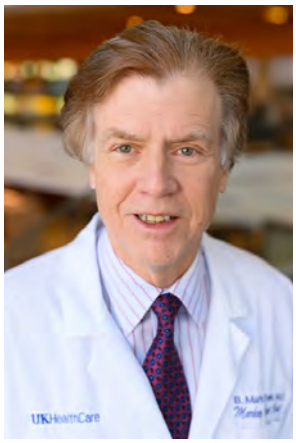

Markey
Cancer Center
An NCI Comprehensive Cancer Center
15TH
ANNUAL
MARKEY CANCER CENTER
RESEARCH DAY
PRESENTED BY THE MARKEY CANCER FOUNDATION
Discover the Latest Advances in Cancer Research
May 13, 2025 | 8 a.m. – 4 p.m.
UK Gatton Student Center

Markey Cancer Center
An NCI Comprehensive Cancer Center



May 13, 2025

Dear Colleagues and Friends,

Welcome to our 15th Annual Markey Cancer Center Research Day.

We gather at a time of both great promise and real challenge for cancer research. And yet, our work moves forward.

This day is a powerful reminder of why it must. It showcases the ingenuity, determination, and collaboration that define Markey. It reflects a simple truth: even in the midst of unknowns, progress continues—driven by the urgency of discovery and the needs of our Commonwealth.

Today's event features more than 100 posters spanning all areas of cancer research—from prevention and control to basic science, clinical care, and survivorship.

We are proud to highlight faculty presentations from two of Markey's exceptional scholars: Laurie McLouth, PhD, and Jin-Ming Yang, MD, PhD. The oral presentations will spotlight the work of four individuals selected for their outstanding abstracts, including the winner of our first-ever *4 Minutes for Cancer Research* competition.

We are also honored to welcome two distinguished keynote speakers. Monica Baskin, PhD—Deputy Director for Research at the Massey Comprehensive Cancer Center, and Professor and Associate Dean for Cancer Innovation at Virginia Commonwealth University—will deliver the Gilbert H. Friedell Memorial Lecture. Robert Vonderheide, MD, DPhil—Director of the Abramson Cancer Center, Vice Dean in the Perelman School of Medicine, and Vice President of Cancer Programs at the University of Pennsylvania—will present the Susan B. Lester Memorial Lecture.

Please join me in thanking our exhibitors, advertisers, and the Markey Cancer Foundation for their continued support in making this day a success.

As you explore the posters, presentations, and conversations that fill today's event, I encourage you to listen deeply, ask questions, and build new connections. The strength of our work lies not only in individual expertise, but in shared purpose. Let this day renew your sense of possibility and remind you that, even in uncertain times, we are never working alone.

Thank you for being here—for bringing your ideas, your energy, and your commitment.

Together, we are working for a cancer-free tomorrow.

Sincerely

B. Mark Evers

B. Mark Evers, MD

MARKEY CANCER CENTER
RESEARCH DAY

Program Contents

On the cover: Uptake of fatty acids induces mitophagy in colon cancer cells. PT130 cells treated with palmitic acid were stained with LAMP1 and COX4 antibodies to reveal the cellular localization of lysosomes (green) and mitochondria (red), respectively. The confocal image shows that fatty acid treatment enhances the interaction between mitochondria and lysosomes as demonstrated by colocalization of COX4 and LAMP1 and the engulfment of fragmentated mitochondria within lysosomal compartments (yellow). ***Credit:*** *Xiaopeng Xiong.*

Oral Presenters

Todd Burus

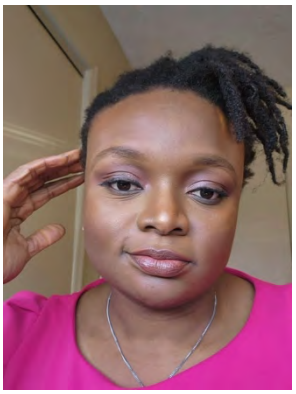
Targeting Lipid Metabolism to Improve Efficacy of BRAF-Targeted Therapy in Colorectal Cancer

Todd Burus is a data scientist in the Community Impact Office at Markey Cancer Center and a 3rd Year PhD candidate in the Epidemiology & Biostatistics program through the College of Public Health. His doctoral research has focused on developing and applying statistical methods in cancer surveillance research, with published works covering topics such as disruptions to cancer diagnoses during the COVID-19 pandemic, current and projected trends in oral tongue cancer, and disparities in geographic access to proton beam therapy. He is also the lead developer on Cancer InFocus—a comprehensive solution for gathering and visualizing publicly-available cancer surveillance data for geographic catchment areas. To date, the Cancer InFocus platform has been adopted by 45 other institutions, including over half of NCI-Designated Cancer Centers. Mr. Burus is a previous graduate of the University of Kentucky, holding bachelor's degrees in mathematics and linguistics, and master's degrees in mathematics and applied statistics.

JungHee Kang

Relationship among Nutrient, Metabolite, and Inflammatory Genotype and Phenotype as Risk Factors of Colorectal Cancer

JungHee Kang is an assistant professor in the College of Nursing at the University of Kentucky. Her research focuses on the role of diet and nutrition in relation to psychological factors, inflammatory mediators, social determinants of health, and sex differences in chronic diseases, such as cardiovascular disease and cancer. She is particularly interested in developing dietary interventions to prevent chronic disease, reduce hospitalization, improve survival, and enhance quality of life. Through training from NIH-supported programs such as the Summer Genetics Institute and the Building Interdisciplinary Research Careers in Women's Health (BIRCWH), along with anticipated microbiome training, she is expanding her work to include genetic, microbial, and sex-specific mechanisms in nutrition-related health outcomes. Dr. Kang earned her BSN from the University of Texas Medical Branch and both her MPH (with a focus in biostatistics) and PhD in Nursing from the University of Kentucky. She recently completed the Applied Nutrition and Culinary Medicine graduate certificate program. She is a member of the Cancer Prevention and Control (CP) Program at the Markey Cancer Center and an associate member of the BIRCWH program at the University of Kentucky.



Jovita Daraezinwa

4 Minutes for Cancer Research

Jovita Daraezinwa is a fifth-year doctoral candidate in Awuah Lab at the department of Chemistry at the University of Kentucky specializing in medicinal chemistry with emphasize on cancer research. Her research career thus far has been defined by a commitment to innovative drug development for cancer research, with a particular focus on overcoming drug resistance in cancer therapies by mechanistic targeting of upstream mediators of SHP2, a key regulatory protein in many cancers. Her core skills includes organic synthesis of small molecules including diazirine probes and peptidomimetics, Structure Activity Relationship(SAR) studies, Molecule docking and structure based drug design, biological testing of lead drug candidates up to mice studies. She is also passionate about science communication. She has won the National Organization for the Professional Advancement of Black Chemists and Chemical Engineers(NOBCCHE) 3 minutes run down research competition and also the University of Kentucky 3-minute thesis competition. In 2024, she represented the University of Kentucky at the regional 3 minutes thesis competition of all the southern universities in America emerging as one of the top contestants from over 58 universities. Her future endeavors hold the promise of making significant contributions to cancer research and science communication to broad audience.

Garrett Anspach

Liver-Specific Deletion of Carnitine Palmitoyltransferase 1a Promotes Tumorigenesis in a Mouse Model of Obesity-Driven Hepatocellular Carcinoma

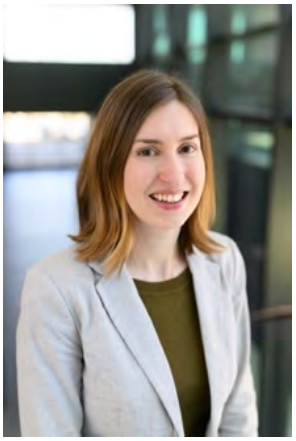
Garrett Anspach is a 2nd year MD/PhD student in Dr. Robert N. Helsley's laboratory within the College of Medicine's Department of Internal Medicine's Division of Endocrinology, Diabetes, and Metabolism at the University of Kentucky. He will enter the Department of Pharmacology and Nutritional Science to begin the Nutritional Science's PhD Program this summer. His research focuses on exploring the role of macronutrient metabolism in the development of hepatic and cardiometabolic disease pathology. More specifically, this includes establishing non-canonical roles of the enzyme Carnitine Palmitoyltransferase 1A, the rate-limiting enzyme of hepatic mitochondrial fatty-acid oxidation. His talk will highlight how hepatic absence leads to increased tumorigenesis of Metabolic Dysfunction-Associated Steatohepatitis (MASH)-driven Hepatocellular Carcinoma (HCC) in murine models, with correlations to human tissue gene expression and metabolite levels. By furthering our understanding of this process, he hopes to understand mechanisms for development and progression that can be targeted therapeutically for the treatment and prevention of HCC, MASH, and other cardiometabolic pathologies. Garrett received his undergraduate degree from the University of Kentucky's College of Health Sciences in Clinical Leadership and Management.

Jin-Ming Yang, MD, PhD

Professor, Toxicology and Cancer Biology
UK College of Medicine

NAC1, a Targetable Orchestrator of Tumor Stemness and Immune Suppression?

Jin-Ming Yang did his post-doctoral fellowship with Dr. William Hait at Yale University School of Medicine from 1989 to 1992. Then, he became a faculty member (assistant professor, associate professor, professor) at Rutgers University/Cancer Institute of New Jersey for 15 years before joining the Penn State University/ Penn State Cancer Institute as a professor in 2008. Dr. Yang's research focuses on the molecular mechanisms of cancer drug resistance. His laboratory applies a multidisciplinary approach to analyze the molecular basis of drug resistance, combining molecular biology, cell biology, and pharmacology tools with animal models and advanced *in vivo* imaging technologies. His work discovered a number of pathways and mechanisms that promote therapeutic resistance in cancer, delineated association between cancer metastasis and drug resistance, and identified several potential targets that modulate therapy resistance, cancer cell fate and tumor progression. Dr. Yang has published over 100 original articles in leading journals including *Cell*, *Cancer Research, PNAS* and *Journal of Clinical Investigation*. Dr. Yang's outstanding achievements have been well recognized, and his research work has long been supported by NIH/NCI, DOD, and other public and private funding agencies.



Laurie McLouth, PhD

Director, Patient-Oriented and Population Science Shared Resource
Markey Cancer Center
Assistant Professor, Department of Behavioral Science
UK College of Medicine

Applying the Science of Hope to Improve Patient and Caregiver-Reported Outcomes in Advanced Cancer

Dr. McLouth is an Assistant Professor of Behavioral Science in the UK College of Medicine, Director of Markey's Patient-Oriented and Population Science Shared Resource, and Director of Markey's Cancer Survivorship Research Initiative. A clinical psychologist by training, her research focuses on developing and testing psychosocial and cancer care delivery interventions to improve the quality of life of patients and their families from the point of cancer diagnosis on. In this presentation, Dr. McLouth will provide an overview of the psychology of hope, its evidence in cancer, the development and testing of a hope intervention for people living withadvanced lung cancer, and the promise of hope interventions for advanced cancer caregivers.

Gilbert H. Friedell, MD Memorial Lecture

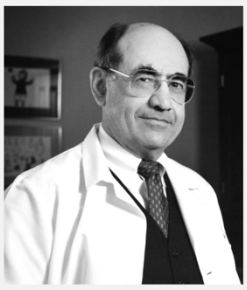
Monica L. Baskin, PhD

Professor and Associate Dean for Cancer Innovation, Virginia Commonwealth University School of Medicine
Deputy Director for Research, Massey Comprehensive Cancer Center

Leveraging Community-Engagement to End Cancer as We Know It

Monica L. Baskin, PhD is a Professor and Associate Dean for Cancer Innovation in the Virginia Commonwealth University School of Medicine and Deputy Director, Research at the Massey Comprehensive Cancer Center. She has previously served in senior leadership roles in other NCI-designated comprehensive cancer centers. Dr. Baskin received her Bachelor of Arts in psychology and sociology from Emory University, and a Master of Science in community counseling and Ph.D. in counseling psychology from Georgia State University. She is a licensed psychologist whose research focuses on advancing health equity related to cancer and other chronic diseases. Her research utilizes community-engaged methods to better understand and address individual, family, and environmental factors associated with the prevention and control of disease. For over two decades, her research program has been funded by the National Institutes of Health (NIH), Robert Wood Johnson Foundation (RWJF), and other regional and local foundations and government agencies. Dr. Baskin is a fellow of the Society of Behavioral Medicine and the Academy of Behavioral Medicine Research (ABMR). She is past president of the Society of Behavioral Medicine (SBM) and currently serves as Chair of the World Cancer Research Fund/American Institute for Cancer Continuous Update Project Global Expert Committee on Cancer Incidence.

About Gilbert H. Friedell, MD



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.

Susan B. Lester Memorial Lecture

Robert H. Vonderheide, MD, DPhil

Director, Abramson Cancer Center
Vice Dean of Cancer Programs
Perelman School of Medicine
Vice President for Cancer Programs
University of Pennsylvania Health Systems

Opportunities for Immune Health, Treatment, and Interception in Cancer

Robert H. Vonderheide, MD, DPhil, is Director of the Abramson Cancer Center, Vice Dean of Cancer Programs for the Perelman School of Medicine, and Vice President for Cancer Programs at the University of Pennsylvania Health System. Dr. Vonderheide is a distinguished laboratory scientist, clinician, and cancer leader who has deciphered mechanisms of cancer immune surveillance and developed novel cancer immunotherapies such as CD40 agonists with a particular focus on pancreatic cancer. He is an expert in the use of genetic mouse models of cancer and a leading architect of immunotherapy clinical trials to understand mechanisms and advance novel therapies. At Penn, Dr. Vonderheide has helped create Penn Medicine's Immune Health® program for immuno-profiling. Funded by the NCI, SU2C-Lustgarten Foundation, and the Parker Institute, Dr. Vonderheide has published senior author manuscripts in *Nature, Nature Medicine, Science*, *Lancet Oncology*, and the *New England Journal of Medicine*. Nationally, Dr. Vonderheide is an elected member of ASCI and AAP, serves on the NCI Board of Scientific Advisers, is a member of the boards of directors for American Association of Cancer Research, American Association of Cancer Institutes, and National Comprehensive Cancer Network.

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.

Susan B. Lester

Abstract List

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Basic Science

ABSTRACT 1

Basic Science

ABL1/2 Kinases Regulate ZEB1 Stabilization via NEDD4L and Enhance MAPK Inhibitor Resistance in Melanoma

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

[1]Pharmacology and Nutritional Sciences, [2]Biostatistics, [3]Markey Cancer Center, University of Kentucky

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. First line therapy for metastatic melanoma is immune-check point blockade (ICB). However, ICB is not effective for some patients, others cannot tolerate the adverse effects, and still others develop resistance to its effects. Constitutive activation of the MAPK pathway which occurs primarily via hyperactive mutations in *BRAF* (50% of cases) or *NRAS* (15-25% of cases), drives metastatic melanoma development. Second-line targeted therapy using BRAF/MEK inhibitors (BRAFi/MEKi) increases survival for *BRAF*-mutant metastatic melanoma patients; however, resistance inevitably develops. For patients with *NRAS*-mutant melanomas, no effective targeted therapy exists as BRAFi and MEKi are ineffective. Importantly, once resistance develops to any regimen, melanomas become highly aggressive and metastatic, and patients rapidly succumb to their disease.

Melanoma invasiveness and metastasis is driven by a shift in different transcriptional states characterized by downregulation of epithelial-mesenchymal transition (EMT) transcription factors (TFs), SNAI2 and ZEB2, and upregulation of TWIST1 and ZEB1. Previously, we showed that ABL kinases, (ABL1/2) are activated during MAPK inhibitor (MAPKi) resistance and drive proliferation, survival, and tumor growth. Here, we show that resistant cells are highly invasive, and silencing/inhibiting ABL1/2 reduces matrigel invasion, and prevents metastatic progression during resistance, *in vivo*. ZEB1 and N-cadherin (NCAD) levels are increased in resistant cells, whereas SNAI2, ZEB2, and in some cases E-cadherin are reduced. Silencing or inhibiting ABL1/2 with nilotinib or highly specific allosteric inhibitors reduces ZEB1 and NCAD expression, and coordinately induces SNAI2. Conversely, expression of constitutively active forms of ABL1/2 into parental melanoma cells induces ZEB1 and NCAD expression and represses SNAI2. Similar to effects with ABL inhibitors/siRNAs, transient/stable silencing of ZEB1 reduces clonogenicity in the presence of MAPK inhibitors and inhibits invasion. Interestingly, ABL1/2 inhibition dramatically induces SNAI2 mRNA but does not impact ZEB1/NCAD mRNAs but instead reduces their protein stabilities. MG-132 (proteasome inhibitor) treatment rescues nilotinib-mediated degradation of ZEB1 but not NCAD, indicating that ZEB1 is degraded by the proteasome whereas NCAD is likely regulated by the lysosome, as has previously been reported. Using the UbiBrowser database, NEDD4L was identified as a predicted ZEB1 E3 ligase. Silencing NEDD4L rescues the ability of nilotinib to reduce ZEB1 protein. We currently are determining whether ABL1/2 phosphorylation: a) inhibits the ability of NEDD4L to ubiquitinate ZEB1; and b) prevents NCAD endocytosis and lysosomal degradation. We also are investigating the mechanism by which ABL1/2 represses SNAI2 mRNA.

Importantly, the pathway we identified is clinically relevant as sample-wise GeneSet Enrichment Analysis demonstrates that ABL1/2 kinase activity (assessed using downstream target gene sets sourced from the IPA Ingenuity Knowledge Base) is positively correlated with ZEB1 transcriptional activity and SNAI2 mRNA levels in RNAseq from patient samples. In summary, our work identifies a new role for ABL1/2 in regulating the proteins that have critical roles not only in invasion and metastasis but also resistance to therapy.

ABSTRACT 2

Basic Science

An *In Vivo* Model for Studying Molecular Regulations of Neuroendocrine Lineage Plasticity in Prostate Cancer

Meng Wu

Toxicology and Cancer Biology, University of Kentucky

Prostate cancer (PCa) is prevalent among men in the United States, with neuroendocrine prostate cancer (NEPC) being a particularly aggressive subtype. NEPC may arise de novo or, more commonly, from existing prostate adenocarcinoma following the use of androgen deprivation therapy (ADT). NEPC are generally defined by the expression of neuroendocrine markers including synaptophysin (SYP+) and loss of androgen receptor (AR-) signaling. Lineage plasticity is a recognized hallmark of prostate cancer progression and can influence therapeutic outcomes. However, due to the lack of suitable *in vivo* NEPC animal models, the cellular and molecular mechanisms underlying lineage plasticity during PCa progression remain poorly understood.

In this study, we present an *in vivo* model designed to investigate the molecular determinants of neuroendocrine lineage transformation. Prostate tumors were developed by sub-renal capsule transplantation of primary mouse prostate cells acutely engineered with clinically relevant driver alterations ($Rb1^{-/-}$; $Trp53^{-/-}$; $cMyc^{+}$). Tumors in an androgen-rich environment (host mice with exogenous time-release testosterone pellets) developed as $AR^{+}SYP^{-}$ adenocarcinomas. Conversely, tumors in a no-androgen environment (castrated host mice) progressed to $AR^{-}SYP^{+}$ NEPC.

Immunohistochemical analysis revealed that castration-induced $AR^{-}SYP^{+}$ NEPC tumors contain two major subtypes: $ASCL1^{+}FOXA2^{+}NeuroD1^{-}BRN2^{-}$ and $ASCL1^{-}FOXA2^{-}NeuroD1^{+}BRN2^{+}$. Furthermore, forced expression of ASCL1 in primary mouse prostate cells was sufficient to induce $AR^{-}SYP^{+}$ NEPC tumors in an androgen-rich environment. Interestingly, we show this lineage transition requires a native *in vivo* microenvironment not replicated by conventional prostate organoid culture.

This model highlights the significance of therapies targeting lineage plasticity and provides a valuable platform for identifying additional drivers of lineage plasticity in prostate cancer.

ABSTRACT 3

Basic Science

c-Myc Lactylation Drives Prostate Cancer Progression by Influencing Gene Transcription

Mithu Howlader, Amos Akinyemi, Md Rakibul Alam, Jianlin Wang, Lixiang Gu, Zhiguo Li

Toxicology and Cancer Biology, University of Kentucky

Prostate cancer (PCa) is a leading cause of cancer-related morbidity and mortality, characterized by complex metabolic reprogramming that supports tumour growth and progression. Recently, lactate has emerged as a critical factor in PCa metabolism, produced via aerobic glycolysis and influenced by oncogene/tumour suppressor gene mutations, androgen signalling, and hypoxia. Glycolytic cancer cells and cancer-associated fibroblasts can secrete lactate, fostering "symbiotic" interactions with oxidative cancer cells through lactate shuttling. Lactate accumulation subsequently promoting the lactylation of proteins which is a novel post-translational modification involving proteins that is induced by lactate. The Warburg effect emphasizes the importance of lactylation in cancer biology, influencing gene transcription and supporting rapid tumour growth. Excessive lactate production and rapid lactate transport in cancer cells are mainly driven by the upregulation of c-Myc which is a master regulator of multiple biological programs. Its persistent activation results in abnormal expression of glycolytic enzymes and lactate transporters, contributing to PCa progression. While c-Myc undergoes various post-translational modifications, the potential for c-Myc lactylation and its role in PCa development remain unexplored. Preliminary data indicate that sodium lactate induces lactylation in PCa cells and that c-Myc is subject to this modification in androgen-independent cell lines. The lactylation of c-Myc not only promotes tumour growth but also contributes to the metabolic flexibility of PCa, enabling them to thrive in hypoxic and nutrient-deprived environments. In this study, we aim to investigate the role of lactate-induced lactylation of c-Myc in androgen-independent PCa progression. Specifically, we will identify lactylation sites on c-Myc and explore their impact on PCa progression. The findings could lead to the development of novel therapies targeting the lactate-c-Myc signalling axis, overcoming resistance to ADT, and improving treatment outcomes in advanced PCa. Additionally, this research could have broader implications for other cancers characterized by metabolic alterations, as lactylation represents a fundamental link between cellular metabolism and protein function. Through advanced models, including genetically modified mouse models, we aim to provide valuable insights into how lactate-mediated modifications influence tumour growth and metastasis. Ultimately, this work has the potential to inform the development of personalized diagnostic and therapeutic strategies in PCa and other malignancies. Understanding the molecular mechanisms underlying c-Myc lactylation provides new insights into PCa biology and opens potential avenues for therapeutic intervention targeting metabolic pathways.

ABSTRACT 4

Basic Science

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

Jianlin Wang[1,2], Xinyi Wang[1,2] Jia Peng[1,2], Jinghui Liu[1,2], Xiongjian Rao[1,2], Min Zhang[1,2], Md Rakibul Alam[1,2], Daheng He[2], Yifan Kong[1,2], Tadahide Izumi[1], Chi Wang[2], Zhiguo Li[1,2]*, Xiaoqi Liu[1,2]*

[1]Toxicology and Cancer Biology, [2]Markey Cancer Center, University of Kentucky

Radiation therapy (RT) or radiotherapy is used to treat up to 50% of cancer patients and manage 40% of patients who are cured. Accordingly, strategies for improving radiotherapy with the aim of increasing the killing of tumor cells and decreasing damage to surrounding normal tissues are urgently needed. Growing evidence, including our previous study, indicates that PLK1 overexpression suppresses the DNA damage response (DDR) pathway and enhances sensitivity to ionizing radiation. Here, we identify ASF1A as a novel PLK1-interacting protein. PLK1 directly phosphorylates ASF1A at S166, a process enhanced by CDK1-mediated phosphorylation at S16, which facilitates PLK1 recruitment to ASF1A. Notably, CDK1/PLK1-dependent phosphorylation promotes ASF1A degradation in a proteasome-dependent manner via FBW7 recognition. Furthermore, CDK1/PLK1-mediated ASF1A phosphorylation weakens the DNA damage checkpoint and impairs DNA repair. Cells and tumors expressing phosphomimetic ASF1A exhibit greater sensitivity to radiation than those expressing wild-type ASF1A, suggesting that patients with elevated CDK1/PLK1 expression may benefit from radiotherapy and other DNA damage-induced treatments. Finally, activation of the CDK1/PLK1/ASF1A pathway with microtubule inhibitors enhances the efficacy of subsequent radiotherapy, supporting a rationale for the sequential administration of docetaxel and radiation in cancer treatment. These findings provide a mechanism for PLK1-induced suppression of the DDR pathway and chromosomal instability, highlighting potential therapeutic opportunities for improving the efficacy of radiotherapy and other DNA damage-induced treatments in cancer therapy.

ABSTRACT 5

Basic Science

Chronic Perfluorooctanesulfonic Acid Exposure Promotes Proliferation of Colorectal Cancer Cells

Jerika Durham[1], Josiane Weber Tessmann[1], Bernhard Hennig[2], Yekaterina Y. Zaytseva[1]

[1]Toxicology and Cancer Biology, [2]Animal Sciences, University of Kentucky

Perfluorooctanesulfonic acid (PFOS) is a “forever chemical” frequently detected in drinking water leading to its high absorption through the gastrointestinal tract. Recent studies demonstrate that PFOS exposures promote intestinal inflammation and gut barrier dysfunction. However, how a long-term PFOS exposure affects colorectal cancer (CRC) progression remains elusive. Therefore, the purpose of this study is to delineate the effect of PFOS exposure on CRC cell proliferation and to test the potential mitigation strategy for the harmful effects of PFOS. SW480 and HCT116 cells have been treated with 1 ug/mL PFOS for 1 month, 2 months, and 3 months followed by a Presto Blue Cell Viability Reagent fluorescence assay to measure proliferation. Proliferation markers were assessed by qPCR and Western blot and a colony formation assay was performed. We show that chronic, low-dose PFOS exposure promotes proliferation of SW480 and HCT116 cell lines starting at 3 months since the first exposure. The increase in proliferation is associated with upregulation of Cyclin D and reduction of DEFA5. We also show that PFOS promotes alterations in lipid metabolism via overexpression of FASN and CD36. Our studies suggest that chronic PFOS exposure promotes proliferation of CRC cells by increasing pro-carcinogenic gene expression and signaling and by reducing expression of DEFA5. Furthermore, our findings suggest that PFOS promotes alterations in lipid metabolism associated with CRC progression and worse disease prognosis. Our studies warrant further investigation of the mechanisms behind the effect of PFOS exposure on cancer progression.

ABSTRACT 6

Basic Science

Disruption of Mitochondrial Dynamics Alters DNA Repair Capacity in Colon Cancer

Marion D Creech III[1], Tianyan Gao[1,2]

[1]Molecular and Cellular Biochemistry, [2]Markey Cancer Center, University of Kentucky

Mitochondrial dynamics refers to a collection of mitochondrial movements, including fission, fusion, and transport. Cancer cells are known to adjust mitochondrial dynamics to provide the metabolic plasticity needed for cell growth, proliferation and migration. We have shown previously that Drp1, a key regulator of mitochondrial fission, plays an important role in mediating fatty acid-induced activation of Wnt/-catenin signaling in colon cancer. In this study, we determined the functional interaction between mitochondrial dynamics and DNA damage response in colon cancer cells. To disrupt mitochondrial fission, inducible Drp1 knockdown colon cancer cell lines were generated using lentivirus-mediated RNAi. Control and Drp1 knockdown cells were treated with radiation or chemotherapy drug irinotecan to induce DNA damage response. The extent of DNA damage as well as the activation of DNA repair signaling were analyzed by western blot and RT-qPCR. We found that Drp1 was activated upon irinotecan or radiation treatment as shown by increased phosphorylation at S616 site. Interestingly, the expression of γH2AX, a DNA damage marker, induced by irinotecan or radiation treatment was decreased in Drp1 knockdown cells, whereas the expression and activation of DNA damage sensing proteins (e.g., ATM and ATR) remain unchanged. Moreover, we showed that irinotecan-induced γH2AX expression was potentiated in cells cultured in fatty acid-enriched media; however, this increase in DNA damage was attenuated in Drp1 knockdown cells. Functionally, silencing Drp1 rendered colon cancer cells resistant to irinotecan-induced apoptosis. Taken together, our results establish a possible link between mitochondrial fission and DNA damage response in colon cancer cells.

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

Subin Kim[1], Allisa Pack[1], Nathan L. Vanderford[1,2], Courtney M. Townsend, Jr.[4], B. Mark Evers[1,3], Piotr Rychahou[1,3]

[1]Markey Cancer Center, [2]Toxicology and Cancer Biology, [3]Surgery, University of Kentucky; [4]Surgery, University of Texas Medical Branch

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 inhibitor doxorubicin. DNA-PK activity was inhibited with peposertib. Clonogenic and proliferation assays were used to evaluate the efficacy of peposertib in combination with 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 and with western blot using cleaved PARP bands. To further validate the role of DNA-PK in the response to treatments, DNA-PK knockdown cell lines were established, and confocal microscopy and western blotting were used to assess DNA damage and apoptosis. Data was analyzed using GraphPad Prism and expressed as a mean ± standard deviation.

Results. DNA-PK inhibition resulted in enhancement of doxorubicin cytotoxic therapy (BON IC50 DOX = 194.73± 26.52 nM, DOX+Pep = 89.87± 17.6 nM; QGP IC50 DOX = 108.37± 23.97 nM, DOX+Pepo = 30.27± 4.80 nM) in both BON and QGP-1 cell lines. Immunofluorescence analysis detected γH2AX foci increase in the doxorubicin and peposertib combination groups. DNA-PK inhibition in combination with doxorubicin treatment significantly increased caspase-3 cleavage and PARP cleavage in BON and QGP-1 cell lines. Similar trends in DNA damage and apoptosis were observed across both drug combination and knockdown groups.

