as an efficient therapeutic strategy for breast cancer treatment but will also justify PGK1 as a new ferroptosis modulator which enhances ferroptosis activity for the treatment of cancers. The information obtained from this study would be foundational for various uses in clinical trials and translational medicine.

Abstract 97

The Sodium Hydrogen Exchanger-1 (NHE1) Drives T-Cell Acute Lymphoblastic Leukemia Self-Renewal by Modifying Mitochondrial Metabolism

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T-cell Acute Lymphoblastic Leukemia (T-ALL) patients who experience relapse have a dismal prognosis, with 5-year overall survival rates of 10% for adults and 36% for children. Relapse is attributed to cytotoxic chemotherapy's inability to target Leukemia Initiating Cells (LICs) that have the potential to re-populate the cancer. There is a need for novel therapeutic strategies to target LIC self-renewal in T-ALL with improved safety profiles and better chances of transition to clinical development.

This project utilized a transgenic zebrafish model that accurately recapitulates the most aggressive form of the human T-ALL. This model offered several advantages, including a high frequency of LICs and suitability for drug testing. Over 770 FDA-approved drugs were screened in vivo using ≥2, 500 syngeneic CG1 zebrafish. Further secondary screening in human T-ALL cells narrowed down the potential hits into 4 compounds that specifically interfere with self-renewal. Finally, the top hit Amiloride was confirmed by a limiting dilution assay (LDA), resulting in a more than 4-fold decrease in the LIC frequency ($p=0.0026$). Amiloride, which reduced the frequency of LICs in zebrafish and human T-ALL cells, is an inhibitor of the Sodium Hydrogen Exachanger-1 (NHE1).

The inhibition of this exchanger was found to impair mitochondrial function in cancer cells, offering a possible mechanism for the inhibition of LICs. In our human T-ALL cells, we found that Amiloride significantly reduced mitochondrial basal respiration ($p=0.0074$) and ATP production ($p=0.0008$). When probing for different genes associated with mitochondrial processes, PARKIN expression was constantly upregulated by more than 8-fold in Amiloride-treated cells, compared to DMSO control ($p< 0.0001$). PARKIN is an E3 ubiquitin ligase and a well-established mediator of mitophagy, which led us to investigate the impact of Amiloride on mitochondrial morphology in T-ALL cells. We found that Amiloride treatment reduced mitochondrial footprint ($p<0.0001$) and mitochondrial branching ($p=0.0028$) in T-ALL cells. Importantly, we found that high mitochondrial content was significantly associated with self-renewal in T-ALL cells, suggesting LICs may be highly sensitive to metabolic disruption.

To validate the NHE1 as the target responsible for the effect on self-renewal seen by Amiloride treatment, we used shRNA knock down (KD) of NHE1 gene expression. We found more than 70% decrease ($p<0.0001$) in in vitro self-renewal with the KD cells compared to the scrambled control and a concurrent decrease in gene expression of Nanog ($p<0.0001$), CD7 ($p<0.0001$) and CD44 ($p=0.0079$), among other genes associated with stem cell self-renewal. A mitochondrial stress test in KD cells revealed reduced mitochondrial energy metabolism and JC-1 staining showed a significant decrease in mitochondrial membrane potential ($p<0.0001$), which is associated with mitochondrial fragmentation.

Overall, this project capitalized on the high frequency of LIC in the Myc-derived zebrafish T-ALL model and the strengths of zebrafish in drug discovery to generate a physiologically relevant hit, Amiloride, in vivo. Following that, further investigations in human cells identified the NHE1 as the target for the inhibition of self-renewal and the impaired mitochondrial function as a possible underlying mechanism.

Abstract 98

Time from Diagnosis to Treatment of Patients with Lung Nodules Evaluated by Interventional Pulmonology at Markey Cancer Center vs. Not

