NIH NATIONAL CANCER INSTITUTE

20th Annual CCR and DCEG

Staff Scientist and Staff Clinician Retreat

"Interdisciplinary Collaborations in Cancer Research"

April 26, 2024

NCI Shady Grove, Rockville, MD 20850

U.S. Department of Health & Human Services | National Institutes of Health

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Welcome Retreat Participants!

On behalf of the Organizing Committee, it is our pleasure to welcome you to the 20th Annual Staff Scientist and Staff Clinician (SS/SC) Retreat of the National Cancer Institute's Center for Cancer Research (CCR), Division of Cancer Epidemiology and Genetics (DCEG), and Frederick National Laboratory for Cancer Research (FNLCR). We are excited to take this opportunity to share information, learn from each other, spark new ideas, foster collaborations, in our combined efforts to help improve the prognosis, and reduce the pain and suffering of those affected by cancer.

The theme for this year's SS/SC retreat is: Interdisciplinary Collaborations in Cancer Research. Today's events highlight the amazing research performed by the dedicated Staff Scientists and Clinicians working within the CCR/DCEG and FNLCR, we are excited to have short talks selected from the top-ranked abstracts submitted to this year's retreat. In addition, we have organized small-group discussions as well as two poster sessions to help foster discussions and collaborations within the SS/SC community. This year's retreat also features keynote addresses from two world-class researchers, Dr. Frank McCormick, and Dr. Tyler Jacks.

Dr. McCormick is internationally recognized cancer researcher for developing novel cancer treatment methods based on counteracting abnormal genetic changes, which either activate oncogenic signaling pathways or eliminate activities of tumor suppressors. Dr Jacks is a world leader in the field of cancer genetics and is perhaps best known for his ground-breaking work on the development of genetically engineered mouse models of cancer. We would like to also highlight planned remarks from the newly appointed NCI Director, Dr. Kimryn Rathmell along with a special address from Ms. Sue Scott, a cancer survivor who benefited from an experimental therapy developed within the CCR.

We highly appreciate the continued support of the NCI/CCR/DCEG/FNLCR leadership to SS/SC. This event would not be possible without the invaluable help from our colleagues at the CCR Center for Cancer Training (CCT), Office of the Director (OD), and Office of Training and Education (OTE), who work to support and enhance intramural cancer research and training experiences. The Organizing Committee greatly appreciates the continuing help and support from Dr. Oliver Bogler, Erika Ginsburg, Angela Jones, and Maria Moten.

We hope that our 20th Annual Staff Scientist and Staff Clinician Retreat will be intellectually stimulating and provide the opportunity to highlight the exciting work being done by all the NCI Staff Scientists and Staff Clinicians.

Best regards and many thanks for your participation,

Dr. Duane Hamilton and Dr. Brajendra Tripathi
Co-Chairs 2024 NCI SS/SC Retreat Organizing Committee
along with the entire 2024 NCI SS/SC Retreat Organizing Committee

2024 NCI SS/SC Retreat Organizing Committee *CO-CHAIRS*

Brajendra Tripathi, Ph.D. and Duane Hamilton, Ph.D.

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AGENDA

20th Annual CCR and DCEG Staff Scientists and Staff Clinicians Retreat

"Interdisciplinary Collaborations in Cancer Research"

Joseph F. Fraumeni Jr., M.D. Conf. Rm TE406/408/410

8.45-9.00 a.m.	Registration and posters setup
9:00-9:15 a.m.	Welcome address by Dr. Stephen Chanock, Director, DCEG Moderator: Dr. Duane Hamilton
9:15-9:30 a.m.	NCI Director's remarks by Dr. Kimryn Rathmell Moderator: Dr. Brajendra Tripathi
9:30-10:15 a.m.	Keynote address I by Dr. Frank McCormick (UCSF) Moderator: Dr. Brajendra Tripathi Title: RAS proteins in human disease
10:15-10:25 a.m.	Coffee break for 10 min
10:25-11:25 a.m.	Oral Presentation Session I: SS/SC selected speakers Moderators: Dr. Brajendra Tripathi and Dr. Zhihui Liu
	Dr. Bing-Rui Zhou Title: Pioneer transcription factors Ascl1/E12a and TFAP2a use distinct modes of nucleosome binding to expose DNA in chromatin
	Dr. Binwu Tang Title: A novel imaging approach suggests a critical role for cancer stem cells as sensitive sensors of microenvironmental quality in metastatic progression
	Dr. James Madigan Title: Multiple layers of epigenetic regulation conspire to silence expression of somatostatin receptor type 2 in pancreatic neuroendocrine tumors
	Dr. James Phelan Title: Response to BTK inhibitors in aggressive lymphomas linked to chronic selective autophagy
11:25-11:30 a.m.	Staff Scientists Organization Introduction by Dr. Swati Choksi
11:30 a.m.-12:15 p.m.	Poster Session I (odd numbered abstracts)
12:15-1:15 p.m.	Lunch and small group discussions

1:15-2:00 p.m.	Poster Session II (even numbered abstracts)
2:00-2:45 p.m.	Keynote address II by Dr. Tyler Jacks (MIT) Moderator: Dr. Duane Hamilton Title: Investigating tumor-immune dynamics using genetically engineered mouse models of cancer
2:45-3:00 p.m.	Coffee break for 15 min
3:00-3:45 p.m.	Oral Presentation Session II: SS/SC selected speakers Moderators: Dr. Brajendra Tripathi and Dr. Zhihui Liu Dr. Sofia Gameiro Title: Harnessing the potential of epigenetic modulation in combination with tumor-targeted IL-12 to overcome tumors refractory to PD-1/-L1-targeted therapy Dr. David Milewski Title: Engineered adoptive T cell transfer in combination with TGF-beta signaling blockade for immunotherapy against osteosarcoma and other malignancies Dr. Dimitris Stellas Title: Combination treatment of hetIL-15 and Fenofibrate affects the metabolic fitness of tumor infiltrating CD8+T cells, severely impacting tumor growth in breast and pancreatic cancer mouse models
3:45-4:30 p.m.	Oral Presentations Session III: SS/SC Selected speakers Moderators: Dr. Duane Hamilton and Dr. Sukhbir Kaur Dr. Lene Veiga Title: Thoracic soft tissue sarcoma risk following breast cancer treatment in two US retrospective cohorts Dr. Masashi Watanabe Phase II trial evaluating the association of peripheral blood immunologic response and therapeutic response to immune checkpoint inhibition in patients with newly diagnosed glioblastoma and gliosarcoma Dr. Stacy Doran Tumor-Infiltrating Lymphocytes (TIL) for Metastatic Human Papillomavirus (HPV)-Associated Cancers
4:30-4:45 p.m.	Ms. Sue Scott Cancer survivorship speaker (Office of Advocacy Relations) Moderator: Dr. Duane Hamilton

4:45-5:00 p.m.	Awards ceremony and closing remarks by Dr. James Gulley, Clinical Director, CCR Moderator: Dr. Brajendra Tripathi
5:30-8:00 p.m.	Happy hour at Rio Lakefront, Gaithersburg, Maryland (in front of Uncle Julio's tavern)

Invited Speakers

Kimryn Rathmell M.D., Ph.D., M.M.H.C. Director, National Cancer Institute

Dr. Kimryl Rathmell is a renowned expert in renal cancer and an influential leader in cancer research and patient care. Before joining as a NCI Director in December 2023, she was the Hugh Jackson Morgan Professor and Chair of Medicine at Vanderbilt University Medical Center (VUMC), and Physician-in-Chief for Vanderbilt University Adult Hospital and Clinics. Dr. Rathmell obtained her undergraduate degrees in biology and chemistry from the University of Northern Iowa. She earned her Ph.D. in biophysics and M.D. from Stanford University. Following completion of her M.D., she did an Internal Medicine internship at the University of Chicago. In 2015, Dr. Rathmell joined VUMC as Professor of Medicine and Director of the Division of Hematology and Oncology, with a secondary appointment in the Department of Biochemistry. In 2019, she was named Deputy Director for Research Integration and Career Development at Vanderbilt-Ingram Cancer Center. She served in this role until her appointment as Chair of the Department of Medicine in 2020. In 2022, she completed her Master of Management in Health Care from the Vanderbilt University Owen Graduate School of Management. Prior to Vanderbilt, she was a faculty member at the University of North Carolina at Chapel Hill, where she was co-director of the HHMI Graduate Training Program in Translational Medicine, Associate Director for Training and Education at UNC Lineberger Comprehensive Cancer Center, and Associate Director and Alexander Family Chair in Translational Research for the Medical Scientist Training Program. As a physician-scientist, Dr. Rathmell's specialty is the research and treatment of complex and hereditary kidney cancers. She also focuses on underlying drivers of kidney cancers using genetic, molecular, and cell biological approaches to develop interventions to improve patients' lives. As a member of The Cancer Genome Atlas, she has contributed to the discovery that mutations in mitochondrial DNA may in part be responsible for the development of a rare form of renal cancer called chromophobe renal cell carcinoma. More recently, her genomic studies have led to the identification of novel molecular biomarkers to help detect and treat renal cancers earlier. Dr. Rathmell is also an advocate for research into rare forms of renal cancer, including renal medullary carcinoma (RMC), a rare type of renal cancer predominantly affecting young adult and adolescent patients of African ancestry who carry one copy of the sickle cell hemoglobin gene. She co-founded the nonprofit RMC Alliance to support patients with renal medullary carcinoma. In addition to publishing hundreds of scientific articles on her research, Dr. Rathmell, has published on ethical issues such as cancer drug shortages and conflicts of interest in scientific publishing. Beyond her research interests, she is committed to mentoring and developing the next generation of physician-scientists. Dr. Rathmell is a recipient of the 2019 Louisa Nelson Award for Women of Achievement, Vision and Inspiration, the 2019 Eugene P. Schonfeld Award from the Kidney Cancer Association and the Paragon Award for Research Excellence from the Doris Duke Foundation and the 2020 American Association for Cancer Research Team Science Award. She has served on the NCI Board of Scientific Advisors and the Forbeck Foundation Scientific Advisory Board. She has held leadership positions for the American Society of Clinical Oncology, Kidney Cancer Research Foundation, and the American Society for Clinical Investigation serving as the Secretary-Treasurer, and later President of that organization. She was elected to the National Academy of Medicine in 2022 and the American Academy of Arts and Sciences in 2023.

Tyler Jacks, Ph.D., David H. Koch Professor of Biology, MIT

Dr. Tyler Jacks is the David H. Koch Professor of Biology at MIT and the President of the Break Through Cancer Foundation. He is a world leader in the field of cancer genetics and is known for his ground-breaking work on the development of genetically engineered mouse models of cancer (GEMMS). Dr. Jacks obtained his BA in Biology from Harvard College and Ph.D. from the University of California, San Francisco. His postdoctoral work was at the Whitehead Institute, where he developed several GEMMs, including the Rb, p53 and Nf1 mice. In 1992, Dr. Jacks joined the Department of Biology at MIT as an assistant professor. He served as director of the MIT Center for Cancer Research and its successor, the Koch Institute from 2000 to 2021. He was a Howard Hughes Medical Institute Investigator from 1994-2021 before stepping down to take on his current role as President of the Break Through Cancer Foundation. The Jacks lab has focused on developing new methods for the construction and characterization of GEMMs of human cancer. His group has produced GEMMs with constitutive and conditional mutations in several tumor suppressor genes, oncogenes, and genes involved in cell cycle control and apoptosis. Using these strains, the laboratory has developed models of various human tumor syndromes and cancer types, including Li-Fraumeni Syndrome, neurofibromatosis type 1, astrocytoma, retinoblastoma, pancreatic cancer, invasive colon cancer, soft tissue sarcoma, endometriosis, ovarian cancer, and lung adenocarcinoma. These GEMMS have been used to examine the mechanism of tumor initiation and progression, to uncover the molecular, genetic, and biochemical relationship to the human diseases, as tools to study response and resistance to chemotherapy, and to explore methods in molecular imaging and early detection of cancer. His laboratory has also extensively studied human tumor-derived cells, determined protein function in these cells, and related gene expression patterns in human cancers to their studies in mouse models. In recent years, the Jacks lab has utilized GEMMs to understand the interactions between the immune system and cancer. These models offer a unique platform for investigating how anti-tumor immune responses shape tumor gene expression, how tumor evasion mechanisms contribute to the development of advanced disease, and they are invaluable preclinical models for testing immunotherapeutics. Over the course of his academic career, Dr. Jacks has published over 200 peer- reviewed papers along with numerous review articles and book chapters. He has served on the Board of Scientific Advisors of the National Cancer Institute and the Board of Directors of the American Association of Cancer Research (AACR); he is also a past President of the AACR. He is currently serving as director of the Blue Ribbon Panel, which provides expert advice for the National Cancer Moonshot Initiative. He also serves as an advisor to several biotechnology and pharmaceutical companies. He is the cofounder of T2Biosystems and Dragonfly Therapeutics. Dr. Jacks has received numerous awards and honors for his contributions to the study of cancer genetics including the AACR Outstanding Achievement Award, the Amgen Award from the American Society of Biochemistry and Molecular Biology, the Chestnut Hill Award for Excellence in Medical Research, the Paul Marks Prize for Cancer Research, 2013 honoree of the MGH Cancer Center's One Hundred celebration, the Hope Funds for Cancer Research Award for Excellence, the Sergio Lombroso Award in Cancer Research, and MIT's Killian Award. He was elected to both the National Academy of Sciences and the Institute of Medicine of the National Academies in 2009, as well as the American Academy of Arts and Sciences in 2012 and the inaugural class of Fellows of the AACR Academy in 2013.

Frank McCormick Ph.D., FRS Professor, UCSF Helen Diller Family Comprehensive Cancer Center, Scientific Director, Ras Initiative, Frederick National Laboratory for Cancer Research

Dr. McCormick holds the David A. Wood Chair of Tumor Biology and Cancer Research at UCSF. He is internationally recognized for developing novel cancer treatment methods based on counteracting abnormal genetic changes, which either activate oncogenic signaling pathways or eliminate tumor suppressors. He has led the National Cancer Institute's Ras Initiative at the FNLCR overseeing the national effort to develop therapies against Ras-driven cancers which include pancreatic cancers, colorectal and lung cancers, and are amongst the most difficult cancers to treat. A native of Cambridge, England, Dr. McCormick received his BSc in biochemistry from the University of Birmingham and his PhD in biochemistry from the University of Cambridge. He carried out his postdoctoral work at the State University of New York at Stony Brook and in London at the Imperial Cancer Research Fund. Prior to joining the UCSF faculty, Dr. McCormick pursued cancer-related work with several Bay Area biotechnology firms and held positions with Cetus Corporation (Director of Molecular Biology, 1981-1990; Vice President of Research, 1990-1991) and Chiron Corporation, (Vice President of Research from 1991-1992). In 1992 he founded Onyx Pharmaceuticals, a company dedicated to developing new cancer therapies, and served as its Chief Scientific Officer until 1996. At Onyx Pharmaceuticals, he initiated and led drug discovery efforts that led to the approval of Sorafenib in 2005 for treatment of renal cell cancer, and for liver cancer in 2007, and the approval of ONYX-015 in 2006 in China for treatment of nasopharyngeal cancer. In addition, Dr. McCormick's group led to the identification of the CDK4 kinase inhibitor, Palbociclib, approved for treating advanced breast cancer. His lab continues to focus on Ras and identifying new ways of treating disease driven by Ras proteins. They have identified the molecular basis of the effects in which K-Ras proteins promote stem-cell properties in cancer cells, enable them to establish metastatic tumors with high efficiency, and become drug resistant. The McCormick lab has identified small molecules that binds irreversibly to K-Ras and prevent prenylation. With a developed collection of highly potent and specific siRNAs that target K-Ras and the components of the pathways, combinations of these siRNAs have resulted in tumor destruction in vivo. They developed a system of expressing single Ras isoforms in a “Ras-less” MEF background as a discovery tool to identify specific biochemical properties of individual Ras proteins in a clean background. Additionally, major progress has been made in understanding the mechanism underlying Neurofibromatosis Type 1 (NF1). The gene responsible for NF1 encodes a protein that negatively regulates Ras and loss of the gene results in hyperactivation of Ras. The NF1 protein depends on another protein, SPRED1, which is mutated in another familial syndrome called Legius Syndrome. He has authored over 400 publications and holds 20 issued patents. He became a Fellow of the Royal Society in 1996. He was on the Board of Directors of the American Association for Cancer Research from 2002-2005 and served as its President from 2012-2013. He was elected as a member of the National Academy of Sciences in 2014. Dr. McCormick is the recipient of numerous awards including the Novartis Drew Award in Biomedical Research (2002), the AACR-G.H.A. Clowes Memorial Award (2002), the Simon M. Shubitz Award, University of Chicago Cancer Research Center (2003), Salute to Excellence Award, American Liver Foundation (2008), Science of Oncology Award, American Society of Clinical Oncology (2010) and the Centenary Award, Biochemical Society (2018).