Conclusions. High DNA repair activity is commonly associated with cancer drug resistance. The combination of DNA-PK inhibition with DNA-damaging therapies impaired 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.

NOTE: ACTION R25 CA221765

ABSTRACT 8

Basic Science

Dual Role of Neurotensin in Intestinal Injury: Promoting Inflammation during Damage and Facilitating Repair during Recovery

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Acute intestinal injury poses a significant clinical challenge, driven by complex mechanisms that govern both tissue damage and subsequent recovery. Neurotensin (NT), a neuropeptide involved in gastrointestinal homeostasis, has been associated with inflammatory responses and tissue repair. Dextran sulfate sodium (DSS) is a widely used chemical agent for inducing acute intestinal injury in experimental colitis models, mimicking key features of human colonic inflammation; however, its precise role during both the injury and recovery phases remains unclear. This study aimed to elucidate the role of NT in acute intestinal injury and recovery, focusing on its effects on inflammation, tissue damage, and repair.

Methods: To investigate the role of NT in acute intestinal injury and recovery, 10-wk-old NT wild-type (NTWT) and NT knockout (NTKO) mice were divided into four groups: NTWT-Normal water (control), NTWT-DSS, NTKO-Normal water, and NTKO-DSS. (i) In the first cohort, an acute colonic injury model was induced by administering 3% DSS in drinking water for 7 d to the DSS groups, while the control groups received ordinary drinking water. Body weight was monitored daily to assess the onset and degree of weight loss. After 7 d, colonic length was measured, and tissues were collected for histological analysis. (ii) In the second cohort, a recovery model was established by treating DSS groups with 3% DSS for 7 d, followed by normal drinking water for 5 d to allow recovery. Body weight was tracked throughout the recovery phase, colonic length was reassessed, and tissues were collected. (iii) Histological analysis using hematoxylin and eosin (H&E) staining was performed for both cohorts to assess submucosal inflammation, tissue damage, and recovery.

Results: (i) During DSS treatment, NTWT mice exhibited earlier and more severe weight loss compared to NTKO mice. By day 3 of DSS administration, NTWT mice began losing weight, whereas NTKO mice showed significant weight loss onset only by day 5. (ii) Colonic length measurements revealed significant differences between the groups. After 7 d of DSS treatment, NTWT mice showed shorter colonic lengths (mean 6.03 cm) than NTKO mice (mean 6.57 cm, $P < 0.05$). Following 5 d of recovery, NTWT mice demonstrated greater improvement in colonic length (mean 7.88 cm) compared to NTKO mice (mean 7.6 cm). (iii) Histological analysis showed that NTWT mice experienced more severe colonic tissue damage during DSS treatment compared to NTKO mice. However, during recovery, NTWT mice exhibited a more pronounced tissue repair, as evidenced by improved histological scores and reduced inflammation.

Conclusions: Our findings suggest that the presence of NT results in distinct effects during different phases of acute intestinal injury. While NT exacerbates inflammation and tissue damage during the injury phase, it promotes tissue repair and accelerates recovery during the healing phase. These findings suggest that targeted modulation of NT signaling could provide a novel therapeutic strategy for managing intestinal injury.

ABSTRACT 9

Basic Science

Epithelial-to-mesenchymal transition coordinates REEP2 to drive pro-metastatic membrane trafficking program in lung cancer

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Membrane trafficking governs the transport of molecules to both intracellular and extracellular locations, thereby maintaining cell homeostasis. During cancer progression, alterations in membrane trafficking are frequently observed. Nevertheless, no effective drugs target membrane trafficking currently and the many mechanisms underlying the dysregulation of membrane trafficking in cancer progression remain a significant unresolved question. Our recent research discovered that the epithelial-to-mesenchymal transition (EMT) employs a membrane trafficking program to coordinate cancer cell invasion and immunosuppression in the tumor microenvironment (TME) in lung adenocarcinoma (LUAD). Importantly, blocking the EMT-driven membrane trafficking program re-sensitizes LUAD to immune checkpoint inhibition, suggesting significant therapeutic potential for this pathway. To dissect the pro-metastatic membrane trafficking program, we initiated a CRISPRi *in vivo* screen to assess more than 2,000 trafficking-related genes in syngeneic mouse LUAD models for their requirements for tumorigenesis. Our preliminary studies identified REEP2 as a novel regulator of the EMT-dependent membrane trafficking program, which is linked to poor prognosis and an immunosuppressive TME in LUAD patients. Our findings here show that the EMT-activating transcription factor ZEB1 upregulates REEP2 by relieving its silencing mediated by miR-193a, thereby facilitating endoplasmic reticulum (ER) polarization to the Golgi apparatus. The REEP2-driven ER polarization promotes pro-metastatic secretory trafficking. Blockade of REEP2 suppresses LUAD cell proliferation, migration, invasion *in vitro*, and tumor metastasis in the syngeneic LUAD mouse tumor model. Our findings elucidate the molecular mechanisms underlying LUAD metastasis and support the development of REEP2 inhibitors to prevent EMT-driven cancer metastasis.

Keywords: EMT, LUAD, REEP2, ER, secretory trafficking

Establishing a Zebrafish Patient-Derived Xenograft Model for Pediatric Sarcomas

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Rhabdomyosarcoma (RMS) is a high-risk pediatric sarcoma with poor prognosis, especially in metastatic or recurrent cases. Patient-derived xenograft (PDX) models in zebrafish offer rapid and cost-effective alternatives for preclinical drug screening. This study evaluates the feasibility of RMS xenografts in zebrafish larvae and assesses chemotherapy responses in vitro at 34°C, a temperature tolerated by zebrafish, before transitioning to in vivo testing. To establish the model, xenotransplantation methods were optimized using cultured RD cells, identifying the dorsal perivitelline space (PVS) as optimal due to its avascular nature, improved tumor retention, and injection ease. RD cells injected into zebrafish larvae at 48 hours post-fertilization demonstrated an engraftment rate of 61% at 24 hours post-injection, with larvae viable for up to four days post-injection. Chemotherapeutic agents tested included dactinomycin (DAC), vincristine (VIN), and cyclophosphamide (CYC) at 34°C compared to standard 37°C conditions. DAC showed a dose-response curve with IC_{50} values of 4.3 nM (37°C) and 3.5 nM (34°C), though with greater variability at lower temperatures. VIN displayed considerable variability between temperatures, whereas CYC showed no cytotoxic effects at any tested concentration. Zebrafish toxicity assays confirmed resistance to CYC and significant lethality with DAC and VIN only above 300 nM. These results support zebrafish RMS xenografts as a functional in vivo model. Future studies will expand this approach using patient-derived primary tumor cells, assessing drug responsiveness in vivo to enhance the clinical relevance of this preclinical testing model.

Evaluation of ICAM-1 Overexpressing Macrophage Engineered Vesicles for Treatment of High Grade Serous Ovarian Cancer

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The National Cancer Institute estimates that 19,680 patients received a new diagnosis of ovarian cancer in 2024. Ovarian cancer is frequently diagnosed at an advanced stage and has poor survival outcomes. 5-year survival is estimated at a rate of 50.9% from 2014-2020. There is a need for further development of novel therapies for treatment of recurrent ovarian cancer. Tumor Associated Macrophages (TAMs) are macrophages associated with tumor tissue and occur in both M1 and M2 phenotypes. Macrophage engineered vesicles (MEVs) have been shown to be able to repolarize M2 macrophages into an M1 state in vitro. When co-cultured with ovarian cancer cells, application of the MEVs resulted in reduced cancer cell viability. Further work is needed to evaluate the effect of MEV treatment on ovarian cancer cells and to optimize MEVs to deliver the most effective treatment. ICAM-1 is a transmembrane glycoprotein that mediates immune responses. ICAM-1 deficiency has been shown to increase M2 macrophage polarization during tumor progression. Thus, we proposed developing MEVs from macrophages overexpressing ICAM-1 and evaluating the response to treatment with these vesicles *in vitro*.

Methods. ICAM-1 overexpression was induced in Raw 264.7 murine tumor associated macrophage cells in our lab via plasmid transfection. Prior to production of MEVs, macrophages were repolarized to M1 state using 20ng/mL LPS and IFN-gamma. Macrophages were also repolarized to M2 state using 20ng/mL IL4 for characterization. MEVs were produced from both Raw cell lines via double nitrogen cavitation followed multiple centrifugations for purification. MEVs were characterized by Nanoparticle Tracking Analysis after resuspension.

Results. Western Blot analysis of lysates from Raw 264.7 and Raw 264.7 cells in the M0, M1, and M2 states shows that M1 polarized macrophages produce higher levels of ICAM-1 than those in the M0 or M2 states. Across all states of polarization, the Raw 264.7-ICAM-1 cells produced higher levels of ICAM-1 than the parent Raw 264.7 cells, indicating successful transfection. Next, MEVs created from M1 polarized Raw 264.7 and Raw 264.7-ICAM-1 cells were analyzed via Western Blot. This showed that ICAM-1 remains present at a higher level after MEV production for the MEVs produced from ICAM-1 overexpressing cells. Media from M2 Raw cells treated with ICAM-1 overexpressing MEVs was collected at 24 and 48 hours post treatment and analyzed via ELISA for production of TNF-alpha, a marker of M1 macrophage conversion. Treatment with ICAM-1 overexpressing M1 MEVs resulted in a 150-fold increase in TNF-alpha production over untreated Raw cells at 24 hours. Raw cells treated with ICAM-1 overexpressing M0 MEVs produced a more modest 24-fold increase in TNF-alpha production.

Conclusions. Raw cells overexpress ICAM-1 after transfection and this overexpression holds after MEV production. This shows that we can create MEVs that overexpress important targeting proteins. Conversion to M1 phenotype is not inhibited by the presence of overexpressed ICAM in the MEVs. This may increase repolarization but further study needs to be completed to determine the extent of this effect. Further *in vitro* study is needed to determine the effect of the ICAM-1 overexpressing MEVs on the viability of ovarian cancer cells both *in vitro* and *in vivo*. We plan to continue to characterize the effects of MEVs produced from ICAM-1 overexpressing Raw 264.7 cells. We will further investigate their effects *in vitro* using different cell culture assays to determine any effect and difference from MEVs produced from the parent Raw 264.7 cells. Further steps after this include *in vivo* treatment of mice intraperitoneal ovarian cancer xenografts and in a syngeneic murine ovarian cancer model.

ABSTRACT 12

Basic Science

Expression of PGK1 in Breast Cancers Alters Their Sensitivity to Ferroptosis Induction via Metabolic Reprogramming

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Therapeutic resistance and recurrence are among the major contributors to poor outcome for patients with breast cancer. Induction of ferroptosis, a form of cellular death characterized by toxic lipid peroxide overload, has emerged as a promising therapeutic strategy against breast cancers including triple-negative breast cancer (TNBC). Nevertheless, certain types of cancer are impervious to induction of ferroptosis and the underlying mechanisms remain incompletely clear. In this study, we show that phosphoglycerate kinase 1 (PGK1), an important enzyme in glycolysis, is highly expressed in breast tumors, and the elevated levels of PKG expression correlate with advanced tumor stages, poor prognosis and ferroptosis insensitivity, particularly in TNBCs. Using genetic or pharmacological inhibition, we demonstrate that knockdown or inhibition of PGK1 enhances ferroptosis sensitivity in both TNBC and luminal breast cancer cell lines. We further demonstrate that depletion of PGK1 destabilizes glutathione peroxidase 4 (GPX4), an anti-ferroptotic defense peroxidase, thereby disturbing cellular redox homeostasis and promoting lipid peroxidation. Moreover, targeting PGK1 disrupts glycolytic metabolism and sensitizes breast cancer cells to ferroptosis induction in tumor cells subjected to glucose deprivation or treated with glycolytic inhibitors. In orthotopic TNBC models, loss of tumoral PGK1 augments the action of the ferroptosis inducer, imidazole ketone erastin (IKE), in inhibiting tumor growth and metastasis, and enhances CD8+ T cell-mediated anti-tumor immunity. These results indicate that PGK1 has a critical role in modulating breast cancer invulnerability to induction of ferroptosis, implying that this kinase may be exploited as a therapeutic target to sensitize breast cancers, especially, TNBC, to ferroptosis inducers.

Extracellular Vesicles from Irradiated Glioblastoma Alter Astrocytic Ca^{2+} Dynamics and Metabolic Activity

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Background: Glioblastoma (GBM) is an aggressive brain tumor associated with therapy-induced cognitive impairment, significantly impacting the quality of life in survivors. Extracellular vesicles (EVs), lipid bilayer-bound particles released by cells, reflect the physiological and pathological status of their cells of origin. Radiation therapy (RT) has been shown to increase the release of EVs, which may contribute to neurotoxicity. Astrocytes, the most abundant glial cells in the central nervous system, are essential for neuronal support and rely on tightly regulated intracellular calcium (Ca^{2+}) signaling for homeostatic functions. Dysregulation of astrocytic Ca^{2+} dynamics by GBM-derived EVs may disrupt the brain microenvironment, impair neuronal function, and promote tumor progression. This study investigates how GBM-derived EVs influence astrocytic Ca^{2+} signaling, mitochondrial respiration, and glycolytic activity.

Methods: EVs were isolated from irradiated GBM cell lines (GL261 and LN18) using membrane affinity spin columns. Particle concentration and size were quantified by nanoparticle tracking analysis. Mitochondrial respiration and glycolytic function of normal human astrocytes (NHA) were assessed using Seahorse XF analysis. In vivo astrocyte Ca^{2+} dynamics were visualized using two-photon microscopy in awake mice following cranial window implantation and AAV5-Gfa104-lck-GCaMP6f transduction in the somatosensory cortex. Baseline Ca^{2+} transients were recorded after whisker stimulation, followed by orbital intravenous administration of GBM-EVs ($2x10^9$) and re-imaging at 0 and 1-hour post-EV exposure. ImageJ and GraphPad Prism were used for calcium transient analysis and statistical comparisons. EV brain delivery was confirmed by intranasal administration of NIR-labeled EVs and IVIS imaging.

Results: GBM-derived EVs reduced mitochondrial respiration and glycolysis in NHA cells in a dose-dependent manner and induced >50% cell death at higher concentrations ($2.5x10^8$). In vivo, GBM-EVs significantly increased the amplitude of astrocyte Ca^{2+} transients, reduced rise time, and prolonged decay time, indicating acute dysregulation of Ca^{2+} signaling.

Conclusion: GBM-derived EVs impair astrocyte metabolic function and disrupt calcium signaling dynamics, potentially contributing to a tumor-supportive and neurotoxic microenvironment. Future studies will explore the downstream impact of EV-activated astrocytes on neuronal viability and GBM cell proliferation using co-culture systems.

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Glycogen Metabolism is Critical for Cancer-Associated Fibroblasts Proliferation and Immunosuppressive Activity in Colorectal Cancer

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Metabolic reprogramming is a characteristic feature of both cancer cells and their neighboring cells in the immunosuppressive tumor microenvironment (TME). Cancer-associated fibroblasts (CAFs) are the major sources of secreted cytokines contributing to the immunosuppressive activity in the TME. Dysregulation of glycogen metabolism is integral to cancer cell proliferation and metastasis. The purpose of this study was to identify whether glycogen metabolism is important for CAF proliferation and cytokine production.

Methods: CAFs were isolated from colorectal cancer (CRC) patient samples and cultured in conditioned medium (CM) derived from HCT116 human CRC cells. The expression of glycogen phosphorylase liver form (PYGL), glycogen phosphorylase brain form (PYGB), hexokinase 2 (HK2), lactate dehydrogenase A (LDHA), IL6, CLCF1, LIF, and CXCL6 was determined by either real time (RT)-PCR or western blot. Glycogen levels were determined using a Glycogen Analysis Kit. Cell proliferation was determined using WST-1.

Results: (**i**) Data set analysis identified an elevated glucose metabolism in CAFs compared to normal fibroblasts. (**ii**) Treatment of CAFs with CM derived from HCT116 cells resulted in activated glycogen metabolism as noted by elevated protein expression of glycogen synthase, PYGL, PYGB, and the reduced level of glycogen in CAFs. In addition, CM increased glucose metabolism in CAFs as noted by significantly increased expression of HK2 and LDHA. Moreover, CM increased the expression of IL6 family cytokines such as IL6, CLCF1, LIF and CXCL6 in CAFs. (**iii**) Inhibition of glycogen metabolism by treatment with the PYG inhibitor, CP-91149, significantly repressed CAF proliferation and reduced the expression of these cytokines. Consistently, knockdown of PYGB or PYGL significantly decreased the level of these cytokines in CAFs. (**iv**) Inhibition of glycogen synthase by treatment with the glycogen synthase inhibitor, MZ-101, or knockdown of glycogen synthase 1 decreased the expression of these cytokines in CAFs.

Conclusion: Our results demonstrate that glycogen metabolism is crucial for promoting CAF proliferation and cytokine production. Importantly, our findings suggest that targeting glycogen metabolism in CAFs reshapes the immunosuppressive TME and may enhance the efficacy of immunotherapy for CRC.

Glycosaminoglycan Modification of NRP1 Exon 4-Skipping Variant Drives Colorectal Cancer Metastasis via Endosomal-Exosomal Trafficking

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Metastasis is the leading cause of colorectal cancer (CRC)-related deaths, underscoring the urgent need to identify critical drivers of CRC metastasis as therapeutic targets. Neuropilin-1 (NRP1), a transmembrane glycoprotein, functions as a co-receptor for multiple growth factors and plays diverse roles in cancer progression and metastasis. We recently discovered a novel human NRP1 splice variant, NRP1-ΔE4, which results from exon 4 skipping. This variant is frequently expressed in CRC, strongly correlates with disease progression, and drives metastasis more potently than wild-type NRP1. Here, we demonstrate the critical role of glycosaminoglycan (GAG) modification in regulating NRP1-ΔE4's cellular trafficking and oncogenic activity. NRP1-ΔE4 undergoes constitutive internalization into endosomes, followed by exosomal release from CRC cells. Exosomal NRP1-ΔE4 enhances the migration and invasion of both donor and recipient CRC cells. Genetic or pharmacological inhibition of exosome secretion, or immunodepletion of exosomal NRP1-ΔE4, markedly reduces its metastatic potential. Notably, GAG modification at the O-glycosylation site Ser612 is essential for NRP1-ΔE4's endosomal trafficking and exosomal release. This modification also promotes the formation of a trimeric complex with Met and β1-integrin, leading to their co-internalization and accumulation in endosomes, which activates FAK signaling and drives CRC metastasis. These findings reveal GAG modification as a key regulatory process that governs the endosomal-exosomal trafficking of NRP1-ΔE4 to facilitate CRC cell dissemination.

Hepatic Aster-C Deficiency Protects Against Diet-induced Hepatic Steatosis in Mice

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Objective: Aster family of proteins (Aster-A, -B, -C), which are involved in non-vesicular cholesterol transport from the plasma membrane (PM) to the endoplasmic reticulum (ER). Disruption of Aster protein function impairs PM-to-ER cholesterol transport in the liver, ovary and adrenal gland. Notably, Aster-C is expressed selectively in hepatocytes, the primary cell type responsible for lipid accumulation in metabolic dysfunction-associated steatotic liver disease (MASLD). In this study, we investigated the effects of Aster-C on lipid accumulation in hepatocytes in a mouse model of diet-induced hepatic steatosis.

Approach and Result: AAV8-Cre and AAV8-Control were injected in Aster-C$^{Flox/Flox}$ (F/F) mice, then fed 3 weeks of Western diet. Result shows that hepatic Aster-C gene expression was significantly abolished in AAV-Cre injected mice. Perform liver lipid assay, we found that liver cholesterol and triglyceride levels were significantly decreased in AAV-Cre injected mice compared to AAV-Control group (liver cholesterol, 81.86 ± 4.0 vs. 177.38 ± 24.1 μg/mg, P<0.01; liver triglyceride, 149.18 ± 1.9 vs. 292.28 ± 32.6, P<0.01). We also generated Aster-C hepatocytes specific knockout mice (LKO) by crossing Aster-C$^{Flox/Flox}$ mice with albumin-Cre mice. There is no significant difference on the levels of liver cholesterol and triglyceride when mice were fed Chow diet (LKO vs. F/F: liver cholesterol, 12.21 ± 1.6 vs. 10.96 ± 1.4 μg/mg, P=0.562; liver triglyceride, 386.06 ± 21.8 vs. 432.48 ± 36.6 μg/mg, P=0.301). However, with 10 weeks of Western diet feeding, hepatic Aster-C deficient mice show significant decrease in liver cholesterol (31.7 ± 4.1 vs. 70.4 ± 10.1 μg/mg, P<0.01) and triglyceride (1407.0 ± 433.7 vs. 2913.2 ± 404.2 μg/mg, P<0.05) compared to control mice. Liver histology images show less lipid drops and macrophages accumulation in hepatic Aster-C deficient mice. What's more RNA sequencing result show that ablation of Aster-C improves nonalcoholic steatohepatitis (NASH) relative gene expression in liver.

Conclusion: Loss of Aster-C in hepatocytes protects against steatosis by reducing hepatic cholesterol and triglyceride accumulation, ameliorating liver damage, and decreasing expression of inflammatory genes following Western diet feeding.

Identification and Characterization of NGFR Positive Cells in Human Neuroma Biospecimens

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The low affinity NGF receptor or NGFR, also known as p75NTR, is a ubiquitous membrane marker of peripheral nervous system (PNS) Schwann cells from spinal nerves, nerve roots, and ganglia. Recently, we reported that additional populations of NGFR positive cells distinct from Schwann cells, the main PNS-resident glial cells, are present in the epineurium, perineurium, and endoneurium of normal human nerves from donors of various ages. Here, we extended our immunohistochemical image analysis of PNS biospecimens with the goal to reveal the identity and localization of NGFR positive cells in peripheral nerve tumors. Our detailed immunohistochemical analysis of formalin-fixed paraffin-embedded samples uncovered the existence of at least three novel populations of NGFR positive cells in the stroma and perivascular areas of the endo-, peri- and epineurium of all neuroma and neurofibroma samples analyzed so far. Of particular importance were the abundant NGFR positive cells found in association with the hyperplastic perineurial layers of human neuromas. These perineurial NGFR positive cells expressed high levels of Glut1, a classical perineurial cell marker. However, they lacked the expression of S100β, a typical Schwann cell marker, SMA, a pericyte marker, and CD34, a fibroblast marker. To conclude, we provide immunohistochemical evidence supporting the widespread occurrence of hyperplastic NGFR positive cells in human nerve tumors primarily within the connective tissue layers. Further studies are needed to understand their possible roles in tumor initiation, progression, and maintenance.

Keywords: NGFR, Schwann cells, fibroblasts, neuromas, neurofibromas, IHC, markers

Abbreviations: Endo: Endoneurium; Epi: Epineurium; Peri: Perineurium; PNS: Peripheral Nervous System

Impact of NTS/NTSR1 Signaling and High-Fat Diet on the Tumor Immune Microenvironment in Colorectal Cancer

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Objective: Colorectal cancer (CRC) presents significant challenges in the Appalachian region (APPKy) due to high rates of obesity driven by long-term high-fat, high-sugar diets and limited healthcare access. Clinical data suggest that low neurotensin (NTS) and NTS receptor 1 (NTSR1) expression levels in colorectal tumors are associated with improved survival outcomes, with 100% 5-year survival observed in patients whose NTS and NTSR1 expression were below the median. This study aims to (i) investigate the role of NTS/NTSR1 signaling in CRC tumor progression and the immune microenvironment, and (ii) elucidate its interaction with a high-fat diet (HFD) in shaping tumor-associated immune responses.

Methods: We established a mouse model to investigate the effects of NTS/NTSR1 signaling on CRC. Both wild-type (NTSWT) and NT knockout (NTSKO) mice were fed an HFD for 20 weeks and subsequently inoculated subcutaneously with MC38 cells, a mouse colorectal cancer cell line. Tumor growth was measured three times per week, and tumor tissues were collected on day 21 for analysis. Flow cytometry was performed to quantify key immune cell populations, including NKT cells, CD8+ T cells, and NK cells, in tumors from both NTSWT and NTSKO mice.

Results: Sixty percent of NTSKO mice exhibited complete regression of MC38-tumor growth within 10 days post-injection, with no tumor recurrence observed. In contrast, NTSWT mice showed significantly larger tumor size and weight compared to NTSKO mice ($P < 0.01$). Flow cytometry demonstrated that intratumoral immune cell populations of NKT cells, CD8+ T cells, and NK cells were significantly elevated in NTSKO mice compared to NTSWT mice ($P < 0.05$).

Conclusion: NTS/NTSR1 signaling, in conjunction with a HFD, suppresses tumor-infiltrating immune cell populations in the CRC tumor microenvironment, thereby promoting tumor progression. The knockout of NTS/NTSR1 restores anti-tumor immune cell activity, particularly NKT, CD8+ T, and NK cells, leading to tumor regression. These findings highlight the potential of targeting the NTS/NTSR1 pathway in CRC, especially in high-risk populations with dietary and metabolic susceptibilities. This study provides a foundation for developing therapeutic strategies to modulate tumor immunity by disrupting NTS/NTSR1 signaling in CRC.

Integrin α6β4 Regulation of Laminin Expression and Deposition in Breast Cancer

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Integrin α6β4 is a major contributor of the aggressive properties of select carcinomas through its ability to alter the transcriptome. To function, integrin α6β4 must bind one of its ligands, which includes multiple isoforms of the extracellular matrix protein, laminin. The β4 subunit (ITGB4) binds only the α6 subunit (ITGA6), thus is representative of the integrin α6β4 expression. Using cBioPortal analysis of TCGA breast cancer database, we find that the genes that correlate best with ITGB4 include the expression of subunits of its primary ligand, Laminin 5 (also known as Laminin-332), which contains laminin subunits α3 (LAMA3), β3 (LAMB3), and γ2 (LAMC2). RNA Seq evidence suggests that integrin α6β4 can alter the transcription of its ligands, suggesting that the correlation between ITGB4 and laminin genes may be causative. The objective of this study is to determine how integrin α6β4 regulates the expression of laminin subunits in triple negative breast cancer (TNBC) cells by assessing the change in expression of the individual subunits that make up the various laminin isoforms when expression of integrin α6β4 is knocked out in cells that endogenously express it or knocked in within cells that do not. Our hypothesis is that integrin α6β4 upregulates the expression of select laminin subunits that lead to an increase in certain isoforms of laminin. Expression of laminin isoforms was assessed using RT-qPCR analysis of mRNA extracted from the knockout constructs generated from MDA-MB-231 and HCC 1806 cells and the knock in constructs generated from BT549 cells. Results from MDA-MB-231 cells showed the expression of laminin subunits LAMA3, LAMA4 and LAMC2 decreased significantly when the integrin β4 subunit was knocked out. Results from BT549 knock in cell lines demonstrated that the introduction of the integrin β4 subunit led to an increase in LAMA3, LAMA5, and LAMB3 subunits. This indicates that integrin β4 may alter gene expression in these cell types, with subtle differences between the two that may be attributed to their varying degrees of stemness. Western blotting confirmed the expression of LAMC2 at the protein level in MDA-MB-231 cells, while RNA seq data indicates that BT549 cells have low expression rates for this particular subtype. To supplement this data, analysis from cBioPortal of the TCGA breast cancer database showed a positive correlation between integrin β4 (ITGB4) expression and laminin subunits LAMA1, LAMB3, LAMC2 and LAMC3 in breast cancer patients. To determine the significance of LAMC2 to integrin α6β4 function, LAMC2 was knocked out in MDA-MB-231 cells. Initial characterization of these cells investigated the loss of laminin γ2 chain on ER stress, which suggests ER stress may be induced by loss of only one laminin subunit. Taken together, these results indicate that cells that express integrin α6β4 upregulate the expression of their primary ligand, laminin 5 (also known as laminin 332), which contains laminin subunits α3, β3, γ2. This observation suggests that integrin α6β4 controls expression of its primary ligand, and therefore does not need externally supplied laminin to activate it. This observation is consistent with the challenges associated with using blocking antibodies to disrupt integrin α6β4 interaction with its laminin ligand. Future investigation of the impact of the secretion of laminins on integrin α6β4 function will require immunocytochemical analysis investigations into the possibility that the laminin 5-integrin α6β4 interaction initiates within the ER prior to cellular secretion. This could further support the idea that these proteins are secreted together to the cell surface as a function of ECM remodeling.