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Background and Significance: Cancer is the second leading cause of death worldwide and lung cancer is the leading cause of cancer-related death. (1) The USPSTF recommends low dose CT chest as a screening test for lung cancer on the basis of data that suggest reduction in lung cancer mortality and all-cause mortality, with a number needed to screen of 130 over 10 years of follow-up. (2) After detection of a screening CT chest abnormality that warrants tissue sampling, patients can undergo biopsy with TTNA or via bronchoscopy with various adjuncts such as EMN, linear and radial EBUS, and rapid onsite evaluation. The additional tools available with bronchoscopic sampling of lung nodules allow mediastinal staging during the same procedure. After a diagnosis of lung cancer has been established, it is imperative that treatment be initiated as soon as possible. There are data that suggest a diagnosis to treatment interval of less than 35-45 days is associated with improved survival. (3, 4). An analysis of data from 599 patients with non-small cell lung cancer suggested one- and 5-year overall survival decreased when time from diagnosis to treatment was >50 days. We sought to evaluate the time from diagnosis to treatment in patients with lung nodules who were seen at UK through the interventional pulmonology clinic at Markey Cancer Center (TDTIP) vs. those who were not seen through this clinic.

Interim analysis: We identified 508 patients who met the inclusion criteria. An interim analysis of 66 patients revealed a trend towards a shorter time from diagnosis to treatment in the group of patients who were seen through the IP clinic at Markey Cancer Center ($p=0.054$, CI -.059 to .955).

Discussion: A longer time from diagnosis to treatment of lung cancer has long been associated with worse outcomes. With our study, we seek to compare these numbers at our institution which will help guide change moving forward if needed in the form of a dedicated nodule clinic in attempts to keep the time from diagnosis to treatment of lung cancer as short as possible.

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Abstract 99

Tobacco Use Status, Stigma and Lung Cancer Screening Intentions

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Background: Lung cancer is the leading cause of cancer death globally. Further investigation is needed to understand barriers to lung cancer screening (LCS) uptake among high-risk individuals. Stigma surrounding tobacco use and lung cancer presents significant obstacles for those diagnosed with lung cancer. Social support, while vital in reducing stigma's impact, may inadvertently normalize tobacco use. Our study examines LCS intention based on tobacco use behaviors and eligibility, as well as the impact of stigma.

Theoretical Background: The Health Belief Model (HBM) suggests that people's health behavior changes are driven by their beliefs about the risks and benefits of certain actions. It also emphasizes the role of cues to action, which can prompt individuals to take preventive measures. We hypothesize that tobacco use and stigma shape perceptions and behaviors associated with LCS intentions.

Methods: Secondary data analysis (N=73) participants in a community-based LCS education intervention. We used the following measures: Demographics, Cancer Stigma Scale, LCS intention.

Data Analysis: Descriptive statistics, Chi-square and Mann-Whitney U-test, A logistic regression analysis was used to determine factors that contribute to stigma.

Results: Of the 73 participants, most were female (70%), non-Hispanic White (47.9%), Black (34.2%) and 20.5% Hispanic/Latino. Nearly 51% had ever used tobacco; 37% were current users; 14.0% were former users, 18.9% were heavy tobacco users, and 18.9% were poly tobacco users. Thirty-three participants are exposed to secondhand smoke. Of them, fourteen participants were non-smokers. Chi-square and Mann-Whitney U-test analyses found no significant difference in LCS intentions based on tobacco use behaviors or stigma. Logistic regression revealed that stigma explained only 1.0% of LCS intention variance across all participants and 3.4% among those LCS eligible.

Discussion and Implications: We found that approximately one-fourth of eligible participants expressed no intent to undergo LCS. Tobacco use did not substantially influence LCS intentions, and stigma played a limited role. Further efforts are needed to investigate additional barriers that may impact LCS intentions. Based on our results, we recommend conducting interviews or focus groups with non-intenders to explore their attitudes and reasons. Policymakers need to develop strategies aimed at increasing access to and uptake of LCS among those at high risk of lung cancer. We recommend developing and evaluating interventions that aim to increase LCS intentions among individuals who are initially disinclined.

Abstract 100

Toxicological and Biological Properties of Reference and Commercial Cigar Products