Sue Scott


Sue Scott is a native Washingtonian; a Realtor in DC and MD; and a metastatic cervical cancer survivor. After Sue's initial diagnosis in October 2011, she received the standard course of treatment for her Stage 1B2 cervical cancer. In summer of 2012, a few months after completing herinitial treatment regimen, Sue was told that her cancer had returned, spread to her lymph nodes and was metastatic. After being told by her team of oncologists that there was no known treatment for her advanced cancer, Sue began to search for clinical trials to participate in. Sue spoke with many research doctors at various hospitals on the East Coast. Although there were no responders yet, Sue chose to participate in the immunotherapy/TIL replacement trial at NIH, led by Dr. Christian Hinrichs. Sue was denied as a patient for the trial twice, due to complications from her very advanced disease. Nevertheless, she persisted. Sue felt very strongly about taking part in this specific clinical trial and after a few months of delays and corrective treatments and procedures, she was accepted as a patient in Dr. Hinrich's trial. In March 2013, Sue was infused with 74.5 billion of her own targeted and expanded TIL cells. Just 2 months later Sue received the news that all the tumors throughout her abdomen and pelvis had disappeared and were no longer detectable on scans. Sue was one of 16 patients in that clinical trial for HPV-caused cancers and is one of only two patients that had a full response to the treatment. 11 years later Sue is still cancer-free. Sue is extraordinarily grateful to be alive today because of the awe-inspiring work and expert care of her team of research doctors, nurses, and staff at NIH. Sue's gratitude for her cancer freedom has led her to share her "guinea pig success story" with the hope it will help more cancer patients find a path to life-saving clinical trials, healing, and life after cancer.

Stephen J. Chanock, M.D. Director DCEG, National Cancer Institute

Dr. Stephen Chanock is the Director of the NCI Division of Cancer Epidemiology and Genetics (DCEG). He is a leading expert in the discovery and characterization of cancer susceptibility regions in the human genome. Dr. Chanock received his M.D. from Harvard Medical School and completed clinical training in pediatrics, pediatric infectious diseases, and pediatric hematology/oncology and research training in molecular genetics at Boston Children's Hospital and the Dana-Farber Cancer Institute, Boston. From 2001-2007, he was a tenured investigator in the Genomic Variation Section of the Pediatric Oncology Branch in the NCI Center for Cancer Research. He also served as co-chair of NCI's Genetics, Genomics and Proteomics Faculty for five years. In 2001, he was appointed as Chief of the Cancer Genomics Research Laboratory (formerly Core Genotyping Facility), and in 2007 as Chief of the Laboratory of Translational Genomics, both within DCEG. From 2012 to 2013, he also served as Acting Co-Director of the NCI Center for Cancer Genomics. Dr. Chanock was appointed Director of DCEG in August 2013. Since 1995, Dr. Chanock has served as the Medical Director for Camp Fantastic, a week-long recreational camp for pediatric cancer patients, which is a joint venture of the NCI and Special Love, Inc. His research focuses on the identification and characterization of cancer susceptibility alleles, the scope of genetic mosaicism and its contribution to cancer risk, the genetic architecture of cancer

susceptibility and how germline variation informs our understanding of somatic alterations in cancer. His team produces genome-wide association studies (GWAS) that help researchers understand cancer risk. NCI GWAS research covers topics such as sex-related effects on immunotherapy response, new genetic loci associated with susceptibility to Ewing sarcoma and prostate cancer, and multiple risk factors for renal cell carcinoma. He has received numerous awards for his scientific contributions including the Niehaus, Southorth, Weissenbach Award in Clinical Cancer Genetics, the NIH Directors Award, the Jeffrey M. Trent Lecture, and the AARC-American Cancer Society Award for Research Excellence in Cancer Epidemiology and Prevention. Dr. Chanock is an elected member of the Association of the American Physicians, the American Epidemiology Society, and the Society for Pediatric Research.

Dr. James Gulley, M.D., Ph.D. Clinical Director, CCR, National Cancer Institute

Dr. James Gulley is an internationally recognized expert in immunotherapy for cancer. He graduated from Loma Linda University in California with a Ph.D. in microbiology in 1994 and an M.D. in 1995. As part of this eight-year MD/PhD Medical Scientist Training Program, he completed a dissertation on tumor immunology. He completed his residency in Internal Medicine at Emory University in 1998, followed by a Medical Oncology fellowship at the National Cancer Institute.
Dr. Gulley serves within the Center for Cancer Research of the National Cancer Institute as Co-Director of the Center for Immuno-Oncology, and also serves as the Clinical Director, CCR, NCI. He has been instrumental in the clinical development of multiple immunotherapeutic agents and has led multiple first-in-human immunotherapy studies through phase 3 clinical trials. He was the coordinating PI of an international trial of avelumab that led to regulatory approval. He was the PI of the first-in-human international study of a first in class agent, bintrafusp alfa, which targets PDL1 and TGF-beta. He also leads a number of rationally designed, cutting edge combination immunotherapy studies. Dr. Gulley is the Interim Editor-in-Chief of JITC and the Vice President of SITC. He serves on many national and NIH boards and committees. He has been an investigator on over 200 clinical trials and has authored over 350 scientific papers or chapters which have been cited over 25,000 times. He has made hundreds of scientific presentations at universities or national / international meetings. He has had multiple awards including the 2010 Presidential Early Career Award for Scientists and Engineers, the highest award bestowed by the US President on investigators early in their careers. He also was awarded the 2018 Hubert H. Humphrey Award for Service to America for contributing to the health, safety, and well-being of the nation by helping to get FDA approval for avelumab for Merkel cell carcinoma and urothelial carcinoma and has received numerous NCI or NIH Director's Awards. Dr. Gulley has recently been appointed to the interm position of co-Director of the CCR.

Brajendra Tripathi Ph.D. Staff Scientist, Laboratory of Cellular Oncology, Center for Cancer Research, National Cancer Institute

Dr. Tripathi has an abiding interest in the clinical applications of basic research. He obtained his Ph.D. in Biochemistry from Jawaharlal Nehru University, India, where he was a recipient of the Council of Scientific and Industrial Research Fellowship. Dr. Tripathi carried out his postdoctoral research in the Laboratory of Molecular and Developmental Biology at the National Eye Institute, National Institutes of Health, where his research focused on signal transduction mechanisms that regulate epithelial cell adhesion and migration in the lens and cornea. He has developed animal models for studying corneal wound healing and evaluated the therapeutic potential of inhibitors of protein kinases, which regulate cell adhesion and cell migration. Subsequently, Dr. Tripathi joined the Laboratory of Cellular Oncology, headed by Dr. Douglas Lowy, at the National Cancer Institute as a Research Fellow and was promoted to Staff Scientist. His current research focuses on how tumor suppressor proteins, which are downregulated or inactivated in several cancer types by distinct mechanisms, can be stabilized, reactivated, and exploited for cancer treatment. Specifically, Dr. Tripathi is exploring new functions of the tumor suppressor protein, Deleted in Liver Cancer 1, and the kinases and methyltransferases that regulate its functions in normal physiology and in cancer. Using lung cancer as a model system, Dr. Tripathi and colleagues discovered that GTP bound RAS protein is required for the efficient export of nuclear proteins to the cytoplasm by Exportin 1 (XPO1) and identified key mechanistic aspects of the nuclear export process. His recent research expands the fundamental understanding of RAS activity in nuclear protein export process, its role in cancer pathogenesis, and the potential clinical utility of RAS inhibition for treatment of cancers that harbor mutant RAS. Dr. Tripathi has published many first and corresponding author peer-reviewed research articles and is a co-inventor in 14 patents. He is a recipient of the NCI Director's Innovation Award, NCI Federal Technology Transfer Award, Distinguished Achievement Award, the Fellow Award for Research Excellence, the American Society of Cell Biology Travel Award, the National Eye Institute Scientific Director's Outstanding Mentor Award, and has been featured in the 'People and Ideas' section of the *Journal of Cell Biology*. He has supervised and mentored many postbaccalaureate fellows and postgraduate research trainees. Dr. Tripathi presently serving as an Associate Editor and Editorial Board member of several scientific journals. He is the current Editor-in-Chief of the Staff Scientist and Staff Clinician (SS/SC) newsletter (The Dossier) and Co-Chair of the Staff Scientist and Staff Clinician (SS/SC) communications sub-committee.

Duane Hamilton Ph.D. Staff Scientist, Center for Immuno-Oncology, Center for Cancer Research, National Cancer Institute

Dr. Hamilton is a Staff Scientist and Head of the Immunomodulation Group of the Center for Immuno-Oncology, NCI. He received his Ph.D. degree in Immunology from the University of Saskatchewan, Canada. He subsequently joined the NIH as a Postdoctoral Fellow in the (former) Laboratory of Tumor Immunology and Biology, Center for Cancer Research, National Cancer Institute, and was appointed as a Staff Scientist in 2015. Dr. Hamilton's research interests include cellular immunotherapy, tumor immunology, and cancer vaccines. The Immunomodulation Group is working to identify and characterize tumor-specific neoepitopes and their use in combination therapies. The group is also involved in the genetic modification to tumor cells to identify mechanisms of action of specific immunotherapeutic agents. The group evaluates techniques to identify tumor antigens unique to a patient's own tumor. It is this group's belief that vaccinating patients with neoepitopes uniquely expressed by their tumor will improve the breadth of anti-tumor immunity generated by the lab's vaccine platforms, and result in greater immunological control of tumor growth. He is an active member of the Staff Scientist and Staff Clinician community in NCI.

ABSTRACTS

Oral Presentations

5. Bing-Rui Zhou, Ph.D., Laboratory of Biochemistry and Molecular Biology, CCR

Bing-Rui Zhou[1]*, Edgar Luzete-Monteiro[2,3]* Hsin-Yao Tang[4], Meilin Fernandez Garcia[3], Mariel Coradin[3], Naomi Takenaka[3], Megan Frederick[3], Benjamin Garcia[3], Kenneth S. Zaret[3]*, Yawen Bai[1]*

"Pioneer transcription factors Ascl1/E12a and TFAP2a use distinct modes of nucleosome binding to expose DNA in chromatin"

1. Laboratory of Biochemistry and Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA
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3. Perelman School of Medicine, University of Pennsylvania, Philadelphia-PA, USA
4. Proteomics & Metabolomics Facility, The Wistar Institute, Philadelphia-PA, USA

30. Binwu Tang, Ph.D., Laboratory of Cancer Biology and Genetics, CCR

Binwu Tang, Jacob Minin, Victoria Gonzalez, Zoya Khan, Yuval Raviv, Lalage M. Wakefield

"A novel imaging approach suggests a critical role for cancer stem cells as sensitive sensors of microenvironmental quality in metastatic progression"

Cancer stem cells (CSCs) are a minor subpopulation of tumor cells that drive tumorigenesis, metastasis, and resistance to therapy. We developed a lentiviral-based fluorescent sensor that enables real-time monitoring of CSC dynamics, using an artificial enhancer element (SORE6) that responds to the master stem cell transcription factors Oct4 and Sox2. To investigate CSC dynamics during early metastatic lung colonization, MDAMB231-LM2 human metastatic breast cancer cells transduced with the sensor were injected into the tail vein of nude mice. Quantitative imaging of lungs harvested at different time points after tumor cell injection, showed distinct dynamics for CSCs and non-CSC tumor cells during early lung colonization. CSCs had a ~5-fold higher early survival advantage compared to non-CSCs. Subsequent development of metastatic lesions to the 8-cell stage exclusively involved symmetric and asymmetric self-renewing divisions within the CSC population. Beyond the 8-cell stage, non-CSC progeny began to proliferate and drive lesion expansion, while the number of proliferating CSCs plateaued. Live-cell imaging of tumor cell cultures in vitro unexpectedly revealed a similar pattern of early expansive CSC self-renewal followed by a plateau. Using single cell fate mapping, we showed that CSCs switch from expansive self-renewal to a state of balanced self-renewal and differentiation at a critical cell density. Consequently, CSC numbers plateau early while non-CSCs continue to expand. Multiple factors contributing to the CSC plateau included nutrient/growth factor depletion and cell contact/shape change. Notably, CSCs exhibit a more rapid response than non-CSCs to changes in the tumor cell microenvironment,

suggesting that CSCs may serve as sensitive cellular "sensors" of microenvironmental quality for the entire tumor cell population. Using pharmacologic inhibitors and genetic knockdown approaches, we identified several input pathways converging on the YAP/TAZ transcriptional co-regulators that affect CSC self-renewal. Importantly, while chemotherapy treatment (Paclitaxel) inhibited only non-CSCs, combined treatment of Paclitaxel with the ROCK1/2 inhibitor Y27632 to modulate YAP/TAZ reduces both non-CSCs and CSCs both in vitro and in vivo. Thus, combined pharmacologic inhibition of YAP signaling with chemotherapy effectively targets both chemo-resistant and chemo-sensitive cancer cells.

Lab of Cancer Biology and Genetics, Center for Cancer Research, National Cancer Institute, Bethesda, MD, USA

43. James Madigan, Ph.D., Surgical Oncology Program, CCR

James P. Madigan, Stephen G. Andrews, Steven D. Forsythe, Srujana V. Yellapragada and Samira M. Sadowski

"Multiple Layers of Epigenetic Regulation Conspire to Silence Expression of Somatostatin Receptor Type 2 in Pancreatic Neuroendocrine Tumors"

Background and Hypotheses: Pancreatic neuroendocrine tumors (P-NETs) are a rare cancer with increasing incidences worldwide. Low-grade P-NETs are unique in that they express high levels of Somatostatin Receptor Type 2 (SSTR2), which represents a target for both tumor imaging and therapeutics. P-NET grade inversely correlates with SSTR2 tumor staining, and higher tumor grade is associated with poor patient prognosis. Unfortunately, application of SSTR2-targeted treatment options is currently limited in high-grade P-NETs, due to loss of SSTR2 expression. Beyond understudied promoter CpG DNA methylation and nebulous histone deacetylation events, little is known regarding the full scale of actual epigenetic events that conspire to negatively control SSTR2 expression. The goal of our ongoing studies is to obtain a more comprehensive understanding, at the molecular level, of the epigenetic events and players which control SSTR2 expression. A better understanding and enhanced knowledge of the epigenetic events which regulate SSTR2 gene expression may allow for identification of potential novel epigenetic-based, SSTR2-specific P-NET imaging/treatment modalities, with the possibility for improved patient toxicity profiles.