Integrin α6β4 Upregulates IDO1 Expression and Decreases IFNγ-Mediated T Cell Growth in ER-Negative Breast Cancer

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The kynurenine metabolic pathway degrades tryptophan into immunosuppressive metabolites following pro-inflammatory cytokine stimulation and is upregulated in ER-negative (ER(-)) breast cancers, where it is associated with a worse prognosis. The laminin receptor integrin α6β4 is expressed in a majority of hormone receptor negative breast cancers, where it is known to promote an aggressive phenotype by regulating cellular signaling and epigenetics. Here, we seek to identify how integrin α6β4 regulates the IFNγ-mediated induction of the first kynurenine pathway enzyme, IDO1, and its impact on immunosuppression in ER(-) breast cancer. Integrin β4 only pairs with integrin α6; thus, expression of the β4 subunit is representative of integrin α6β4 when integrin α6 is expressed. We demonstrate that stable expression of integrin β4 in BT549 cells regulates IDO1 expression by altering methylation of the IDO1 promoter and gene body compared to empty vector (EV) control, which resulted in increased IDO1 expression determined using high throughput sequencing, qPCR, and immunoblot analyses. Likewise, knockdown of integrin β4 by shRNA or knockout by CRISPR in HCC1806 and HCC1954 cells decreased IDO1 expression. Gene set enrichment analysis demonstrated an increased IFNγ response in BT549 cells expressing integrin β4 compared to EV control. Moreover, using the TCGA Breast Invasive Carcinoma dataset, we show that expression of IFNγ, a known inducer of IDO1, is positively associated with IDO1 expression in breast cancer. Upon IFNγ stimulation, the expression of integrin β4 resulted in a more dramatic upregulation of IDO1 at the mRNA and protein level. Furthermore, integrin α6β4 signaling significantly increased secretion of the immunosuppressive metabolite kynurenine following IFNγ stimulation, effects which were abrogated by pharmacologic IDO1 inhibition. Using conditioned media transfer onto Jurkat cells, we show that conditioned media from integrin β4-expressing cells stimulated with IFNγ significantly reduced T cell growth compared to media from IFNγ stimulated EV cells or unstimulated β4 cells. In summary, our data suggest that integrin α6β4 increases IDO1 induction in ER(-) breast cancer cells, amplifies kynurenine secretion, and suppresses T cell growth in the presence of the inflammatory cytokine IFNγ. This study suggests a novel role of integrin α6β4 in limiting T cell-mediated antitumor immunity and highlights the need to elucidate the detailed mechanisms governing integrin α6β4-mediated immunosuppression in ER(-) breast cancers.

Investigating p53 Mutations Using Molecular Dynamics to Reveal Structural and Functional Insights in Monomeric and DNA-Bound Forms

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p53, often called the “guardian of the genome,” is a crucial tumor suppressor protein that helps regulate cell cycle progression and apoptosis. However, mutations in p53 are common in various cancers and can severely compromise its protective role. In this study, we used all-atom molecular dynamics (MD) simulations to examine how these mutations influence p53 structure and function in both its monomeric DNA-binding domain (DBD) and its DNA-bound tetrameric form. Our simulations reveal that certain mutations disrupt key secondary structural elements and alter critical protein-DNA interactions, leading to changes in the stability of both the monomeric and tetrameric states. We also observed shifts in inter-residue contacts that can diminish the overall DNA binding affinity of the mutated tetramer. These findings offer a detailed molecular perspective on the ways p53 mutations can undermine its normal tumor-suppressive activities, and they underscore the potential value of targeting these structural disruptions in future therapeutic strategies aimed at restoring or compensating for lost p53 function.

Investigating the Role of SETDB1-RHOB Axis in Prostate Cancer

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Prostate cancer (PCa) is the most common cancer diagnosed in male and the second cancer-related death in the United States. Although patients response to the androgen deprivation therapy (ADT) initially, here are still a large proportion of patients develop more aggressive PCa. The cause of PCa progression has remained elusive. In addition to genetic alterations, epigenetic aberrations such as histone modification also play a pivotal role. SETDB1 is a well-known protein lysine methyltransferase which can methylate histone H3 at lysine 9 to transcriptionally silence expression of its target gene. SETDB1 is implicated as an oncogene in various cancers including prostate cancer, its overexpression predicts poor prognosis. But the underlying mechanism has not been well explored. To elucidate the mechanism of SETDB1 promoting PCa progression, we performed ChIP-seq and RNA-seq in PCa cell line, results identified Ras homolog family member B (RHOB), a tumor suppressor, is the epigenetic repressed target of SETDB1. Previous studies showed that downregulated expression of RHOB has often been detected in many human cancers, which lead to uncontrolled tumor progression. But the role of RHOB in prostate cancer is still ambiguous and the exact mechanism remains unexplored. Our RNA-seq result showed RHOB negatively regulates epithelia-mesenchymal transition (EMT). Our central hypothesis is SETDB1 silences RHOB expression through its methyltransferase activity to activates EMT process, eventually contributing to PCa progression. The goal of this study is to identify a therapeutic approach to abolish SETDB1 epigenetic function and restore RHOB expression in PCa.

Keywords: SETDB1, RHOB, EMT, prostate cancer

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Investigation and Optimization of Isoeugenol Dimers as Novel Drug Candidates in the Treatment of Triple Negative Breast Cancer

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Lignans are small, polyphenolic compounds that play a role in plant defense against pathogens and predators. Recently, lignan machilin D was reported to be effective as an anti-tumorigenic agent in triple negative breast cancer (TNBC) tumor-bearing mice. Previous studies have relied on plant-extracted material, which limits scalability and diversification of the natural scaffold. Herein, we describe a generalizable one-pot synthesis of machilin D and its derivatives via an iron chloride-induced dimerization of isoeugenol. Employing this synthetic methodology allowed for a robust diversification campaign to access seven (7) new lignan derivatives of the machilin D family with superior anti-proliferative properties in 2D and 3D TNBC models. In addition, the starting material isoeugenol can be derived from lignin, an abundant biopolymer that is wasted byproduct of many industries. This provides a path away from petrochemical-based pharmaceuticals towards “greener” medicine. Overall, this work enables lignan natural product-based drug discovery to elucidate novel biological targets, develop therapeutics for aggressive cancers such as TNBC, and promote sustainable pharmaceutical discoveries.

Liver-Specific Deletion of Carnitine Palmitoyltransferase 1a Promotes Tumorigenesis in a Mouse Model of Obesity-Driven Hepatocellular Carcinoma

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Background/Purpose: Metabolic dysfunction-associated steatotic liver disease (MASLD) is the fastest-growing etiology of hepatocellular carcinoma (HCC). The primary goal of this project is to determine the contribution of carnitine palmitoyltransferase 1a (CPT1a)-mediated fatty acid oxidation (FAO) to MASLD-driven HCC.

Methods: Paired HCC tumor (n=8) and adjacent non-tumor samples (n=8) from patients with suspected MASLD-HCC were obtained from the Biospecimen Procurement and Translational Pathology Shared Resource Facility at the University of Kentucky Markey Cancer Center. All patients met cardiometabolic MASLD criteria and were negative for viral hepatitis. Hematoxylin and eosin (H&E) staining was used for pathological determination of tumor and adjacent nontumor tissue by a pathologist. Lipids were extracted using a methyl-tert-butyl ether extraction method and subjected to lipidomics by the West Coast Metabolomics Center. RNA was isolated and bulk sequencing conducted. For murine studies, four-to-five-day old CPT1a$^{F/F}$ and liver-specific CPT1a KO (LKO) pups were treated with 7,12-dimethylbenz[a]anthracene and fed GAN diet (40% kcal fat; Research Diets) until 34 weeks of age. Mice were necropsied after a 24-hour fast, liver images were captured for gross assessment, and tissues collected. Data were analyzed using paired nonparametric analyses via a Wilcoxon or Mann-Whitney test, as appropriate.

Results: H&E staining showed significant lipid vacuole accumulation in HCC tumors relative to nontumor tissue. Lipidomic analysis revealed significant increases in long-chain nonesterified monounsaturated fatty acids (MUFAs; C16:1, C18:1, C20:1) and MUFA-enriched phospholipids (PC30:1, PC32:1, PE32:1, and PC36:1) in tumors relative to nontumor tissue. On the contrary, both MUFA- (AC14:1, AC18:1) and PUFA-enriched acylcarnitines (AC18:2, AC18:3) were collectively reduced in human tumors. Consistent with lipid profiles, RNA sequencing revealed fatty acid oxidation genes (*CPT1A, CPT2, ACADL, ACADM, ACADS, HADHA*) were significantly reduced in tumor versus nontumor tissue. In mice, CPT1a deletion increased liver weight to body weight ratios by 50% (P=0.0003) and increased overall tumor by >3-fold in male mice (3.7 vs. 12.4 average nodules per mouse; P=0.0055). H&E analysis suggests that tumors in mice replicate the histopathology of human samples.

Conclusions: These results suggest human HCC tumors exhibit a reduced capacity to undergo mitochondrial β-oxidation resulting in accumulation of free- and esterified-MUFAs with a concomitant reduction in MUFA-carnitines. Complimentary mouse studies show that CPT1a deletion in hepatocytes promotes HCC in male mice. Future studies are underway to identify mechanisms governing these differences. These findings identify the FAO pathway as a potential therapeutic target for MASLD-HCC prevention and treatment.

Microbial Metabolites Increase Key Hallmarks of Colorectal Cancer

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Alterations in the gut microbiome and its metabolites are associated with colorectal cancer. However, little work has been done to understand the mechanisms of how the interactions between the gut microbiome, metabolites, and intestinal epithelial cells may lead to carcinogenesis. As colorectal cancer remains a major public health concern, especially in Kentucky, it is necessary to understand the underlying factors that may be driving cancer formation and progression. Levels of spermidine, a member of the polyamine class of microbial metabolites, were significantly enhanced in a colorectal cancer model. Although intracellularly produced polyamines are well characterized, we do not completely understand the impact of exogenous, microbially-derived polyamines on intestinal epithelial cells in the context of colorectal cancer. We hypothesized that polyamine transporters expressed in intestinal epithelial cells play a significant role in importing exogenous polyamines inside intestinal epithelial cells, which then promotes the proliferation and migration of colorectal cancer epithelial cells. To test this hypothesis, we first identified spermidine as an increased microbial metabolite in a murine AOM/DSS model of colorectal cancer. We further examined the effects of polyamines on cell proliferation, migration, and mutation, hallmark processes important in cancer development and progression. An EdU proliferation assay demonstrated the ability of exogenous spermidine to increase the proliferation of intestinal epithelial cells, even at a low dose. Further investigations via transwell plate migration assay showed that exogenous polyamines also increased colorectal cancer epithelial cell migration, a critical cellular function which promotes cancer metastasis. We then determined the ability of the metabolic products of spermidine metabolism to induce DNA damage in cells, a process which can induce mutations if the DNA damage is not properly repaired. By using specific inhibitors against polyamine transporters and intracellular polyamine production, we finally determined a potential mechanism of action for spermidine to affect intestinal epithelial cells via these polyamine transporters. Our investigations into the mechanism and effects of exogenous, bacterially derived polyamines show the importance of the microbiome and its metabolites as a key factor in colorectal carcinogenesis.

Nanoparticle Formulation for Targeted Chemoradiotherapy of Metastatic Neuroendocrine Tumors

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Neuroendocrine tumors (NETs), once consider rare, has seen increased incidence over recent decades without improvement to survival rates due to limited treatment options. Targeted chemoradiotherapy may address these issues because it can selectively kill cancer cells while sparing healthy tissues. Triapine (3-AP), a small molecular inhibitor of the M2 metal binding site of ribonucleotide reductase (RR), is a potent drug that demonstrates strong antitumor activity and enhances radiosensitivity against various cancer cells including NETs, yet its clinical application has been limited due to pharmaceutical issues such as poor aqueous solubility, short plasma half-life, and systemic toxicity. This study aims to develop nanoparticle formulations that can: 1) enhance the aqueous solubility of 3AP by encapsulating the drug through polyion complexion between 3AP-cyclodextrin (CD) and MPEG5K-b-PLE100 nanoparticle complexes producing particles with a diameter less than 100 nm, achieving a clinically practical concentration of over 1 mg/mL; and 2) optimize the pharmacokinetic properties of 3AP-loaded nanoparticles (3AP/NP) and initiate in-vivo experiments in collaboration with clinicians. The nanoparticles will be characterized for size, charge, drug loading, release kinetics, and in vivo suitability. The expected outcome for this study is that the formation of 3AP-polymer will enhance the aqueous solubility, blood half-life, and tumor accumulation of 3AP improving the efficacy and safety of chemoradiotherapy which can be further developed as an additional therapy option for the treatment of metastatic NETs. Successful completion of this research will create a novel targeted chemoradiotherapy.

Oligomerization of Collagen Lysyl Hydroxylases Regulates Their Functions

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Collagen, the most abundant protein in vertebrates, serves as the building block for connective tissues, such as the vasculature, bone, and skin. What allows collagen to function properly in these different connective tissues is the hydroxylation and glycosylation of lysine residues. In humans, bifunctional enzymes named lysyl hydroxylases (LH1-3) catalyze the collagen lysyl hydroxylation and glucosylation modifications. The importance of LH-mediated collagen post-translational modifications is emphasized by human diseases. LH mutations lead to connective tissue disorders involving vasculature, bone, and skin. Aberrant LH overexpression contributes to the progression of fibrosis and cancer. Yet, how collagen lysyl hydroxylases are structurally regulated is still not clear. The objective of this study is to gain structural insights into lysyl hydroxylases' mechanistic and regulatory functions. By using crystallography and CryoEM, we show that lysyl hydroxylases form oligomer assemblies. The crystal structures of the interacting domain identify critical residues involved in oligomer formation. The dimer interface residues are highly conserved among LH family members, suggesting LHs may form hetero-oligomers. Site-directed mutagenesis of the interface residues modulates dimer formation and LH enzymatic activity. The biological significances of these oligomer assemblies are supported by previously identified LH lethal mutations on the oligomer interfaces. These structural insights inform collagen lysyl post-translational modifications and human disease pathology. Our results show that collagen modifications by Lysyl Hydroxylases are, in part, regulated by oligomerization.

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Overexpression of Fatty Acid Synthase Increases Exosomes Secretion in Colorectal Cancer

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More than 50% of colorectal cancer (CRC) patients develop metastases over the course of their disease, with the liver being the most common site of metastasis. Fatty acid synthase (FASN), a key enzyme of de novo lipid synthesis, is overexpressed and strongly associated with metastasis and poor prognosis in CRC. FASN catalyzes the biosynthesis of palmitate, an essential component of structural lipids. Exosomes are composed of a lipid bilayer membrane and carry a wide range of bioactive molecules, including lipids, proteins, and nucleic acids. Tumor-derived exosomes play a critical role in establishing a pre-metastatic niche (PMN) to support metastasis. Hepatic stellate cells (HSCs) can be activated by tumor signals and transformed to cancer-associated fibroblast, thus, contributing to PMN formation. However, the mechanisms by which exosomes promote HSCs activation and their subsequent contribution to PMN formation during CRC liver metastasis are not fully understood. Therefore, this study aims to investigate the contribution of FASN in exosomes formation, release, and HSCs activation in CRC. To delineate the role of FASN in exosomes formation, FASN-knockdown (FASN KD), FASN-overexpression (FASN OE), and pharmacological inhibition of FASN in CRC cells were utilized. Cells were cultured in media containing exosome-free FBS for 48h. Exosomes were isolated from cell culture media using ultracentrifugation. The concentration and size of exosomes were measured using nanoparticle tracking analysis. Exosomes markers were assessed using western blot. Proteomics analysis was conducted to assess the impact of FASN on exosome cargo. To investigate the functional impact of CRC-derived exosomes, HSCs LX-2 was used. The purity of isolated exosomes was confirmed by assessing the expression of CD9, Alix, TSG101, and syntenin. Secretion of exosomes by FASN-KD cells ($Exo^{FASN-KD}$) and by cells treated with FASN inhibitors was significantly reduced as compared to control cells. Moreover, FASN expression was decreased in $Exo^{FASN-KD}$. In contrast, secretion of exosomes by FASN-OE cells ($Exo^{FASN-OE}$) was significantly increased as compared to control cells. Consistently, we observed an increase in expression of FASN in $Exo^{FASN-OE}$. Proteomic analysis revealed that FASN modulates exosome cargo, with changes in inflammatory response, metabolism, exocytosis, and adhesion pathways. To test if FASN expression in CRC cells affects the ability of secreted exosomes to activate HSCs, LX-2 cells were incubated with exosomes from control and FASN-KD CRC cells. We found that the exposure to exosomes from control CRC cells leads to robust activation of LX-2 cells as determined by FAP and α-SMA expression; however, the level of LX-2 activation was significantly reduced when cells were exposed to $Exo^{FASN-KD}$. In summary, our data suggests that FASN promotes exosomes secretion in CRC. Importantly, the association between the level of FASN expression in CRC cells and activation of LX-2 cells by secreted exosomes suggests a potential role for FASN in PMN formation. Further studies are needed to elucidate the functional importance of FASN in PMN formation via exosomes secretion in CRC.

Pdcd4/mTORC2 Axis Regulates Tumorigenesis through PFKFB3 in NSCLC

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Programmed cell death 4 (PDCD4) is a tumor suppressor whose expression is frequently downregulated in various cancers, including lung cancer. PDCD4 has been shown to suppress tumor cell proliferation, migration, invasion, and metastasis in cultured cells and in mouse models. It has also been suggested to play a role in regulating glucose metabolism; however, the detailed mechanisms remain unclear. Using reverse phase protein array, we found that knockdown of PDCD4 upregulated 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3), a critical enzyme in regulation of glycolysis. PFKFB3 converts fructose-6-phosphate to fructose-2,6-bisphosphate to activate the rate-limiting enzyme, 6-phosphofructo-1-kinase, in glycolysis. PFKFB3 expression is upregulated in lung cancer and correlates with tumor stage progression. Inhibition or down-regulation of PFKFB3 in lung cancer cells induces cell apoptosis and inhibits tumor growth in nude mice. Given that PDCD4 inhibits mTORC2 activity, we next examined how mTORC2 affects PFKFB3 expression. We found that mTORC2 interacted with PFKFB3 in NSCLC cells. Using In vitro kinase assays demonstrated that can phosphorylate PFKFB3. Further analysis pinpointed a Ser residue as the target site, leading to the generation of phosphorylation-deficient (S to A) and phospho-mimetic (S to D) mutants. Stability assays revealed that phosphorylation at this specific Ser is essential for maintaining PFKFB3 protein stability; as the phosphorylation-deficient mutant underwent rapidly degradation, whereas the phospho-mimetic mutant showed increased its half-life. Functional assays showed that WT PFKFB3 promoted high proliferation rates, whereas the phosphorylation-deficient mutant impaired colony formation and proliferation. Taken together, these findings uncover a key mechanism of metabolic reprogramming in NSCLC, in which mTORC2 drives glycolysis by directly phosphorylating PFKFB3, highlighting the therapeutic potential of targeting the PDCD4/mTORC2/PFKFB3 axis in NSCLC treatment.

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Perfluorooctanesulfonic Acid Promotes Cellular Proliferation and Activates EGFR Signaling in Primary Colorectal Cancer Cells

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Perfluorooctanesulfonic acid (PFOS), a subset of per-and polyfluoroalkyl substances (PFAS), has been recognized as an emerging ecological contaminant due to its widespread environmental persistence, being detected in 45% of USA drinking water. The gastrointestinal tract is directly exposed to environmental pollutants via contaminated drinking water and food. Given its resistance to natural degradation, PFOS can accumulate in intestinal tissues, potentially influencing homeostasis under both physiological and pathological conditions. Despite extensive research on PFOS's impact on various health conditions, including its potential role in tumor promotion, its contribution to colorectal cancer (CRC) progression remains poorly understood. Therefore, this study aims to investigate the effects of long-term exposure to PFOS *in vitro*. To access the effects of PFOS exposure on CRC cell proliferation, a primary CRC cell line, PT130, was exposed to 1µg/mL of PFOS for 3 months. We performed a PrestoBlue assay to evaluate cell proliferation and conducted western blot analysis to assess changes in protein expression. We observed an increase in cell proliferation in PFOS-treated PT130 cells compared to control cells. Additionally, PFOS exposure led to the activation of EGFR, STAT3, and ERK along with increased protein levels of Cyclin D and Survivin, key markers associated with cell proliferation. These findings suggest that PFOS exposure increases CRC cell proliferation, possibly through EGFR pathway activation. Ongoing studies will explore the effects of PFOS on additional downstream proteins involved in CRC progression. Further studies are essential to investigate PFOS's impacts and to develop strategies to mitigate its harmful effects on CRC.

Pharmacologic Induction of cAMP to protect melanocytes against ultraviolet radiation damage and mutagenesis

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Objective: The melanocyte stimulating hormone (MSH)- melanocortin 1 receptor (Mc1r) signaling axis protects melanocytes against UVR damage by up-regulating production of UVR-protective eumelanin pigment and by enhancing the repair of UV photolesions, which are known to be an oncogenic driver for melanoma. The Mc1r is a G protein-coupled receptor that functions by activating adenylyl cyclase that mediates production of the second messenger cAMP. Loss-of-function variants in Mc1r are a bona fide melanoma risk factor in humans and are characterized by blunted cAMP signaling responses. Our lab has been interested in the capacity of pharmacologic cAMP induction to protect melanocytes against ultraviolet radiation (UVR) damage and mutagenesis. We are studying whether pharmacologic cAMP induction by the direct adenylyl cyclase activator forskolin, which has the capacity to bypass dysfunctional Mc1r, impacts the clearance of [6,4]-photoproducts and cyclopyrimidine dimers (CPD's), two mutagenic UV photolesions.

Methods: To determine this, we treated A375 amelanotic melanoma cells with sublethal doses of UVR and measured the amount of [6,4]-photoproducts over the next several hours. To determine the kinetics of forskolin's impact on cAMP signaling in the cell, we quantified levels of phospho-CREB, a well-characterized indicator of cAMP signaling, by immunoblotting in A375 cells treated for 1h with 10 uM forskolin.

Data: We found that while forskolin exposure had little effect on total CREB levels, it caused significant increases in phospho-CREB levels by 3 hours. The highest levels of phospho-CREB were observed at 6h, and the effect of the drug appeared to wane thereafter.

Conclusion: We conclude that forskolin is highly effective in transiently activating cAMP signaling pathways in A375 melanocytes and hypothesize that pharmacologic activation of cAMP signaling in melanocytes may be of potential benefit as a melanoma-preventive strategy by enhancing the efficiency of melanocytes to clear mutagenic UVR DNA injury.

PLK1 Mediated BRN2 Phosphorylation Contributes in NEPC

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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.

PLK1-Catalyzed CHAF1A Phosphorylation Contributes to AR Pathway Maintenance in PCa

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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[1]. Polo-like kinase 1 (PLK1), a highly conserved serine/threonine kinase, assumes a pivotal role in orchestrating cell mitosis. Emerging evidence underscores its involvement in promoting malignancy and conferring drug resistance in a spectrum of cancer types, including PCa[2]. 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, and actively involves in multiple types of cancer, but its role in PCa remains unelusive[3]. 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 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-deficient mutation (CHAF1A-T591A) into C4-2 cells, we have illuminated the functional consequences of this phosphorylation event. Our results demonstrate that the CHAF1A-T591A variant attenuates the expression of PSA in C4-2 cells and enhances their responsiveness to enzalutamide treatment. Inhibition of PLK-mediated phosphorylation of CHAF1A leads to increased deposition of CHAF1A on chromatin without altering its nuclear localization, which may represent a potential mechanism for PSA expression suppression, given CHAF1A's established role as a marker for heterochromatin.

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.

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

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Prostate cancer (PCa) remains a leading cause of cancer-related mortality in men, particularly in its advanced stages. While androgen deprivation therapy (ADT) and next-generation androgen receptor (AR)-targeted therapies have improved outcomes, castration-resistant prostate cancer (CRPC) continues to pose significant therapeutic challenges due to tumor heterogeneity and resistance mechanisms, including AR splice variants like AR-V7. Emerging evidence suggests that lipid metabolism plays a crucial role in CRPC progression, with de novo lipogenesis (DNL) and lipid accumulation contributing to tumor aggressiveness and resistance to AR-targeted treatments. FASN and SREBPs are key mediators of this process, regulated by AR signaling. HOXB13, a homeobox transcription factor essential for prostate development, has been implicated in PCa progression through its interaction with AR and its role in reprogramming the AR cistrome. However, the molecular mechanisms underlying HOXB13 regulation remain largely unexplored. Our study aims to investigate the post-translational modification of HOXB13, specifically its phosphorylation by polo-like kinase 1 (Plk1), a mitotic kinase frequently overexpressed in PCa. We hypothesize that Plk1-mediated phosphorylation of HOXB13 alters its stability and enhances its interaction with HDAC3 through the MEIS domain, thereby influencing lipid metabolism and CRPC progression. To test this hypothesis, we will pursue three specific aims: (1) determine whether HOXB13 is a substrate of Plk1 and identify its phosphorylation site; (2) assess the functional significance of HOXB13 phosphorylation in regulating lipogenesis and CRPC progression through gene expression analysis, RNA sequencing, and lipid metabolism assays; and (3) evaluate the physiological relevance of HOXB13 phosphorylation using xenograft model and performing prostate reconstitution in SCID mice to investigate its impact on tumor formation and metastasis. By elucidating the regulatory role of Plk1 in HOXB13-mediated lipid metabolism and CRPC progression, this study will provide critical insights into the molecular mechanisms driving prostate cancer aggressiveness and may identify novel therapeutic targets for advanced disease.

PLK1-Phosphorylation of OCT4 Regulates Trans Differentiation from Castration-Resistant Prostate Cancer to Neuronal Endocrine Prostate Cancer

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Prostate cancer (PCa) stands as the most prevalent malignancy and a leading cause of cancer-related deaths among males in the United States. Recently, a novel and aggressive subtype of prostate cancer, known as neuroendocrine prostate cancer (NEPC), has emerged. NEPC is characterized by rapid disease progression and resistance to conventional therapies, including second-generation anti-androgen inhibitors. This drug resistance highlights the urgent need to develop novel therapeutic targets and expand the repertoire of biological tumor markers to better understand and manage the disease. The phenotypic switch to NEPC is associated with an increase in stemness and neuroendocrine (NE) markers, contributing to aggressive behavior and poor prognosis.

PLK1 (Polo-like Kinase 1) is a serine/threonine kinase known for its critical role in cell cycle regulation, particularly in mitotic entry. It has also been implicated in the progression of prostate cancer and has been shown to phosphorylate and regulate various proteins, thereby influencing multiple signaling pathways. One of these pathways involves the transcription factor OCT4 (POU5F1), which is essential for maintaining pluripotency and has been linked to cancer cell proliferation and stem cell-like properties in tumor cells.

Our study investigates the interaction between PLK1 and OCT4 and its implications for NEPC progression. We demonstrate that PLK1 phosphorylates OCT4, leading to its degradation via CHIP E3 ligase. This finding is significant because OCT4 plays a crucial role in maintaining stemness in cancer cells. Using CRPC cell models treated with enzalutamide, we observed an initial increase in both stemness markers and NE markers. However, prolonged treatment results in the maintenance of high levels of NE markers while stemness markers begin to decline. This suggests that continuous treatment contributes to a phenotypic shift toward NEPC.

In NEPC cell models, we found that OCT4 knockdown or targeted degradation resulted in elevated expression of MYC and NE markers, reinforcing the link between OCT4 modulation and NEPC characteristics. These results underscore the potential of OCT4 as a key player in NEPC progression and reveal PLK1's role in mediating OCT4's stability. Our findings provide insight into the molecular mechanisms driving NEPC transformation and suggest that targeting the PLK1-OCT4 axis could offer a novel therapeutic strategy to combat NEPC and improve patient outcomes.

PRL Proteins in Zebrafish Development and Cancer Progression

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Phosphatases of Regenerating Liver (PRL1-3) proteins are upregulated in various forms of cancer, and their over-expression is linked to poor patient outcomes. While phosphatases have been prominent targets for cancer therapeutic development for over two decades, additional studies are needed to fully harness their clinical potential. In particular, PRLs do not have a well-defined mechanism linking their overexpression to increased cancer metastasis, proliferation, poor patient prognosis, and other malignancy-associated traits. Additionally, the normal physiological functions of PRLs are not completely understood. To begin addressing these gaps, we utilized a zebrafish developmental model to study PRL function in vivo. Zebrafish embryos undergo rapid cell proliferation and migration during development, processes analogous to those implicated in PRL-associated cancer progression. We used morpholino oligonucleotides (MO) designed to specifically hybridize mRNA encoding for the four PRL genes (panPRL), blocking their expression. This treatment produced a distinctive phenotype characterized by uneven blastoderm cap distribution during gastrulation, followed by pronounced dorsalization at 24 hours post-fertilization. Rescue experiments and replication of the phenotype in p53 knockouts confirmed the specificity of this phenotype. To investigate the basis of this dorsalization, we conducted whole mount in situ hybridization (ISH) to visualize the localization and intensity of genes implicated in dorsoventral patterning. PanPRL MO-injected embryos displayed an expanded localization of dorsal factors and a reduction in ventral factors compared to controls. We also examined the yolk syncytial layer (YSL), known for its role in cell differentiation and dorsoventral patterning. In panPRL MO-injected embryos, we observed a significant change in yolk syncytial nuclei shape and size due to panPRL MO injection. Additionally, because actin constriction and microtubule pull-down of the blastoderm cap is crucial for gastrulation, as it internalizes the yolk, we are exploring how PRL knockdown affects cytoskeletal organization. Preliminary actin staining results have shown insignificant differences in enveloping layer actin between panPRL and control MO-injected embryos at the shield stage. However, future observation of actin organization in epiboly will provide more conclusive results. Our initial microtubule staining findings demonstrate a potential centromere abnormality, as nuclei in panPRL MO-injected embryos were seen to frequently be associated with multiple or in some cases no centromeres. Our findings indicate that PRL proteins are integral to normal developmental processes, including dorsoventral patterning, cell fate determination, and cytoskeletal organization. Because these factors contribute to cancer progression, elucidating PRL function in development may provide key insights into their role in malignancies and guide therapeutic strategies.