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Lung cancer has the highest cancer mortality in the United States, and Appalachian Kentucky has the highest lung cancer rates in the nation. Cigarette and cigar smoking are responsible for the vast majority of lung cancers and contribute significantly to the burden of head and neck cancers, and other cancers. Filtered cigars are a product that is inhaled much like cigarettes, but have been poorly studied to this point. Large cigars and cigarillos, although not inhaled into the lungs, do contribute to head and neck and other cancers and have not been studied in any extensive way. We obtained condensates from both reference and commercial filtered cigars, large cigars and cigarillos from the Center for Tobacco Research Products at the Kentucky Tobacco Research and Development Center in the UK College of Agriculture, who also carried out chemical analysis of these products. Our lab performed toxicological and biological studies on the condensates of these products in immortalized bronchial and oral epithelial cell models and an AHR reporter cell model. The results from experiments on the reference and commercial filtered cigars were compared to results with the reference cigarette, 1R6F, as well as with each other. Results obtained with commercial large cigars and cigarillos were compared with their reference counterparts. Our findings suggest that filtered cigars may be more toxic than even cigarettes to lung epithelial cells and induce higher levels of AHR-mediated gene expression. Data with commercial large cigars and cigarillos are variable between products and in comparison with their reference products. This is expected to be the case, as these products are somewhat unique in composition. Nevertheless, they show high levels of induction of AHR-mediated gene expression and have toxic effects on oral epithelial cells. Our findings provide the basis for understanding some of the carcinogenicity and toxicity of these tobacco products.

Abstract 101

ABSTRACT WITHDRAWN

Abstract 102

Tumor Associated Macrophages Promote Immunosuppression in Gastric Cancer

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Gastric cancer remains a lethal global health concern. It is the 3rd most common leading cause of cancer related mortality worldwide. Several studies have shown that macrophages promote tumorigenesis in pancreatic, liver and colon cancer. However, the role of macrophages in gastric cancer is poorly understood. Using mouse models of primary and metastatic gastric cancer, we show that tumor associated macrophages (TAMs) are a predominant cell type in the microenvironment. These macrophages exhibit an activated phenotype expressing MHCII and CD206. Conditioned media from tumor cells polarize naïve macrophages into alternatively activated phenotype by inhibiting TNF-a, IL1b and upregulating Arg-1. Functionally, TAMs inhibit naïve polyclonal CD4+ T cell activation in-vitro. Accordingly, macrophage depletion inhibited tumor growth in-vivo. Taken together, these data show that tumor-macrophage crosstalk promotes T cell anergy and gastric cancer tumorigenesis. Further studies will investigate mechanistically how TAMs inhibit T cell function and elucidate potential therapeutic vulnerabilities in gastric cancer.

Abstract 103

Using EZH2 Inhibitor as a Tool for Targeting Lung Disease

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Lung disease, including chronic obstructive pulmonary disease, fibrosis and cancer are major health problems and together lead to deaths of millions of individuals each year. Pulmonary fibrosis is a life-threatening respiratory disease caused by lung tissue inflammation that leads to scarring and damage. Smokers with fibrosis have more than 2-fold higher risk of developing lung cancer 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 EPZ6438, tazemetostat is an epigenetic drug that specifically targets and inhibits EZH2. FDA-approved in 2020, EPZ6438 serves as an oral drug that 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. Pro-inflammatory cytokines such as Transforming Growth Factor Beta (TGFB), Interleukin-1-Beta (IL1B), and Tumor Necrosis Factor-alpha (TNF-a) have been identified as key drivers of fibrosis progression in both human and animal cell lines with TGFB being the master regulator for fibrosis. For our current study, we use both normal and fibrotic human lung fibroblasts cells and treated them with the cytokine TGFB-1 in the presence of EZH2 inhibition. So far, our findings suggest that in human fibroblasts TGFB-1 increases the expression of fibrotic markers such as alpha smooth muscle and vimentin and EZH2 inhibitor reduces expression. We have also tested inhibiting the fibrotic response to bleomycin in mouse lungs with EZH2 inhibitor and observed reduction in bleomycin induced fibrotic phenotypes, which may be due in part to a reduction in inflammatory response. Lastly, mouse lung mesenchymal cells have been co-cultured with tumor and other immune cells to observe the effect these interacting cells have on tumor growth in the presence of EZH2 inhibition and immunotherapy. We observed that combined therapy reduces tumoroid number in culture. 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 104

Utilizing Mitochondria Modulating Agents to Sensitize Prostate Cancer to Radiation Treatment