Study Design and Methods: Two high-grade P-NET cell lines, BON1 and QGP1 (both with low SSTR2 expression), and one low-grade P-NET cell line, NT-3 (high SSTR2 expression), were employed in our studies. Both small molecule inhibitors/drugs and validated shRNA, targeting various epigenetic enzymes, were utilized in functional assays to determine their potential effects on SSTR2 expression. Western blot analysis was used to gauge potential increased expression of SSTR2, along with changes in global expression levels of selected epigenetic marks. Chromatin immunoprecipitation (ChIP) was used to measure levels of specific histone-based epigenetic marks located within SSTR2 gene regulatory elements. Bisulfite Next-Generation Sequencing was employed to determine,

both qualitatively and quantitatively, levels of CpG methylation in the SSTR2 gene promoter.

Results and Conclusions: We have demonstrated that DNMT3B is the sole DNA methyltransferase responsible for silencing SSTR2 expression in P-NETs. Additionally, we identified Class I HDACs as the main histone deacetylases important for SSTR2 expression regulation. Furthermore, we discovered that Polycomb Repressor Complexes 1 and 2 (PRC-1 and PRC-2) are central to SSTR2 silencing. Removal of activating histone H3K4 methylation marks is an additional mechanism for silencing SSTR2 expression, through specific histone lysine demethylases, such as LSD1 and KDM5A. Moreover, we identified the HDAC and LSD1-containing NuRD (Nucleosome Remodeling and Deacetylase) repressor complex as vital for epigenetic silencing of SSTR2 in P-NETs. Finally, we identified the chromatin remodeling enzyme, Lymphoid-Specific Helicase (LSH), as a negative regulator of SSTR2 expression. We conclude that multiple inhibitory epigenetic mechanisms cooperate to silence expression of SSTR2 in P-NETs. Knowledge gained from our studies will assist in formulation of novel epigenetics-based intervention strategies, to increase expression of SSTR2, for improved imaging and therapeutic treatment of high-grade P-NETs.

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25. James Phelan, Ph.D., Lymphoid Malignancies Branch, CCR

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Diffuse large B cell lymphoma (DLBCL) is a molecularly heterogeneous cancer that presents a challenge for precision medicine. Inhibitors of Bruton tyrosine kinase (BTK) block B cell receptor (BCR)-dependent NF-ÎºB signaling and are particularly effective in DLBCL with mutations in the BCR subunit CD79B and MYD88 (MCD DLBCL). MCD tumors are enriched for a multiprotein supercomplex, termed the My-T-BCR, that is nucleated by MYD88L265P, TLR9 and the BCR, and serves as a central hub of NF-ÎºB signaling. The integrity of the My-T-BCR complex is rapidly compromised following BTK inhibition, but the molecular mechanisms responsible for this remain elusive.

To investigate the mechanisms regulating the My-T-BCR, we used CRISPR-Cas9 screens in MCD DLBCL cell lines treated with inhibitors of BTK (BTKi) and other survival pathways. We identified several drug resistance genes encoding negative regulators of BCR, NF-ÎºB, and PI3 kinase signaling that are recurrently inactivated in DLBCL biopsies. Unexpectedly, autophagy-related genes involved autophagosome formation (ATG9A, ATG101, ATG13,

RB1CC1 and ATG14) and autophagosome membrane expansion (ATG2A, WIPI2, WDR45) also counteracted the toxicity of BTKi in these screens.

To gain further insight, we generated BTKi-resistant cell lines deficient in ATG9A or ATG101 (ATG KO) and performed CRISPR screens and RNA-seq with or without BTKi. We observed 11 ATG genes that displayed epistatic interactions, no longer conferring BTKi resistance in ATG KO MCD cells. We also observed the buffering of NF-ÎºB negative regulators, and the increased sensitivity to loss of NF-ÎºB positive regulators. Furthermore, gene expression studies showed decreased expression of BCR, MYD88 and NF-ÎºB signatures in control BTKi-treated cells, whereas ATG KOs did not. ATG KOs displayed higher levels of nuclear NF-ÎºB upon BTKi treatment. Amongst primary tumors, MCD patients have the highest levels of NF-ÎºB gene expression. Interestingly, MCD patients also displayed the lowest gene expression for ATG genes, suggesting this pathway is counter-selected during pathogenesis.

As autophagy promotes protein degradation, we assessed global protein levels and localization using mass spectrometry and BioID. In ATG KOs, selective autophagy receptors TAX1BP1, NBR1 and p62 were significantly upregulated and were found near MYD88L265P suggesting this mutant proteoform is targeted for degradation by selective autophagy. To test this, we engineered autophagy reporters (GFP-RFP fusions) for TAX1BP1, NBR1, p62, or MYD88L265P. The autophagic flux of each reporter was blocked in ATG KOs.

To identify genes controlling autophagic flux of MYD88L265P, we performed CRISPR screens for changes in turnover of the MYD88L265P autophagy reporter. Deletion of the above epistatic interactors of ATG9A also blocked MYD88L265P autophagic degradation. Conversely, deletion of BTK, mTORC1-related genes and IRF4 promoted MYD88L265P autophagic degradation. We validated these findings using targeted inhibitors of each gene alone, or in combination, and observed synergy for promoting MYD88L265P autophagic degradation.

Collectively, we identified a non-canonical form of selective autophagy that degrades MYD88L265P and is promoted upon BTK inhibition. Our findings help to elucidate the exceptional benefit of BTK-targeted therapies in MCD DLBCL and offer a rationally designed combination therapy regimen that specifically degrades this oncogenic allele of MYD88.

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60. Sofia R Gameiro, Ph.D., Center for Immuno-Oncology, CCR

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"Harnessing the potential of epigenetic modulation in combination with tumor-targeted IL-12 to overcome tumors refractory to PD-1/-L1-targeted therapy"

Clinical benefit from immune checkpoint blockade (ICB) targeting the PD-1/PD-L1 axis remains elusive for most patients with solid malignancies that are poorly inflamed or display tumor-intrinsic loss of MHC class I (MHC-I) and aberrations in antigen processing machinery (APM) and interferon gamma (IFNï) pathways. Here we demonstrate, by using comprehensive single-cell transcriptome, proteome, and immune cell analysis, that the epigentic modulator Entinostat, a class I histone deacetylase inhibitor, facilitates accumulation of the necrosis-targeted immune-cytokine NHS-IL12 in ICB-refractory murine colon tumors (CT26) and poorly immunogenic breast carcinomas (EMT6) in which the therapy was curative. Combination therapy reprograms the tumor innate and adaptive immune milieu to an inflamed landscape, where the concerted action of highly functional CD8+ T cells and activated neutrophils drive a dramatic macrophage M1-like polarization, leading to complete tumor eradication in 41.7%-100% of cases. Further, combination therapy overcame multiple anti-PD1/PDL1-resistant murine tumor models harboring MHC-I, APM, and IFNg response deficiencies [TC-1/a9 (lung, HPV16 E6/E7+), lung CMT.64, and RVP3 sarcoma]. Potent anti-tumor activity and survival benefit observed with combination therapy were driven by prolonged activation and tumor infiltration of cytotoxic CD8+ T cells. Combination therapy promoted M1-like macrophages and activated antigen presenting cells (APCs) while decreasing M2-like macrophages and Tregs in a tumor-dependent manner. This was associated with increased levels of IFNg, IL-12, CXCL9 and CXCL13 in the TME. Combination therapy synergized to promote MHC-I and APM upregulation, and enrichment of Jak/STAT, IFNg-

response and antigen processing-associated pathways. A biomarker signature of the mechanism involved in these studies is associated with patients' overall survival across multiple tumor types. Conclusions: Our findings provide a rationale for combining the tumor targeting NHS-IL12 with the HDACi entinostat in the clinical setting for patients unresponsive to anti-PD1/PDL1 and/or with innate deficiencies in tumor inflammation, MHC-I, APM expression, and IFNï signaling.

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80. David Milewski, Ph.D., Genetics Branch, CCR
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"Engineered adoptive T cell transfer in combination with TGF-beta signaling blockade for immunotherapy against osteosarcoma and other malignancies"

Background and Hypotheses: Osteosarcoma is an aggressive bone sarcoma with only a 60% 5-year survival rate despite multimodal chemotherapy regimens. One of the hallmark features of osteosarcoma is the presence of significant structural chromosomal abnormalities which generates a large number of somatic mutations. Our group and others have found that osteosarcomas frequently have high immune cell infiltration including CD8+ T cells, and patients with the high intra-tumoral T cell infiltration have improved survival. For reasons that remain unclear, however, immunotherapies trying to exploit this have failed to show any significant benefit. We recently identified TGF-beta, a pleiotropic immune-suppressive growth factor, is highly overexpressed in osteosarcoma tumors. The etiology of this overexpression and the therapeutic relevance in osteosarcoma are unknown. We hypothesize that co-targeting TGF-beta may substantially improve the efficacy of immunotherapy such as engineered adoptive T cell transfer.

Study Design and Methods: RNAseq from adult TCGA tumor samples (n=9,880) and pediatric extracranial solid tumors (n=642) were analyzed for expression of TGFB1. A clinical-grade TCR targeting the cancer germline antigen PRAME (HLA-A2*01) was co-expressed with a dominant-negative TGFB receptor 2 (dnTGFBR2) gene or an irrelevant truncated EGFR gene as a negative control. Normal donor T cells were transduced with either of these constructs or mock transduced and infused into mice bearing osteosarcoma cell line and patient derived xenografts. Efficacy was measured using volumetric and bioluminescence measurement. Tumor infiltrating lymphocytes (TIL) were isolated at day 10 and profiled using single cell RNAseq with CITEseq staining for 16 phenotypic T cell markers. TIL were also profiled using bulk ATACseq and bulk RNAseq to evaluate changes in T cell phenotypes.

Results and Conclusions: We identified that TGF-beta ligand expression is higher in osteosarcoma among nearly any known tumor. We evaluated the efficacy of anti-PRAME T cells against osteosarcoma solid tumor models using a single low-dose infusion of T

cells. While these T cells displayed a moderate effect in tumor control, co-transduction with a dominant-negative TGFBR2 (dnTGFBR2) dramatically improved the efficacy and lead to multiple mice achieving a complete and durable response. Expression profiling of TILs revealed a significant expansion of dnTGFBR2 expressing CD8+ T cells and favorable markers of T cell activation. Specifically, PRAME-TCR positive T cells in the dnTGFBR2 group had a 50% reduction in dysfunctional terminally exhausted CD8+ T cells (P=.003), a doubling of effector memory CD8+ T cells (P=.0067), and three times as many central memory T cells (P=.0046). These changes were associated with a 30-fold increase in global chromatin accessibility, which may be related to a more favorable and plastic T cell state. In summary, the efficacy of adoptive T cell therapy against the osteosarcoma tumor antigen PRAME was greatly improved by combining with dnTGFBR2 expression on the therapeutic T cells. Protecting T cells from chronic exposure to high levels of TGF-beta, such as co-expressing a dnTGFBR2, has considerable potential in unlocking anti-tumor immune responses in osteosarcoma. This construct will be further developed for clinical trials in patients with cancers expressing PRAME and TGB-beta.

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73. Dimitris Stellas, Ph.D., Human Retrovirus Pathogenesis Section, Vaccine Branch, CCR

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"Combination treatment of hetIL-15 and Fenofibrate affects the metabolic fitness of tumor infiltrating CD8+T cells, severely impacting tumor growth in breast and pancreatic cancer mouse models"

Background and Hypothesis: Tumor-infiltrating cytotoxic CD8+T cells frequently undergo an altered state of differentiation referred to as exhaustion and, as a result, they fail to control tumor growth. Interleukin 15 (IL-15) is a cytokine which stimulates the generation, proliferation, and cytotoxic function of tumor specific CD8+ T cells and NK cells. We have produced the native heterodimeric form of IL-15 (hetIL-15) which has advanced in clinical trials. Fenofibrate (FF) activates the PPAR-Î±, that leads to transcription of multiple metabolic genes, increasing the fatty acid (FA) Î²-oxidation (FAO) as an alternative to glucose-derived pyruvate, metabolic pathway. The scope of this study was to overcome the exhaustion of the tumor-infiltrating CD8+ T cells and increase their cytotoxicity, using Fenofibrate in combination with hetIL-15 in mouse models of breast and pancreatic cancer.

Study design and Methods: We have evaluated the therapeutic efficacy of hetIL-15 immunotherapy as a monotherapy and in combination with FF in the murine EO771 orthotopic breast cancer model and KPC heterotopic pancreatic model. The effects of hetIL-15 and/or FF on immune cells were analyzed in tumors by flow cytometry, transcriptomics, metabolomics, immunohistochemistry, and multiplex serum protein profiling. The metabolic profile of tumor infiltrating T cells was assessed by the Seahorse assay.

Results and Conclusions: hetIL-15 monotherapy resulted in tumor eradication in 40% of animals, increased survival in the breast cancer model and resulted in significant growth delay in the pancreatic cancer model. Seahorse analysis of the tumor-infiltrating cytotoxic CD8+T cells revealed an increase in oxygen consumption rate (OCR) with substantial increase of spare respiratory capacity. The tumor infiltrating CD8+T cells presented elevated extracellular acidification rate (ECAR) and showed a pronounced shift in the OCR to ECAR ratio compared to controls, confirming their increased proliferating status. Consistent with the above finding, tumor infiltrating CD8+T cells from hetIL-15 treated mice showed increased mitochondrial potential and/or mass and fatty acid (FA) uptake, as evidenced by increased MitoTracker staining and Bodipy, respectively. Furthermore, transcriptomic analysis revealed increased expression of genes involved in several metabolic pathway such as oxidative phosphorylation, FA axidation and glycolysis, of the tumor infiltrated CD8+T cells. Monotherapy with FF had no effect in tumor growth delay on the breast model, but deladed the pancreatic tumor growth. The combination of both compounds reshaped the tumor microenvironment (TME) and increased the mitochondrial function, FA uptake and OCR, revealing a more metabolically active phenotype, resulting in complete eradication of the tumors in 85% of mice in the breast model and showed additive effects regarding the regression of pancreatic cancer. Our data show that hetIL-15 supported a favorable metabolic profile of intratumoral effector lymphocytes, important for their function. We hypothesize that metabolic reprogramming of tumor-specific CD8+T cells using the combination of hetIL-15 and FF is a promising strategy to overcome T cell exhaustion and promote survival in a metabolically hostile TME.

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48. Lene Veiga, Ph.D., Division of Cancer Epidemiology and Genetics, CCR
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"Thoracic soft tissue sarcoma risk following breast cancer treatment in two US retrospective cohorts"

Survivors of breast cancer who received radiotherapy are at high risk of developing subsequent thoracic soft tissue sarcomas (STS). Compared to other radiation-related subsequent malignancies, these cancers occur with a shorter latency period and risk did not decline with increasing age at exposure, which raises interesting questions about

potential co-factors. We evaluated the risk of subsequent thoracic STS among breast cancer survivors in two complementary cohorts.

Eligibility criteria in both cohorts were female breast cancer cases (stage I-III) aged 20-84 years who had survived at least 1 year since diagnosis. The Kaiser Permanente (KP) cohort included 16,004 women diagnosed 1990-2016, with detailed treatment data, and co-morbidities (including hypertension and diabetes at or before breast cancer diagnosis). The SEER 13 registries cohort included 457,300 women diagnosed 1992-2016 with initial treatment data. The outcome of interest was any second thoracic STS (angiosarcomas and other subtypes). Risk factors for thoracic STS were assessed using multivariable Poisson regression models.