Protein Tyrosine Phosphatase Receptor Type F Negatively Regulates c-Src Kinase through the Dephosphorylation of the Activation Loop

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Kinases and phosphatases play a dynamic role in the regulation of signaling propagation. c-Src is an oncogenic kinase that plays multifunctional roles in the initiation and progression of many cancers, including breast, colorectal and lung cancers. The activity of c-Src kinase is tightly regulated by the phosphorylation status of two key tyrosine residues (Tyr419 and Tyr530 in human c-Src) that controls its propensity for entering an active conformation. Phosphorylation of Tyr530 at the C-terminus of c-Src locks the kinase in an inactive confirmation whereas phosphorylation of Tyr419 in the activation loop is required to activate c-Src. A number of tyrosine phosphatases have been identified to activate c-Src by dephosphorylating Tyr530. However, the phosphatases controlling the Tyr419 site remain elusive. In this study, we investigated the role of protein tyrosine phosphatase receptor type F (PTPRF) in regulating the phosphorylation of c-Src. PTPRF is characterized by an extracellular domain, a single transmembrane motif, and two tandem intracellular phosphatase domains (including a catalytically active D1 domain and a catalytically inactive D2 domain). We show that overexpression of PTPRF, but not a phosphatase inactive mutant, results in a decrease in Tyr419 phosphorylation of both wild-type and Y530F constitutively active c-Src in 293T cells. On the other hand, CRISPR-mediated knockout of PTPRF increases the phosphorylation of c-Src and signaling downstream. Co-immunoprecipitation experiments indicate that PTPRF interacts with c-Src through its D2 pseudo phosphatase domain. Consistent with the notion that the kinase activation reduces c-Src protein stability, we find that the expression of c-Src is increased when co-expressed with PTPRF, suggesting that PTPRF-mediated dephosphorylation stabilizes c-Src protein. Taken together, this study identifies PTPRF as a novel phosphatase that negatively regulates c-Src kinase activity through the dephosphorylation of Tyr419 in the activation loop.

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. Deletion of PTPRF using CRISPR/cas9 in 293T 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. PTPRF downregulation promotes signaling propagation through the RAC1/PAK axis. Functionally, PTPRF-loss markedly increases cell migration. These exciting new findings identify GAB1 as a possible novel substrate of PTPRF.

REEP2-Driven Pro-Metastatic Secretion Promotes Lung Cancer Progression

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Background: Membrane trafficking, critical for cellular function, is disrupted in cancer, promoting metastasis, yet remains untargeted therapeutically. In lung adenocarcinoma, the epithelial-to-mesenchymal transition activator ZEB1 drives a pro-metastatic membrane trafficking program, but the underlying molecular mechanisms are unclear. Our goal is to elucidate these mechanisms and identify targets to prevent lung adenocarcinoma metastasis.

Methods: We performed a CRISPRi *in vivo* screen of 2,099 membrane trafficking regulators for their functions in tumorigenesis in a syngeneic mouse model, which develops lung adenocarcinoma due to epithelial-to-mesenchymal transition. We assessed the prognostic values of the candidate hits using The Cancer Genome Atlas database analysis and evaluated their functions in membrane trafficking and lung adenocarcinoma progression *in vitro* and *in vivo* using human and murine lung adenocarcinoma cell lines and syngeneic mouse models.

Results: We found that REEP2, an endoplasmic reticulum shaping protein, is associated with poor prognosis in lung adenocarcinoma patients, and significantly correlated with a z-normalized 16-gene epithelial-to-mesenchymal transition score (P = 1.32e-27) and with an immunosuppressive tumor microenvironment. Further mechanistic studies demonstrate that the epithelial-to-mesenchymal transition activator ZEB1 upregulates REEP2 expression, which drives endoplasmic reticulum polarization to promote pro-metastatic secretion. REEP2 depletion suppresses lung adenocarcinoma cell cycle progression and invasive ability *in vitro* and reactivates anti-tumor immunity in the tumor microenvironment to repress lung adenocarcinoma growth and metastasis in syngeneic mouse models.

Conclusion: Our findings elucidate the molecular mechanisms underlying lung adenocarcinoma metastasis and provide a rationale for targeting REEP2 to prevent epithelial-to-mesenchymal transition -driven lung adenocarcinoma metastasis.

Regulation of Lipogenesis Pathway through Phosphorylation of HOXB13 by Plk1

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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.

Regulation of PRMT5 in DNA Damage Response and During Inflammatory Response in Human Monocytes Leukemia Cell Line

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Protein arginine methyltransferase 5 (PRMT5) catalyzes symmetric dimethylation (SDMA) of arginine residues in proteins, including histones H3 and H4. The epigenetic modification regulates DNA repair genes and contributes to resistance against ionizing radiation (IR) in cancer cells including those in prostate cancer. PRMT5 also promotes immune evasion by repressing antigen presentation genes, i.e., MHC class I/II, CD80, and CD86 in prostate cancer (Genes, 2023, 36980950; Ann Transl Med, 2021, 34268368).

In this study, we report a novel observation in the human monocytic leukemia cell line THP-1. Upon differentiation with phorbol 12-myristate 13-acetate (PMA) followed by lipopolysaccharide (LPS) treatment, PRMT5 protein levels were significantly reduced. This reduction was observed for both endogenous and constitutively expressed PRMT5 and was accompanied by the appearance of high molecular weight species in immunoblotting, suggestive of degradation and/or post-translational modification. In contrast, LPS treatment alone did not affect PRMT5 levels in undifferentiated cells. These results suggest a macrophage-dependent mechanism that down-regulates PRMT5 in response to inflammatory stimuli, potentially de-repressing immune genes and reversing PRMT5-mediated immune tolerance. This study highlights a dual role of PRMT5 in cancer: regulating DNA repair and modulating tumor immune evasion. Targeting both pathways controlled by PRMT5, sensitizing cells to DNA damage and boosting anti-tumor immunity via the cGAS-STING pathway and recognition of damage-associated molecular patterns (DAMPs), is crucial for enhancing radioimmunotherapy in prostate cancer.

Role of Obesity in Regulation of Acute Myeloid Leukemia Progression

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Acute myeloid leukemia (AML) accounts for 15-20 percent of childhood leukemia. Although improved treatment options have increased the overall survival for AML, approximately 30% of the cases tend to relapse. The management of AML patients is an ongoing challenge due to the relapse rates and treatment related mortality. Obesity is a leading cause of preventable death in the United States. Clinical outcome is linked to the degree of genetic heterogeneity of AML and higher body mass index (BMI) at diagnosis. As these underlying factors are associated with poor survival for AML, we are addressing the role and mechanism of obesity-associated factors in progression of AML in mouse models.

Since obesity during pregnancy and childhood obesity may be associated with increased risk of developing AML, we tested the growth of the mouse AML cell line C1498 (ATCC.org) in the progeny of obesity-prone C57BL/6J *db/db* and *ob/ob* mice or normal (wild-type) C57BL/6J mice. Our data indicated that both *ob/ob* mice that lack leptin and *db/db* mice that lack the leptin receptor were prone to increased AML growth and showed significant increase in CD33-positive cells compared to wild-type C57BL/6J mice. Kaplan-Meier analysis of survival curves showed statistically significant differences between obese mice and wild type control C57BL/6J mice. Since obesity may change the immune phenotype and thereby affect AML growth, we performed blood cell immunophenotyping in C57BL/6J, *db/db* and *ob/ob* mice before C1498 engraft. We found that the T-cell numbers were significantly lower in the blood of *db/db* and *ob/ob* mice compared to the wild type control C57BL/6J mice. In contrast, myeloid-linage cell numbers were high in the blood of *db/db* and *ob/ob* mice than wild type C57BL/6J mice. The same pattern of immunophenotyping was found in post-AML blood as in pre-AML blood. Pearson Correlation analysis showed that survival of the mice positively correlated with the T-cell population and negatively correlated with myeloid population in blood.

As diet-induced obesity (DIO) is the most common cause of obesity in humans, we used C57BL/6J mice that were fed with normal chow diet or high-fat diet (Research Diets, Inc., Catalog # D12492) and tested them for AML growth. Our data showed that obesity associated with high-fat diet also promoted AML growth with decreased survival when compared to normal chow-fed C57BL/6J mice.

Taken together, our studies performed in mice with genetically driven obesity or high-fat diet induced obesity indicate that obesity promotes the growth of AML cells and is associated with decreased survival in mice. Our results suggest that T-cell and myeloid cell populations that are modulated by obesity may play an important role in AML progression.

Targeting DCN1-Mediated Neddylation to Disrupt Oncogenic Signaling in BRAFV600E Melanomas

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Background: Melanomas harboring the BRAFV600E mutation exhibit constitutive MAPK and NF-κB signaling, which contributes to aggressive tumor behavior and resistance to therapy. Emerging evidence implicates neddylation, a post-translational modification that activates Cullin-RING ligases (CRLs) as a key process in sustaining oncogenic signaling. DCUN1D1 (DCN1), a co-E3 ligase that promotes cullin neddylation, is overexpressed in various cancers. However, its role in melanoma remains underexplored.

Objective: To investigate whether pharmacologic inhibition of DCN1 disrupts neddylation-dependent degradation of regulatory proteins, thereby attenuating NF-κB and MAPK pathway activity in BRAFV600E melanoma cells.

Methods: Using melanoma cell lines gotten from the NCI-60 cancer cell panel with characterized BRAF and DCN1 inhibitor sensitivity, we are conducting NF-κB luciferase reporter assays, immunoblotting for phosphorylated ERK, JNK, and IκBα, and viability assays post-treatment with a DCN1-specific inhibitor. Mechanistic studies include DUSP (dual-specificity phosphatase) stabilization assays, TAK1 neddylation analysis, and DCUN1D1 overexpression to evaluate impacts on SCF E3 ligase activity.

Anticipated Results: We expect that DCN1 inhibition will stabilize IκBα and DUSPs, leading to reduced NF-κB transcriptional activity and attenuated ERK/JNK phosphorylation. DCN1 inhibitor-sensitive cell lines, particularly those with constitutive BRAFV600E signaling, are anticipated to show diminished viability and a strong response to pathway suppression. Additionally, we predict that DCUN1D1 overexpression will accelerate IκBα degradation and enhance NF-κB activity, confirming DCN1's regulatory role.

Conclusion: This work aims to define a novel mechanistic link between neddylation and survival signaling in melanoma. Targeting DCN1 may offer a unique therapeutic strategy to overcome resistance in BRAF-mutant tumors by simultaneously modulating MAPK and NF-κB pathways.

Keywords: Melanoma, BRAFV600E, NF-κB, DCN1, Neddylation, MAPK signaling, DUSPs, Cancer Therapeutics

Targeting EZH2 to Overcome Osimertinib Resistant Non-Small Cell Lung Cancer

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Non-Small Cell Lung Cancer (NSCLC) is a continual issue across the United States as the leading cause of cancer-related mortalities. Within NSCLC, Epidermal Growth Factor Receptor (EGFR) mutations have emerged as a main genetic driver. Treatment for EGFR mutated lung cancers has evolved over the past two decades, starting with the EGFR tyrosine kinase inhibitors gefitinib and erlotinib, and more recently moving to osimertinib. These treatments are typically successful initially, but resistance to EGFR inhibitors is inevitable for many patients. Findings ways to overcome the myriads of resistance mechanisms that can occur is the key to improving treatment outcomes. Our lab studies the Polycomb Repressive Complex 2 (PRC2) that contains the subunit Enhancer of Zeste Homolog 2 (EZH2). PRC2 is an epigenetic methyltransferase that facilitates histone H3 lysine 27 tri-methylation (H3K27me3) to silence gene expression. EZH2 has been implicated in many cancers may potentially drive treatment resistance. Our hypothesis is that inhibition of EZH2 will overcome osimertinib resistance by blocking the oncogenic signaling driven by the transcription factor c-MYC. To test our hypothesis, PC9, PC9GR4, and HCC4006 were all made resistant to osimertinib with IC_{50}s of 1μM and above. Following generation of acquired resistance, we found that treatment with the EZH2 inhibitor tazemetostat (EPZ6438) was able to resensitize cell lines to osimertinib. In PC9GR4OR this re-sensitization was accompanied by increased apoptosis. We next tested two cell lines, PC9GR4OR and HCC4006OR, as *in vivo* xenografts, and showed that combination of osimertinib and EPZ6438 led to significant tumor regression that was not achieved by either drug alone. Next, to understand mechanisms of resistance, we performed RNA sequencing on both parental and osimertinib resistant lines treated with vehicle, EPZ6438, osimertinib, or both. GSEA analyses showed that resistance mechanisms for the three lines were not the same. PC9OR upregulated cell cycle targets and c-MYC targets, HCC4006OR upregulated oxidative phosphorylation and c-MYC targets, and PC9GR4OR had an increase in cholesterol homeostasis. RT-qPCR analyses also showed that combination treatment increased antigen presentation (*HLA-A*) and lowered PD-L1 (*CD274*). This combination of changes may produce tumors that are more susceptible to immune surveillance, or that respond better to immune checkpoint inhibitors. Overall, this work highlights the ability of inhibiting EZH2 to overcome multiple osimertinib resistance mechanisms in human lung cancer cells and suggests that this drug combination may prolong the treatment benefit for many lung cancer patients. This work was funded by: Markey Women Strong, Markey STRONG Scholars Program through the American Cancer Society IRG-22-152-34 (HY), T32 CA165990 (DRP), R01 CA237643 + Cure Supplement (CFB, CMG), R01 HL170193 (CFB), P30 CA177558 (Markey Shared Resources).

Targeting Fatty Acid Synthase to Improve the Efficacy of BRAF-Targeted Therapy in Colorectal Cancer

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Mutations in proto-oncogene B-Raf (BRAF) occur in about 10% of colorectal cancer (CRC) patients and $BRAF^{V600E}$ is the most common type. This subset of CRC is associated with a reduced response to chemotherapy and poor prognosis compared to BRAF wild-type CRC. While the FDA-approved encorafenib plus cetuximab therapy for BRAF-mutant CRC provides a significant benefit, only 22% of patients respond to this therapeutic approach and resistance ultimately develops in the majority of patients. Therefore, development of new efficacious strategies based on further characterization of resistance mechanisms is needed to improve outcomes for $BRAF^{V600E}$ CRC. We found that development of resistance to PLX8394, a second generation BRAF inhibitor, is associated with an increase in cellular proliferation, invasion, and lipid metabolism. We identified fatty acid synthase (FASN), a key enzyme in lipid synthesis, as a major lipogenic enzyme overexpressed in PLX8394-resistant cells. Therefore, the goal of this study is to test if inhibition of FASN will enhance the efficacy of PLX8394 in BRAF-mutant CRC.

Methods. HT29, PT130, and PT24249pt, parental and PLX8394-resistant, CRC cells were utilized. The effect of PLX8394 alone and in combination with FASN inhibitors (TVB3664 and C75) was tested using cell viability and colony formation assays. The synergy score was determine using a BLISS synergy model (SynergyFinder 3.0). The development of resistance to PLX8394 in the presence or absence of TVB3664 was evaluated using IC50 analysis, flow cytometry, and western blot.

Results. We found that PLX8394-resistant cells exhibit an increase in FASN expression, oxidative phosphorylation, and triglyceride storage. Combination of PLX8394 and FASN inhibitors led to a significant decrease in cell viability and colony formation as compared to each drug alone. Consistently, we found a significant synergy between PLX8394 and C75 using the BLISS synergy model. FASN-knockdown cells exhibited higher sensitivity to PLX8394 as compared to control cells supporting the role of FASN in resistance to PLX8394. Importantly, an addition of TVB3664 to PLX8394 treatment postpones the development of resistance to PLX8394. Analysis of cell cycle by flow cytometry confirmed a suppression of cell cycle progression via a decrease in a number of cells entering the S phase when cells are treated with combination of PLX8394 and TVB3664. Consistently, we observed a decrease in expression of proteins associated with the cell cycle such as cyclin D, pRB, and E2F expression in this group.

Conclusion. Collectively, these data show that combination of PLX8394 with FASN-targeted therapy at treatment initiation reduces $BRAF^{V600E}$ CRC cell proliferation via inhibition of their cell cycle progression. These findings suggest that targeting FASN could enhance the efficacy and delay the development of resistance to PLX8394 in BRAF-mutant CRC.

Targeting KDM3A to Treat Enzalutamide-Resistant Castration Resistant Prostate Cancer

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Prostate cancer (PCa) is the second leading cause of cancer-related mortality among man in the United States. Androgen receptor (AR) plays a crucial role in PC development and progression. Many patients received androgen deprivation therapy (ADT) through surgery (e.g., orchiectomy, medical castration) or drug treatment (e.g., AR inhibitors). Most of patients had initial response to ADT but developed castration-resistant prostate cancer (CRPC) after few years. Recently, Enzalutamide, a second generation of AR inhibitor, was approved by FDA to treat both non-metastasis and metastasis CRPC. Unlike other AR inhibitors, which only inhibit the binding of androgen to AR, enzalutamide also inhibits the nuclear localization, DNA binding and co-activator recruitment. Enzalutamide improved the overall survival of CRPC patients. However, a high percentage of patents are primarily resistant or acquire resistant after Enzalutamide treatment. The resistant mechanisms include AR overexpression, amplification, mutation, or expression of AR variants, particularly ARV7. Activation of other signaling pathways, such as Wnt/β-catenin and PI3K pathways also contribute to Enzalutamide resistance.

To overcome enzalutamide resistance, we screened a panel of commercial and home-made compounds, and identified a family of histone lysine demethylases 3A (KDM3A) inhibitors that significantly enhance the therapeutic efficacy of Enzalutamide on 22RV1 PC cells, which express the ARV7 variant. KDM3A regulates H3K9 methylation, which represses gene expression. Inhibition of KDM3A increases H3K9 methylation and blocks transcription. We originally developed KDM3A inhibitors to inhibit the Wnt/β-catenin signaling. We identified a carboxamide-substituted benzhydryl amine (hereinafter abbreviated as CBA-1) as an epigenetic regulator that inhibited KDMs possessing the Jumanji C (JmjC) domain. Based on structure-activity relationships (SAR) in conjunction with molecular docking studies, we identified a new CBA-1 derivative, CBA-2, that inhibited 22RV1 PCa xenografts growth in mouse models.

KDM3A is overexpressed in PCa and has been recognized as an important drug target for PCa. We hypothesize that KDM3A inhibitors enhanced Enzalutamide efficacy by multiple mechanisms: 1) inhibits the expression of ARV7 variant; 2) inhibits AR target gene expression; and 3) inhibits other signaling pathways that contribute to Enzalutamide resistance. Although several histone demethylase inhibitors have entered Phase I/II clinical trial, most of these inhibitors target other members of the histone demethylase, particularly LSD1. These histone demethylases have different targets and different functions. No KDM3A inhibitor has been approved by FDA for clinical studies. To address this unmet need, we will further optimize KDM3A inhibitors and evaluate the leading KDM3A inhibitors in monotherapy and combinatorial therapy with Enzalutamide.

Targeting Mesenchymal Cells with Epigenetic Therapy in Lung Cancer and Lung Disease

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Lung diseases, including fibrosis and cancer, are major health problems that together lead to millions of deaths each year. Non-small-cell lung cancer (NSCLC) has high morbidity and mortality rates, and patient prognosis and survival are very poor. Similarly, pulmonary fibrosis is a life-threatening respiratory disease characterized by scarring and damage caused by increased abundance of collagen-secreting myofibroblasts. An autopsy study showed that as many as one third of non-small cell lung cancer patients have some amount of pulmonary fibrosis1, suggesting that these two diseases frequently co-exist. Our laboratory has also found that higher abundance of mesenchymal cells in tumors predicts a poor prognosis2. 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 lung disease. Several EZH2 inhibitors (tazemetostat and Valemetostat) provide a reliable and therapeutically tolerable treatment option for diseased patients. Our central hypothesis is that EZH2 inhibition can prevent or reverse fibrosis via targeting myofibroblasts. To test this hypothesis, we have established pulmonary mesenchymal cell lines from both normal and diseased human lung. We observed that EZH2 inhibition slowed cell proliferation with or without TGF-beta, a cytokine known to drive a myofibroblast fate. In vivo, we have observed that EZH2 inhibition attenuates the fibrosis that occurs after exposure to bleomycin. Next, we are testing the roles of these mesenchymal cells in responses to cancer therapies, including immunotherapy. For our initial experiments, we used murine pulmonary mesenchymal cells grown with tumor cells and immune cells in a 3-dimensional Matrigel co- cultures. This culture system mimics aspects of the tumor immune microenvironment in vitro and has allowed us to test therapies including EZH2 inhibition and anti- PD1 immunotherapy in a tractable system. Our preliminary results suggest that EZH2 inhibition can alter cells to be more susceptible to anti-PD1, and we are now examining the contributions of mesenchymal cells to these phenotypes. Together our results suggest that targeting EZH2 may improve aspects of both pulmonary fibrosis and lung cancer, and be a way to target these co-occurring diseases to improve patient outcomes. 1doi: 10.7196/ajtccm.2020.v26i1.050, 2doi: 10.1016/ j.labinv.2023.100176. Funded by R01 CA237643, R01 HL170193, and P30 CA177558 (Markey Shared Resources).

Targeting PGK1 to Control Metabolic Plasticity in Prostate Cancer Progression

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Prostate cancer (PCa) undergoes dynamic metabolic reprogramming during progression, shifting from citrate oxidation in early-stage tumors to glycolysis in advanced, treatment-resistant states. Phosphoglycerate kinase 1 (PGK1), a glycolytic enzyme, has emerged as a key metabolic regulator with additional roles in oncogenic signaling and therapeutic resistance. However, its precise role in driving metabolic adaptation and resistance in PCa remains unclear. This study aims to elucidate PGK1's role in driving metabolic adaptation in PCa and assess its potential as a therapeutic target to overcome treatment resistance. Our preliminary data demonstrate that PGK1 inhibitors significantly reduce cell viability in 22Rv1 and N2P1 PCa cell lines, as shown by MTT assays. Additionally, colony formation assays reveal impaired long-term proliferative capacity upon PGK1 inhibition, while transwell assays indicate a marked reduction in cell migration and invasion. These findings suggest that PGK1 is critical for maintaining the aggressive phenotype of late-stage PCa. To further elucidate the molecular mechanisms underlying PGK1-mediated metabolic adaptation, we will investigate its role in regulating key metabolic and survival pathways. Future studies will explore PGK1 inhibition in vivo to assess its therapeutic efficacy in overcoming treatment resistance. By targeting PGK1-driven metabolic reprogramming, this study aims to provide novel insights into the metabolic vulnerabilities of advanced PCa and establish PGK1 as a promising therapeutic target.

Targeting the Inhibition of PLK1 and HIF1A Signaling to Combat Prostate Cancer

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Hypoxia-inducible factor 1 (HIF1) signaling pathway plays a key role in cancer progression by enhancing glycolysis through activating the transcription of glycolytic genes. The stability and activity of HIF-1alpha are regulated by various post-translational modifications, such as hydroxylation, acetylation, and phosphorylation. Polo-like kinase 1 (PLK1) is an evolutionary conserved Ser/Thr kinase which is known for its roles in cell cycle regulation and it is predominantly expressed in G2/S and M phase. PLK1 is overexpressed in many cancers, which is associated with poor prognosis, making PLK1 an attractive target for cancer therapy. Herein, we demonstrated that the phosphorylation of HIF1A by PLK1 promotes the degradation of HIF1A in a dose dependent manner. In the presence of PLK1, the WT and 3D mutant are not as stable as 3A mutant as demonstrated by the CHX experiment. The dual luciferase experiments also indicated that 3A can increase more the HRE element activity compared with WT and 3D. The cell lysates stable expressing HIF1A (WT, 3A, 3D) were harvested for Western Blot and the results demonstrated that, AR signaling pathway is inhibited in 3A mutant cells. SYP, OC2 and PLK1 expression level are increased in 3A mutant compared with WT and 3D mutant cells. In the C4-2 PLK1 OE or shPLK1 Tet induced cells, overexpression of PLK1 inhibit HIF1A expression, while knockdown PLK1 increased the HIF1A protein levels. And real-time PCR also performed that HIF1A mRNA levels have no changes while PLK1 OE or knockdown. Meanwhile, pharmacologically inhibit PLK1 kinase activity, increased the protein levels of HIF1A in 22RV1 and C4-2 cells. Moreover, the proliferation assay shows that compared with the WT and 3D mutant, the HIF1A 3A has a higher proliferation rate when the cells were treated with Enzalutamide. The OCAR assay also indicated that, compared with 3A mutant, WT and 3D mutant have an increased oxygen consumption rate which means stable expressing 3A cells have a higher glycolysis and an inhibited mitochondria function in C4-2 cells. Taken together, PLK1 inhibition alone would have side effect on targeting prostate cancer therapy as it increases the protein levels of HIF1A. Our findings highlight the promise of PLK1 inhibitors alongside with HIF1A inhibition as a potential prostate cancer treatment avenue, warranting the clinical investigation.

Targeting the Pros1/Mer/PTP1b Axis to Improve the Efficacy of Chemotherapy and Radiotherapy in Melanoma

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Resistance to chemotherapy and radiotherapy is a substantial challenge in the treatment of solid tumors. Although new checkpoint inhibitors have dramatically changed the therapeutic landscape of advanced melanoma, many patients still receive other forms of therapy after failing to respond to immunotherapy. Targeting the innate immune response to improve therapy effectiveness is a promising, yet understudied area. Recently, we identified cancer-secreted Pros1 as a mediator to limit immune activation inside the tumor microenvironment. By sequestering Stat1 in the cytoplasmatic Mer/Ptp1b complex, Pros1 prevents macrophage activation by Damage Associated Molecular Patterns (DAMPs) released by chemotherapy and radiotherapy. We found that Pros1-deficient murine syngeneic melanoma tumors are more sensitive to cisplatin, likely because of higher levels of immune activation within the tumor microenvironment. By targeting the Pros1/Mer/Ptp1b signaling axis using the inhibitor BVT948, macrophage ability to respond to chemotherapy and radiotherapy-released DAMPs was substantially increased. BVT948 was able to increase the efficacy of cisplatin, vemurafenib and radiotherapy in multiple preclinical melanoma models, leading to 70-90% reductions in tumor growth. Increased immune infiltration was detected following cisplatin and BVT948 combination therapy, with an overall increase in pro-inflammatory (M1) macrophages. Depleting phagocytes, like macrophages, within the tumor using clodronate liposomes eliminated the combination therapy suppression of tumor growth. In conclusion, we have identified a novel mechanism through which cancer cells suppress innate immune activation inside the tumor. Co-treatment with a Ptp1b inhibitor may represent a strategy to increase the efficacy of chemotherapy and radiotherapy in patients with advanced melanoma.

The Effect of Fatty Acid Synthase Inhibition on mTOR Signaling in Colorectal Cancer

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Cancer cells undergo metabolic alterations to support their increasing needs for growth and proliferation. Fatty acid synthase, FASN, a key enzyme in the fatty acid biosynthesis pathway responsible for convering acetyl-CoA and malonyl-CoA to palmitate is frequently upregulated in cancer. Previous studies have shown that upregulation of FASN and de novo lipid biosynthesis promote tumor progression and metastasis, thus providing a strong rationale for targeting FASN using small molecule inhibitors for cancer therapy. In this study, we determined the effect of FASN inhibition on modulating mTOR/Akt signaling in colon cancer. To inhibit FASN activity, we treated colon cancer cells with small molecule inhibitors of FASN including TVB-2640 and TVB-3664. Interestingly, FASN inhibition induced a marked increase in malonylation of cellular proteins as a consequence of increased cellular pool of malonyl-CoA. Comparing to other post-translational modifications, only a small number of substrates have been reported and the functional importance of protein malonylation remains largely unknown. Here, we showed that mTOR becomes malonylated upon FASN inhibition in colon cancer cells. The malonylation of mTOR decreases its kinase activity as shown by decreased phosphorylation its substrate p70S6K. As S6K functions as a negative feedback regulator of AKT, we found that FASN inhibitor-mediated inhibition of the mTOR/p70S6K signaling axis removes its negative regulation imposed on AKT. The activation of AKT also leads to increased phosphorylation and activation of ACLY, a known substrate of AKT, in FASN inhibitor treated colon cancer cells. Functionally, we demonstrated that combining FASN inhibitors with inhibitors of AKT or ACLY synergistically inhibits cell growth *in vitro*. Together, this study identifies a novel mechanism by which of FASN inhibition regulates mTOR and AKT signaling through protein malonylation. Our findings suggest that co-targeting AKT and ACLY may enhance the efficacy of FASN inhibitors in treating colorectal cancer.

The Phosphorylation-Dependent Activity of Deubiquitinase USP7 in the Shoc2 Signaling Complex

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The ERK1/2 signaling cascade is a tightly regulated fundamental signaling cascade. The Shoc2 scaffold protein plays a pivotal role in modulating ERK1/2 signaling by facilitating the assembly of signaling complexes and amplifying ERK1/2 activity. Our previous studies revealed a dynamic interaction between Shoc2 and the deubiquitinase USP7. USP7 binds to Shoc2 when the ERK1/2 pathway is activated and acts as a molecular switch to control the E3 ubiquitin ligase HUWE1 activity in the Shoc2 complex. Here, we demonstrate that activation of the ERK1/2 pathway leads to Src-mediated tyrosine phosphorylation of USP7, enhancing USP7 interaction with Shoc2.

The genetic mutations in the USP7 gene result in a rare neurodevelopmental disorder, Hao-Fountain Syndrome (HAFOUS). In fibroblasts derived from a HAFOUS patient harboring variant M225I, we found that USP7 was constitutively bound to Shoc2. Furthermore, the phosphorylation levels of the USP7 variant M225I were also markedly elevated. Our additional studies revealed that additional USP7 HAFOUS variants within the catalytic domain of USP7 also impact the USP7-Shoc2 interaction and the levels of the USP7 phosphorylation. These findings suggest that dysregulation of the USP7-Shoc2-HUWE1 axis potentially contributes to the pathogenesis of HAFOUS.