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Prostate Cancer (PCa) is the second leading carcinoma in men. Although treatment has improved vastly in recent years, recurrence and resistance to radiation treatment (a leading PCa treatment option beside proctectomy) persists as a problem. Our laboratory studies have shown that prostate cancer cells can increase mitochondrial utilization and quantity to evade cell death from radiation treatment and progress metastasis and recurrence. This is primarily facilitated by a process known as mitochondrial biogenesis, where cells use their pre-established mature mitochondria to produce new mature mitochondria. The increase in mitochondrial number is correlated with upregulation of mitochondrial metabolic pathways in radiation resistant prostate cancer (RR-PCa) cells with Stable Isotope-Resolved Metabolomics (SIRM) techniques. Here in, we propose to target radiation resistant prostate cancer by inhibiting their mitochondrial function and impairing their metabolism. To accomplish this, we developed radioresistant RR-PCa cells that mimic clinical treatment (33 treatments of 2Gy radiation) with ability to form tumor in immunocompetent mice (RR-RM-1). The surviving RR-RM-11 cells exhibited changed morphology (increased surface area, etc) and growth patterns. We also observed that these cells had increased mitochondrial quantity and mass compared to parental cells via the use of Mitotracker Green (a mitochondria targeting dye), mitochondrial isolation, flow cytometry, and photo-spectroscopy. The cells were confirmed to be more radiation resistant via colony survival assay post-radiation treatment, compared to parental RM-1 cells. Our laboratory has recently found that FDA approved antibiotic, Azithromycin, has dual properties to increase levels of reactive oxygen species in the mitochondria and inhibit translation of mitochondrial proteins. Using the Seahorse Mito-Stress Test assay, we observed that Azithromycin inhibited the basal respiration, maximal respiration, and ATP-linked respiration of prostate cancer cells (PC3, RM-1). Azithromycin has also shown to alter mitochondrial fuel flexibility. The impairment of mitochondrial function is correlated with a decrease in cell viability of RM-1 cells and RR-RM-1 cells. This effect was even more compounded when used in conjunction with radiation therapy, and even under hypoxic conditions (1% O2), a significant obstacle to the efficacy of radiation in cancer treatment. To mimic the heterogeneity, cell interaction, and hypoxic gradients found in patient tumors, these radiation resistant prostate cancer cells (RR-PC3, RR-RM-1) and their respective parental PCa cell lines were 3D-Cultured. Using PrestoBlue and photo-spectrometry, we found that Azithromycin was effective at decreasing cell viability in both cell line spheroids. With the guidance of Markey Cancer Metabolism Core, our next step is to employ SIRM technique to investigate the effect of Azithromycin on mitochondrial metabolism under hypoxic condition 3D-culture. Overall, we surmise that we can use compounds, such as Azithromycin, to inhibit prostate cancer's use of mitochondria for treatment evasion and improve radiation treatment viability.

Abstract 105

What We Missed: Quantifying Undiagnosed Cancer Cases in the US during COVID-19, March-December 2020

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Purpose: The COVID-19 pandemic disrupted the normal course of cancer screening and detection in the U.S. A nationwide analysis of the extent of this disruption and any resulting disparities in underdiagnosis using cancer registry data has not been conducted.

Methods: We used time-series forecasting methods (seasonal ARIMA) to calculate expected cancer incidence rates for March-December 2020 from pre-pandemic trends (January 2018-February 2020). We measured the relative difference between observed and expected cancer incidence rates and numbers of potentially missed cases. We analyzed the impact on the total U.S. population and on subgroups by age, sex, race, urbanicity, and length of state-level stay-at-home orders. We also considered disruptions based on stage of diagnosis.

Results: Nationwide, observed rates of all sites cancer incidence were 28.6% (95% prediction interval [PI], 25.4%-31.7%) lower than expected during the height of shutdown (March-May 2020), 6.3% (95% PI, 3.8%-8.8%) lower in June-December 2020, and overall 13.0% (95% PI, 11.2%-14.9%) lower during the first 10 months of the pandemic, resulting in potentially 134, 395 (95% PI, 112, 544-156, 680) undiagnosed cancers. Prostate cancer accounted for the largest number of potentially missed cases (22, 950), followed by female breast cancer (16, 870) and lung cancer (16, 333). Screenable cancers saw a total rate reduction of 13.9% (95% PI, 12.2%-15.6%) versus expected. Rates of female breast cancer showed evidence of recovery to previous trends following the first three months of the pandemic, but levels remained suppressed for colorectal, cervical, and lung cancers. From March-May 2020, states with more restrictive COVID responses (stay-at-home orders ≥42 days) had significantly greater disruptions; yet, these differences were non-significant by December 2020 except for lung, kidney, and pancreatic cancer. Other disparities in disruption were observed based on age, sex, race and urbanicity.