In the KP cohort there were 19 thoracic STS (11 angiosarcomas, 8 other sub-types) after a median follow-up of 9.3 years. The median latent period for developing a thoracic STS was 5.5 years (IQR:3·8–7·5) and most (95%) thoracic STS occurred in women treated with radiotherapy (RR=8.1; 95%CI:1.1-60.4). We found no relationship with radiotherapy prescribed dose, number of fractions, or receipt of boost radiation. For thoracic angiosarcomas, anthracyclines were associated with a 3.6-fold increased risk (RR=3.6, 95%CI:1.0-13.3) and history of hypertension and diabetes were associated with approximately 5-times increased risk (RR=4.8, 95%CI:1.3-17.6 and 5.3, 95%CI:1.4-20.8, respectively). In the SEER cohort there were 430 subsequent thoracic STS (268 angiosarcomas) after a median follow-up of 8.3 years. The median age at thoracic STS diagnosis was 74 years (IQR:62–79) and most (78%) cases occurred after radiotherapy (RR=3.0; 95%CI: 2.4-3.8), with an increased risk of thoracic angiosarcoma for patients who underwent breast-conserving surgery+radiotherapy vs mastectomy+radiotherapy (RR=1.9: 95%CI:1.1-3.3). By 10 years after radiotherapy, cumulative incidence of thoracic STS was 0.20% in the KP cohort and 0.15% in SEER.

Radiotherapy was the strongest risk factor for thoracic STS in both cohorts with a short latency for development. Of clinical relevance, we observed an increased risk of thoracic angiosarcoma with anthracycline receipt and with a history of hypertension or diabetes at or before breast cancer diagnosis. The potential role of these comorbidities warrants further investigation, to reveal possible targets for future prevention strategies and increased surveillance for this highly fatal disease.

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61. Masashi Watanabe, Ph.D., Neuro-Oncology Branch, CCR

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"Phase II Trial Evaluating the Association of Peripheral Blood Immunologic Response and Therapeutic Response to Immune Checkpoint Inhibition in Patients with Newly Diagnosed Glioblastoma and Gliosarcoma"

Background and Hypotheses: Glioblastoma and gliosarcoma, both Grade IV primary CNS malignancies, are nearly always fatal. Current standard treatment (surgical resection, radiotherapy, chemotherapy) only provides modest survival benefits. Despite success in other cancers, immune checkpoint inhibition (ICI) therapy has failed to demonstrate efficacy against the malignant gliomas. Due to the immunosuppressive microenvironment of these tumors, the infiltration of peripherally activated T lymphocytes is likely required to achieve an effective intracranial anti-tumor immune response. We therefore hypothesized that a clinical response to ICI therapy necessitates a detectable immune response in the peripheral blood. To test this question, patients with newly diagnosed glioblastoma or gliosarcoma are actively enrolling on a Phase 2 clinical trial where all patients receive combinatorial ICI immunotherapy for peripheral blood analysis (NCT04817254). Extensive monitoring of systemic, longitudinal immune response allows for a comparative analysis of overall survival between patients who have a peripheral blood immune response and those who do not.

Study Design and Methods: A total of forty-eight glioblastoma and gliosarcoma patients will receive Ipilimumab and Nivolumab ICI therapy with adjuvant Temozolomide chemotherapy standard treatment with surgical resection and combined radiation and chemotherapy. Peripheral blood samples (PBMC and plasma) are collected before, during, and after treatment for immunological analysis. To predict patients T lymphocyte responsiveness to ICI therapy, a bead-based in vitro stimulatory assay was developed. Beads are conjugated with 1) anti-CD3 antibody for stimulating TCR and 2) human CD80-Fc recombinant protein stimulating CD28 (providing a co-stimulatory signal) and CTLA-4 (providing a co-inhibitory signal). In this assay, we postulate that a response of patient's blood T cells to Ipilimumab-mediated blockade of CTLA-4 predicts clinical response to ICI therapy. For an extensive immune profiling, patient's blood samples are assessed through 39-color high-dimensional flow cytometry, multiplexing cytokine and chemokine assays, single-cell-RNA- and TCR-sequencing, and functionality measurements. Conducting such analysis at multiple timepoints followed by a comprehensive bioinformatics analysis with clinical data would highlight associations among immune biomarkers and clinical trends and outcomes.

Results and Conclusions: As accrual is ongoing, a full analysis of clinical findings is not yet available. Interim efficacy analysis was passed. Preliminary analysis has confirmed feasibility of proposed methods including anti-CD3/CD80-Fc beads successfully stimulate T lymphocytes to proliferate in vitro. Cytokine profiling has been conducted on

preliminary patients multiple timepoints samples, confirming unique patterns among patients, and differing from those of healthy donors. Such results highlight the physiological relevance and predictive potential of these assays as it pertains to ICI response. Our initial data confirm feasibility of accrual and sample acquisition and analysis in a prospective clinical trial with extensive correlative studies. The biological correlates of ICI response identified by this clinical trial could better inform population enrichment of future trials, potentially improving the treatment effect size and therefore establishing ICI treatment efficacy. As a result, our novel in vitro assay and others could be used to screen for ICI responders, non-responders, or those at risk for immune toxicities. The treatment of many difficult-to-treat cancers could therefore be more patient-personalized.

Neuro-Oncology Branch, CCR, NCI

BASIC SCIENCE RESEARCH

1. **Altruistic feeding and cell signaling during bacterial differentiation actively enhances phenotypic heterogeneity**

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Starvation triggers bacterial spore formation, a committed differentiation program that transforms a vegetative cell into a dormant spore that can survive stress environments. Cells in a population, though, enter the sporulation pathway non-uniformly to secure against the possibility that favorable growth conditions, which puts sporulation-committed cells at a disadvantage, may resume. This heterogeneous behavior is initiated by a passive mechanism: stochastic activation of a master transcriptional regulator. Here, we identify a novel genetic system that enables a cell-cell communication pathway that actively promotes phenotypic heterogeneity, wherein Bacillus subtilis cells in a population that start sporulating earlier secrete glycerol, which acts both as a signaling molecule and a nutrient to delay non-sporulating cells from entering sporulation. This pathway produced a population that was better poised to take advantage of a sudden influx of nutrients, compared to populations that generated heterogeneity via stochastic gene expression alone. Thus, although conflict systems are prevalent among microbes, genetically encoded cooperative behavior in unicellular organisms can also boost population fitness.

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2. **Exogenous DNA enhances DUOX2 expression and function in human pancreatic cancer cells by activating the cGAS-STING signaling pathway**

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Cytokines upregulate the expression of DUOX2, which adversely affects the survival of PDAC patients. Because the cGAS-STING pathway is known to initiate pro-inflammatory cytokine expression following uptake of exogenous DNA, we examined whether activation of cGAS-STING could play a role in the generation of reactive oxygen species by PDAC cells. Here, we found that a variety of exogenous DNA species markedly increased the production of cGAMP, the phosphorylation of TBK1 and IRF3, and the translocation of phosphorylated IRF3 into the nucleus, leading to a significant, IRF3-dependent enhancement of DUOX2 expression, and a significant flux of H2O2 in PDAC cells. However, unlike the canonical cGAS-

STING pathway, DNA-related DUOX2 upregulation was not mediated by NF-ÎºB. Although exogenous IFN-Î² significantly increased Stat1/2-associated DUOX2 expression, intracellular IFN-Î² signaling that followed cGAMP or DNA exposure did not itself increase DUOX2 levels. Finally, DUOX2 upregulation subsequent to cGAS-STING activation was accompanied by the enhanced, normoxic expression of HIF-1Î± and VEGF-A as well as DNA double strand cleavage, suggesting that cGAS-STING signaling may support the development of an oxidative, pro-angiogenic microenvironment that could contribute to the inflammation-related genetic instability of pancreatic cancer.

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3. Structural study of cancer associated RhoA mutants L69R and L69P

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Background and Hypotheses: The Ras genes, which are the prototype for the Ras superfamily of small GTPases, encode guanine nucleotide binding proteins that are frequently mutated in cancer. The superfamily has five major branches: Ras, Rho, Rab, Ran, and Arf. Its wild-type members function as molecular switches that are active when GTP-bound and inactive when GDP-bound. Previously analyses of cancer-associated point mutants of RhoA indicated that L69P and L69R substituted proteins do not bind to Rhotekin and instead, bind strongly to Citron, a Rho-dependent kinase implicated in cytokinesis and cell cycle regulation [1]. L69P and L69R RhoA had high GDP-binding and low GTP-binding activity, similar to WT. Purified GDP-bound L69P and L69R RhoA formed a complex with Citron more efficiently than did GTP-bound or GDP-bound WT RhoA [1].

Study Design and Methods: We crystalized RhoA L69R and L69P substituted protein by using microbatch-under-oil methodology. The crystals were screened, and data were collected at the Advanced Photon Source at Argonne National Laboratory. NMR signals were previously assigned for GDP bound-RhoA [2] and we used this information as well as AlphaFold2-Multimer to study the interaction of RhoA L69R and L69P with Citron.

Results and Conclusions: We solved the crystal structures of RhoA L69R and L69P to ~1.5 resolution, with the R69 and P69 sidechain atoms well resolved. For both L69R- and L69P-substituted RhoA, the Switch II helix is broken and interactions with Switch I disrupted due to P69/R69 flipping away from the protein core. The substitutions at codon 69 also cause the coordination of a bound Mg2+ ion at Switch I and the nucleotide to be reconfigured. Our NMR data indicate that Citron binds to GDP-bound L69P- and L69R- substituted RhoA, but not to GDP-bound WT RhoA, and further highlight dynamic properties that are also observed in the crystal structures. A model of RhoA L69P or L69R with Citron is proposed based on the NMR data and AlphaFold2-Multimer.

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4. Single molecule analysis of CENP-A chromatin by high-speed atomic force microscopy

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Chromatin accessibility is modulated in a variety of ways to create open and closed chromatin states, both of which are critical for eukaryotic gene regulation. At the single molecule level, how accessibility is regulated of the chromatin fiber composed of canonical or variant nucleosomes is a fundamental question in the field. Here, we developed a single-molecule tracking method where we could analyze thousands of canonical H3 and centromeric variant nucleosomes imaged by high-speed atomic force microscopy. This approach allowed us to investigate how changes in nucleosome dynamics in vitro inform us about transcriptional potential in vivo. By high-speed atomic force microscopy, we tracked chromatin dynamics in real time and determined the mean square displacement and diffusion constant for the variant centromeric CENP-A nucleosome. Furthermore, we found that an essential kinetochore protein CENP-C reduces the diffusion constant and mobility of centromeric nucleosomes along the chromatin fiber. We subsequently interrogated how CENP-C modulates CENP-A chromatin dynamics in vivo. Overexpressing CENP-C resulted in reduced centromeric transcription and impaired loading of new CENP-A molecules. From these data, we speculate that factors altering nucleosome mobility in vitro, also correspondingly alter transcription in vivo. Subsequently, we propose a model in which variant nucleosomes encode their own diffusion kinetics and mobility, and where binding partners can suppress or enhance nucleosome mobility.

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6. Itaconic acid underpins lipid metabolism of peritoneal tumors and fatty liver disease

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Itaconate, the product of the decarboxylation of cis-aconitate, regulates numerous biological processes. We and others have revealed itaconate as a regulator of fatty acid beta-oxidation, generation of mitochondrial reactive oxygen species and the metabolic interplay between resident macrophages (preSMA) and tumors. Peritoneal tumors elicited significant upregulation of both oxidative (OXPHOS) and glycolysis in preSMA, in a manner largely attributed to an Irg1-mediated increase in fatty acid oxidation. Our findings are consistent with the hypothesis that IRG1 is important for macrophage utilization of fatty acids as a fuel for OXPHOS-derived production of reactive oxygen species and antimicrobial activity. Here we show further that Irg1-dependent itaconic acid regulates lipid homeostasis in human non-alcoholic steatohepatitis and a mouse model of non-alcoholic fatty liver disease. Mice bearing macrophage-, but not hepatocyte-specific Irg-1 deficiency have

exacerbated lipid accumulation in the liver, glucose, and insulin intolerance and mesenteric fat deposition. Treatment of mice with the itaconate derivative, 4-OI, reverses dyslipidemia associated with high fat diet feeding. Mechanistically, itaconate treatment of primary hepatocytes reduce lipid accumulation and increases their oxidative phosphorylation in a manner dependent upon fatty acid oxidation. We propose a model whereby macrophage-derived itaconate acts in trans upon hepatocytes to modulate the livers' ability to metabolize fatty acids. These findings suggest that interventions to alter itaconate levels may hold therapeutic potential to regulate metabolic disease and dyslipidemia. Taken together, our findings indicate metabolic changes in the peritoneal cavity and liver, particularly Irg1-mediated catabolism of cis-aconitate, underlie important differences in the function of preSMA during homeostasis, inflammation, and peritoneal tumor progression. Metabolit targeting may be a means by which liver metabolic disease and tumors may be treated.

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7. Mouse ribosomal DNA arrays are composed of highly diverged rDNA units due to differences in the intergenic spacer (IGS)

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Ribosomal DNA (rDNA) repeat units are organized in the most abundant repetitive gene clusters in eukaryotic cells. In the human genome, tandemly repeated arrays reside on the short arms of all five acrocentric chromosomes: 13, 14, 15, 21, and 22. It was demonstrated that the human ribosomal DNA arrays are composed of highly homogenized tandem clusters. In mice, the tandem clusters are sited on at least 8 chromosomes: 4, 12, 13, 15, 16, 17, 18, and 19 and they are almost exclusively pericentromeric. Mouse rDNA clusters show extensive diversity in numbers between different laboratory strains, ranging from 74 to 419 in different strains. In this study, we have focused on characterizing the structure and variation of rDNA units in mouse nucleolar organizer regions (NORs). To analyze intra- and intergenomic variations in mouse rRNA gene copies, we applied TAR (Transformation-Associated Recombination) cloning that allowed selective isolation of individual rDNA units. Further accurate long-read sequencing revealed substantial variations in the size of rDNA units. The rDNA-containing clones fell into three size classes, i.e., 35-40 kb, 40-45 kb and 45-50 kb. Sequence comparison among them and with the published mouse consensus sequence revealed that size differences result from differences in the intergenic spacer (IGS). The size heterogeneity of the IGS region is due to insertions and deletions of SINE, LINE, LTR, and other repetitive elements. In addition, TAR clones revealed XXX variant positions in the 45S transcription unit, XXX in promoters, and transcribed 5'- and 3'ETS regions. The identified difference between rDNA units raises the possibility that they are regulated differentially among tissues, and the variants provide a route to investigate possible effects on nucleolar formation and correlations with the proposed involvement of altered ribosomes in pathology.

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8. Blocking cystine and leucine import overcomes the melanoma BRAF inhibition resistance

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Background and Hypotheses: Oncogenic BRAF mutation induces tumorigenesis and promotes the transformed cell's uncontrolled growth. Targeting this oncogenic mutant BRAF kinase by BRAF inhibiter (BRAFi) could inhibit tumor proliferation, leading to tumor cell apoptosis or ferroptosis and the activation of ER stress signaling pathways. However, tumor cells could develop an alternative route to support their survival under the drug treatment condition, therefore becoming resistant, resulting in tumor recurrence, and causing cancer mortality. Hypothetically, the altered new route will support tumor cell proliferation capacity as a 'fitness cost' for resistant melanoma, which becomes resistant melanoma's vulnerability to novel therapy.

Study Design and Methods: To identify the altered new pathways or candidate molecular targets in BRAFi-resistant melanoma for therapeutic utility, we designed and performed the following experiments: 1) establish the acquired BRAFi-resistant human melanoma models. 2) determine the pathways or candidate molecular targets in BRAFi-resistant melanoma models using RNA sequence. 3) Confirm the candidate genes from the RNA sequence data using Nanostring, qPCR, and western blot. 4) validate the function of molecular targets or pathways for inhibition of BRAFi-resistant melanoma using small molecular inhibitors and knockout technology. 5) Evaluate the relationship between the candidate genes' expression in human cancers (available data sets such as TCGA); 6) Evaluate the therapeutic effects of potential inhibitors for candidate genes in vitro and in vivo.