Our research provides novel insights into the molecular mechanisms underlying the regulation of ERK1/2 signaling and the pathogenesis of HAFOUS. These studies may have implications for the development of therapeutic strategies targeting HAFOUS.

ABSTRACT 54

Basic Science

The Role of Eukaryotic Elongation Factor 2 Kinase in Sex Disparities in Melanoma Immune Response and Tumor Persistence

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Melanoma exhibits strong sex disparities, with males experiencing higher incidence, more aggressive disease, and worse outcomes. Our meta-analyses of national cancer databases reveal that eEF2K expression distribution differs by sex, with males exhibiting a broader range and general higher expression capacity. High eEF2K expression is associated with poorer survival in males, whereas females show a relative survival advantage, highlighting its role in sex-differential melanoma progression. Functionally, we show that dihydrotestosterone (DHT) upregulates eEF2K expression, while androgen receptor (AR) inhibition reduces its levels, suggesting AR signaling as a driver of these differences. Additionally, eEF2K suppresses melanoma antigenicity, potentially contributing to immune evasion and sex-specific survival disparities. Ongoing treatments of a YUMM1.7 wildtype and eEF2K- knockdown syngeneic mouse model with enzalutamide will further evaluate AR-eEF2K interactions *in vivo*. These findings position eEF2K as both a prognostic marker and a therapeutic target in melanoma sex disparities.

The Role of Glucose-6-Phosphate Dehydrogenase (G6PD) in Platelet Activation and Hemostasis

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Introduction: Glucose-6-phosphate dehydrogenase (G6PD) is a key enzyme in the pentose phosphate pathway (PPP), generating NADPH to counteract oxidative stress and producing pentoses for nucleotide synthesis[1]. G6PD deficiency—the most prevalent enzymopathy worldwide—affects over 400 million people and is primarily linked to hemolysis due to reduced NADPH in red blood cells (RBCs)[2]. While its impact on RBCs is well established, emerging evidence suggests G6PD deficiency may also influence platelet function, particularly in older individuals with cardiovascular disease[3]. However, its specific role in platelet activity remains underexplored.

Aim/Objective: This study investigates how G6PD deficiency affects platelet function using a G6PD Mediterranean (Med) mutation conditional knock-in mouse model and G6PD knock-out (KO) mice.

Methods: We examined platelet function by inhibiting G6PD activity in wild-type mouse platelets and assessing Ca^{2+} influx. Additionally, platelet count, morphology, Ca^{2+} influx, clot contraction, and thrombin-stimulated activation were analyzed in G6PD KO mice. Tail bleeding time was also measured. Similar parameters were evaluated in G6PD Med-mutant mice.

Results and Discussion: G6PD inhibition reduced Ca^{2+} influx in a dose-dependent manner. G6PD KO mice exhibited impaired platelet function, with decreased Ca^{2+} influx, reduced clot contraction, and slightly prolonged tail bleeding time, suggesting weakened platelet activation. In contrast, G6PD Med-mutant mice displayed enhanced platelet function, characterized by increased clot contraction and shortened bleeding time.

Conclusion: G6PD deficiency compromises platelet function by reducing Ca^{2+} influx and clot contraction, while the G6PD Med mutation appears to enhance platelet activity, potentially increasing cardiovascular risk. These findings highlight the role of G6PD in platelet physiology and its implications for thrombotic disorders.

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

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Relapsed T-cell Acute Lymphoblastic Leukemia (T-ALL) patients have a dismal prognosis, with 5-year overall survival rates of 10% for adults and 36% for children. Relapse is attributed to chemotherapy's inability to eliminate Leukemia Stem Cells (LSCs). Investigating LSCs in T-ALL is particularly challenging due to the absence of well-defined surface markers that distinguish LSCs and their low frequencies in both murine models and patient samples.

Here, we leveraged a transgenic zebrafish model of *rag2-myc* driven T-ALL to overcome these limitations. Our lab previously generated leukemia lines from this model with an LSC frequency of ~ 10%, markedly higher than the <0.01% observed in murine models. Using this system, we screened over 770 FDA-approved drugs *in vivo* using >2,500 syngeneic CG1 zebrafish to identify compounds that target self-renewal. From this screen, we identified Amiloride, an inhibitor of the Sodium Hydrogen Exachanger-1 (NHE1), as a potent LSC-targeting drug. Validation studies in human T-ALL cells and a Limiting dilution assay *in vivo*, confirmed Amiloride significantly reduce the frequency of LSCs *(p=0.0026)*. Notably, NHE1 has not been previously linked to self-renewal in hematologic malignancies, presenting a novel therapeutic strategy to inhibit LSCs.

Clinical relevance was supported by the analysis of patient datasets, which revealed that high expression of *SLC9A1* (encoding NHE1) correlates with worse survival (HR = 2.05, $p = 0.07$). Additionally, *SLC9A1* expression is significantly elevated in relapsed T-ALL samples compared to primary cases ($p = 0.0005$), further implicating NHE1 in leukemia progression. Functional validation using shRNA knock down (KD) of NHE1 in human T-ALL cells led to a >70% decrease ($p< 0.0001$) in self-renewal capacity and a concurrent downregulation of key self-renewal genes, including *Nanog* ($p< 0.0001$), *CD7* ($p< 0.0001$) and *CD44* ($p= 0.0079$). NHE1 inhibition also induced a significant G1 cell cycle arrest and downregulated critical leukemia self-renewal pathways, including MYC targets, KRAS, Hedgehog, and Notch signaling, as revealed by RNA sequencing.

Mitochondrial function emerged as a key mechanism underlying NHE1's role in LSC self-renewal. Comprehensive multi-omics profiling, including proteomics, transcriptomics, and metabolomics of the NHE1 KD cells identified a strong downregulation of oxidative phosphorylation and mitochondrial translation. Functional validation using seahorse mitochondrial stress test confirmed that Amiloride inhibited mitochondrial function in several T-ALL cell lines while reducing mitochondrial footprint ($p< 0.0001$) and branching ($p=0.0028$). Similar mitochondrial defects were observed in the NHE1 KD cells, suggesting that NHE1 inhibition disrupts leukemia metabolism.

To investigate the *in vivo* impact of NHE1 KD, we xenografted immunodeficient mice with human T-ALL KD or Scr control cells. Mice injected with KD cells exhibited significantly reduced bone marrow engraftment of human CD45+ cells ($p < 0.0001$) and increased apoptosis, as evidenced by elevated cleaved caspase-3 (CC3) levels ($p < 0.0001$). This increase in apoptosis was likely driven by the observed mitochondrial dysfunction in KD cells ($p < 0.0001$).

Overall, this study capitalizes on a high LSC frequency zebrafish model to identify Amiloride as a physiologically relevant LSC-targeting agent. Moreover, we are the first to establish NHE1 as a critical regulator of self-renewal in hematologic malignancies, potentially through its role in cellular energy homeostasis. These findings provide a strong rationale for targeting NHE1 as a novel therapeutic strategy in T-ALL.

The SRG RAT Supports in vivo Human Cell Xenotransplantation through Enhanced Tumor Microenvironment Interactions

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WITHDRAWN

The SRG Rat, an Immunodeficient Model for Orthotopic Glioblastoma, Diffuse Intrinsic Pontine Glioma (DIPG) PDX, and Intracranial Metastatic Breast Cancer PDX Tumors

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WITHDRAWN

Toxicity of Reference and Commercial Large Cigars, Cigarillos, and Filtered Cigars

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Tobacco smoke from cigarettes and various types of cigars is a mixture of compounds that have been linked to lung and oral cancers as well as other cancers. While the carcinogenicity of cigarette smoke has been well known for years, the other products that generate tobacco smoke have not been well studied. Part of the reason for the lack of research is the large variability in other products such as cigars. The Center for Tobacco Products at the Kentucky Tobacco Research and Development Center in the University of Kentucky College of Agriculture has recently produced and made available reference large cigars, cigarillos, and filtered cigars. The KTRDC has also performed chemical and physical analysis of these reference cigars along with comparable commercial products. Our lab in the Department of Toxicology and Cancer Biology in the University of Kentucky College of Medicine has performed toxicology experiments using particulate phase condensates (generated by the KTRDC) of these reference and commercial cigar products. So far, cell proliferation and viability and AHR gene reporter assays have been done. As expected, these experiments show a range of effects on cell proliferation and viability and AHR-related gene expression based on the total particulate matter (TPM) and chemical composition of the tobacco smoke generated. In examining the toxicity of filtered cigars, which some studies indicate are consumed like cigarettes, we found the reference and at least some commercial filtered cigars to be more toxic than the reference cigarette. This suggest that additional regulation of these commercial filtered cigars may be warranted.

TP53 Mutant Cooperates with H3K27M to Drive Radioresistance and Tumor Progression in DIPG

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Background: Diffuse Intrinsic Pontine Glioma (DIPG) is an aggressive pediatric brainstem tumor, characterized by the presence of H3K27M mutation, which disrupts histone methylation and leads to widespread epigenetic dysregulation, and recurrent TP53 mutations, which contribute to radioresistance. Analysis DIPG patients' samples identified several TP53 mutations with a high recurrence, most frequently occurring in the DNA binding domain of p53. *The overall objective of this study is to determine how TP53mut and H3K27M collaborate to drive DIPG survival after treatment.* As a first step, we assessed whether p53 mutants retain DNA binding capacity or exhibit gain-of-*function* under DNA damage conditions.

Methods: We generated expression constructs of TP53 mutants for protein expression analysis in HEK293 cells. These constructs were also co-transfected with histone H3.3 or H3.3K27M to study changes in TP53 RNA expression levels by RT-PCR. We performed ChIP-qPCR to determine the highest TP53wt response under DNA damage by irradiating transfected HEK293 cells. The TP53-DNA binding capacity was then tested for all the mutants by isolating nuclear fraction from transfected HEK293 cells and assessed by EMSA assay. Lastly, we used ChIP-qPCR to determine TP53mut-DNA binding capacity under DNA damage.

Results: TP53wt and mutants have a different protein expression profile, and RNA levels vary in the presence of histone mutant H3.3K27M compared to H3.3. TP53wt binds to its target genes p21 and MDM2 under DNA damage after 6hs post irradiation using 8Gy dose. EMSA assay shows a significant binding of TP53wt and mutants (R248W, R273C, V157F), while R175H, R342X, S241F have either low or no DNA binding capacity. Binding activity for some mutants varies under DNA damage, such as S241F, which increases the binding activity when compared to R175H which does not bind DNA under normal or DNA-damage conditions.

Conclusions: DIPG patients have recurrent TP53 mutations, which influence expression and DNA binding activity. TP53wt and mutant proteins have a different expression profiles. The strongest TP53wt response after DNA damage is achieved after 6hs post irradiation at an 8Gy dose. EMSA assays shows strong binding capacity for TP53wt, and R248W, R273C, a low binding capacity for R342X, S241F, and non-binding for R175H. Under DNA damage conditions, TP53wt and the mutants R248W, R273C, S241F bind to DNA as a canonical response, while this interaction decreases significantly when compared with non-stressed HEK293 cells. The TP53 mutant R175H does not bind DNA under DNA damage conditions in HEK293 cells, but its function is still under study to elucidate its cooperation with H3K27M in radioresistant patients.

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Tumor Derived SPP1 Promotes Macrophage Mediated Gastric Carcinomatosis

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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. Peritoneal spread or gastric carcinomatosis is the primary cause of death in majority of patients, with a median survival of 6-9 months. Several studies have shown that macrophages promote tumorigenesis in pancreatic, liver and colon cancer. However, the role of macrophages in gastric carcinomatosis is poorly understood. SPP1 (osteopontin) is a glycoprotein involved in diverse biological functions. It is secreted by benign and malignant cells. It's role in macrophage polarization and function is context dependent and not described in gastric carcinomatosis.

We show that tumor associated macrophages (TAMs) are a predominant cell type in the microenvironment in both mouse and humans. These macrophages exhibit an activated phenotype expressing MHCII, Arg-1 and CD206. Targeted macrophage depletion diminished tumor growth. Conditioned media from tumor cells polarized naïve macrophages into alternatively activated phenotype by inhibiting TNF-α, IL1β, CD86 and upregulating Arg-1. RNA sequencing revealed differential expression of genes related to chemotaxis, wound healing and anti-inflammatory phenotype. SPP1 is upregulated in gastric cancer and is associated with diminished overall survival. SPP1 promotes macrophage migration and polarization. Further studies will investigate mechanistically how SPP1 alters macrophage activity and explore potential therapeutic vulnerabilities in gastric cancer.

ABSTRACT 62

Clinical

Assessment of Patient Reported Clinical Outcomes of Risk-Adapted Stereotactic Body Radiotherapy (SBRT) Treatment of Peripherally Located Lung Tumors

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Purpose: Lung SBRT became a standard of care for localized non-small cell lung cancer (NSCLC) or metastatic lung cancer patients. However, radiation-induced chest wall pain (CWP) or rib fracture (RF) is a concern for peripherally located lung SBRT patients. Herein we report our long-term tumor local control (TLC) rates and toxicity profiles in lung SBRT patients treated per RTOG0813 protocol's criteria and 6MV flattening filter free (FFF) volumetric modulated arc therapy (VMAT) plans.

Materials and Methods: In this IRB approved retrospective study, we have included 123 inoperable lung SBRT patients with either primary NSCLC (n=92) or isolated thoracic metastatic lesion (n=31) treated with a risk-adapted 50–55 Gy in 5 fractions prescribed to 70-80% isodose line. Patients were immobilized on VacLoc bag and 4D-CT based highly conformal SBRT plans were generated via 6FFF beam, non-coplanar partial VMAT arcs and AcurosXB algorithm. Treatments were delivered every other day via pre-treatment CBCT guidance and *6dof* couch corrections. Plan quality and delivery efficiency were reported. Outcomes reported include TLC rates, chest wall pain, rib fracture and pulmonary toxicity on physical exam followed by post SBRT diagnostic CT scans per CTCAE v5 criteria. Median follow-up interval was 16.2 ± 12.8 (3–66) months.

Results: Mean planning target volume (PTV) was 33.2 ± 31.1 (4.7–201.3) cc. All SBRT plans met RTOG0813 criteria for tumor coverage, conformity, and organs at risk (OAR) sparing: Average maximum dose to skin (18.5 Gy), ribs (46.7 Gy) and dose to 1 cc of ribs (35.0 Gy). Mean values of conformity and gradient indices, D_{2cm} and lung V_{20Gy} were 1.02 ± 0.05, 4.2 ± 0.9, 51.8 ± 5.8% and 2.6 ± 2.2%. Average beam on time was 4.5 ± 1.2 min. All patients tolerated lung SBRT treatment. Mean couch time including CBCT imaging and set up correction was < 15 min. Of 123 lung SBRT patients treated, 112 had an adequate post-treatment chest CT scan to assess treatment response; among them TLC was achieved in 101/112 (90.2%) as reported their tumor shrinkage in follow up CT; 18 (16%) patients presented with locoregional nodal failure, 19 (17%) metastatic patients had new distant metastasis. 17 (15.2%) patients who were smoker, had COPD showed asymptomatic radiographic changes in lungs with no Grade 3+ toxicity; 6 (5.4%) patients reported chest wall pain within 3 months of SBRT and 4 (3.6%) patients presented with rib fracture at 4.0–48.0 months; these patients were managed with steroids or gabapentin.

Conclusions: Risk-adapted SBRT delivery of peripheral lung lesions via 6FFF VMAT was safe, fast, efficacious, and convenient treatment with promising TLC rates, acceptable acute and late toxicity profile. Rapid delivery of VMAT lung SBRT could reduce intrafraction motion error due to shortness of breath, coughing/back pain, making geographic miss unlikely improving patient comfort and clinic workflow. Detail data analysis of Kaplan Meier curves for longer follow up times including dosimetric factors associated with rib fracture or chest wall pain in this cohort is underway.

Disparities among Breast Cancer Patients in Appalachian Kentucky: Are We Making Progress?

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Background: Despite lower rates of breast cancer in Appalachian counties in Kentucky (KY) compared to the rest of KY, the mortality rate for Appalachian breast cancer patients has historically been significantly worse. We aimed to analyze differences in patient, tumor, and treatment factors in breast cancer patients from KY Appalachian counties compared to those from non-Appalachian counties.

Methods: The KY Cancer Registry, a population-based central cancer registry, was queried for all patients with a new diagnosis of stage I-IV breast cancer between 2010 and 2020. Demographic, clinicopathologic, treatment, and outcomes data were analyzed.

Results: Of the 4,100 KY patients with invasive breast cancer, 2,090 (50.98%) were from an Appalachian county. Appalachian patients were more likely to be white, have Medicaid/Medicare, have a higher BMI, and use tobacco (all $p<0.05$). They were less likely to present with local breast cancer (60.86% vs 63.98%) and more likely to present with locally advanced (30.19% vs 28.56%) or metastatic breast cancer (8.52% vs 7.36%) ($p=0.038$). Hormone receptor status was similar between groups. When stratified by stage, there were no significant differences in receipt of chemotherapy, endocrine therapy, or radiation for Appalachian versus non-Appalachian patients. Appalachian patients were significantly less likely to get surgery for localized disease (66.2% vs 69.4%) but more likely to get surgery for locally advanced (30.8% vs 28.6%) or metastatic disease (2.6% vs 2.1%) ($p=0.021$). Despite an increase in receipt of surgery in more advanced stages, Appalachian patients were not more likely to undergo more aggressive axillary surgery. The mastectomy rate was significantly higher in Appalachian patients (52.3% vs 45.7%, $p < 0.0001$). There was also a higher rate in the use of neoadjuvant chemotherapy in Appalachian patients (31.3% vs 23.8%, $p < 0.001$). Unadjusted survival analysis indicated that the risk of death was 17% higher for Appalachian patients ($p=0.019$); however, after adjusting for stage there was no significant survival difference between cohorts.

Conclusions: Appalachian patients are more likely to present with more advanced breast cancer. However, survival in this study population now matches those of patients in non-Appalachian counties when adjusting for stage suggesting some progress may have been made in the quality of treatment for Appalachian breast cancer patients. Further investigation into interventions that decrease the number of Appalachian patients presenting with later stage disease continues to be needed.

How effective are HPV Vaccination Interventions Among Young Adults (18-26 years)

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Background. Human Papillomavirus (HPV) is the most common sexually transmitted infection. Persistent infections can develop into HPV-related cancers including cervical and Penile cancer. HPV vaccination could prevent more than 90% of cancers caused by HPV from ever developing. HPV vaccination was recently expanded to all persons through age 26 years, who had not been previously vaccinated, but uptake remains low.

Aim: This literature review evaluates HPV vaccination interventions targeting young adults (18-26 years).

Methods. We searched PubMed, CINAHL, and Google Scholar using a combination of Mesh terms "human papillomavirus," "HPV," "vaccine/s," "interventions," "effectiveness," "prevention," "uptake," and "young adult" for intervention studies published between 2014-2024. Two members of the team reviewed each article. Studies were included if they focused on HPV vaccination promotion and were conducted in the United States.

Results. We identified 12 articles that met inclusion criteria, nine were randomized control trials, two pilot projects and one was a quasi-experimental study. Our review found that various interventions have improved HPV vaccination uptake including decision support tools, printed educational materials, psychosocial interventions, informational, web-based, social media campaigns, and information-motivation-behavioral skills. Interventions took place in different settings including community and care clinics, college campuses, and through social media. Participants showed improved awareness of primary and secondary cancer prevention approaches. However, not all interventions showed significant effects on HPV vaccination uptake.

Conclusions. Interventions that combined multiple methods such as psychosocial and education strategies, tended to be more impactful. A multifaceted approach is recommended to increase HPV vaccination among young adults.

Impact of Social Support on the Quality of Life of Patients Diagnosed with Prostate Cancer: A Systematic Review

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Background: Prostate cancer diagnosis and treatment impact patient's well-being. The diversity of treatments leads to unfavorable experiences increasing psychological strain and threatening their quality of life. However strong social support has been associated with improved quality of life.

Aim: This review aimed to evaluate the impact of social support on the quality of life of prostate cancer patients.

Methods: Following PRISMA guidelines, we searched PubMed, CINAHL, and PsycINFO databases and included studies from 2001 to 2024 that assessed social support's impact on prostate cancer patients' quality of life, excluding those with other conditions. The JBI critical appraisal checklist was used to evaluate the studies' methodological quality.

Results: Seven (6 quantitative and 1 qualitative) articles were included in the review. The review highlighted that higher baseline social support was significantly associated with better quality of life. Satisfaction with social support was strongly correlated with a higher quality of life. Prostate cancer patients with stronger partner support and more diverse support networks reported better sexual and physical quality of life While patients with unmet support needs had worse hormonal, sexual, and mental health. Findings from the qualitative study emphasized that patients valued unstructured social support from families.

Conclusion: This review underscores the significant role of social support in improving the quality of life for prostate cancer patients. Social support should be an integral part of treatment plans and providers should refer patients to support services. Strengthening family connections through communication and involvement can improve emotional support and quality of life for prostate cancer patients.

In the Eye of the Beholder: Utilizing Lean Process Improvement of Uveal Melanoma Brachytherapy Service Line to Expand Rural Oncology Equity

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Purpose/Objective(s): Uveal melanoma (UM) is categorized as a NIH rare cancer, with an estimated 3,500 yearly cases in the United States (1). At diagnosis, one third of patients present with an asymptomatic, morphologically evolving nevus (2). Plaque brachytherapy is an effective but complicated modality in the management of non-metastatic UM. Successful plaque brachytherapy requires collaboration of an ophthalmic oncologist, medical physicist, and ocular radiation oncologist.

Multi-disciplinary management of UM is complicated by social determinants of health in our tri-state region with under- or uninsured citizens, low health literacy, and transportation obstacles. We aim to improve the metrics of patient compliance, accurate information exchange for planning, and reduce overall treatment time to enhance rural oncology equity.

Materials/Methods: We performed a comprehensive procedural analysis under radiation oncology leadership to identify areas of improvement. We consolidated the complex workflows via a Kaizen approach to operationalize work-up efficiency and enhance compliance. Subsequently, we instituted 1) same day staging CT and MRI, 2) MRI orbital protocol with dedicated neuroradiologist interpretation, 3) newly designed and standardized schematic for ocular measurements, and 4) same day safety consult with radiation physicist.

Results: In patients for whom this revised lean workflow has been implemented, we have seen nearly 100% compliance from consult to brachytherapy execution. Time from initial consultation to plaque insertion is now often within 4 weeks.

Conclusion: A UM plaque program with this new lean approach allows teams to streamline care by decreasing medical errors and miscommunications. Streamlining is a promising start to increase compliance, reduce trips for patients, and compressed treatment time for a potentially life-limiting disease, which when treated accurately and expediently has 5-year overall survival 85% (1). This lean approach significantly improved efficiency and access to UM brachytherapy for our rural patients. Kaizen incremental improvement method was taken for continual improvement with further goals including same day multi-disciplinary consult, tele-health, standardized radiology report, patient educational modules, and transition from COMS-based plaque planning to plaque simulator planning system. Stepwise streamlining also opens the door for in-house trial generation, cooperative trial enrollment in a region that historically has close to zero protocol enrollment. Future goals include expanding eye health outreach and mobile screening programs for rural communities. To the best of our knowledge, this is the first formally reported workflow refinement in the literature with regards to eye plaque brachytherapy in the treatment of non-metastatic UM.

Intraoperative Optical Imaging of Tissue Hemodynamic Variations in Mastectomy Skin Flaps for Identifying Ischemic/Hypoxic Tissues

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Background: Breast cancer is the most commonly diagnosed cancer among women worldwide, with over 2.3 million new cases each year. In breast cancer treatment, mastectomy followed by reconstruction is a common surgical approach aimed at restoring form and quality of life. However, one of the most frequent complications is mastectomy skin flap necrosis (MSFN), which affects 5-30% of patients and is primarily caused by insufficient tissue perfusion during or after surgery. Intraoperative fluorescence angiography (SPY-PHI) has been used for predicting MSFN. However, several issues limit its wide acceptance, including allergic reaction, short time-window for observation, and high cost for equipment and supplies. We report an innovative, inexpensive, dye-free, and depth-sensitive multiple wavelength speckle contrast diffuse correlation tomography (MW-scDCT) that enables noncontact imaging of tissue hemodynamic variations during surgery.

Methods: Six patients undergoing mastectomies were imaged sequentially by the SPY-PHI and MW-scDCT. The MW-scDCT utilizes scanning laser point sources at 690 nm and 830 nm and a CMOS camera to capture intensity images at multiple source positions. Tissue blood flow maps were reconstructed by quantifying diffuse laser speckle contrasts while tissue blood oxygenation maps were reconstructed by quantifying light intensity reductions at two wavelengths.

Results: For the first-time blood flow and oxygenation were simultaneously imaged during the surgery to identify ischemic/hypoxic tissue in mastectomy skin flap. The hemodynamic images obtained by the MW-scDCT and SPY-PHI in 6 patients were generally consistent. Particularly, an ischemic skin flap in one patient (P6) was detected by both SPY-PHI and MW-scDCT during surgery, indicating the risk of post-surgery necrosis. As a result, the implant was not performed in P6.

Conclusions: The MW-scDCT offers a groundbreaking noninvasive imaging method that simultaneously measures blood flow and oxygenation, enabling the identification of ischemic or hypoxic tissues during surgery. With broader adoption and more patient data, MW-scDCT has the potential to become a cost-effective and noninvasive tool for intraoperative evaluation of skin flap viability, aiding in the prediction and prevention of MSFN.

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Leveraging Mitochondrial Metabolic and Energetic Differences to Target Radiation and Hypoxic Adaptation

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Prostate Cancer (PCa) is the second leading carcinoma in men and radiation therapy (RT) is one of the primary treatment options. Despite its clinical prevalence, post-radiotherapy recurrence and radiation resistant PCa (RR-PCa) remain significant challenges. Studies indicate PCa cells utilize tumor microenvironmental conditions, such as hypoxia, and upregulation of mitochondrial oxidative stress management enzymes, such as Peroxiredoxin 3 (PRX3), to evade treatment efficacy. Our western blot analysis show RR-PCa increased expression of PRX3. PRX3 is a major scavenger of mitochondrial ROS which directly links it to the mitochondrial metabolism through the TCA cycle enzyme, Aconitase 2, which can be deactivated by mitochondrial reactive oxygen species (mt-ROS). RR-PCa may use hypoxia to resist treatment by upregulating HIF-1a, which has been implicated in treatment resistance, or by decreasing mtROS generated from oxygen metabolism. HIF-1a upregulation has also been linked to PRX3 overexpression. This indicates that overexpression of mtROS regulators (ex: PRX3) may facilitate mitochondrial metabolism adaptations, and hypoxia induced radiation resistance. Our human biopsy data suggests that PCa increase mitochondrial quantity through mitochondrial biogenesis (mito-biogenesis) to facilitate re-population after RT. To investigate this, we developed RR-PCa from human (PC3) and mouse (RM-1) PCa cell lines (RR-PC3, RR-RM-1). Mouse derived PCa and RR-PCa cells (RM-1 and RR-PM-1, respectively) can form allographic tumors in immunocompetent mice. RR-RM-1 cells demonstrate lower sensitivity to RT, altered morphology, increased glucose dependency, and higher mitochondrial quantity and mass compared to parental RM-1 cells. Furthermore, we conducted 2D Stable Isotope Resolved Metabolomics (SIRM) analysis on RR-PC3 and PC3 cells, using 13C-glucose. The results suggest that RR-PC3 cells have differential metabolic rewiring associated with mitochondrial metabolic pathways (TCA Cycle, glutaminogenesis, etc). RR-PC3- and RR-RM-1-derived 3D spheroids also show enhanced growth under 1% O2, indicating correlations between treatment resistance and hypoxic adaptation. To overcome RR-PCa, we propose that treatment with mt-ROS generating agents consecutively with RT will facilitate mtROS overload and increased RT efficacy. Azithromycin (AZM), Thiostrepton (TS), and the novel AuPhos-89 all generate mtROS and mitochondrial disruption via alternative pathways. Using the Seahorse Mito-Stress Test, they significantly inhibited basal, maximal, and ATP-linked respiration in RR-PCa cells correlating with decrease in cell viability. They also show synergy with RT at decreasing PCa and RR-PCa cell viability, even under 1% O2. We believe that using these compounds as a proof of concept, we can show that by targeting mitochondrial oxidative stress regulation and biogenesis, we can sensitize even hypoxia adapted RR-PCa to RT. To further this concept, we plan to use the DTAG system to degrade PRX3, temporarily and selectively, before RT. This may increase RT effectiveness by priming mitochondria for oxidative stress before RT via elevated mtROS levels. We also plan to run 3D SIRM experiments consecutively with RT and redox agent treatment. 3D culture spheroids closer mimic the tumor microenvironment that contributes to hypoxia, treatment variance, etc. These SIRM experiments will give us a greater understanding of the metabolic changes that facilitate radiation resistance and how these treatments may affect those changes. Overall, our findings indicate that modulating mtROS and mitochondrial metabolism can disturb RR-PCa's ability to regrow after RT, even under hypoxic conditions.