Conclusions: A substantial disruption to cancer diagnoses occurred in the U.S. during the first 10 months of the COVID-19 pandemic. Our findings on the overall and differential impacts inform where the U.S. healthcare system should be looking to make up ground in cancer screening and detection.

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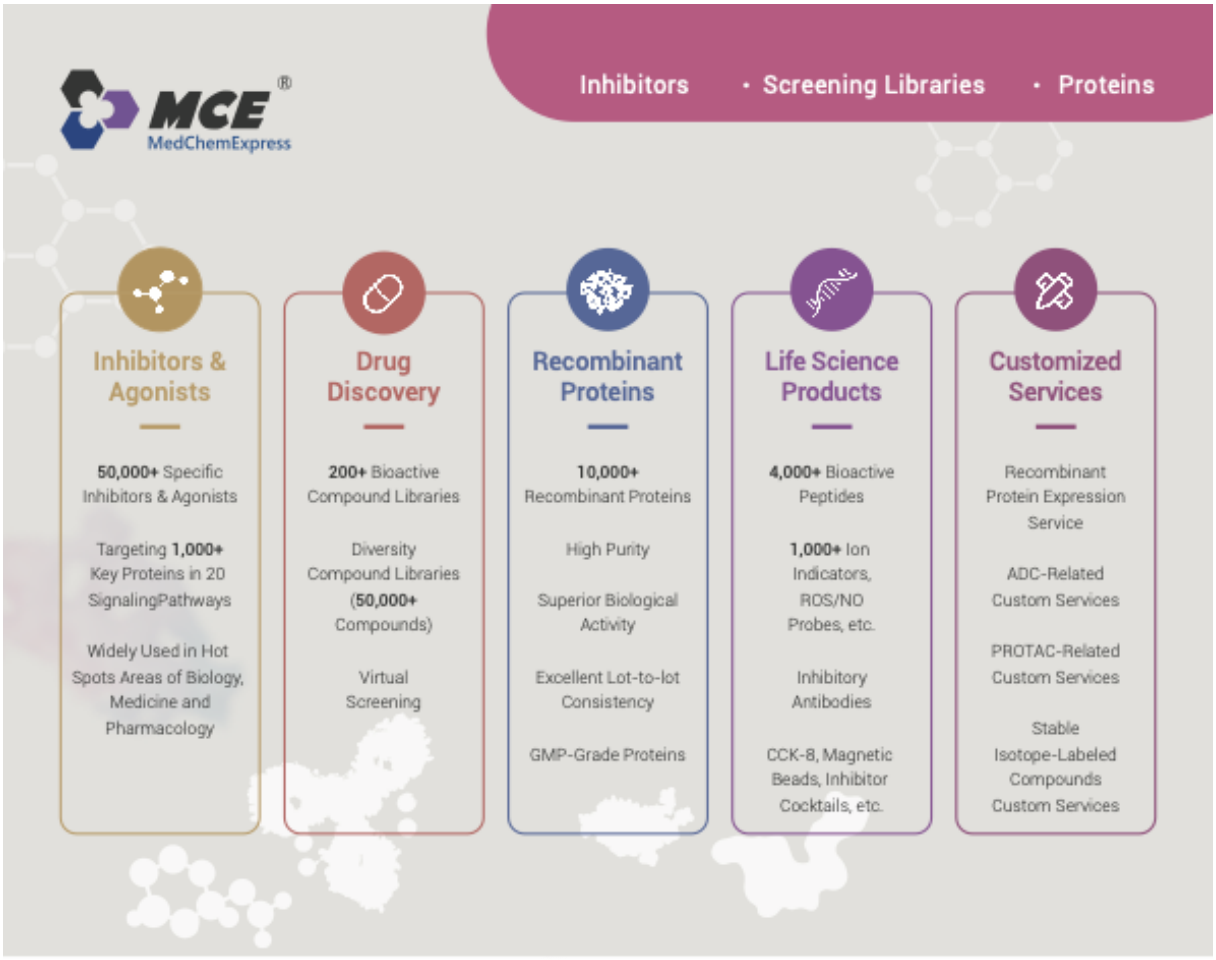

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