Results and Conclusions: By comparing the transcriptional profile between all BRAFi-resistant and parental melanomas using RNA sequence analysis, we identified rewired metabolic routes in BRAFi- resistant melanomas to reach their needs in energy and biosynthetic precursors, both essential for escaping the BRAFi attack and vulnerability for treating BRAFi-resistant melanomas. The amino acids of cystine and leucine import were found to be significantly increased in BRAFi-resistant melanoma by their amino acid transporters. Interestingly, inhibition of cystine and leucine import by inhibitors or knockout of their transporters enhanced the sensitivity of BRAFi-resistant melanoma and led the BRAFi-resistant melanoma to ferroptosis and apoptosis, whereas less affected the parental melanoma. In contrast, overexpression of their transporters in sensitivity to BRAFi parental melanoma cells promoted the ability of resistance of BRAFi. Moreover, co-targeting both cystine and leucine import could dramatically inhibit the BRAFi-resistant melanoma growth. Notably, to effectively combat the BRAFi-resistant melanoma and overcome this significant challenge, we then first identified the three new compounds that could target the cystine and leucine import, therefore inducing BRAFi-resistant melanoma apoptosis and inhibiting

BRAFi-resistant melanoma growth in preclinical mouse models. Our data showed that blocking cystine and leucine import by new compounds could offer novel therapeutic opportunities.

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9. **Bisubstrate Inhibitors of 6-Hydroxymethyl-7,8-dihydropterin Pyrophosphokinase: A New Generation Exhibiting Cell Permeability**
Genbin Shi, Gary X. Shaw and Xinhua Ji

6-Hydroxymethyl-7,8-dihydropterin pyrophosphokinase (HPPK) is one of the enzymes in folate biosynthetic pathway. It catalyzes the pyrophosphoryl transfer from ATP to 6-hydroxymethyl-7,8-dihydropterin (HP). The enzyme is essential for microorganisms, is absent in humans, and is not the target for any existing antibiotics. Therefore, HPPK is an attractive target for developing novel antimicrobial agents. Using biochemical and biophysical methods, we have previously studied the thermodynamic and kinetic properties of HPPK and fully characterized the structural dynamics of the protein and interactions between the protein and its substrate and products. These results provide sold fundaments for structure-based drug design. First, we synthesized a series of bisubstrate analog inhibitors of the enzyme by linking 6-hydroxymethylpterin to adenosine through 2, 3, or 4 phosphate groups. Then, we developed a second generation of linked purine pterin HPPK inhibitors by replacing the pterin moiety with 7,7-dimethyl-7,8-dihydropterin and the phosphate bridge with a piperidine linked thioether. On the basis of high-resolution crystal structures of the enzyme in complex with the inhibitor, we further modified the structure of the inhibitor in two steps, resulting in a nanomolar bisubstrate inhibitor. Here, we report the structure and activity of a new inhibitor. Exhibiting observable cell permeability toward a Gram-positive bacterium, it represents the fourth generation of bisubstrate inhibitors of HPPK.

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10. **Development of structure-guided hRpn13-targeting molecules as an anti-cancer strategy**
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11. Dicer processing of pre-miR-138-2 is regulated by uridylation

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As a class of small non-coding RNAs, microRNAs (miRNAs) play critical roles in mammalian development and human diseases. Dicer is an essential miRNA biogenesis enzyme that converts hairpin-shaped miRNA precursors (pre-miRNA) to mature miRNAs. It was well established that dysregulation of miRNA biogenesis contributes to tumorigenesis, highlighting the importance of deciphering the mechanisms by which miRNA biogenesis is regulated. miR-138, a tumor-suppressing miRNA, is encoded at two genomic loci: miR-138-1 and miR-138-2. It was reported that pre-miR-138-2 is ubiquitously expressed, but the expression of the mature miR-138-5p is limited to the brain tissue in adult mice, suggesting an unknown regulation on Dicer processing of pre-miR-138-2.

Here, by ectopically expressing pri-miR-138-1 and pri-miR-138-2 separately in HEK293T cells, we find that the former but not the latter is efficiently processed by Dicer. Deep sequencing analysis indicates that more than 60% of pre-miR-138-2 is oligo-uridylated while uridylation of pre-138-1 is barely detectable. By taking advantage of TUT4/TUT7 double knockout cells, we find that pre-miR-138-2 is uridylated by TUT4 and TUT7 in an LIN28-independent manner. These results suggest that the specific uridylation of pre-miR-138-2 underlies its poor processing by Dicer. Supporting this idea, knocking out TUT4 and TUT7 results in the upregulation of mature miR-138. Surprisingly, uridylated pre-miR-138-2 can still be cleaved by Dicer in vitro, suggesting that uridylation per se is not sufficient to block its processing by Dicer. While the cytoplasmic accumulation of uridylated pre-miR-138-2 is evident and can be detected by Northern blot following cell fractionation, no uridylated pre-miR-138-2 is detected in the Dicer-immunoprecipitation samples, suggesting that cellular factors shield the uridylated precursors from being accessed by Dicer. Knocking down 3ï,¢ poly-U-binding protein La diminishes the accumulation of uridylated pre-miR-138-2, which can be rescued by depleting U-tail-specific nuclease DIS3L2. Together, these results support a model where uridylated pre-miR-138-2 is protected by La protein from DIS3L2 degradation and from Dicer processing. This study reveals a new mechanism for how pre-miRNA processing can be regulated.

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12. Lipase mediated gut-brain communication modulates insulin secretion in Drosophila

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Pancreatic beta cells synthesize and secrete insulin in response to elevated glucose to maintain blood glucose levels within the physiological range. Defective insulin secretion is causal in most forms of diabetes. While glucose is the primary stimulus for insulin secretion by the beta cells, it is known that lipids obtained from diet or generated intracellularly can amplify glucose-stimulated insulin secretion (GSIS). How the dietary lipid pool potentiates GSIS, the lipid signals and mechanisms involved in relaying the signals to pancreatic beta cells have not yet been well established. This is a challenging endeavor because maintenance of glucose homeostasis after a meal poses substantial complexity. It requires the coordination of digestion, absorption, relay of signals from other organs, and the final integration of these inputs at the beta cells to determine the extent of insulin release. Thus, inter-organ communication plays a vital role in glycemic control. Drosophila is increasingly being appreciated as a model to dissect mechanisms governing metabolism that involve inter-organ communication. Many metabolic pathways are conserved between flies and humans. This includes the insulin pathway and in Drosophila, there are eight insulin-like peptides, DILP1-8, of which DILP2 is most closely related to human insulin. DILP2 is synthesized and secreted from median neurosecretory cells called the insulin producing cells (IPCs) in the brain. Here, we show, a Drosophila secretory lipase, Vaha, is synthesized and secreted from the midgut in response to dietary fat. It moves to the brain, where it concentrates in the IPCs in a process requiring Lipid Transfer Particle, a Drosophila lipoprotein originating in the fat body. Our results demonstrate Vaha participates in fat amplified insulin secretion. Vaha mutant flies, flies with gut specific knockdown of Vaha or flies lacking Vaha lipase activity, all show decreased release of DILP2 from the IPCs upon feeding after fasting. Vaha mutant flies have reduced DILP2 in circulation and exhibit features of diabetes including hyperglycemia and hyperlipidemia. Our findings suggest Vaha functions as a diacylglycerol lipase in a gut-brain axis modulating DILP2 release to maintain glucose homeostasis in the adult fly.

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13. NCI/CCR/LRBGE Optical Microscopy Core

Tatiana S. Karpova, David A. Ball, Mohamadreza Fazel

NCI/CCR/LRBGE Optical Microscopy Core (OMC) is open to everyone within the NCI, and we also welcome collaborations with investigators from other NIH institutes or beyond. We operate state-of-the-art fluorescence microscopes, confocal, wide-field, and super and

nano-resolution, and custom-built HILO microscopes, that enable a broad spectrum of fluorescence imaging experiments. We provide expert guidance and troubleshooting in the design, execution, interpretation, and publication of these experiments.
Fluorescence microscopy permits in situ imaging of proteins, lipids, DNA and RNA in tissues, cells, and intracellular organelles. The latest technological advances allow imaging of individual molecules. Computerized image analysis is applied to single-molecule biophysics and to building of molecular maps of intracellular organelles. Modern fluorescence markers include fluorescent antibodies, fluorochrome-labeled oligonucleotides, GFP and other fluorescent protein fusions, such as fusions to ligand-bound Halo Tag, Snap Tag, CLIP tag etc., and fluorescent stains for lipids, DNA and RNA. Specific markers are suitable for live cells and for biophysical quantification of diffusion, of binding rates, and of protein dimerization by FRAP, FCS, N&B. Biosensors allow observation of protein interactions and enzymatic activity dynamics by FRET. Resolution in X, Y, and Z may be improved by deconvolution and by super resolution: SIM (Structural Illumination Microscopy), PALM (Photoactivation Localization Microscopy) or STED (Stimulated Emission Depletion Microscopy). The newcomer, MINFLUX, provides nano-resolution, bringing the resolution up to the level of electron microscopy. Image quality may be enhanced by AI.
These exciting technologies are available to NCI and NIH from our Optical Microscopy Core which specializes in single molecule biophysics and nano-resolution, but also provides conventional microscopy imaging. We offer the conventional methods of fluorescence microscopy, including time lapse imaging of cells in 2D or 3D, with computational methods for removal of out of focus light (deconvolution), as well as sophisticated quantitative microscopy: FRAP, FRET, superresolution (SIM and PALM), nano-resolution (MINFLUX), and single molecule tracking (SMT). The staff participates in the development of quantification microscopy technologies. First-time users should contact Dr. Tatiana Karpova (karpovat@mail.nih.gov, 240-760-6637) to discuss the proposed work.

LRBGE/Optical Microscopy Core, CCR, NCI

14. Cortex glial subtypes in the central nervous system differentially regulate seizure susceptibility

Govind Kunduri[1], Usha Acharya[1], and Jairaj K Acharya[1]

Seizures are initiated by a hypersynchronous burst of neuronal activity in a population of cortical neurons of the brain. Abnormal neuronal activity could remain localized to a small area (focal) or alternatively or spread across different parts of the brain (generalized), resulting in a loss of cognitive functions and consciousness. Understanding where seizures originate and how they propagate to different parts of the brain is one of the long-standing questions in epilepsy research. We have previously shown that ceramide phosphoethanolamine synthase (cpes) mutants of Drosophila show light induced seizures (LIS) due to defective cortex glia (CG). Melom, JE. et al have shown that loss of cortex glial specific Na+/Ca2+, K+ exchanger (zyedeco) results in temperature sensitive seizures (TSS). In Drosophila, the CG, also known as neuronal cell body, associated glia, appear as a honeycomb like structure and is uniformly spread throughout the central nervous system (CNS). Recent studies have shown that cortex glia in the optic lobes (OL), midbrain (MB), and ventral nerve cord (VNC) are distinct and could be distinguished with different Gal4

drivers. However, expression patterns of these Gal4 drivers are not clean as their expression spills into other cell types including neurons and other glial types during development. Here we optimized Gal4, Gal80 and split Gal4 drivers to specifically express gene of interest in cortex glial subtypes of OL, MD and VNC. Taking advantage of these drivers, we have asked whether different parts of the brain regulate seizure susceptibility differently in seizure models such as LIS and TSS. Using cortex glial subtype specific rescue experiments we show that OL and MB, but not VNC specific CG, was able to significantly suppress LIS. In contrast, VNC but not OL or MD specific cortex glial rescue was able to suppress TSS. Taken together our findings suggest that cortex glial subtypes differentially regulate seizure susceptibility in seizure models.

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15. Identification of novel regulators of TLK1-ASF1-HIRA pathway

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16. CXCR1/2 inhibition increases the response of HPV-negative head and neck squamous cell carcinoma models to docetaxel

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Head and neck squamous cell carcinoma (HNSCC) cases unrelated to HPV infection present with a poor prognosis and do not typically respond to currently available treatment modalities. To improve the clinical outcome and to prolong survival in this group of patients, novel therapeutic approaches are needed. Our current study investigated the expression of the chemokine IL-8 and its receptors, CXCR1 and CXCR2, in HNSCC to potentially identify novel therapeutic targets and combination therapy strategies for this disease. Overexpression of IL-8 in several tumor types has been shown to promote mechanisms of tumor progression and drive resistance to chemotherapy and immunotherapy by increasing tumor angiogenesis, inducing tumor cellular plasticity leading to increased tumor cell migration, invasiveness, and resistance to cell death, and chemoattracting polymorphonuclear myeloid-derived suppressor cells (PMN-MDSC) to the tumor microenvironment. Our initial analysis of HNSCC tissues via RNA in situ hybridization revealed enhanced expression of IL-8 and its receptors in HNSCC tumors compared with healthy tissues. Data from HNSCC tissues and cell line models revealed a prevalence of IL-8, CXCR1, and CXCR2 expression in HPV-negative compared with HPV-positive HNSCC. Treatment of several HPV-negative HNSCC cell lines in vitro with SX-682, a clinical stage, small molecule inhibitor of CXCR1/2, sensitized the tumor cells to the cytotoxic activity of the microtubule-targeting chemotherapy agent docetaxel. In addition, in vivo treatment of two xenograft models of HPV-negative HNSCC with the combination of SX-682 plus docetaxel led to strong anti-tumor control. This phenomenon was associated with an increase of microRNA-200c (miR-200c) and a decrease of tubulin beta-3, a protein involved

in resistance to microtubule-targeting chemotherapies. Additionally, in vivo treatment of a murine syngeneic model of HNSCC with SX-682 plus docetaxel led to potent anti-tumor efficacy through a simultaneous decrease in suppressive CXCR2+ PMN-MDSC and an increase in cytotoxic CD8 T cells in combination therapy treated tumors compared to controls. Anti-tumor efficacy was further enhanced with the addition of anti-PD-1 to the SX-682 plus docetaxel combination. These findings provide rationale for the use of CXCR1/2 inhibitors in combination with docetaxel for the treatment of patients with HPV-negative HNSCC.

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17. Membrane structure organization at the mother centriole during primary cilia assembly

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Primary cilium biogenesis initiation requires mother centriole docking to membranes. Here, we employ high-resolution microscopy approaches to characterize the ultrastructure of the developing ciliary membrane and elucidate the mechanisms associated with the initiation and progression of ciliogenesis. Three-dimensional reconstructions elucidate the early stages of ciliogenesis, demonstrating the precise process of preciliary vesicle docking to the distal appendages of the mother centriole, followed by vesicle fusion and enlargement, leading to the formation of a tubular, C-shaped vesicle and the emergence of an unprecedented membrane toroid. The shaping of these membranes is under the influence of the membrane-regulating protein EHD1, which orchestrates the assembly of tubular structures from the preciliary vesicles, while RAB8 plays a pivotal role in the completion of the toroid structure. Additionally, the IFT-B protein IFT88 is also implicated in organizing these membranes before axoneme growth occurs. Remarkably, tubulovesicular membranes exhibit a coordinated association with the removal of the CP110/CEP97 cap via a direct linkage to EHD1 and are also associated with recruitment of transition zone proteins. These findings provide a novel architectural framework for understanding ciliogenesis and demonstrate the significance of using volume electron microscopy in investigating the dynamics of organelle biogenesis.

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18. Loss of NAT10 disrupts enhancer organization via p300 mislocalization and suppresses transcription of genes necessary for metastasis progression

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Acetylation of protein and RNA represent a critical event for development and cancer progression. NAT10 is the only known RNA acetylase that catalyzes the N4-actylcytidine (ac4C) modification of RNAs. Here, we show that the loss of NAT10 significantly decreases

lung metastasis in allograft and genetically engineered mouse models of breast cancer. NAT10 interacts with a mechanosensitive, metastasis susceptibility protein complex at the nuclear pore. In addition to its canonical role in RNA acetylation, we find that NAT10 interacts with p300 at gene enhancers. NAT10 loss is associated with p300 mislocalization into heterochromatin regions. NAT10 depletion disrupts enhancer organization, leading to alteration of gene transcription necessary for metastatic progression, including reduced myeloid cell-recruiting chemokines that results in a less metastasis-prone tumor microenvironment. Our study uncovers a distinct role of NAT10 in enhancer organization of metastatic tumor cells and suggests its involvement in the tumor-immune crosstalk dictating metastatic outcomes.