Outcomes of Elderly Multiple Myeloma Patients Who Underwent Transplantation Prior to Stratification Based on Frailty Status

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Palumbo et al. conducted a study using the Myeloma Frailty Score Calculator to determine how frailty affected 3-year overall survival (OS) in elderly patients with multiple myeloma (*Blood*, 2015;125:2068). The calculator generates a score based on age, selected co-morbidities, ability to perform activities of daily living, and ability to perform instrumental activities of daily. Score possibilities include 0 (fit), 1 (intermediate-fitness), or ≥2 (frail). In an analysis of 869 patients, the 3-year OS was 84% for fit patients, 76% for intermediate, and 57% for frail patients (Palumbo, 2015). These results indicate how a geriatric assessment can be used as a prognostic marker to guide clinicians when considering aggressive treatments such as chemotherapy or autologous stem cell transplantation in this population (Palumbo, 2015).

The objective is to calculate the frailty score of patients ≥65 years old with multiple myeloma who underwent autologous stem cell transplantation at the UK Markey Cancer Center prior to the routine implementation of a frailty score in determining transplant eligibility. The outcomes based on frailty score were compared to expected outcomes published in the International Myeloma Working Group report (Palumbo et al.).

IRB approval was obtained for a retrospective study of patients ≥65 years old with multiple myeloma who underwent autologous stem cell transplantation or immune effector cell therapy at UK from 2013 to 2023. Demographic and outcomes data were collected through a retrospective chart review. Patients were retroactively assigned a frailty score using a frailty calculator and stratified according to score (frailty score 0 vs 1). All patients were given an ADL score of 6 and an IDL score of 8 when calculating frailty score, as these were institutional requirements for transplantation. Statistical analysis was conducted through the UKMCC Division of Cancer Biostatistics. Fisher's Exact Test was used for survival outcomes and a 2-sample t-test was used for KPS pre-transplant, KPS post-transplant, age, BMI and distance.

112 patients were identified for the study. Patients were stratified by frailty score. 40 received a score of 1 (F1); 72 received a score of 0 (F0). No frail patients proceeded to transplantation. The average age of patients was 68.15 (F1) vs 67.93 (F0); average BMI was 29.46 (F1) vs 28.60 (F0); average distance to treatment center was 52.85 miles (F1) vs 88.29 (F0). Survival at 100 days post-transplant was 100% (F1) vs 97.22% (F0); at 1 y, 97.37% (F1) vs 94.29% (F0); at 3 y, 83.33% (F1) vs 86.54% (F0). The average KPS prior to transplant was 82.89 (F1) vs 83.75 (F0) and 100 days post-transplant it was 81.62 (F1) vs 81.54 (F0).

There was no significant difference in survival between the two cohorts at 100 days post-transplant (F1 100%, F0 97.22%, P= 0.54), 1 y post (F1 97.37%, F0 94.29%, P=0.42), or 3 y post (F1 83.33%, F0 86.54%, P=1.00). Average KPS prior to transplant (F1 82.89, F0 83.75, P=0.55) and 100 days post (F1 81.62, F0 81.54, P=0.97) were similar between groups and not significant. There was no significant difference in the average age (F1 68.15, F0 67.93, P=0.68) or average BMI (F1 29.46, F0 28.60, P=0.42). Although a discrepancy was demonstrated in distance to treatment center (F1 52.85 mi, F0 88.29 mi), it was not significant (P=0.35).

In this population, there was no significant difference in survival at 100 days post-transplant, 1 y, or 3 y post-transplant based on frailty score. When compared to the Palumbo study, UKMCC patients in the F0 and F1 cohorts had improved OS at 3 y. The 3 y OS for F0 patients from UKMCC vs the study population was 86.54% vs 84%, respectively. The 3 y OS for F1 was 83.33% vs 76%, respectively. One source of bias: patients were assigned specific values for ADLs and IDLs based on their documented pre-transplant KPS. However, individual ADLs and IDLs were not used. Additionally, the data collected does not reflect all patients who were referred for transplantation. The study suggests that prior to routine inclusion of the myeloma frailty score in determining transplant eligibility, appropriate patients were selected with comparable outcomes.

Presenting Patient Reported Clinical Outcomes of Gamma Knife Stereotactic Radiosurgery for Pituitary Adenomas and Dosimetric Factors Associated with Post-Radiosurgery Pituitary Function

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Purpose/Objectives: Gamma Knife Stereotactic Radiosurgery (GK SRS) for pituitary adenomas is an adjuvant treatment option to surgical resection. We present our long-term clinical follow up outcomes in patients with pituitary adenomas and post radiosurgery incidence of new onset associated with dosimetric variables treated via GammaPlan (TMR10) on Leksell GK Perfexion^{TM}.

Materials/Methods: In this IRB approved retrospective study, a total of 90 patients who underwent GK SRS treatment to pituitary adenomas: functioning/non-functioning pituitary adenoma (FPA/n-FPA) between 2010 and 2024 were included. Utilizing high resolution contrast enhanced MRI scan, highly conformal GK SRS plans were generated manually or via lightening dose optimizer. Average tumor size was 0.99±1.46 (0.06–10.29) cc. Mean marginal prescription dose to FPA & n-FPA was 24±3 (14–30) Gy and 19±3 (12–25) Gy; prescribed to 50% isodose line. GK SRS plans were evaluated for Paddick's conformity, gradient indices (PCI, GI), maximum dose to optic apparatus and brainstem and normal tissue volume (NTV) around pituitary sella receiving V_{8Gy}, V_{10Gy}, V_{12Gy}, and V_{14Gy}. Patients were followed up for treatment response and hypopituitarism post GK in 3-month intervals.

Results: For highly conformal GK SRS plans, PCI and GI were 0.58±0.08 (0.45–0.77) and 3.02±0.41 (2.11–4.18). Average maximum dose to optic apparatus and brainstem were 6.5 Gy (maximum up to 9.1 Gy) and 5.9 Gy (18.0 Gy). Mean NTV around pituitary sella receiving V_{8Gy}, V_{10Gy}, V_{12Gy}, and V_{14Gy} were 5.29, 3.60, 2.57, and 1.85 cc. 74/90 patients had clinical outcome with mean follow up intervals of 48±41 (4–162) months. A total of 77 treatments with a 25:51 male to female ratio were evaluated; 3 repeat GK SRS. Median age was 47±14 (18–77) years. Of these, 34 (43.4%) were treated for FPA and 37 (48.7%) treated for n-FPA. Tumor local control was achieved for a total of 64 (83.1%) patients with GK, while 13 (16.9%) did not respond. 10 of 13 were FPA patients and all females. 3 of 13 underwent a second course of GK (in 6-24 months) were all female with FPA and gained local tumor control. One patient had blurred vision post GK; no patients presented with brainstem toxicity. Follow up reports showed onset of all 3 axes of hypopituitarism in 18 (24.3%) FPA patients at on average 48 months post GK; they were managed with hormone replacement. 7 of 18 FPA patients reporting hypothyroidism were female. Linear correlation between hypothyroidism and maximum dose to pituitary tumor (p=0.03) but no correlation with NTV was seen.

Conclusion: Our long-term clinical follow up results of GK SRS to pituitary adenomas is a highly effective treatment with less radiation induced hypopituitarism including reirradiation. Majority of FPA were female patients who also developed hypopituitarism after GK and related to maximum tumor dose. These clinical findings will be useful to future pituitary GK SRS plan optimization. Detail data analysis of pituitary deficit post Gamma Knife SRS in all 3 hormonal axes and Kaplan Meier prediction curves for local control, acute and late effect is warranted.

Phase II Clinical Trial of Cesium-131 Low-Dose Rate Interstitial Brachytherapy as an Organ-Preserving Irradiation Technique for Recurrent Cervical and Uterine Cancer

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Introduction: For patients with advanced stage cervical and uterine cancer with vaginal relapse, the standard of care is pelvic exenteration which is a highly morbid procedure. As these patients have often had external beam radiation as part of their curative intent treatment, external beam radiotherapy could be contradicted due to normal tissue tolerance. On the other hand, brachytherapy is a procedural irradiation technique which allows delivery of highly conformal radiation and can be used to treat recurrent cervical and uterine cancer patients with vaginal relapse. A prospective study of the use of salvage cesium 131 low dose rate brachytherapy as a technique for organ preservation in this population has not been conducted.

Objective: The primary objective is to determine the 1-year organ preservation rate, defined as avoidance of pelvic exenteration and organ sacrificing surgeries including total vaginectomy, cystectomy and anterior perineal resection. Secondary objectives include assessment of 1-year local control rate, 1-year progression free survival rate, acute toxicity (<= 6 months), chronic toxicity (> 6 months), and comprehensive financial cost of procedure. Additional exploratory objectives include Molecular markers for tissue hypoxia (HIF-1a) and prognosis (p16) via BPTP in-house IHC staining, and radiation sensitivity markers (MMR and BRCA1/2) via send-out CARIS testing, using the tissue biopsy specimen of the recurrent lesion, collected 4-8 weeks prior to Cesium-131 LDR.

Methods: Following IRB approval, 10 participants will be recruited. Eligibility criteria include age (1) adult women (≥18 years old) with (2) appropriate ECOG performance status (≤ 2), (3) histologically confirmed vaginal relapse visualized on standard pelvic exam amendable for salvage Cesium-131 LDR brachytherapy from (4) recurrent cervical and uterine cancer and (5) a history of curative-intent pelvic irradiation. Participants will have diagnosis of recurrence established with CT Chest Abdomen Pelvis and biopsy of recurrence. Treatment will be a 1-time Cesium 131 LDR brachytherapy implant at our brachytherapy suite under appropriate anesthesia. Following treatment, surveillance will include history and physical every 3 months and CT Chest Abdomen Pelvis every 6 months for 2 years to assess acute and chronic toxicities.

Results: Analysis of the objectives as stated above will be conducted in conjunction with the Biospecimen Procurement and Translational Pathology, and Biostatistics and Bioinformatics Shared Resource Facility.

Discussion: This study will answer a clinically important question of utilizing brachytherapy to achieve organ preservation in recurrent cervical and uterine cancer, and could potentially be practice-changing to replace pelvic exenteration as first-line therapy.

Acknowledgements: This research will be supported by pilot funding provided by the University of Kentucky Markey Cancer Center's Cancer Center Support Grant (P30 CA177558). Support from the CCSG will also enable services from the Biospecimen Procurement and Translational Pathology, and Biostatistics and Bioinformatics Shared Resource Facility, whose services will be used in the conduct of this research.

Quality of Life and Psychosocial Determinants of Cancer Patients' Decision to Quit Smoking without Assistance

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Introduction: Quitting smoking after cancer diagnosis is associated with better treatment outcomes and improved quality of life. Many cancer patients who smoke decline tobacco treatment, often citing a desire to quit on their own. However, unassisted quit attempts are a well-cited reason for quit failure. This longitudinal mixed-methods study aims to determine the role of modifiable quality of life and psychosocial factors in cancer patients' desire to quit smoking without assistance. These modifiable factors can inform future interventions to increase tobacco treatment uptake among cancer patients.

Method: The sample was 35 adult cancer patients (69% female, 86% White, non-Hispanic, 57% unemployed due to disability, 54% rural residents, 28% gynecologic cancer) who reported smoking 12.2 ± 7.8 cigarettes per day at baseline. Patients were eligible if they declined tobacco treatment offered at a cancer center due to the desire to quit without assistance. Participants completed two semi-structured interviews and three surveys across 60 days. The sample answered interview questions about how quality of life and psychosocial functioning impact their decision to quit without assistance. Participants responded to the single-item Distress Thermometer (0-10 range) and PROMIS Global Health (10-item) measure. PROMIS domain scores are reported as a T-score, normed to the US population. Higher scores on each measure indicate higher levels of that construct.

Results: Themes that emerged from the interviews included physical health (e.g., pain, fatigue), psychological functioning (e.g., distress, anxiety), social constraint, and practical problems (e.g., finances), all being barriers to tobacco treatment acceptance. Across the 60-days study, distress scores were moderate (5.2 ± 2.5). Average scores on PROMIS Global Health scales were 35.8 (SD 6.8) for physical health, 36.3 (SD 5.9) for mental health.

Conclusions: Cancer patients who smoke experience significant quality of life (e.g., physical health symptoms) and psychosocial functioning (e.g., distress, social constraint) barriers to accepting tobacco treatment when offered at a cancer center. Given the adverse health outcomes associated with persistent smoking post- cancer diagnosis and low success of unassisted quit attempts, interventions to increase uptake of tobacco treatment are sorely needed. These interventions should look to improve quality of life and psychosocial functioning barriers identified in this study.

The Role of Hope and Goal Interference in Symptoms of Depression, Anxiety, and Quality of Life among Advanced Cancer Caregivers

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Introduction: Caregivers of people undergoing treatment for advanced cancer often experience significant role strain and personal goal disruption, with potential detriment to their mental health and quality of life. Hope, a positive motivational state arising from goal-directed thinking and planning, may be a promising intervention target to address role strain and goal disruption, but its relation to caregiver mental health and quality of life is not well-characterized.

Method: A cross-sectional study was conducted with caregivers of patients with advanced cancer (N=60). Participants completed psychometrically validated measures of goal interference, hope, depression, anxiety, positive affect, and quality of life. Pearson correlations and multiple regression mediation analyses using SAS PROC CAUSALMED were used to examine the relationships between these variables.

Results: The study sample included 60 caregivers (M age = 54.6 ± 13.38) who primarily identified as White (95%), Female (65%), currently married (78%), and working full time (50%). The primary reported relationship of caregiver to patient was spouse (57%). Caregivers reported a mean depression T-score of 55.33 (SD = 4.20) and mean anxiety T score 59.52 (SD = 9.97). Goal interference was positively correlated with both depression ($r = .502$, $p < .01$) and anxiety ($r = .652$, $p < .01$), and negatively correlated with hope ($r = -.613$, $p < .01$) and quality of life ($r = -.673$, $p < .01$). Higher levels of hope were associated with lower depression ($r = -.568$, $p < .01$), lower anxiety ($r = -.585$, $p < .01$), and greater quality of life ($r = .612$, $p < .01$). In regression analyses, goal interference partially mediated (24%) the relationship between hope and depression ($p = 0.1103$) as well as the relationship between hope and anxiety (48%; $p = 0.0007$). However, goal interference did not mediate the relationship between hope and positive affect (17%; $p = 0.16$).

Conclusions: These findings highlight the importance of addressing both goal interference and hope in interventions for caregivers of advanced cancer patients. Caregivers who experience less goal interference and higher levels of hope reported better psychological outcomes. Interventions focused on enhancing hope and managing goal interference may reduce depressive symptoms and improve overall quality of life for this population. Future research should focus on adapting hope-enhancing interventions for caregivers in this context.

Ultrasound Screening Utilizing Endometrial Thickness Measurements for Detection of Endometrial Cancer in Asymptomatic Women

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Objective: To understand the utility of screening for endometrial cancer in asymptomatic women

Methods: Individuals enrolled in the University of Kentucky Endometrial Cancer Screening Protocol (IRB # 69429) from 6/2021 to 3/2025 were evaluated. Eligibility criteria included biological female, age 45 and older or age greater than 25 with a family history of endometrial cancer or Lynch syndrome. Study participants completed a questionnaire including medical history, surgical history, menopausal status, hormone use, and family history of cancer. Each participant underwent transvaginal ultrasonography (TVUS) utilizing a standardized approach. A total of 6 ultrasonographers performed the TVUS. Images were reviewed by two physicians. The protocol specified that endometrial thickness be determined, presence of a mass be recorded and that the subject be queried about vaginal bleeding. An endometrial thickness of greater than 1.0 cm was categorized as "thickened." All screening data were entered into a networked database (MEDLOG Systems). Individuals who had normal screen results were scheduled for a follow-up screen in 12 months. Descriptive statistics were used to describe endometrial thickness measurements of the population. Performance parameters (sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV)) were calculated for assessing the ability of serial ultrasound screening based on endometrial thickness to detect endometrial cancer.

Results: A total of 12,091 participants presented 29,002 evaluable screens during the study collection period. The mean age of participants was 66.8$\pm$0.06 yrs. Average BMI was 27.2$\pm$0.04. Median parity was 2.14 and median gravidity was 2 ($IQR_{20\text{-}80}$ = 1 for both). The endometrium could not be visualized in 812 participants and 1730 screens. 10847 participants and 26,423 screens had an endometrium $\leq$ 1 cm and 432 participants and 849 screens had a thickened endometrium $\geq$ 1 cm. The mean endometrial thickness of the participants was 0.39 cm. Endometrial lining measurements were higher with increasing BMI with a mean of 0.316, 0.349, 0.400, 0.435, 0.469, and 0.541 cm for underweight, normal weight, overweight, obesity class I, obesity class II, and obesity class III participants respectively. Six malignancies (TP) have been detected with a mean endometrial thickness of 1.13$\pm$0.39 cm, age = 69.5$\pm$2.94, BMI = 32.2$\pm$3.12, and a third of which were nulliparous. The sensitivity, specificity, PPV, and NPV for endometrial screening to detect malignancy were 60%, 99%, 35%, and 99% respectively. Prior use of hormone therapy (HRT) did not seem to affect endometrial thickness with 3.2% of participants who never received HRT having an endometrium $\geq$ 1 cm and 2.6% of participants who received HRT (either estrogen alone, progesterone alone, or a combination of both hormones) having an endometrium $\geq$ 1 cm.

Conclusion: In the first 3 years of operation, the University of Kentucky Markey Cancer Center endometrial cancer screening protocol accrued 12,000+ women, who received > 29,000 serial screens, detecting 6 malignancies and performing with high specificity. With a historic incidence of 22 endometrial cancers/100,000 and considering a rising epidemic of endometrial cancer cases, the approach taken by this protocol is feasible and effective.

Variants of Unknown Clinical Significance (VUSs) in Pediatric Cancer Patients at the University of Kentucky

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Inherited cancer risk due to pathogenic variants affects approximately 8-10% of pediatric cancer diagnoses. Germline cancer genetic testing may identify genes that cannot be categorized as benign or pathogenic based on current knowledge and are therefore labeled as variants of uncertain significance (VUSs). Determining relationships between VUSs, pathogenicity, and health disparities supports secondary prevention for families with inherited cancers.

Our study used focused exome sequencing and selective analyses of 81 pediatric cancer predisposition-associated genes. To date, over 225 patients with pediatric malignancies treated at the University of Kentucky have been enrolled. Variants were classified as benign, likely benign, VUS, likely pathogenic, and pathogenic, with patient demographic data extracted from electronic health records.

Results indicate that VUSs and pathogenic variants are more commonly observed in early childhood and teenage patients. Both were identified across central and eastern Kentucky, with 55% of VUSs originating from Appalachia. Most counties with VUSs have socioeconomic data below Kentucky's averages. Several genes were associated with four or more VUSs. In contrast to national trends, a higher percentage of VUSs were found in patients with lymphoma, renal, soft tissue, and bone cancers.

Variants of uncertain significance are present in Kentucky's pediatric cancer population, with socioeconomic differences between affected communities and state averages. If certain VUSs are re-designated as pathogenic, socioeconomic factors will likely influence cancer surveillance and outcomes. Future work will focus on developing a pipeline to identify VUSs warranting closer monitoring.

ABSTRACT 76

Informatics / Information Technology

Bridging the Gap: AI-Powered Query Translation to Unlock the Full Potential of Local Data Commons

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Local data commons (LDCs) [BMC Bioinformatics 2022, 23(Suppl 12):386] are critical infrastructure for translational oncology, enabling the integration of genomic, proteomic, imaging, and clinical data at institutional levels. As technologies such as next-generation sequencing (NGS), mass spectrometry, and whole slide imaging (WSI) rapidly evolve, data is continuously and automatically generated at multiple levels and modalities [Cancer Res 2021, 81(16):4188-4193; N Engl J Med 2016, 375(12):1109-1112; Science 2015, 347(6220):1260419]. However, despite automated pipelines delivering this data, integration, and query workflows remain largely manual, relying heavily on human effort.

This human-centered management introduces several vulnerabilities. If a key data steward leaves their role, institutional knowledge and data usability can decline sharply. Many LDCs accumulate hundreds of poorly documented or hidden tables, complicating efforts to retrieve information, even for experienced analysts [Kaczmarzyk J. R. et al. ArXiv 2025]. At the same time, researchers often follow a familiar multi-step pattern when requesting data. A clinician might begin with a question like, *"How many pancreatic cancer cases are in the U.S.?"* and iteratively refine the query by geographic region, age, disease stage, or treatment history. Each refinement typically requires a new query and repeated communication with a database analyst, creating significant bottlenecks when only a few analysts support many researchers.

While tools like cBioPortal [Cancer Discov 2012, 2(5):401-404] offer interactive data exploration, they require training and time, which are often unavailable to clinicians balancing patient care with research. In parallel, the growth of generative AI and Large Language Models (LLMs) offers promising new directions. However, current applications of LLMs in biomedicine are often generic, raising concerns about data privacy, intellectual property, and model transparency. Moreover, these models are typically not fine-tuned to the specific schema or semantic context of biomedical databases, leading to inconsistent and unreliable outputs when applied to structured data management tasks [PLOS Digit Health 2024, 3(1):e0000417].

In this project, we introduce an alternative approach: using fine-tuned LLMs as natural language-to-SQL translators, tailored specifically to local database structures. By training LLMs to understand the schema, field definitions, and relationships in an LDC, we enable researchers to ask natural language questions and receive accurate, executable SQL queries in response. For example, a user can ask, *"List breast cancer cases with high Ki-67 expression and RNA-seq data,"* and receive a validated SQL query targeting relevant image annotations and omics records.

We demonstrate this approach through ImagePath, a cloud-based platform for managing pathological WSIs. Our local prototype, built with Python, FastAPI, PostgreSQL, and LLMs deployed via Ollama and Open WebUI, supports file ingestion, annotation tracking, and AI-assisted query translation. This framework not only improves data accessibility but also enhances database sustainability by identifying unused tables, resolving hidden relationships, and encouraging schema maintenance.

Ultimately, our work provides an intuitive and scalable solution to a longstanding bottleneck in biomedical research: enabling equitable, rapid access to complex data systems without requiring specialized query language skills. This AI-powered framework can help unlock the full potential of LDCs in translational oncology by bridging the gap between structured databases and everyday biomedical inquiry.

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Population-Based / Behavioral

Demand and Availability of Financial Legal Navigation Services Targeting Cancer Patients and Caregivers in Languages Other than English: A Systematic Review

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Cancer patients and their caregivers are vulnerable to experiencing financial toxicity due to the rising cost of cancer care and associated out of pocket expenses.[1-3] Abrams et al.[5] define financial toxicity (FT) as the financial burden and distress caused by cancer treatment, often leading to material (such as bankruptcy and debt), psychological, and behavioral consequences due to financial hardship from cancer.[4,5] More so, the immigrant patient population experience unique social determinants that serve as barriers to health, such as restrictions to accessing healthcare coverage, fear of immigration enforcement, homelessness, and poverty.[6] Consequently, immigrant cancer patients and their caregivers are prone to experiencing financial toxicity. Oncology financial navigation (OFLN) services target FT amongst patients by connecting patients with financial assistance resources for out-of-pocket costs, coaching patients to utilize health insurance, apply for disability benefits, and navigate healthcare systems.[7,8] The accessibility of OFLN programs addressing FT amongst immigrant patient and caregivers undergoing cancer treatment, particularly populations with limited fluency in English, is relatively unexplored. This systematic review investigates the abundance and need for OFLN services for cancer patients and caregivers that are available in languages other than English. The inclusion criteria for selected studies were based on the following: studied an OFLN intervention or addressed financial or legal services in a non-English language; participants were cancer patients or their caregivers; published in English, in countries within the Anglosphere, and before 2010. From searching the databases PubMed, MEDLINE, and Embase, 31 studies were included: 19 implemented OFLN services in languages other than English, 11 conducted qualitative interviews and analyzed data of existing OFLN programs with non-English speaking cancer patients and caregivers, and 1 utilized financial toxicity screening tools in non-English languages. Recommendations for culturally competent, bilingual cancer financial and legal navigation services, health and social disparities experienced by non-native English-speaking patients and caregivers, and minimal existing OFLN services for populations with limited English proficiency were common themes found within the studies. While OFLN interventions are tailored to address financial toxicity of cancer patients and caregivers, their availability and effectiveness amongst non-English speaking populations is largely unknown. Further research is required to investigate barriers to implementation of such interventions and the potential impact on health outcomes of cancer patient and caregiver populations fluent in languages other than English.

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A Randomized Controlled Trial of a Hope-Based Intervention to Reduce Depression Symptoms and Improve Quality of Life among Patients Undergoing Treatment for Advanced Stage Lung Cancer: Pathways Study Protocol

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Background: Despite advances in cancer treatment, over 35% of people with advanced stage lung cancer suffer depression symptoms. Access barriers constrain psychosocial treatment engagement, particularly in rural, socioeconomically disadvantaged areas that bear a disproportionate burden of the disease. We previously demonstrated the feasibility of "Pathways," a brief, nurse-led intervention at the point of cancer care to increase hope and reduce depression symptoms. The aim of the current study is to determine the efficacy of Pathways, test mediators, and evaluate intervention equity.

Method: This two armed, randomized controlled trial will recruit 234 people with moderate to high distress levels who are 3-12 weeks into systemic, infusion-based treatment for advanced stage lung cancer at an academic medical center in rural Central Appalachia. Participants complete baseline assessment and are randomized to intervention (Pathways) or control ("Resource Guide"). Pathways consists of a resource guide and 5 sessions (two in-person; three phone; 2.5-3 hrs. total) in which patients discuss personal values, goals, and goal pathways with an advanced practice provider, primarily during infusion. The Resource Guide consists of a one-time visit with study staff to review guide content (e.g., symptom management strategies) and how to apply and reference it during treatment. The primary outcome measure is the 6-item Patient Reported Outcomes Measurement Information System (PROMIS) Depression Short Form, which is administered at baseline, mid- and post-intervention, and 6- and 12-week follow-up visits along with secondary outcomes (i.e. anxiety, demoralization, quality of life, etc.) and mediators (goal interference, goal adjustment). Equity is evaluated by intervention completion and benefit among sociodemographic strata (age, residence, and sex).

Results: Enrollment began on November 23, 2023. To date, 66 eligible patients have been approached and 49 (74%) have enrolled, 44 (89.8%) of whom have completed baseline and been randomized (M_{age}=65.02 (SD=7.9), 36.4% male, 70.4% rural; 93% white; n = 23 Pathways; n = 26 control). Retention is 85% at post-assessment, 88% at 6-week follow-up, and 100% at 12-week follow-up.

Conclusion: Results from this study will determine the impact of a novel, highly disseminable psychosocial intervention among a large, underserved patient population. If efficacious, Pathways could easily be translated for other advanced cancer populations.

Characterizing Tobacco Use Behaviors by Lung Cancer Screening Status among Older Black Adults with a 20+ Year Tobacco Use History

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Background: Racial disparities in lung cancer incidence and survival persist, potentially driven by differences in tobacco product use and lung cancer screening (LCS) behaviors. Black adults, who are disproportionately affected by lung cancer and have established varying patterns of tobacco product use behaviors than their White counterparts, have diverse reasons for seeking and receiving LCS. Understanding tobacco product use behaviors among Black adults, stratified by LCS status, can help identify factors driving this disparity and highlight opportunities for intervention.

Methods: We used data from a sequential mixed-methods pilot study aimed to understand the barriers and facilitators to LCS among older Black adults in Lexington, Kentucky with a 20+ year tobacco use history (n=100, mean age: 61.6, female gender: 63.0%). This study surveyed 100 adults on their tobacco use behaviors, lung cancer perspectives, and health and lived experiences and conducted semi-structured interviews on 25 of these adults, stratified by LCS status, to further glean their perceived barriers and facilitators to LCS. For this analysis, we descriptively characterized tobacco use behaviors, such as past-year use by product and cessation outcomes, among the sample, stratified by LCS status. We compared distributions of selected characteristics by LCS status using Student's t-tests and chi-square/Fisher exact tests.

Results: Slightly more than half of the sample (56.0%) has ever received an LCS. LCS varied by age (p=0.006) and insurance status (p=0.03). Among the entire sample, past-year cigarette smoking (65.0%) was the most common use behavior, followed by using e-cigarettes (19.0%), cigarillos (14.0%), premium cigars (6.0%), filtered cigars (3.0%), and hookah/waterpipe (2.0%) and traditional pipe (2.0%). Product use differed by LCS status for cigarillos (p = 0.001), with a higher proportion of adults who used cigarillos not receiving an LCS. About two-thirds of the sample (70.0%) made a quit attempt in their lifetime, while only 37.0% were able to completely quit for one year or longer, and these factors differed by LCS status (p=0.005 and p=0.023, respectively). Further, LCS status did not differ by how soon people wanted to quit tobacco products for good (p=0.93). Among resources to help adults quit, the most requested were help of advice from a doctor (52.0%), followed by products that help people quit such as nicotine patches (49.0%), help of advice from family/friends (42.0%), and help from a tobacco treatment specialist (12.0%).

Conclusions: Continued tobacco use among adults who undergo or are eligible for lung cancer screening remains a significant concern. Various factors determine LCS access and availability to Black adults in the United States. One of them may be that other combustible tobacco products, such as cigarillos, are not considered in determining screening eligibility. LCS offers early detection and may serve as a motivator for quitting, as supported by our findings that cessation outcomes varied by LCS status. Additionally, this study identifies potential avenues for tobacco cessation tailored to Black adults. Future directions include a more detailed assessment of tobacco product use behaviors by considering the duration, frequency, and intensity of use to approximate pack-years. We also aim to explore lung cancer screening attitudes, perceptions, and knowledge, along with the health and lived experiences of the sample, through an integrated quantitative and qualitative analysis.