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19. Histone demethylase Jhd2 mediated demethylation of Dam1 contributes to centromere function and chromosome segregation

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20. TGF-beta induces alternative mRNA splicing in human colon cancer cells

Christina H. Stuelten, Hannah Kim, Ting Chen, Ying E. Zhang

LCMB, CCR, NCI

21. Vaccine-induced innate protection against intrarectal SIV challenges

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Background and Hypotheses
Identifying immune correlates of protection is a major challenge in AIDS vaccine development. Anti-Envelope antibodies have been considered critical against SIV/HIV acquisition. However, adoptive transfer study in macaque models failed to demonstrate protection against viral challenges. This motivates a search for immune mechanisms of protection other than anti-Envelope antibodies. We hypothesized that CD8+ cell responses and/or innate immunity might be able to mediate sterile protection against intrarectal SIV acquisition in the absence of anti-Env antibody responses.

Study design and Methods
We immunized 12 macaques with live attenuated SHIVSF162P, and 18 months later, challenged the macaque with eight low-dose rectal SIVmac251viral challenges. Since there was no cross-reactivity between SIV and SHIV Envelope antibodies, the role of antibody was excluded. We further ran in vivo CD8 depletion and RNAseq to evaluate and dissect the protective mechanisms.

Results and Conclusions
After eight low-dose rectal challenges with SIVmac251, 12 SHIV-vaccinated animals demonstrated 83% efficacy, compared to six naive controls, suggesting protection could be achieved in the absence of anti-Envelope antibodies. Interestingly, CD8+ T cells (and some NK cells) were not essential for the preventing viral acquisition, as none of the CD8-depleted macaques was infected by SIVmac251 challenges.
Further investigation of protective innate immunity revealed that protected animals had elevated pathways related to platelet aggregation/activation, and reduced pathways related to interferon and responses to virus. Moreover, higher expression of platelet factor 4 (PF4) on circulating platelet-leukocyte aggregates was associated with reduced viral acquisition. The newly described mechanisms of protection might be leveraged by new HIV vaccines, and potential cancer vaccines in the future. Our data highlighted the importance of innate immunity and may provide new opportunities for novel vaccines or therapeutic strategy development.

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22. Age-induced altered hematopoiesis is normalized by regulating PD-1H/SHP2 in aged murine hematopoietic stem cells

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23. Metabolism-focused CRISPR screen unveils Mitochondrial Pyruvate Carrier 1 as a critical driver for PARP inhibitor resistance in lung cancer

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Homologous recombination (HR) and poly ADP-ribosylation are partially redundant pathways for repair of DNA damage in normal and cancer cells. In cell lines that are deficient in HR, inhibition of poly (ADP-ribose) polymerase (PARP1/2) is a proven target with several PARP inhibitors (PARPi) currently in clinical use. Resistance to PARP inhibitors often develops, usually involving genetic alterations in DNA repair signaling cascades, but also metabolic rewiring particularly in HR-proficient cells. We surmised that alterations in metabolic pathways by cancer drugs such as Olaparib might be involved in the development of resistance to drug therapy. To test this hypothesis, we conducted a metabolism-focused CRISPR knockout (KO) screen to identify genes that undergo alterations during treatment of tumor cells with PARP inhibitors. Of about 3000 genes in the screen, our data revealed that mitochondrial pyruvate carrier 1 (MPC1) is an essential factor in desensitizing NSCLC lung cancer lines to PARP inhibition. In contrast to NSCLC lung cancer cells, triple-negative breast cancer cells do not exhibit such desensitization following MPC1 loss and reprogram the TCA cycle and oxidative phosphorylation pathways to overcome PARP inhibitor treatment. Our findings unveil a previously unknown synergistic response between MPC1 loss and PARP inhibition in lung cancer cells.

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24. KSHV promotes oncogenic FOS to inhibit nuclease AEN and transactivate RGS2 for AKT phosphorylation

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Background and Hypotheses: Kaposi's sarcoma-associated herpesvirus (KSHV) is a human oncogenic virus associated with various malignancies, including Kaposi's sarcoma, primary effusion lymphoma, and multicentric Castleman disease. The expression of viral products is essential for initiating and sustaining KSHV-induced tumors. KSHV ORF57, a viral RNA-binding protein, plays a crucial role in regulating viral gene expression at the posttranscriptional level by promoting viral RNA stability, splicing, and translation. However, the impact of ORF57 on host gene expression and its role in virus-mediated cell transformation remains elusive.

Study Design and Methods: To identify the host targets of KSHV ORF57, we utilized the patient-derived KSHV-transformed B cell line BCBL-1. Induction of ORF57 expression in BCBL-1 cells was achieved by initiating KSHV lytic infection using valproic acid treatment. Subsequently, we conducted UV cross-linking immunoprecipitation (CLIP) followed by RNA-seq analysis of the CLIPed RNA fragments (CLIP-seq). The obtained reads were aligned to the human genome to identify ORF57 binding sites within host transcripts. To delineate the functional consequences of ORF57 binding to cellular RNAs, we performed differential gene expression analysis on BCBL-1 and HEK293T cells with or without ORF57 expression using

RNA-seq. The intersection of these datasets pinpointed functionally relevant ORF57 regulation of host mRNAs.

Results and Conclusions: We identified ORF57-bound transcripts from 544 host protein-coding genes. By comparing RNA-seq profiles from BCBL-1 and HEK293T cells with and without ORF57 expression, we identified FOS and CITED2 mRNAs as two common ORF57-specific RNA targets. FOS was selected for further investigation of molecular mechanisms and the role of ORF57 binding to host RNAs. FOS, when forming a dimer with JUN, constitutes an AP-1 transcription factor involved in cell proliferation, differentiation, and transformation. The knockout of the ORF57 gene from the KSHV genome led to the loss of FOS expression during KSHV lytic infection, which could be restored by lentiviral-driven ORF57 expression. Within the FOS RNA, ORF57 binds to a unique 13-nt RNA motif near the FOS RNA 5Ê[1] end and prevents a host nuclease, AEN to bind to the same motif thus extending FOS mRNA half-life by 7.7 times. The elevated FOS also inhibits AEN transcription but transactivates RGS2, a regulator of G-protein coupled receptors, by binding to the conserved AP-1 site in the RGS2 promoter. The enhanced RGS2 expression consequently leads to AKT phosphorylation, which has previously been shown to promote viral replication. Our findings reveal that KSHV ORF57 specifically disregulates host gene expression to induce pathogenesis during KSHV lytic infection.

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26. Synergism between NRAS and IL7R drives acute lymphoblastic leukemia Kerstin Wenqing Li[1], Hila Winer[1], Gisele Rodrigues[1], Tim Gower[2], Thomas Joshua Meyer[3], Julie Hixon[1] and Scott Durum[1]

Diffuse large B cell lymphoma (DLBCL) is a molecularly heterogeneous cancer that presents a challenge for precision medicine. Inhibitors of Bruton tyrosine kinase (BTK) block B cell receptor (BCR)-dependent NF-ÎºB signaling and are particularly effective in DLBCL with mutations in the BCR subunit CD79B and MYD88 (MCD DLBCL). MCD tumors are enriched for a multiprotein supercomplex, termed the My-T-BCR, that is nucleated by MYD88L265P, TLR9 and the BCR, and serves as a central hub of NF-ÎºB signaling. The integrity of the My-T-BCR complex is rapidly compromised following BTK inhibition, but the molecular mechanisms responsible for this remain elusive.

To investigate the mechanisms regulating the My-T-BCR, we used CRISPR-Cas9 screens in MCD DLBCL cell lines treated with inhibitors of BTK (BTKi) and other survival pathways. We identified several drug resistance genes encoding negative regulators of BCR, NF-ÎºB, and PI3 kinase signaling that are recurrently inactivated in DLBCL biopsies. Unexpectedly, autophagy-related genes involved autophagosome formation (ATG9A, ATG101, ATG13, RB1CC1 and ATG14) and autophagosome membrane expansion (ATG2A, WIPI2, WDR45) also counteracted the toxicity of BTKi in these screens.

To gain further insight, we generated BTKi-resistant cell lines deficient in ATG9A or ATG101 (ATG KO) and performed CRISPR screens and RNA-seq with or without BTKi. We observed 11 ATG genes that displayed epistatic interactions, no longer conferring BTKi resistance in ATG KO MCD cells. We also observed the buffering of NF-ÎºB negative regulators, and the increased sensitivity to loss of NF-ÎºB positive regulators. Furthermore, gene expression studies showed decreased expression of BCR, MYD88 and NF-ÎºB signatures in control BTKi-treated cells, whereas ATG KOs did not. ATG KOs displayed higher levels of nuclear NF-ÎºB upon BTKi treatment. Amongst primary tumors, MCD patients have the highest levels of NF-ÎºB gene expression. Interestingly, MCD patients also displayed the lowest gene expression for ATG genes, suggesting this pathway is counter-selected during pathogenesis.

As autophagy promotes protein degradation, we assessed global protein levels and localization using mass spectrometry and BioID. In ATG KOs, selective autophagy receptors TAX1BP1, NBR1 and p62 were significantly upregulated and were found near MYD88L265P suggesting this mutant proteoform is targeted for degradation by selective autophagy. To test this, we engineered autophagy reporters (GFP-RFP fusions) for TAX1BP1, NBR1, p62, or MYD88L265P. The autophagic flux of each reporter was blocked in ATG KOs.

To identify genes controlling autophagic flux of MYD88L265P, we performed CRISPR screens for changes in turnover of the MYD88L265P autophagy reporter. Deletion of the above epistatic interactors of ATG9A also blocked MYD88L265P autophagic degradation. Conversely, deletion of BTK, mTORC1-related genes and IRF4 promoted MYD88L265P autophagic degradation. We validated these findings using targeted inhibitors of each gene alone, or in combination, and observed synergy for promoting MYD88L265P autophagic degradation.

Collectively, we identified a non-canonical form of selective autophagy that degrades MYD88L265P and is promoted upon BTK inhibition. Our findings help to elucidate the exceptional benefit of BTK-targeted therapies in MCD DLBCL and offer a rationally designed combination therapy regimen that specifically degrades this oncogenic allele of MYD88.

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27. Molecular mechanisms of intron retention in long non-coding RNAs

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Intron retention (IR) is a form of alternative splicing in which an intron that should be spliced out from a precursor transcript, is retained in the mature RNA after the splicing is completed. Although there is emerging evidence of widespread IR in protein-coding genes and long noncoding RNAs (lncRNAs), the underlying molecular mechanisms remain largely unclear. Here, we report the discovery of novel transcripts from the p53-induced lncRNA

PURPL, in which intron 2 is retained. To determine the molecular mechanism(s) of IR in PURPL, we conducted a CRISPR-based screen in 3 different cell lines. For this purpose, we utilized a genome-wide guide RNA library expressing a reporter minigene containing the sequence of PURPL intron 2 and its flanking exons. Considering the Percent Intron Retention as readout, we unexpectedly identified proteins of the basal splicing machinery as potential promoting factors of PURPL IR, including the U2-Auxiliary Factor 2 (U2AF2) splicing factor which was one of the top hits of the screen. We next analyzed ENCODE eCLIP-seq datasets to identify RNA binding proteins that could regulate intron 2 and decided to focus on U2AF2 which showed the highest number of binding sites and strongest eCLIP signals within PURPL intron 2 sequence. We validated the effect of U2AF2 by knocking it down, confirming that U2AF2 is a positive regulator of PURPL intron 2 retention as opposed to its canonical function. To determine the global impact of U2AF2 knockdown, we performed Iso-Seq and RNA-seq upon U2AF2 depletion. We found that although U2AF2 predominantly acts to promote the splicing of introns in most cellular transcripts, it promotes intron retention in a subset of transcripts. One of these targets is MALAT1, a lncRNA known to play role in splicing by interacting with splicing factors in nuclear speckles. Interestingly, U2AF2 depletion results in MALAT1 exclusion from nuclear speckles. We are currently in the process of characterizing the effect of U2AF2 in the function of the two lncRNAs. These data provide mechanistic insights on PURPL and MALAT1 splicing and function and reveal a previously unrecognized non-canonical function of U2AF2 in promoting intron retention.

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28. Investigation of RNA Polymerase & CI Interactions in Bacteriophage Lambda: Promote PRM Auto-regulation

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Background: One of the best understood systems in genetic regulatory biology is the so-called genetic switch This determines the choice the phage-encoded CI repressor makes by binding cooperatively to tripartite operators, OL (OL1, OL2 & OL3) and OR (OR1, OR2 & OR3), in a defined pattern. Transcription at two lytic promoters, PL and PR, is blocked, while transcription at lysogenic promoter, PRM, is activated and repressed at low CI and high CI concentrations, respectively. The autoregulation of PRM is dependent on the interaction of RNA polymerase (RNAP) binding to the PRM promoter and CI binding to OR2.

Methods: By using a purified in vitro transcription system, we analyzed the activation complex between RNAP at PRM and CI at OR2 by DNA and protein mutations. We inserted 5-bp or deleted 1-bp DNA between OR2 & OR3 to change the angular rotation

and distance between RNAP and CI. We also mutated E34K of CI which interacts with RNAP during the activation of PRM.

Results: We obtained unexpected findings. First, a 1-bp DNA deletion of -34A of PRM resulted in the repression of PRM at the same CI concentration for the repression of PL and PR. This repression is depending on DNA looping and the binding of CI to OR2. Second, a 5-bp DNA insertion between the PRM promoter site and OR2 site resulted in the repression of PRM at the same CI concentration for the repression of PL and PR. Third, mutating E34K of CI which is involved in the activation complex resulted in the repression of PRM at the same CI concentration for the repression of PL and PR. In all three cases, PRM is repressed at the same CI concentration for the repression of PL and PR. Finally, DNA looping enhances PRM activation and repression.

Conclusion: Disruption of the activation complex between RNAP at PRM and CI at OR2 by mutating CI or inserting or deleting base pair to change the angular orientation and distance between RNAP and CI led to the repression of PRM. These unexpected results suggest that maybe RNAP is creating negative contacts with CI at OR2 preventing RNAP from escaping and repressing PRM. Future studies are being conducted to understand the molecular mechanisms where these changes result in the repression of PRM.

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29. Investigations into the Mechanism and Activity of the SAMT-247 Small Molecule Covalent Inhibitor of HIV Gag Polyprotein

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Although antiretroviral therapy for HIV has advanced, there remains a critical need for new therapeutics targeted at resistant viral strains. Covalent modification of target proteins is a well-established mechanism for therapeutics. However, selectivity is critical, as off-target effects can be more persistent and deleterious than reversible inhibitors. SAMT-247 was developed as a covalent inhibitor of HIV Gag polyprotein and shows potent antiviral activity. As it is a small, highly reactive molecule, it is unlikely that Gag is its sole target. Yet, low cytotoxicity is observed in cell- and animal-based studies. RNA seq experiments investigating the effects of SAMT-247 treatment in HIV infection suggest that it could target a zinc finger protein, ZC3H7A, involved in miRNA biogenesis and inflammation. Additionally, activation of M1 macrophages has been indicated as a mechanism of action, driving inflammation and glycolysis.

Mass spectrometry was used to investigate potential off-target effects in both directed and unbiased experiments. First, SAMT-247 reaction with the zinc fingers of ZC3H7A was analyzed using isolated peptides and recombinant protein. Second, a global proteomic approach was used to screen for other potential targets of SAMT-247 in a human leukemia monocytic cell line. Thermal proteome profiling uses quantitative mass spectrometry to determine the thermal melting profile of proteins on a proteome-wide scale by quantifying

the amount of soluble protein following heating from 37°C to 67°C. By comparing the change in thermal melting for each protein in the untreated and treated states, direct and indirect targets can be identified. Additionally, Seahorse metabolic flux assays were used to identify any changes in OXPHOS and glycolysis following treatment with SAMT-247 and when combined with a macrophage-activating cocktail (PMA).

Analysis of potential reaction and covalent modification of peptides representing the zinc fingers (ZF) of ZC3H7A demonstrated that three of the four zinc fingers were all modified. Further, when recombinant ZC3H7A (440-971) was incubated with SAMT-247, multiple sites of reaction were observed on both cysteine and lysine residues throughout the protein. Initial, thermal proteome profiling analysis of SAMT-247 effects in THP-1 cells comparing the control-treated and SAMT-247-treated conditions identified 7 proteins of which 2 were metabolically relevant. Following co-stimulation of SAMT-247 and PMA, 170 genes were identified with a significant shift in melting point including several zinc finger-containing proteins and many metabolically relevant genes.

Seahorse metabolic flux assays identified significant decreases in OXPHOS including basal respiration and ATP production. Concurrent increases in glucose utilization of THP-1 cells were also measured. These changes were significantly increased following the addition of PMA to activate the macrophages. Calculation of the metabolic parameters showed a particular increase in the proton leak from the electron transport chain.
SAMT-247 is a potent covalent inhibitor of the zinc-coordinating domains of the HIV Gag polyprotein that has been shown to modify both cysteine and lysine residues of Gag. Here, we have used targeted and unbiased mass spectrometry approaches to examine off-target effects of this inhibitor in immune cells and identified metabolically relevant targets that represent new avenues to exploit and potential new uses for SAMT-247 beyond its antiviral effects.

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31. Progression of pre-malignant lesions in live animals

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Head and neck cancer is the 6th leading cause of cancer-related death in the US. Early detection is key to successful treatment of head and neck cancer. Therefore, understanding the mechanism of tumor initiation and early-stage tumor development is of pivotal importance for head and neck cancer treatment. However, majority of the current studies of head and neck cancer using animal models focus on the behavior of established tumors and their response to therapies. In this study, we develop a minimally invasive procedure

which enables the long-term invtravital microsocpy imaging on mouse oral cancer initiation and progression induced by chemical carcinogen. For the first time, we discover heterogenous fates of each lesion observed throughout the 6 months study on the same animal. With artificial intelligence neural network, we quantitatively characterize tumor cells morphology and dynamics during early tumor initiation and progression. Moreover, we acquire images for mitochondrial metabolism, immune cell response, autophagy, EGFR signaling, etc., thus providing insight into the mechanism controlling tumor progression in vivo which can serve as a springboard to develop novel therapies.

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32. Structural Insights into HIV-1 Vif Degradation of Cell Cycle Regulator PPP2R5A

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HIV-1 virion infectivity factor (Vif) recruits host cullin-RING-E3 ubiquitin ligase and core binding factor beta (CBFbeta) to degrade the cellular APOBEC3 (A3) antiviral proteins and avoid inhibition of viral replication by A3-mediated cytidine deamination and lethal hypermutation. Besides antagonizing A3G, A3F, and some A3H haplotypes, Vif also induces G2/M cell cycle arrest by utilizing the same complex to target the regulatory subunits PPP2R5(A to E) of cellular protein phosphatase 2A for degradation. Despite detailed information on Vif-A3 protein interactions, it is unclear how Vif can recognize PPP2R5(A to E) proteins which bear no functional or structural resemblance to the APOBEC3 proteins.

Here we report the cryo-electron microscopy (cryo EM) structure of PPP2R5A in complex with HIV-1 Vif/CBFbeta/elongin B/elongin C at a 3.8 Ã… resolution. Biochemical and mutational analyses support this structure and reveal that the Vif interaction surface of PPP2R5A spans the entire Vif molecule, contacting both the Vif alpha and alpha/beta domains and partially overlaps with interfaces shared by other A3 proteins.

These results increase our understanding of the structural basis of Vif-mediated PPP2R5A substrate recognition and degradation. Development of inhibitors that disrupt the Vif-A3 and/or Vif-PPP2R5A binding interfaces could serve as a new therapeutic strategy to combat HIV-1 infection.

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33. Oncohistone H3 mutations facilitate CENP-A mislocalization

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Background & Hypothesis: Centromeric localization of histone H3 variant, CENP-A (Cse4 in S. cerevisiae), is essential for faithful chromosome segregation. Overexpressed CENP-A leads to its mis-localization to non-centromeric chromatin and contributes to aneuploidy in yeasts, flies, and humans. Overexpression and mislocalization of CENP-A is observed in

many cancers and this correlates with poor prognosis. Understanding how CENP-A is deposited into non-centromeric regions is essential for our understanding of the mechanisms that govern genome maintenance and genomic instability. Our previous studies have defined a role for histone stoichiometry in preventing mislocalization of Cse4. We determined that gene dosage of histone H4 contributes to mislocalization of Cse4 in budding yeast and human cells (Au et al., Genetics, 2008, Eisenstatt et al., Genetics, 2021, Shreshta et al., J. Cell Biol. 2017, 2021, Ohkuni et al., Nucleic Acids Res., 2022). Our recent studies have shown that the interaction of Cse4 with histone H4 facilitates a conformational state of Cse4 in vivo from an enclosed to an open state and this contributes to Cse4 mislocalization (Ohkuni et al., Nucleic Acids Res., 2024). Since histone H3 is an obligate partner of histone H4, we hypothesized that reduced gene dosage or mutants of histone H3 will facilitate Cse4-H4 interaction and promote mislocalization of Cse4. Using budding yeast as a model, we examined if oncohistone H3 mutations in the histone hold domain, which are frequently seen (hotspots) in many cancers, affects the interaction of Cse4 with histone H4, and if this leads to mislocalization of Cse4. It will help us to understand the function of histone hold domain of oncohistone H3 in humans.

Study Design & Methods: We used histone H3 deletion (hht1âE† and hht2âE†), structurally defective (hht1 Y99A), and oncohistone (hht1 E97A and hht1 E105A) H3 mutants to examine Cse4-H4 interaction, Cse4 structure and localization of Cse4. Genetic and biochemical approaches were used to examine how the Cse4 interaction with histone H4 due to defective H3-H4 interaction affects the structural conformation of Cse4 in vivo and the consequences of this on mislocalization of Cse4.

Results & Conclusions: Our results showed that reduced gene dosage of histone H3 (hht1âE† and hht2âE†) and oncohistone H3 mutants promote Cse4-H4 interaction facilitating an open state of Cse4 in vivo and this contributes to Cse4 mislocalization. These results support our hypothesis that defect of H3-H4 interaction increases Cse4-H4 interaction, and this facilitates conformational change in Cse4 and mislocalization of Cse4. Based on our yeast study, we propose that oncohistone H3 mutants in the histone hold domain indirectly contributes to CENP-A mislocalization in humans and that CENP-A at noncentromeric regions functions as an oncohistone H3, a histone H3 hypermutations.

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34. Structure of the LRRK2:14-3-32 complex

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Leucin-rich repeat kinase 2 (LRRK2) is a large multidomain protein with both GTPase and kinase activity. LRRK2 is involved in endosomal and lysosomal trafficking through the phosphorylation of a subset of Rab proteins. In vivo regulation of LRRK2 is a complex process that presumably involves changes in phosphorylation, and oligomerization as well as complex formation with regulatory proteins. Members of the 14-3-3 family are known LRRK2 interactors that regulate cellular localization and kinase activity. Cancer and Parkinsons Disease (PD) associated mutations not only increase kinase activity but also

impair the interaction with 14-3-3. Here we study the LRRK2:14-3-3 complex to understand the molecular basis of LRRK2 regulation by 14-3-3 proteins.

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35. Covalent-modification of Tyrosine 204 on Tyrosyl-DNA Phosphodiesterase 1 Catalytic Groove Using SuFEx-mediated Access to Quinolone Fluorosulfates

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Covalent modification of proteins has been found to be a very useful technique in many biological and therapeutic settings. Site-selective protein modification by small molecules can provide invaluable insights into mechanisms of biological function. Sulfur (VI) fluoride exchange (SuFEx) has emerged as a new genre of biocompatible Click Chemistry reactions. Fluorosulfate-containing agents, which are easily prepared, can react with multiple natural amino acids, in particular, with the sidechain hydroxyls of Tyr residues. SuFEx-derived molecules are currently being widely applied to rationally design covalent ligands that target specific residues in a diverse array of enzymes. Recently, using an X-ray crystallographic fragment screen, we identified quinolone Hot Spot-binding moieties within the active site of tyrosyl-DNA phosphodiesterase 1 (TDP1). Quinolones are important pharmacophore structures that have been widely used in antibiotics. X-ray crystal structures of TDP1 in complex with the quinolone fragments reveal that the oxygen and carboxylic acid on the quinolone forms hydrogen bonds with the key catalytic His-Lys-Asn residues (HKN motifs) at the TDP1 catalytic site. Substituents at the 8-position of the quinolones are directed toward the relatively narrow and positively charged DNA-substrate-binding pocket. The evolutionary conserved residues, Tyr204 and Phe259, are located proximately in the catalytic groove. Our current presentation will detail the development of a series of substituted quinolones that have sulfonyl fluoride moieties tethered at the 8-position of the quinolone scaffold. Importantly, the X-ray crystal structures of a subset of these quinolones in complex with TDP1, confirm the formation of sulfonyl adducts to the Tyr204 residue within the catalytic pocket. In certain covalent complexes with TDP1, the tethered aromatic functionality projects toward the key Phe259 residue. The structural information obtained from these crystal structures may be helpful to inform the optimization of these covalent inhibitors to transform them into more potent agents.

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36. Histone deacetylase maintains a critical density of modified histones essential for histone-templated heterochromatin and epigenetic memory

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Background and Hypothesis: The organization of the eukaryotic genome into heritable heterochromatin structures plays a crucial role in repressing lineage-inappropriate genes and maintaining cellular identity. Genome organization relies on histone-modifying activities, such as those that introduce the heterochromatin-defining histone modifications, H3 lysine-9 and H3 lysine-27 methylation. Heterochromatin formation is initiated by sequence-dependent factors, such as DNA binding proteins or RNA, which target histone methyltransferases to the initiating region and trigger the spreading of histone methylation to boundary elements. Spreading is enabled via the read-write activity of the methyltransferase, which can both recognize the pre-existing histone methyl marks and deposit new marks. Once established, a high local concentration of histone methyltransferase bound to pre-methylated histones is sufficient to maintain heterochromatin domains even in the absence of the initiating activity. Although chromosomal context affects the epigenetic stability of different heterochromatin domains, the chromatin features responsible for the variability are not well studied. Here we utilize the fission yeast Schizosaccharomyces pombe to investigate chromatin features that play a role in maintaining the critical density of H3 lysine-9 methylated histones.

Study Design and Methods: Our previous work revealed that self-templated formation of heterochromatin at the fission yeast mating-type locus is associated with a high-level of the H3 lysine-14 histone deacetylase (HDAC) Clr3. The most prominent peaks of Clr3 are found at specific sites, called Clr3 attracting sequences (CAS), where DNA binding factors mediate Clr3 recruitment. We hypothesized that high-level Clr3 localization primes the heterochromatin domain for histone-templated propagation. To test this, we first mapped Clr3 localization genome-wide and identified DNA regions that mediate Clr3 localization. We then examined the effect of these novel CAS-like sequences on the propagation of self-templating heterochromatin. By introducing CAS sequences to a heterochromatin domain assembled at the tetO-ade6+ reporter by tethering a hybrid of tetracycline repressor and Clr4/Suv39h histone H3 lysine-9 methyltransferase (TetR-Clr4), we were able to monitor heterochromatin propagation upon cessation of TetR-Clr4 targeting.

Results and Conclusions: We demonstrated that the presence of HDAC targeting DNA elements pre-conditions chromatin for the propagation of epigenetic domains. A high level of chromatin-bound HDAC Clr3 stabilizes epigenetically marked nucleosomes and supports the accumulation of a critical amount of H3 lysine-9 methyltransferase Clr4/Suv39h. We propose that histone deacetylation, in addition to unblocking lysine residues for repressive methylation, protects epigenetic memory by precluding access to acetyllysine-binding machinery that reduces the density of pre-methylated histones and compromises heterochromatin propagation.

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37. Secondary amino-acid substitutions contribute to the emergence of HIV protease inhibitor resistance as directly as primary amino-acid substitutions

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38. DNA methylation patterns are influenced by Pax3::Foxo1 expression and developmental lineage in rhabdomyosarcoma tumors forming in genetically engineered mouse models

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Rhabdomyosarcoma (RMS) is a family of pediatric soft tissue sarcomas, which shows features of developing skeletal muscle and consists of two major subtypes, fusion-positive (FP; PAX3::FOXO1 most commonly) and fusion-negative (FN). In humans, FP and FN RMS are characterized by distinct DNA methylation patterns. Additional DNA methylation-defined subsets are found within the FN and FP RMS categories and are associated with mutational differences. To investigate the mechanisms responsible for DNA methylation differences within this family of tumors, we profiled DNA methylation in 31 RMS tumors derived from genetically engineered mouse models (GEMMs) in which various driver mutations were introduced into different myogenic lineages. Our unsupervised analyses of DNA methylation patterns among these mouse tumors yielded two major clusters, which correspond to high and no/low expression of Pax3::Foxo1. These findings mirror the results in human FP and FN RMS tumors and support a role of PAX3::FOXO1 in establishing the DNA methylation pattern associated with FP RMS. Two distinct methylation-defined subsets were found within the cluster of mouse RMS tumors with no/low Pax3::Foxo1 expression, one subset enriched in tumors established in the Pax7 lineage and a second subset enriched in tumors established in the Myf5 lineage. This latter finding reinforces the important contribution of developmental lineage to the DNA methylation patterns in FN RMS subsets. Our integrative analysis of DNA methylation and transcriptomic expression data in GEMMs of RMS and human RMS revealed a common group of differentially methylated and differentially expressed genes that are associated with PAX3::FOXO1 transcriptional targets and developmental pathways, highlighting the presence of a conserved set of genes functioning in both human RMS and murine models of RMS. In conclusion, this study of GEMMs of RMS provides mechanistic insight into the establishment of DNA methylation patterns in FP and FN RMS, and supports the utilization of these systems to investigate the epigenetic landscape for novel therapeutic targets in RMS.