Children Eating Well (CHEW) Mobile App: Parent Opinions of App Utility, Usability and Satisfaction

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Obesity and associated behaviors including unhealthy diet, lack of physical activity, and poor sleep are associated with diverse chronic diseases including cancer, heart disease, stroke, and type II diabetes. Obesity prevalence in the U.S. continues to rise to approximately 40% of adults and roughly 20% of children. Obesity in childhood is associated with an increased risk for adult obesity as well as early onset high blood pressure, high cholesterol, asthma, sleep apnea, and joint problems. Early intervention to encourage healthy lifestyle habits may reduce obesity risk across the lifespan. Evidence-based approaches to reducing obesity exist including individually guided goal setting and behavior tracking. The CHEW study is a two-arm parallel cluster-randomized control trial that evaluates the use of parent-targeted, guided goal-setting and subsequent behavior tracking for childhood obesity risk reduction via a mobile app, for parents of preschool-aged children. Leveraging data from the CHEW study, the goal of this poster is to examine the patterns of parents' opinions about the CHEW app in terms of utility of the core app functions, usability of the app, and satisfaction using the app. The analysis was conducted on n=75 parents in the intervention arm that used the CHEW app and completed the follow up survey. For each item, the response options were a Likert scale from 1 (most negative) to 5 (most positive). Results indicate that parents overall had neutral to positive opinions about the app, but they did not differ based on demographic characteristics. The items that scored the highest related to the ease of learning and using the CHEW app as well as helpful tips provided through the app to encourage healthy eating. The items that scored the lowest related to participants' projected frequency of using the CHEW app, frustration while using the app and assistance provided by the app for buying healthy food. Future research should explore if perceptions of app utility, usability, and satisfaction are related to how much parents used and engaged with the app.

College Women's Knowledge of Pap and HPV Tests by Age

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Background. College-aged people are at the highest risk of contracting Human papillomavirus (HPV) infections. HPV is the leading cause of cervical cancer—the fourth most common cancer in women globally. The HPV vaccine, administered to youth as early as age 9, is one frontline defense against cervical cancer. Other methods of cervical cancer prevention and early detection include the Pap and HPV tests that are recommended for young women starting at ages 21 and 25, respectively. Recent studies however show lags in prevention screening across demographics and among young women aged 21 to 29. Research also shows that knowledge gaps are significant barriers to screening compliance.

Purpose. This study compared cervical cancer screening knowledge of college women across distinct age groups that represent periods at which screening guidelines change: 18-20 (non-screening), 21-24 (Pap test), and 25-29 (HPV test or Pap/HPV test). Specifically, this study examined the relationship between age, HPV vaccination status, and Theory of Planned Behavior constructs (i.e., attitude, perceived behavioral control, and behavioral intention) toward cervical cancer prevention screening.

Method. A survey questionnaire was administered through a university-based sampling pool (N=340). SPSS version 30 was utilized to analyze data. Thematic analysis was performed for open-ended questionnaire responses. The Institutional Review Board approved study procedures.

Results. The majority of respondents were of the age group 18-20 (74.7%; n=254), followed by 21-24 (23.8%; n=81) and 25-29 (1.5%; n=5), and the majority were White (83.8%; n=285). Juniors accounted for the most represented college year (32.1%; n=109) while sophomores (27.9%; n=95) and freshmen (27.6%; n=94) were roughly equally represented. The majority of respondents had received at least one dose of the HPV vaccine (76.2%; n=259) and were sexually active (63.5%; n=216). One-way ANOVA tests revealed no significant effect of age on behavioral intention, $F(2, 335) = 1.127$, $p=.33$ or perceived behavioral control to undergo screening, $F(2, 329) = 1.369$, $p=.26$. A two-way ANOVA was conducted to explore the impact of age and HPV vaccination status on attitude toward prevention screening. The interaction effect was not statistically significant, $F(2, 332) = 4.112$, $p=.20$. Open-ended survey questions gathered respondents' knowledge of the recommended ages to begin Pap and HPV tests and general knowledge on prevention screening. Knowledge gaps pertained to the recommended start age for Pap tests (i.e., "I have no clue probably around 25 or sooner if I get super active"); attributing the start of sexual activity as the starting point for Pap tests (i.e., "I believe its when you first become sexually active"); and uncertainty about test purpose (i.e., "21 because you are fully mature down there"). Knowledge gaps for HPV tests include lack of awareness about the test (i.e., "I know nothing. I think it's something you get if you're genetically pre-disposed to HPV, but I could be wrong") and conflating the HPV vaccine and HPV test (i.e., "I remember getting [it] early high school, maybe even middle school... so probably around 14 or 15").

Discussion. This study contributed to literature on college women's understanding of cervical cancer detection methods. Preliminary statistical analysis shows no significant effect of age on screening behaviors. Further analysis is needed to identify other possible factors that affect screening behaviors. Qualitative analysis reveals knowledge gaps about Pap tests and broad misunderstanding about HPV tests, suggesting opportunities for awareness messaging targeted to college-aged women that incorporates both test types.

Evaluating Mobile Health Interventions for Addressing Financial Toxicity in Oncology: A Systematic Review

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Financial toxicity (FT), or the economic burden associated with cancer care, significantly affects patients and caregivers, contributing to poorer outcomes and decreased quality of life. Mobile health (mHealth) applications are emerging as potential tools to support patients in managing these financial challenges. This systematic review aims to evaluate the effectiveness of mHealth applications in reducing or managing FT among cancer patients and their caregivers. A literature search across PubMed, CINAHL, and Embase yielded 46 articles, which were then narrowed to 3 based on the following inclusion criteria: U.S.-based interventional studies utilizing an app to address finances of adult cancer patients or caregivers, conducted in English within the past 10 years. The final 3 studies are app-based interventions relating to cost communication, financial navigation, and the collection of social determinants of health data to identify and address financial vulnerabilities. Preliminary review of these articles suggests mHealth tools may improve financial awareness and access to resources, though further analysis using the Risk of Bias in Non-Randomized Studies Tool (ROBINS-I) will clarify risk of bias and overall intervention effectiveness. Future research should explore broader implementation and integration of financial-focused mHealth interventions in oncology care.

Examining the Influence of Race and Skin Tone on Melanoma Risk Perception: The Role of Knowledge, Attitudes, and Behaviors

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Skin cancer is the most diagnosed cancer in the United States, with melanoma-associated mortality rates disproportionately affecting individuals of lower socioeconomic status and people of color. Despite melanoma having an increased mortality rate in these populations, especially among Latinx and Black individuals, the diagnosis and awareness of melanoma often falls off, sequentially leading to poorer outcomes and evident presentations later in life. Melanoma can be divided into various subtypes, with acral lentiginous melanoma (ALM) being the most frequently diagnosed subtype among Latinx and Black populations presents on anatomical areas of the skin that are not exposed directly to sun, such as the palms, soles, and nails, distinguishing it from the more common cutaneous melanoma. Although melanoma mortality rates have decreased in non-Latinx White individuals in the United States since 2014, similar improvements are not observed within Latinx, Black, or rural communities. In addition to this disparity, delayed diagnoses of melanoma are particularly prevalent in Black and Hispanic populations, this could be due to the misconception that individuals with darker skin tones are less susceptible to melanoma. This prevailing notion and limited recognition of malignant lesions throughout these communities, further slows early detection efforts. Furthermore, cultural beliefs, such as the idea that darker skin provides protection against sun damage and skin cancer, shape the attitudes and behaviors of minority groups, leading to a reduced focus on melanoma prevention. Our study aims to explore the relationship between race and skin-tone complexion with the perception of melanoma skin cancer risk along with the mediating effects of knowledge, attitudes, and behaviors on this relationship. It examines the mediating effects of knowledge, attitudes, and behaviors in shaping these perceptions. Understanding these factors may uncover how ingrained beliefs about self-identity affect melanoma risk awareness, ultimately highlighting the need for tailored education and intervention strategies to reduce melanoma-related health disparities.

Pharmacogenomics of Liquid and CNS Tumor Pediatric Cancer Patients

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Background: Pediatric cancer patients with certain genetic mutations (particularly SLCO1B1, UGT1A1, VKORC1, and TPMT mutations) have negative pharmacologic consequences. These mutations have implications for toxicities and therapeutic effects when considering chemotherapies or other treatment options, however much of the literature is based on adult pharmacogenomic data. More data from the pediatric population is necessary to fully understand the impacts of various genetic mutations on the pharmacologic aspects of care for cancer patients.

Methods: Pediatric patients with leukemia, lymphoma, and central nervous system malignancies at the Kentucky Children's Hospital were given the opportunity to undergo genetic testing of a panel of 16 genes with known pharmaceutical effects. The pharmacogenomic panel results were analyzed for possible correlations with diagnosis, age, race, biologic sex, and county of residence for each patient. County of residence was also analyzed for possible correlations with diagnosis, mean family income, and average number of mutations per patient.

Preliminary results: Out of the 121 participants, 114 (94%) had at least one mutated gene included on the panel. Of these 114 participants, 45% were diagnosed with ALL, 44% with a CNS malignancy, 8% with with APL or AML, and 4% with another form of liquid tumor. One third of the participants residing in Kentucky were from a county defined as a Small Town or Rural by the USDA. The majority (58%) of the participants were male, 89% were White, and 91% were not of Hispanic, Latino/a, or Spanish ethnicities. The average participant age was 13 years old. Our findings suggest the pressing need to study the impact of pharmacogenomic mutations on chemotherapeutic toxicity in children.

Relationship among Nutrient, Metabolite, and Inflammatory Genotype and Phenotype as Risk Factors of Colorectal Cancer

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Introduction. Systemic inflammation is a significant risk factor and a hallmark of colorectal cancer (CRC) and poor prognosis. We examined whether sex and rs1205 genotype moderate the indirect effect of gut microbiota choline metabolism on C-reactive protein (CRP), a marker of inflammation, through Trimethylamine N-Oxide (TMAO) metabolite. Gut microbiota metabolizes nutrients, such as dietary choline, an essential nutrient found in various foods, including eggs and meat, to form its metabolite, TMAO. Researchers found higher serum level of TMAO in healthy males than healthy females. In addition, a recent meta-analysis indicated the association of high level of TMAO metabolites with increase in CRP concentrations. CRP gene rs1205 polymorphism, particularly its major C allele, is associated with higher serum levels of CRP and presence of low-grade chronic inflammation. However, complex relationships among diet, metabolites, sex, and inflammatory genotype and phenotype are not known.

Methods. The secondary analysis was performed using baseline data from the HeartHealth study. Participants included rural-dwelling Kentuckians (N=274); majority were female (75%). Blood samples were analyzed for the serum levels of CRP. We genotyped participants for the rs1205 polymorphism (CC vs. CT/TT). Choline intake was assessed using a web-based Food Frequency Questionnaire. TMAO was extracted from plasma and analyzed via high-performance liquid chromatography with electrospray ionization tandem mass spectrometry. The multiplicatively moderated mediation analysis was conducted using PROCESS macro for SPSS (Ver. 4.3; Model=11). The TMAO and CRP were log-transformed. We used age, body mass index, education, financial status, marital status, and insurance status as covariates.

Results. We found a significant three-way interaction among choline (X), rs1205 genotypes (Z), and sex (W) [B=.0026, t(260)=2.572, P=.011], indicating that the moderating effect of sex on the effect of choline on CRP depended on rs1205 genotypes (R^2=.190; P<.001). There was a significantly positive association between choline and CRP in females compared to males in the rs1205 CT/TT genotype group [$\theta_{XW\rightarrow Y}|(Z$=CT/TT)=.0012; F(1;260) =3.947; P=.048]. In contrast, the association was not statistically significantly moderated by sex in the rs1205 CC genotype group. The index of moderated mediation was .0003 (95% bootstrap confidence interval: <.0001-.0010).

Conclusions. Our findings provide initial evidence that CRP-related genetic variation and sex moderate an association between diet and CRP. Females with high choline diet and CRP rs1205 CT/TT genotype may be at higher risk of a low-grade chronic inflammatory status. The findings indicate a complex interaction between inflammatory genotype and sex in the relationship of nutrients and metabolites with systemic inflammation. Understanding these associations may inform tailored anti-inflammatory dietary interventions to reduce CRC risks among rural residents.

Research to Inform Interventions to Address Alcohol Use and Cancer Risk in Young Adult Women

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Purpose: Young adulthood is associated with increased alcohol misuse, and women are especially vulnerable to alcohol-related harm. New research emphasizes the link between alcohol and cancer risk, but existing evidence-based alcohol reduction interventions do not address this link. The aim of this study was to better understand the salience of alcohol use as a risk factor for cancer among young adult women to inform adjustments to future programming.

Methods: An online survey was administered to a national sample of women (N=315) who were aged 18-34, had no personal cancer history, and reported past-month alcohol use. To obtain a sample representing a variety of experiences with alcohol-associated cancers, quotas were used so that 1/3 of participants had no family cancer history, 1/3 had a family history of breast cancer, and 1/3 had a family history of colorectal cancer. We also deployed alcohol use quotas, such that ¼ were light drinkers and ¾ were at-risk or high-risk drinkers. Survey questions covered alcohol use, readiness to change, knowledge of the alcohol-cancer link, reactions to information documenting the alcohol-cancer link, and preferences for programming.

Results: Participants averaged 7.54 drinking days, 3.80 drinks per occasion, and 4.26 heavy-episodic drinking occasions over the past month. Over half (53.7%) reported interest in changing their drinking. Less than half (43.8%) believed that cancer could result from alcohol use. Common themes of open-ended feedback in response to education on the alcohol-cancer link were that this information was surprising (or not), worrisome, useful, and motivating to change one's drinking. Most of the sample believed that it was "very" or "extremely" important to receive education about the alcohol-cancer link (76.6%) and would be "very" or "extremely" motivating to learn about as a part of alcohol reduction programming (66.7%).

Conclusion: Results suggest that many heavy-drinking young women are interested in changing their drinking, are unaware of the link between alcohol and cancer, and would find content on this topic motivating to change. After additional analyses are completed exploring outcomes according to drinking status and family history of cancer, results will be used to inform the development of an alcohol reduction intervention for young adult women.

Survival of Patients Diagnosed with Cancer in the United States during the First Year of the COVID-19 Pandemic

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Importance: The effects of COVID-19 pandemic-related disruptions on cancer diagnosis in the United States have been widely observed, but their impact on short-term survival have not been assessed.

Objective: Examine one-year cause-specific survival (CSS) among patients diagnosed with cancer during 2020 using high-quality cancer registry data.

Design: A cross-sectional study of cancer survival for 2015-2020 using the Surveillance, Epidemiology, and End Results 22 registries (SEER-22) database.

Setting: Population-based

Participants: Individuals with an invasive cancer diagnosis and complete follow-up reported to registries included in SEER-22 between January 1, 2020 and December 31, 2020.

Exposure(s): Age, sex, race and ethnicity, urbanicity and stage at diagnosis.

Main Outcomes and Measures: Calculated one-year CSS for patients diagnosed with cancer in 2020 and compared them to one-year CSS among patients diagnosed in 2019. Additional site-specific analyses were performed on common cancer sites identified as having low-survival (5-year relative survival <33%) or high-incidence/high-survival (incidence >20.0 per 100,000 and 5-year survival ≥66%).

Results: Patients diagnosed with cancer in 2020 had a one-year CSS of 83.70% (95% confidence interval (CI), 83.60%-83.80%), which was a significant 1.21 percentage points lower than in 2019 (95% CI, 1.07%-1.35%) and resulted in an estimated 13,517 excess deaths (95% CI, 11,944-15,090)—or roughly 8% more than if CSS had not decreased. All five high-incidence/high-survival cancer sites examined, and three out of five low-survival sites, had significant CSS reductions compared to 2019, ranging from 0.21% lower for female breast cancer (95% CI, 0.04%-0.37%) to 2.77% lower for liver cancer (95% CI, 1.50%-4.03%). Small reductions in CSS for high-incidence cancers led to large percentage increases in deaths within one year of diagnosis, including approximately 20% more melanoma deaths than expected without CSS reductions. The greatest number of excess deaths by cancer site were estimated for colorectal cancer (1,726; 95% CI, 1,285-2,168) and lung cancer (1,262; 95% CI, 545-1,980). Significant one-year CSS reductions were observed regardless of stage at diagnosis.

Conclusions and Relevance: Individuals diagnosed with cancer in 2020 experienced poorer short-term survival than those diagnosed in 2019, suggesting substantial harms related to cancer care disruptions during the first year of the COVID-19 pandemic.

Tobacco Use, Secondhand Smoke Exposure and Breastfeeding Practices among Rural Appalachian Mothers

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Introduction: Women in rural communities have higher rates of tobacco use and secondhand smoke (SHS) exposure as well as lower rates of breastfeeding initiation and duration compared to their urban counterparts. Policy outcomes research shows pregnant women living in communities with strong smoke-free laws have lower rates of preterm birth, but research is lacking on the association of tobacco use, SHS exposure, smoke-free laws and breastfeeding practices in rural communities.

Purpose: To examine tobacco use, SHS exposure and breastfeeding practices among mothers living in rural communities.

Methods: This feasibility study used a cross-sectional retrospective design and purposive cluster sampling with stratification by strength of municipal smoke-free laws and tobacco exposure status. Women between 18-45 years of age currently residing in one of the six identified rural Kentucky counties: Knott, Owsley, Perry (strong smoke-free laws) and Bath, Menifee, Morgan (absent smoke-free laws), who have given birth to a live infant within the past two years were eligible. Measures included demographics; infant feeding practices; tobacco use; SHS exposure; lung cancer screening and worry; depression; anxiety; and alcohol and substance abuse.

Results: All participants (n=13) were white and non-Hispanic. Most participants resided in counties without smoke-free laws (61%) and over half of the participants (54%) reported tobacco exposure during the first year of their child's life. In bivariate analyses, the strength of municipal smoke-free laws was not associated with BF duration.

Discussion: Future plans include a mixed methods study with expanded recruitment to additional rural Appalachian counties using objective measurements of tobacco exposure, air quality monitoring as well as psychosocial factors influencing tobacco use/exposure and breastfeeding practices.

Utilizing Integrative Medicine Service for Yoga to Reduce Cancer Related Fatigue

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Background: Cancer related fatigue (CRF) is one of the most prevalent and distressing side effects experienced by individuals undergoing cancer treatment. In fact, many patients report CRF as more debilitating than pain itself. CRF is characterized by persistent, severe exhaustion that is not alleviated by sleep and is not linked to prior exertion. Despite its widespread impact, the options available to manage CRF are limited, typically including practices like good sleep hygiene, balanced nutrition, mindfulness, and supplements. Integrative medicine, particularly yoga, has emerged as a promising intervention for reducing CRF. However, yoga is underutilized as a therapeutic approach to combat this debilitating symptom.

Purpose: This study aimed to assess the effectiveness of yoga in reducing CRF among patients undergoing active cancer treatment by referring them to an integrative medicine clinic for yoga and measuring outcomes using the Functional Assessment of Chronic Illness Therapy – Fatigue (FACIT-F) scale before and after this intervention.

Conceptual Model: Dorothea Orem's Self-Care Deficit Nursing Theory was utilized as the framework for this study.

Methodology: This study employed a prospective, quasi-experimental design, which involved a non-randomized pre- and post-intervention approach. Participants were chosen based on the following criteria: a cancer diagnosis, receiving active cancer treatment, and willingness to participate in the study. A pre-chart review identified eligible patients. The goal was to enroll at least 25 participants within the designated timeframe. Pre- and post-intervention assessments were conducted using the FACIT-F tool to measure changes in fatigue level.

Results: A total of 23 participants enrolled in the study. Nine completed both the pre- and post-survey. One completed the pre-survey but expired before the post-survey was completed. A paired t-test was conducted to determine whether the difference in scores was statistically significant. The mean difference between pre- and post-test scores was -3.33 (SD=11.9), indicating a slight increase in post-test scores. However, the t-test results showed that this difference was not statistically significant.

Conclusions: Yoga, in this study, was found to be useful in the reduction of CRF in patients receiving active cancer treatment. This study needs to be replicated with a larger, more inclusive sample size to further support the use of yoga in the reduction of CRF.

ABSTRACT 90

Translational

Adverse Effects of Glioblastoma-Derived Extracellular Vesicles after Radiation: Drivers of Neuroinflammatory Responses and Tumor Aggressiveness

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Glioblastoma (GBM) remains an incurable cancer, characterized by high recurrence rates and profound cognitive impairments that are more severe than those seen in other cancers. Our research revealed that glioblastoma produces high levels of ROS, leading to the formation of the toxic lipid peroxidation product, 4-hydroxynonenal (4HNE). Notably, GBM patients exhibit high numbers of extracellular vesicles (EVs), which are enriched in 4HNE-adducted proteins, particularly following radiation therapy (GBM-RT-EVs). Thus, we hypothesize that GBM-RT-EVs could be a key molecular mediator driving both tumor recurrence and therapy-associated neurotoxicity, in GBM patients.

To assess the cognitive impact of GBM-RT-EVs, we administered them intracranially in the somatosensory cortex of immunocompetent mice. Remarkably, these mice exhibited cognitive deficits and anxiety-like behaviors, accompanied by DNA damage in cerebral tissue, decreased neuron markers, and elevated levels of p50 and pro-inflammatory cytokines, like IL-6 and IL-1β. Further mechanistic studies revealed that GBM-RT-EVs are internalized by microglia (HMC3), leading to pronounced microglial activation characterized by morphological transformation to an amoeboid/hyper-ramified phenotype, increased secretion of pro-inflammatory cytokines, elevated H2O2 production, and enhanced glycolytic function. These findings suggest that GBM-RT-EVs promote microglial activation, driving the release of ROS and cytokines as mediators.

To assess the downstream neurotoxic effects of H2O2, GBM-RT-EVs were added to co-culture chambers containing HMC3 cells and neurons (HCN2). Co-culturing studies showed that the viability of neurons was significantly reduced when exposed to microglia activated by GBM-RT-EVs. Consistent with these findings, in vivo studies demonstrated elevated pro-inflammatory cytokines in circulation and CD68 expression—a marker of microglial activation—in the brains of mice with cognitive deficits caused by GBM-RT-EVs.

Proteomics analysis revealed that GBM-RT-EVs are enriched in proteins associated with cell replication and show a marked upregulation of mitochondrial proteins compared to EVs derived from non-irradiated GBM cells. These findings suggest that the cargo proteins in GBM-RT-EVs could contribute to the observed mitochondrial metabolism alterations and NFκB signaling pathway activation in microglial cells.

Since previous studies showed that GBM-derived EVs can increase proliferation, we further determined if GBM-RT-EVs contribute to tumor progression. Direct application of GBM-RT-EVs to GBM cells significantly increased their migration and proliferation capacity, suggesting direct pro-tumorigenic effect of GBM-RT-EVs. Additionally, migration was also increased indirectly through interaction with glial cells as shown by co-culture experiments. The downstream mechanisms by which GBM-RT-EVs contribute to GBM proliferation need further investigation.

Next, we explored if the neurotoxicity and tumor-promoting effects induced by GBM-RT-EV could be mitigated with BMX-001, an MnSOD mimetic currently in Phase II clinical trials for GBM. As anticipated, BMX-001 treatment reduced CD68 expression and pro-inflammatory cytokines like IL-6, IL-1β and TNFα in microglia while sensitizing GBM response to oxidative stress, enhancing radiation-induced cytotoxicity. This highlights the therapeutic potential of redox modulation in both tumor and neural components of brain microenvironment.

Overall, our data indicates that GBM-RT-EVs contribute to GBM proliferation and migration while inducing microglia-mediated neuronal injury and cognitive alterations. These detrimental effects may be mitigated by overloading the pro-oxidant burden in GBM cells while reversing the pro-inflammatory redox state in microglia—using redox-active antioxidant therapies such as BMX-001.

Characterization of TP53 T253I as Likely Pathogenic in an Individual with Adrenal Cortical Carcinoma

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We identified a germline *TP53* c.758C>T (p.T253I) mutation in the *TP53* tumor suppressor gene in a pediatric adrenocortical carcinoma (ACC) patient. Characteristic to pathogenic p53 mutations, we observed upregulation of total p53 protein levels in the patient's ACC and concurrent suppression of the wild-type (WT) *TP53* allele. As ACC can be associated with Li-Fraumeni Syndrome (LFS) and the mutation has not yet been linked to LFS, we sought to characterize the functionality of the T253I mutation. We acquired $p53^{-/-}$ HEK293 cells and stably transduced them with GFP-tagged wild type (T253) or T253I p53 as well as two established pathogenic p53 mutants (C176Y and R213X). Compared to p53 WT, levels of T253I p53 increased while MDM2 levels decreased, suggesting a loss of MDM2-mediated regulation of T253I p53. Additionally, T253I showed a reduction in DNA damage responsive events, diminished DNA binding capabilities, and blunted transactivation capacity. These experimental data lead us to conclude that T253I represents a pathologic variant in *TP53* that may predispose to LFS-associated tumors.

Clinicogenomic Comparison of Early- and Average-Onset Gastric Cancer

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Introduction. In recent decades, there has been an unprecedented rise in gastric cancer among younger individuals, contrasting with a decline among older individuals. However, the biological underpinnings of gastric cancer in younger individuals remain poorly understood. We aimed to elucidate the clinicopathologic and genomic characteristics of early onset gastric cancer (EOGC) compared to average onset gastric cancer (AOGC).

Methods. Using the prospective Oncology Research Information Exchange Network (ORIEN) database, we compared demographic, clinicopathologic, genomic, and survival outcomes between EOGC (≤50 years) and AOGC (>50 years). Kaplan-Meier curves compared overall survival (OS). Whole exome sequencing data were processed to identify significant somatic mutations. RNASeq data facilitated differential gene expression, pathway enrichment, and immune cell deconvolution analyses.

Results. We analyzed 166 patients including 40 with EOGC and 126 with AOGC. The median age at diagnosis was significantly lower in the EOGC group (45 vs 66 years; p<0.0001). There were no differences in body mass index, race, smoking, or alcohol consumption. EOGC patients exhibited significantly higher rates of pain at diagnosis (57% vs 29% p=0.004), but not anemia or reflux. EOGC patients were more likely to present with stage III/IV disease (70% vs 45% p<0.01) and diffuse/signet ring histology (57.5% vs 24% p=0.0002). Consequently, OS was decreased in the younger cohort (HR 0.54; p=0.041). Somatic mutational load was decreased in young patients (p=0.009). Significantly mutated genes in the entire cohort included *CDH1*, *ARID1A*, *P53*, *PIK3CA*, *TGFBR2* and *TYRO3,* but *CDH1* was more frequently mutated in EOGC (47% vs 19% p=0.001). Significant differences in RNA expression were observed, with upregulated epithelial cell signaling in H. pylori, *P53* signaling, and DNA damage repair. Immune deconvolution revealed a predominance of M2 macrophages, mast cells, and $CD4^+$ T cell subsets, but no differences between cohorts.

Conclusion. Young patients with gastric cancer have a unique clinicogenomic signature. They are more likely to present with aggressive disease characterized by pain, advanced stage, diffuse/signet histology, poorer OS, diminished mutational load and higher CDH1 mutational frequency. Pathway dysregulation in EOGC may contribute to tumorigenesis and therapy resistance. This study underscores the need for further research into novel therapies and biomarker discovery in younger individuals.

Establishing a Syngeneic Model to Explore the Biological Function of Integrin α6β4 in Triple Negative Breast Cancer

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Triple negative breast cancer (TNBC) is the deadliest form of breast cancer due to its aggressive nature and lack of effective treatment options. Integrin α6β4 is a laminin receptor and is highly expressed in more than 80% of TNBC cases. Previous work demonstrates that integrin α6β4 has the ability to alter expression of genes that are critical to promote tumor cell survival, migration, invasion, and metastasis. By using established TNBC cell lines and cell line-based xenograft models, work from our lab suggests that integrin α6β4 signaling impacts TNBC cell response to the current standard-of-care therapy for TNBC. Furthermore, a recent study from our lab demonstrated that integrin α6β4 enhances tryptophan metabolism, suggesting its role in promoting an immunosuppressive tumor microenvironment. A syngeneic TNBC model is needed to translate these *in vitro* findings into more physiologically relevant *in vivo* investigations to further study the impact of integrin α6β4 in promoting tumor progression and its role in therapeutic responses. First, the retrovirus constructs encoding human integrin β4 (WT) and its signaling domain mutant as well as the empty vector were transfected into 293T cells and then the packaged virus were collected to infect EMT6 cells that don't naturally express integrin β4. After puromycin selection, cells were collected and immunoblotting was performed to assess integrin β4 expression. Direct cell counting and MTT viability assays were performed to assess how integrin β4 expression impacts cell proliferation. The invasive capacity of these newly engineered EMT6 cells was assessed by measuring the diameter of colonies grown in three-dimensional culture and through clonogenic survival assays. Our data demonstrated that compared to control cells, stably expressing WT integrin β4 but not the signaling domain mutant in EMT6 cells significantly promoted cell proliferation and increased colony size, and a more aggressive growth pattern. Similar experiments were performed on 4T1 β4 knockout cell line generated in our lab. We found that the 4T1 knockout cells showed decreased invasive capacity compared to the control cells. In summary, we successfully generated and characterized EMT6 syngeneic TNBC cell lines for integrin α6β4 studies. Generation of these cell lines provides a useful preclinical model that will be complimentary to cell line-based xenografts used in TNBC studies that focus on integrin α6β4. Future studies will include implantation of these cells into the mammary fat pad of mice to test the impact of integrin α6β4 on tumor aggressive nature such as tumor growth, metastasis, as well as on the standard-of-care therapy for TNBC *in vivo*.

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

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Cancer immunotherapies through immune checkpoint blockade by therapeutic antibodies have achieved remarkable tumor regression in limited cancer patients. Immune checkpoint ligand PD-L1 has been validated as a viable target in different types of cancers. However, poor bioavailability and immune-related adverse effects of monoclonal therapeutic antibodies limit their applications. In contrast, small-molecule inhibitors of the PD-L1 pathways have shown promising immunotherapeutic effects but none have been approved by the FDA to date. The identification of additional small-molecule PD-L1 modulators warrants further effort. We present here the discovery of iminoquione-1 as a novel PD-L1 inhibitor. Through screening our in-house compounds, we identified iminoquinone-1 that selectively induced premature PD-L1 expression in cancer cells in a dose-dependent manner. Mechanism of action studies by RNA-seq analysis revealed that iminoquinone-1 activated unfolded protein response. Iminoquinone-1 inhibited PD-L1/PD1 signaling in a cancer cells/T cells co-culture system and suppressed tumor growth of MC38 colon cancer in a C57BL/6 mouse model. Overall, iminoquione-1 represents a potential PD-L1 inhibitor for small-molecule cancer immunotherapy.

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 LD_{50} 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.

Keywords: Docetaxel, Nrf2, castration-resistant prostate cancer.

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

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Integrin α6β4 is highly expressed in approximately 80% of triple negative breast cancer (TNBC) cases, and its expression associates with more aggressive phenotypes. Currently, Adriamycin (doxorubicin) and Cytoxan (cyclophosphamide) are employed as front-line neoadjuvant chemotherapeutics in TNBC care plans, with platinum agents generally reserved for metastatic cases. Although previous investigations from our lab demonstrate that TNBC cells bearing integrin α6β4 have greater sensitivity to platinum-based chemotherapy, the impact of integrin α6β4 on the current Adriamycin/Cytoxan (A/C) standard-of-care treatment remains untested. Using a breast cancer tissue microarray constructed from cancer tissues obtained from Markey patients, we confirmed that integrin α6β4 is highly expressed in a majority of TNBC cases. Interestingly, we also observed that integrin α6β4 expression associates with greater overall survival for TNBC patients. To investigate this important association, we treated BT549 (EV vs. β4) and MDA-MB-231 (Cont. vs β4 knockout) cells with various 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 AAT Bioquest software to determine IC50 values and SynergyFinder software to calculate ZIP and Bliss synergy scores. We found that integrin α6β4 expression did not notably alter response to either drug alone; however, its expression was associated with significant synergistic sensitivity to doxorubicin/mafosfamide dual treatment in BT549 and MDA-MB-231 cells. Immunoblot assay analysis demonstrated that integrin α6β4 expression in these cells enhanced the activation of the DNA damage response markers γH2AX and phospho-p53 S15 in response to a combination treatment. Immunocytochemical staining was used to quantitatively confirm the activation levels of phospho-p53 S15 and γH2AX in BT549 (EV vs. β4) and MDA-MB-231 (Cont. vs β4 knockout) cells in response to single and dual drug treatments with doxorubicin and mafosfamide. We observed that phospho-p53 S15 and γH2AX were detected in greater abundance following dual drug treatments in the integrin α6β4 bearing cells. Clonogenic survival assays using 4T1 cells (Cont. vs β4 knockout) further supported the result that integrin α6β4 promotes synergy following a combination treatment with doxorubicin and mafosfamide. 3D Colony formation assay results indicated that integrin α6β4 expression in BT549 cells was associated with increased size and response to dual treatment with doxorubicin and mafosfamide. 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 median expression. In summary, these results provide evidence that integrin α6β4 promotes the efficacy of A/C treatment, the current first-line standard-of-care therapy for TNBC. We further suggest that integrin α6β4 could be used as a potential biomarker in predicting benefit of A/C treatment plans for TNBC patients.

Prevalence of Pharmacogenomic Mutations in Pediatric Populations: Insights from the Project Inherited Cancer Risk Study

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Pharmacogenomics has proven to have helped in the advancement of personalized medicine for cancer patients. Mutations of genes like CYP4F2, SLCO1B1, UGT1A1 and VKORC1 all been shown to have negative impacts on pharmacokinetics and pharmacodynamics for drugs commonly used in cancer treatment regimens. Patients with these mutations can be offered alternative treatments and dosages; however, much of the literature is based on adult pharmacogenomic data meaning more data is needed from the pediatric population to validate and report differences between the two populations. Project Inherited Cancer Risk (PICR) is a clinical trial in the DanceBlue Pediatric Oncology clinic designed to identify and manage young people with a hereditary increased cancer risk. As part of the study, variants in 16 pharmacogenomic genes are identified in 95 pediatric oncology patients with solid tumors (excluding brain tumors). Patient demographics were collected using descriptive statistics including age, biological sex, race, ethnicity, and zip code to look for epidemiologic links to mutations. Of the 95 patients, 89 patients (93.7%) had at least 1 gene with a variant. Of the 16 genes studies, 10 genes (62.5%) had at least 1 patient with a variant. The 4 genes that are more commonly presented with variants were CYP4F2 (41.6%), SLCO1B1 (21.8%), UGT1A1 (47.5%), and VKORC1 (55.4%). Much of the patient population were white (89.5%), non-Hispanic (93.7%), male (56.8%), and of the age 15 or older (58.9%). With just over a quarter of the patients having zip codes that fall within Fayette or Madison county (26.3%). Though pharmacogenomics (PGx) is still in its infancy when it comes to treating pediatric cancer, we hypothesize that pharmacogenomic findings can facilitate safer and personalized therapeutic management plans.

Social Drivers of Psychological Distress in Cancer Patients: The Impact of Financial Well-Being, Education, Health Literacy, and Cancer Team Communication

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Background. Kentucky ranks the worst in cancer incidence and mortality in the US. To advance health equity in Kentucky, the Comprehensive Connected Cancer Care (C4) Program aims to improve timely access to high-quality cancer care among underserved populations. This study explores how social drivers of health may influence psychological distress among patients at the Markey Cancer Center. It is hypothesized that lower levels of socioeconomic status, health literacy, interactions with social worker/patient care navigator, and perceived quality of cancer team communication will lead to higher psychological distress during cancer treatment.

Methods. Patients diagnosed with cancer (N = 61) completed surveys at baseline and some (N=44) again four months later. Measures include PROMIS Depression and Anxiety, the worriedness item from FACT-G7: Worry that my condition will worsen, financial well-being (FACIT-COST), education, health literacy, access to social worker/patient care navigators, and perceived quality of cancer team communication (CAHPS). General linear models and multivariate analyses (MANOVA) were conducted to examine the effects of socioeconomic status (financial well-being and education), health literacy, interaction with social workers or patient care navigators within the last 30 days, and quality of cancer team communication on psychological distress (anxiety, depression, and worriedness) at both time points.

Results. The analysis of financial well-being revealed a significant multivariate effect at both the pretest (Wilks' $\Lambda = .010$) and 4-month follow-up (Wilks' $\Lambda = .038$). Univariate analyses at pretest and follow-up showed significant effects of financial well-being on depression ($p < .009$ pretest; $p = .003$ follow-up), anxiety ($p = .012$ pretest; $p = .020$ follow-up), and worry ($p < .003$ pretest; $p = .027$ follow-up). Health literacy and social work contact were not statistically significant predictors of psychological outcomes at either time point. While not statistically significant, baseline health literacy showed a small multivariate effect (Roy's Largest Root = .037), and a corresponding non-significant univariate association with depression ($p = .115$, partial $\eta^2 = .084$). Although cancer team communication at 4 months was not statistically significant overall ($p = .222$), a non-significant multivariate effect was observed (Roy's Largest Root = .083), along with a univariate trend-level association with worry about condition worsening ($p = .092$, partial $\eta^2 = .282$).

Conclusion. Financial well-being consistently predicted psychological distress at both timepoints, with higher financial well-being associated with lower depression, anxiety, and worry that their condition will worsen. These findings reinforce the importance of addressing financial strain in cancer treatment. Although health literacy and social work contact were not statistically significant predictors, exploratory findings suggest a possible association between lower health literacy and increased depressive symptoms ($p = .115$), warranting further investigation. Cancer team communication demonstrated a non-significant multivariate effect (Roy's Largest Root = .083, Wilks' $\Lambda = .222$), but a trend-level univariate association with lower worry that one's condition would worsen was observed ($p = .092$, partial $\eta^2 = .282$), suggesting that patient-provider communication may play a role in reducing patient concerns about condition worsening. Our preliminary findings identify opportunities to strengthen patient-centered healthcare for underserved cancer patients in Kentucky.

Targeting ER Stress Sensors to Overcome Enzalutamide Resistance in Prostate Cancer

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Prostate cancer (PCa) is a significant public health issue, ranking as the second leading cause of cancer-related death in men in the United States, with projections for 2024 estimating 299,101 new cases and 35,250 deaths. A critical driver of PCa progression, the androgen receptor (AR) signaling pathway remains a major therapeutic target, particularly in castration-resistant prostate cancer (CRPC). Although AR-targeted therapies such as abiraterone and enzalutamide were developed to delay the need for chemotherapy, they provide only modest survival benefits of approximately five and two months, respectively, emphasizing the need for more effective strategies. This research investigates a resistance mechanism in CRPC mediated by a kinase-driven phosphorylation of GRP78, a key chaperone protein that aids in cellular survival independent of conventional AR signaling pathways. In this study, we examine how this phosphorylation of GRP78 triggers post-translational modifications that support CRPC cell survival under AR-targeted therapy. Using both in vitro and in vivo approaches, we characterize this adaptive resistance pathway. In vitro assays using CRPC cell lines include RNA sequencing (RNA-Seq) for transcriptomic profiling, cell viability assays, proteasome activity measurements, and Western blot analysis to evaluate protein expression changes. Confocal microscopy and flow cytometry are employed to assess cellular responses, while kinase assays and mass spectrometry elucidate specific GRP78 modifications. Chromatin immunoprecipitation sequencing (ChIP-Seq) further investigates the impact of GRP78 modification on AR's genomic binding. In vivo, CRPC xenograft models are treated with enzalutamide, allowing us to assess tumor progression and validate cellular findings via immunohistochemistry (IHC) and RNA-Seq analysis. Preliminary data indicate that phosphorylation-induced modifications of GRP78 enhance CRPC cell resilience under AR inhibition, supporting a proteostasis mechanism that counters therapeutic pressure. Targeting this adaptive response pathway may offer a novel strategy to disrupt resistance and improve the effectiveness of AR-directed therapies in advanced CRPC. By advancing insights into the cellular reprogramming associated with AR resistance, this work contributes to identifying new therapeutic targets for enhanced PCa management.

Targeting Lung Heterogeneity to Improve Non-Small Cell Lung Cancer Treatments

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Non-small cell lung cancer (NSCLC) is currently the leading cause of cancer related death in the United States. Despite improvements in treatment, including advances in anti-PD1/PD-L1 targeting immunotherapies, treatment success is extremely varied due to tumor heterogeneity. Our laboratory hypothesizes that targeting tumor heterogeneity could improve treatment responses for NSCLCs. Our first method to accomplish this is to inhibit the histone methyltransferase EZH2, which tri- methylates histone 3 lysine 27 to silence genes. Using a syngeneic lung cancer model, we observed that the EZH2 inhibitor valemetostat combined with anti-PD1 immunotherapy drives tumor regression, increased activated T cells and increased antigen expression on the tumor cells. We believe that this switch to an MHC class II high state makes the tumors very susceptible to immunotherapy-mediated cell killing. To closely study tumor-immune cell interactions ex vivo, our lab has optimized 3-dimensional tumoroid model that incorporates the liquid air interface seen within the lung with tumor and immune cells. With this model, we have observed that bone marrow from valemetostat-treated mice led to a dramatic depletion of tumoroids, and a dramatic increase in CD8+ T cells relative to controls. In addition to EZH2 inhibition that is currently in clinical trial, we are also exploring methionine restriction as an alternative way to alter tumor heterogeneity. Methionine is a pre-cursor to S-adenosyl methionine, the methyl donor for many methyl-transferases, including EZH2. Our previous studies showed that dietary methionine restriction improved carboplatin response and slowed tumor progression in an aggressive KRAS/ *Lkb1* mouse model (doi: https://doi.org/10.1101/2024.06.25.599795). Given some controversy around methionine restriction and immunotherapy, we next performed a syngeneic graft assay with KRAS/Lkb1 tumor cells. We observed that methionine restriction coupled with carboplatin or carboplatin/anti-PD1 lead to improved tumor control compared to control chow arms. We also created “multi-culture” experiments that mirror dietary intervention by reducing methionine within the media and treating with chemotherapy and immunotherapy. We observed that tumoroid growth in low methionine was stalled, particularly for KRAS/*Lkb1* tumoroids co-treated with a combination of carboplatin and anti-PD1. We also show that with the low methionine media there were significantly more T-cells present. Together these studies show that modulating EZH2 or methionine levels in NSCLC are effective ways to boost the current treatment options. We also demonstrate use of cutting edge “multi-culture” experiments that can successfully model in vivo environments to better predict cancer response to treatment.

This work was funded by: Work supported by Markey STRONG Scholars Program through the American Cancer Society IRG-22-152-34 (HY), T32 CA165990 (DRP), R01 CA237643 (CFB), R01 HL170193 (CFB), P30 CA177558 (Markey Shared Resources).

Precision Randomized Clinical Trial Comparing MTB Assisted Care to Usual Care (PRiMAL)

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Purpose and Goals: The PRiMAL study is a multicentered, prospective, cluster randomized trial, designed to compare Molecular Tumor Board (MTB) assisted care to usual care. In this study, 10 oncology practices are randomized into the usual care group or MTB assisted care intervention group using a cluster randomized design. The overall purpose and goals of the PRiMAL study is to compare survival and health-related quality of life between individuals with newly diagnosed stage IIB-IV non-small cell lung cancer (NSCLC) who receive MTB assisted care to those who receive usual care. To compare guideline concordant care, specifically to those receiving next generation sequencing (NGS) testing and treatments based on identified mutations who receive MTB assisted care to those who receive usual care. This study is sponsored by Eli Lilly and Company.

Challenges and Needs: At present, there is underutilization of NGS and Precision Medicine (PM) despite being considered usual care for all patients with Stage IIB-IV NSCLC. The PRiMAL study hypothesizes that MTBs may be an effective strategy for increasing PM use. MTBs have demonstrated improved progression free survival and response rates in single arm trials.

Target Population: This trial will compare MTB assisted care to usual care for patients across the state of Kentucky and surrounding Appalachian and non-Appalachian regions who have newly diagnosed histologically or cytologically confirmed stage IIB-IV NSCLC and are planning to undergo treatment for their cancer.

Components: This study utilizes research engagement models to maximize access across the Kentucky catchment area and maximize participation across centers. The study uses an off-site research mechanism to allow enrollment at additional centers without local research infrastructure (site personnel are not engaged in research) via the use of a centralized MCC Study Coordinator. Through the off-site research model, a MCC study coordinator screens the local eMR to identify patients and the local clinical team connects the MCC Study Coordinator with the local patient via an iPad tablet for electronic remote informed consent process and for collection of data and patient reported outcomes (PRO). For sites randomized to the MTB Intervention, the MTB nurse navigator facilitates all aspects of NGS sequencing for patients. MCC MTB Coordinator assembles the clinical data elements for presentation at the MCC MTB meeting. Individualized treatment recommendations are provided by the MCC MTB in the form of a report to the treating physician.

Endpoints: While there is nearly universal agreement that individuals with a targetable mutation should receive the targeted therapy, regarding NGS sequencing itself, many providers still question the need for testing. While population level NGS testing is guideline concordant, this question of value, combined with the complexity of NGS testing and therapy selection remain major barriers to the uptake of PM, especially in community settings. This intervention is designed to overcome both barriers by implementing NGS practice-wide and providing support (nurse navigation) for testing and interpretation. Demonstrating clinical value, specifically improved survival and health-related quality of life, in comparison to usual care will provide strong and compelling evidence for the value of the MTB in supporting use of PM in a real-world setting.

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Core Resources

Application of LAICPMS on Tumor Slices

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Breast cancer is the 2nd most common type of cancer and affects 1/8 of the women in their lifetime [1]. It is also the 2nd leading cause of death among women. To better understand, treat, and prevent breast cancer, new techniques are needed at the microscopic level to assess disease and drug resistance biomarkers. LAICPMS (Laser Ablation Inductively Coupled Plasma Mass Spectrometry) is a powerful tool for simultaneous quantitative imaging of endo- and exogenous metals, metal containing drugs as well as metal-tagged antibodies to biomarkers in tissue sections. It has been applied to a range of biological samples and pathologies [2]. With the newly improved rapid response laser ablation sample cells and analyte transport technologies, the sub ppm range sensitivities at a spatial resolution ≤ 10 μm, or near single cell can be achieved [3-5]. With the multiplex ACCLAIMS (Accumulated Elements and Cyto-Proteomics/Metabolomics by Laser Ablation Imaging Mass Spectrometry) we are developing, contamination metals and the specific designed lanthanide-conjugated antibodies to biomarkers of these tissue can be detected simultaneously for an unprecedented understanding of the mechanism of how these metals impact tumorigenesis, progression, and drug resistance. Compared with other imaging techniques, ACCLAIMS has the advantages of low background, multi-channel simultaneous acquisition, with no signal overlap between channels. Here we demonstrate the approach with images of a pair of cancerous breast tissue vs its benign counterpart showing the distribution of two contaminant metals together with six different cell/functional markers at 10 μm resolution. These images provide valuable spatially resolved information for further study of the interaction between contaminant metals and cancer cell markers in the tumor microenvironment of these patients.

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Exploring and Planning Dissemination of the Quality Implementation of Lung Cancer Screening (QUILS™) System from the Kentucky LEADS Collaborative™

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Introduction. Through the work of the Prevention and Early Detection Component of the Kentucky LEADS Collaborative™, the team developed the Quality Implementation of Lung Cancer Screening (QUILS™) System, including the QUILS™ Index, a rigorous and quantitative approach to evaluating delivery of lung cancer screening (LCS). To improve the quality of programs across the country and to facilitate translation of LCS across diverse community settings, the QUILS™ Group continues to enhance and expand a system and structure to measure, monitor and facilitate quality implementation of LCS. This planning grant provided resources to engage with state-based opportunities to enhance equitable LCS. It also allowed the team to evaluate and identify two states from a list of candidates who would be well-positioned and best suited to participate in a state-based initiative to support broad implementation of equitable and high-quality lung cancer screening.

Methods. A multidisciplinary development team designed the QUILS™ Index 2.0, an evaluation system to assess quality LCS program operations across seven domains. Each domain includes multiple quality elements, creating 21 scored elements. The transition to the revised 2.0 Index included feedback from a multidisciplinary consultant team with expertise in LCS, pulmonology, radiology, community outreach, and addressing health disparities. Changes to the resource portal including updated training materials and web-based access to the QUILS™ Assessment to help facilitate the audit and feedback process and technical assistance. Usability of the revised resource portal was also conducted with 6 lung cancer screening navigators. With access to the revised portal, they were instructed to complete 4 mock scenarios, complete a survey on system usability, and participate in an interview that assessed their knowledge, skills, and attitudes of the portal. Feedback from these usability sessions were aggregated and sent to the development team for incorporation.

The state readiness evaluation process consisted of an (1) initial interest email, (2) a survey, (3) a virtual meetings to discuss the opportunity, (4) an informative webinar, (5) in-person interviews, and (6) final state selection based on the evaluation domains. These domains included state engagement, communications, collaboration potential, current leadership/state of lung cancer screening, opportunities to reach marginalized populations, compatibility with the QUILS™ System, potential for screening programs participating, and logistical considerations surrounding implementation.

Result. Successful revision to QUILS™ 2.0 including a new comprehensive online platform that incorporates a public-facing website, protected Resource Portal and a Learning Community.

A thorough approach of prospective sites narrowed to a focused 5, and ultimately 2 states. Prospective states consistently received high scores on the evaluation domains of engagement, reach, and communications. Differences were noted in collaboration, program willingness to participate, compability with QUILS™, and logistical considerations.

Conclusion. Quality LCS implementation is an essential component to achieving optimal individual and population health benefits promised by rigorous efficacy trials of LDCT.

With additional funding provided by the Bristol Myers Squibb™ Foundation, the QUILS™ Group will implement the QUILS™ System in at least 12 lung cancer screening programs at academic, private, and rural community hospitals across two states and include innovative components of Statewide Coalition Building (to strengthen lung cancer screening infrastructure and encourage policy change) and Clinician Engagement Initiative (for provider education, outreach, and academic detailing) to enhance sustainability and success.

Markey Cancer Center Research Network Coordinating Center

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Established in 2016, the Markey Cancer Center Research Network (MCCRN) is an alliance of physicians conducting clinical research studies in the prevention, early detection, and treatment of cancers. The MCCRN conducts studies on the implementation of oncology health services and quality of life for cancer patients, survivors, and caregivers. The MCCRN conducts studies initiated by our own physicians and scientists, selected industry studies, as well as national studies available through 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 to participate in clinical trials close to home, the MCCRN supports Markey Cancer Center's mission of reducing cancer burden in Kentucky 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 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, offering education and training, study monitoring, budget and billing expertise, and regulatory support.

Markey Investigator-Initiated Trials: Based on collaborative relationships, our investigator-initiated studies are developed with unique insights 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, 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.

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 contributes to removing barriers to research participation and reducing the burden of cancer care by bringing important research opportunities into the communities we serve. MCCRN sites have enrolled 1,218 participants to studies (2016-2024), residing in 65/120 counties (54% of state), expanding Markey's clinical trial footprint by 16 Kentucky counties and 15 additional counties (13 Appalachian) outside the state. In 2024, MCCRN sites enrolled 266 participants (151 therapeutic intervention, 2 screening intervention, 23 health services research intervention, 3 prevention intervention and 87 diagnostic observation).

MCC Research Communications Office

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Markey Cancer Center, University of Kentucky

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 University of Kentucky—free of charge—by:

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

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Helping Markey researchers with editing, graphics, grants and more

NF-κB-Mediated Oxidative Stress Drives Cigarette Smoke-Induced EMT in Human Bronchial Epithelial Cells

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Cigarette smoke is a leading cause of lung cancer and chronic respiratory diseases, primarily due to its ability to induce epithelial-to-mesenchymal transition (EMT), a process critical for tumor initiation and progression. However, the molecular mechanisms linking cigarette smoke exposure to EMT remain incompletely understood. In this study, we investigated the role of oxidative stress and NF-κB signaling in mediating cigarette smoke condensate (CSC)-induced EMT in normal human bronchial epithelial cells (HBECs). Exposure of HBEC cell lines (HBEC3KT, HBEC14, HBEC2) to CSC (20 μg/mL) led to pronounced EMT-like morphological changes, including loss of epithelial characteristics and acquisition of a spindle-shaped mesenchymal phenotype. These changes were accompanied by increased reactive oxygen species (ROS) production and activation of NF-κB signaling, as evidenced by elevated phosphorylation of NF-κB p65 and degradation of IκBα in HBEC3KT. Pre-treatment or post-treatment with N-acetylcysteine (NAC), a potent antioxidant, markedly attenuated CSC-induced ROS generation and inhibited EMT-like changes. RNA-sequencing analysis further revealed significant upregulation of genes associated with oxidative stress and NF-κB-mediated transcription in CSC-treated cells. Collectively, our findings demonstrate that NF-κB-mediated oxidative stress is a key driver of CSC-induced EMT in human bronchial epithelial cells, providing mechanistic insights into early events in cigarette smoke-associated carcinogenesis

Noncontact Diffuse Optical Imaging of Blood Flow and Oxygenation Distributions in Reconstructive Skin Flaps of Rats

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Background: Breast cancer is the second common cancer among women in the United States. Approximately half of all women diagnosed with breast cancer undergo mastectomy. The main complication following mastectomy and breast reconstruction is mastectomy skin flap necrosis (MSFN), caused by insufficient blood flow and oxygenation. Intraoperative imaging of skin flap blood flow and oxygenation provides objective information for assessing ischemic-hypoxic tissues that are associated with post-surgery necrosis.

Methods: We have developed an innovative multi-wavelength speckle contrast diffuse correlation tomography (MW-scDCT) system for noncontact imaging of deep tissue blood flow and oxygenation. A galvo mirror was employed to direct the point light to multiple source positions on the tissue surface for boundary data acquisition. An sCMOS camera was used as a 2D detector array to capture re-emitted lights from the tissue for quantifying spatial diffuse speckle contrasts within the selected region of interest (ROI). MW-scDCT was first evaluated on the tissue phantom and human forearm during artery cuff occlusion. Then it was used for longitudinal imaging of 5 rats with full necrotic, half necrotic, implant, and sham skin flaps over 7 days post-surgery.

Results: The MW-scDCT enabled imaging of Intralipid particle flow contrasts in the tissue phantom at different depths and detected significant variations in forearm blood flow and oxygenation during artery cuff occlusion. In rat skin flaps with full necrosis, blood flow and oxy-hemoglobin concentration decreased, while deoxy-hemoglobin concentration increased over 7 days, demonstrating the sensitivity of MW-scDCT in detecting severe tissue ischemia and hypoxia.

Conclusions: Intraoperative fluorescence angiography has been used for detecting MSFN. However, several challenging issues limit its wide acceptance in clinical settings, including allergic reactions, short time-window for observation, and high cost for equipment and supplies. We report an inexpensive dye-free MW-scDCT that enables noninvasive and longitudinal imaging of blood flow and oxygenation distributions in skin flaps of rats. We are currently testing MW-scDCT for intraoperative imaging of human mastectomy skin flaps in the clinic for detecting MSFN.

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 (QUILS™ 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 QUILS™ System 1.0 in ten LCS programs to evaluate quality of LCS delivery in diverse settings.

Methods: A multidisciplinary development team created the QUILS™ 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 QUILS™ Index 1.0. Three data sources contributed to calculating the QUILS™ 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 QUILS™ 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 QUILS™ Audit and Feedback process, and providing access to the QUILS™ Resource Portal and QUILS™ 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.

Biostatistics and Bioinformatics Shared Resource (BB SR)

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The Biostatistics and Bioinformatics Shared Resource (BB SR) is a cancer center-managed providing essential data science expertise in biostatistics and bioinformatics to support the execution of the Markey Cancer Center's (MCC) basic science, clinical and population research. BB SR 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 SR 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 SR services are coordinated with other MCC SRs via integrated workflows to ensure comprehensive, seamless and non-overlapping support. Key technical strengths of the BB SR include: 1) innovative methods for early phase clinical trials supporting precision medicine, MCC-led multi-center NCI Experimental Therapeutics Clinical Trials Network trials and predictive biomarker development; 2) cutting-edge methods and integration of several omics and high throughput platforms; 3) design of population-based outcomes research, implementation science and behavioral intervention research using advanced methods in pragmatic trials, mediation analysis and cluster randomized designs.

Biospecimen Procurement and Translational Pathology Shared Resource (BPTP SR)

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The Biospecimen Procurement and Translational Pathology Shared Resource (BPTP SR) is a cancer center-managed resource providing biospecimen services that are cost-effective and comprehensive. BPTP SR 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 SRs 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 SRs 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.

Cancer Research Informatics Shared Resource (CRI SR)

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The Cancer Research Informatics Shared Resource (CRI SR) 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 SRs and Research Programs. CRI SR coordinates services with other MCC SRs to ensure complementary and efficient service delivery.

Flow Cytometry and Immune Monitoring Shared Resource (FCIM SR)

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The Flow Cytometry and Immune Monitoring Shared Resource (FCIM SR) 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 SR 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.

Oncogenomics Shared Resource (OG SR)

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The Oncogenomics Shared Resource (OG SR) plays a vital role in supporting the genomic investigations of the three Markey Cancer Center (MCC) Research Programs. The OG SR'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 SR'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 SR assists in choosing the proper platform(s) for genomic investigation and participates in integrated data discussion with MCC members. The service expertise of OG SR has greatly facilitated the genomic, transcriptomic and epigenomic research of the MCC Research Programs. OG SR 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 SRs to support the team science research model of MCC. With genomic service expertise and the newly obtained NGS platforms, OG SR will continue to provide comprehensive and cost-effective genomic services to MCC members. In addition, the cutting-edge sequencing capacity will enable OG SR to catalyze spatial biology and multi-omics research at MCC. The integrated service workflow between OG SR and other SRs and the streamlined process of data review and discussion between OG SR and researchers will continue to contribute to the team science model of the MCC.

Patient-Oriented and Population Sciences Shared Resource (POP SR)

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The MCC Patient-Oriented and Population Sciences Shared Resource (POP SR) supports studies with cancer patients, caregivers, and providers, as well as the general population regarding cancer-related topics. POP SR 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 SR supports population surveys, clinical studies, health services research, community-engaged research and non-therapeutic clinical trials. POP SR 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 SR 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 SR 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 SR with procedural expertise in patient-oriented and population-based research, POP SR 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.

Redox Metabolism Shared Resource (RM SR)

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The Redox Metabolism Shared Resource (RM SR) is a cancer-center managed entity that provides critical services for Markey Cancer Center (MCC) Research Programs. Notably, the RM SR 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 (especially mitochondria), metabolism and protein alterations underlie both tumorigenesis and treatment resistance. RM SR Specific Aims include 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) Proteomics-facilitated protein identification relevant to cancer. Key investments have included obtaining state-of-the-art Seahorse XFpro and XFp and Biotek Cytation cell imaging and proteomic instrumentation. RM SR 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 SR 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 SR 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 SR facilitates research with the other MCC SRs to improve the analytical power of various cutting-edge technologies for understanding the enormous cancer burden in Kentucky.