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39. Identification of ALVAC, Alum and ALFQA immunological signatures by plasma proteome in vaccinated macaques

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40. Mechanisms of Î±-Keto acid dehydrogenase-mediated oxidation in proinflammatory macrophages

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Proinflammatory or M1 macrophages commit to a glycolytic state when endogenous nitric oxide (NO) reprograms mitochondrial metabolism by limiting aconitase (ACO)-2 and pyruvate dehydrogenase (PDH) activities. We previously showed that in murine bone marrow derived macrophages (BMDMs) stimulated with LPS+IFNÎ³ there is a decline in PDH-E3 enzyme, known as dihydrolipoamide dehydrogenase (DLD), that is dependent on NOS2. Here we provide evidence that NO directly targets the PDH complex using the cofactor lipoate to generate nitroxyl (HNO), a radical that interacts with thiols forming modifications resistant to reduction. PDH E2-associated lipoate is indeed modified in NO-rich macrophages while the DLD is irreversibly inhibited via modifications at Cys484. Consequently, we reveal a macrophage signature of proteins with reduction resistant modifications identifying a subset of potential HNO targets. Moreover, recombinant DLD is modified in HNO dependent manner at Cys477 and Cys484, and molecular modeling shows these cause impairment of functional DLD homodimer formation. Interestingly, DLD functions as E3 subunit also in Î±-ketoglutarate and branched-chain amino acid-dehydrogenase (OGDH, BCKDH) and the glycine cleavage system (GCS). Our findings raise the possibility that these mitochondrial dehydrogenases sharing DLD would be similarly targeted in macrophages. Metabolomics from stimulated WT and Nos2-/- macrophages suggest that BCKDH, OGDH and GCS are indeed targeted in a NO-dependent manner. To strengthen this conclusion, we carried out [U-13C6] Leucine tracing and quantified labeling on downstream intermediates to assess BCKDH activity. While equal rates of fractional labeling through proximal transamination into Î± -ketoisocaproate were detected between WT and Nos2-/-, we observed decreased leucine-derived carbon incorporation downstream of BCKDH in BMDMs in NOS2-dependent manner: the isotopologue pattern confirmed indeed disruption of BCKDH. We also looked at glycine cleavage in BMDMs and found that although tendency for NOS2-dependent incorporation of glycine carbon into adenine and N-

formylmethionine was observed, it appears that GCS pathway is not highly active in macrophages under our conditions.

In conclusion, our work demonstrates that HNO is produced physiologically, and its presence is peculiar to the lipoate-rich PDH complex facilitating irreversible modifications critical in NO-dependent metabolic rewiring. We report indeed strong regulation of leucine carbon fueling by NO in primary macrophages during stimulation; distinct NOS2-dependent effects have now been documented on PDH, OGDH, BCKDH and GCS. Our model reveals that targeting of DLD by NO has major repercussions in cellular metabolism.

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41. IL1Î±/Î² and IL-6 Promote Synergistic Up-Regulation of the DUOX2/DUOXA2 Complex in Colon Cancer Cells

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Background and Hypothesis: The interleukin 1 (IL-1) family of cytokines plays a critical role in both maintenance of normal gut homeostasis and in the pathogenesis of gastrointestinal (GI)-related inflammatory disorders, including inflammation-associated colorectal cancer (CRC). This dichotomous role of IL-1 in the induction of inflammation-related disease while simultaneously promoting protection of the intestinal mucosa has contributed to the failure of clinical trials aimed at neutralizing IL-1 or IL-18 in patients with Crohn's disease and ulcerative colitis. New insights into the signaling pathways supported by IL-1 family members in the pathogenesis of colon cancer may provide downstream targets for treatment that minimally perturb intestinal immunity.

Study Design and Methods: Induction of dual oxidase 2 (DUOX2)/DUOXA2 mRNA and protein expression by IL-4/IL-17A was previously reported to increase oxidative stress and DNA damage in colon cancer cell lines by our laboratory. To build on these results, here we report the treatment of Caco2, HT29, Ls513, T84 or Colo205 human colon cancer cells with IL-1Î± or IL-1Î², in cooperation with IL-6, affords dramatic up-regulation of a hydrogen peroxide producing (Amplex Red oxidizing) DUOX2 enzyme complex. Crucial signaling pathway components mediating up-regulation were identified and related through monoclonal antibody antagonists, (Anakinra, Tocilizumab), siRNA and CRISPR techniques.

Results and Conclusions: IL-1Î±/Î² (+ IL-6) stimulation resulted in oxidant production through significant up-regulation of DUOX2 enzymatic activity. Up-regulation of the hydrogen peroxide generating DUOX2/DUOXA2 enzyme complex was directly associated with enhanced histone H2AX phosphorylation (Î³H2AX), a marker of DNA double strand breaks. Interestingly, this concentration- and time-dependent induction of expression and oxidative response was absent in Caco2 cells and was not mediated by other IL-1 family members (IL-

18, IL-33 or IL-37). Investigations with the interleukin-1 receptor antagonist anakinra have established that signaling, both for IL-1Î± or IL-1Î²/IL-6 co-treatments, proceeded through the IL-1 receptor for all cell lines, though minimal receptor is present in T84 and Ls513. Similarly, Tocilizumab, an IL-6R antagonist, has verified that the IL-1Î±/Î² + IL-6 synergistic up-regulation of DUOX2/DUOXA2 is also IL-6 receptor dependent. Perturbation of IL-1 and IL-6 signaling pathway elements IRAK1, STAT1 and STAT3 by siRNA knockdown demonstrated a significant contribution to DUOX2 up-regulation, while dependence on RELA was absent. In support of these in vivo observations, studies from our group have demonstrated significant up-regulation of DUOX2 and DUOXA2, as well as IL-1, in surgically resected colon cancer specimens compared with adjacent normal colonic epithelium. Recently, a correlation of the observed DUOX2 up-regulation with an important therapeutic target, the heterodimeric transmembrane protein MUC-1 was discovered. Supported by gene expression levels in colon tumors versus non-malignant adjacent tissue, DUOX2 and MUC1 demonstrate highly significant correlated expression levels both in publicly available datasets (Pearson >0.5, Colonomics database, n=100) and samples prepared from CHTN acquired tissues (n=20). Future studies will determine if MUC1 can perturb the hydrogen peroxide production of DUOX2, potentially enhancing the status of DUOX2 as a therapeutic cancer target.

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42. Gastruloids: A Versatile Model System for Investigating Embryonic Development and Gene Function

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The successful progression of embryonic development hinges upon a consistent supply of stem and progenitor cells capable of self-renewal and differentiation. During this intricate process, pluripotent Embryonic Stem Cells (ESCs) and Epiblast Stem Cells (EpiSCs) are exposed to pivotal Wnt and FGF signaling pathways, triggering their differentiation into the diverse cell types that make up the three germ layers of the developing embryo an essential process known as gastrulation. To investigate and replicate this critical stage of development, researchers have turned to in vitro modeling techniques. ESCs can be differentiated within 2-dimensional extracellular matrix-coated cell culture dishes or employing 3-dimensional culture systems, particularly ultra-low attachment dishes termed embryoid bodies (EBs) and the more structured gastruloids. Unlike EBs, which originate from thousands of cells and exhibit disorganized, irregular shapes, gastruloids are formed from a more modest number of cells. They initiate as spherical bodies and gradually transition into an elongated shape, closely mirroring the shape changes seen during embryonic development. One of the key advantages of gastruloids is their ability to exhibit distinct, organized early embryonic gene expression domains, providing researchers with a unique platform to study how cells assemble into tissues and organs independently of the embryo context. Similar to embryos, gastruloids demonstrate self-organization, pattern formation, and cell-fate specification, making them an invaluable tool for investigating these

intricate developmental processes. Unlike their embryonic counterparts, gastruloids are highly scalable and readily available, making them an ideal choice for experimentation. Moreover, they can be generated from genetically modified ESCs, enabling loss-of-function and gain-of-function studies for genes of interest specifically during gastrulation. This scalability also facilitates the screening of small molecules, drugs, or CRISPR/Cas9-based assays to explore various self-organization, patterning, and cell-fate specification phenotypes. Another notable advantage of gastruloids is their compatibility with human induced pluripotent stem cells (iPSCs), offering a model system that circumvents the legal, ethical, and technical challenges associated with working on human embryos. This capability allows researchers to model developmental disorders more effectively. Furthermore, the gastruloid assay can be seamlessly integrated with cutting-edge single-cell technologies such as live-cell imaging, single-cell RNA sequencing (scRNA-seq), and high-resolution spatial transcriptomics, enhancing the depth and precision of developmental investigations. As an example, here, we tested if Wnt target gene transcription factors, Sp5/8 are required for patterning and self-organization in gastruloid assay and found that Sp5/8 dko mutant gastruloids failed to elongate with disrupted AP axis likely attributed to the perturbation of the Wnt3a and T/Bra positive feedback loop for specifying posterior progenitors. In conclusion, while in vivo data remains the gold standard for studying gene function, gastruloids serve as a complementary tool, offering a unique platform for investigating and addressing critical questions in embryonic development and gene function. Their scalability, versatility, and compatibility with modern technologies make gastruloids an invaluable asset in the field of developmental biology and regenerative medicine.

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43. Characterization of different 3D culture paradigms to study metabolic reprogramming and vulnerabilities in metastatic breast cancer cells

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Background and hypotheses: It is widely accepted that metabolic reprogramming is a key driver of cancer aggressiveness, and cancer cells may experience a ametabolic plasticity to adapt to different phases of metastasis. Importantly, a subset of cancer cells that is independent of glycolysis and utilizes oxidative phosphorylation (OXPHOS) has garnered attention recently in this regard. Furthermore, it has become increasingly recognized that cell cultures in 2D plastic dishes do not recapitulate physiologically relevant cell biology. This study aims to determine the impact of different 3D culture conditions on tumor cell metabolism, their potential relevance to in vivo physiology, and as tools to discover specific therapeutic vulnerabilities.

Study Design and Methods: a. Approaches: Molecular and metabolic features of metastatic breast cancer cell lines grown in different 3D culture paradigms and their comparison to primary tumors by Proteomics, Metabolomics, Transmission Electron Microscopy, In vivo Experimental Metastasis assays, Gene expression, Seahorse-based metabolic assays, and Cell viability using clinically relevant pathway inhibitors/drugs.

b. Cell lines and mouse models: SUM149 (triple negative breast cancer subtype), and IBC-3 (HRE2+ subtype), Cell-line derived Xenografts (CDX), and Patient-derived xenograft (PDX) mice.

Results and Conclusions: We employed 3D sphere culture that enriches cancer stem cells, 3D emboli culture involving viscous media and fluid shear, 2D culture, and tumor tissues. Proteomics analysis suggested that emboli were more similar to tumor tissue compared to cells in 2D or sphere cultures. In vivo experimental metastasis assays showed that cells from emboli cultures were more efficient at colonizing the lung tissue compared to cells from 2D culture, similar to stem-cell enriched sphere cultures. Analysis of lipid content and metabolites showed that emboli cultures exhibit increased triacylglycerol (TAG) and cholesteryl ester but decreased lactic acid, a surrogate marker for glycolysis. In agreement with these findings, sphere cultures expressed more genes of the glycolysis pathway while emboli cultures increased the expression of some mitochondrial genes. Seahorse metabolic measurements indicated that emboli cultures had a higher basal oxygen consumption rate (OCR) and spare respiratory capacity (a measure of mitochondrial function) than extracellular acidification rate (ECAR) (a measure of glycolytic capacity) and inversely for the sphere culture. Next, we probed the functional relevance of these pathways with inhibitors specific to glycolysis and/or complex 1 of the electron transport chain and found that the emboli cultures were more dependent on OXPHOS whereas the sphere cultures were more sensitive to inhibition of glycolysis. These findings highlight how culture conditions, and by inference microenvironment, can trigger diverse energy metabolic adaptations in metastatic IBC cells. Given the potential role of OXPHOS in cancer cell survival during dissemination, the 3D emboli culture paradigm presented here may be a useful approach to study the signaling pathways that lead to increased malignancy and drug resistance through reprogramming of the metabolism in metastatic breast cancer cells.

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45. Does high-grade aneuploidy in cancer cells develop by gradual or abrupt changes in chromosomal content?

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***Optical Pooled Screening for the study of nuclear biology**

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Background and Hypotheses: We report the implementation of Optical Pooled Screening pairs pooled lentiviral libraries with *in situ* sequencing (ISS) to link genetic perturbations with visual cellular phenotypes at the single cell level for up to millions of cells.

Study Design and Methods: To test OPS, we generated stable pooled cell lines in different backgrounds (hTERT-RPE-Cas9, HCT116-Cas9 and U2OS-Cas9) expressing several different sgRNAs and used either immunofluorescence (IF) or native-FISH as optical phenotype readout. On the chemistry/imaging side, standard OPS protocols for library preparation and 4-color sequencing-by-synthesis (SBS) chemistry were used in all cell lines over 12 cycles of imaging with a 10X objective. On the computational side, we implemented a multi-step image analysis pipeline. First, we segmented nuclei cells using DAPI images, and then aligned ISS images across all 12 sequencing cycles. We then measured intensity variance at the pixel level across sequencing cycles to detect the position of ISS signals. For each cycle, we called a base by measuring fluorescence intensities at the site of ISS signals, and then base calls were chained into 12-nt sgRNA sequence reads. Furthermore, and for each cell, we counted the number of reads for each sgRNA, and selected the sgRNA sequence with the highest number of reads. Finally, we matched sgRNAs to target genes using a predefined codebook to identify the perturbed gene for each cell and used the Biowulf HPC cluster to run this pipeline in a high-throughput format.

Results and Conclusions: We detected an average of 5.5, 6.1 and 4.9 ISS spots/cell for hTERT-RPE-Cas9, HCTT16-Cas9 and U2OS-Cas9, respectively. In addition, 60-83% of the cells could be mapped to an sgRNA sequence read present in the codebook. Next, we tested the association of genetic perturbations to visual phenotypes using IF on pooled cells expressing a mix of control non-targeting or LMNA-targeting sgRNAs. LMNA IF images were captured with either 10X or 20X objectives, and then matched to ISS images for the same cells acquired at 10X. This necessitated the development of an additional computational module in the pipeline to match segmented nuclei objects between images acquired at different magnifications. Nuclei matching was achieved by calculating global coordinates for each nucleus object and then subsequent alignment algorithm. The alignment of multiple magnification levels was optimized by employing a slide-and-fit method to calculate the offsets between fields of view (FOVs). This approach has greatly improved efficiency, as the computed offsets are propagated across adjacent FOVs, resulting in a substantial reduction in computational time. Finally, we developed an assay that combines OPS with native-FISH for the screening of Alternative Lengthening of Telomere (ALT) regulators. Altogether, these results demonstrate that we can successfully implement ISS together with different image-based assays to perform pooled CRISPR screens with optical readouts.

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***A meiotic cyclin causes aneuploidy in mitotic cells by promoting kinetochore disassembly**

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Background and Hypotheses: Apart from its genetic tractability and small karyotype, the fission yeast *Schizosaccharomyces pombe* is an outstanding model for studying chromosome segregation. Specifically, *S. pombe* centromeres, the regions where the kinetochore is assembled, resemble the typical repetitive heterochromatic arrays seen in human cells, and virtually all inner and outer kinetochore components are present and highly conserved when compared to their vertebrate counterparts. We previously found that defective RNA processing (e.g. mutations in RNA interference or RNA elimination

factors) can trigger the chromosomal anomaly uniparental disomy (UPD), a frequent genetic abnormality (~1 of every 2,000 people) that is linked to congenital disorders and various cancers. Importantly, UPD is caused by the untimely expression of gametogenic genes and the loss of meiotic proteins Rec8 or Crs1 is sufficient to suppress UPD in RNA processing mutants (*Folco et al., Nature 2017*). In this work, we investigate how the untimely expression of the cyclin Crs1 in proliferating mitotic cells can trigger chromosome segregation defects such as aneuploidy.

Study Design and Methods: Standard *S. pombe* genetic techniques were used. Chromosome segregation was monitored by using quantitative genetic assays that determine the frequency of UPD as well as various minichromosome loss assays. Centromeric localization of cohesins and kinetochore proteins was determined by ChIP-qPCR and live-cell imaging.

Results and Conclusions: We find that ablating both Crs1 and Rec8 genes in RNA processing mutants has an additive effect on UPD suppression. Moreover, centromeric localization of Rec8 does not depend on Crs1, and Rec8 and Crs1 genes are independently expressed, indicating that both gametogenic factors act in distinct pathways. Interestingly, in haploid cells, the untimely expression of Crs1 is more deleterious than that of Rec8 as assessed by minichromosome loss and growth assays. Remarkably, our phenotypical characterization of inner and outer kinetochore components in various genetic backgrounds revealed that Crs1 causes aneuploidy in mitotic cells by promoting massive disassembly of the kinetochore as inner and outer subunits dissociate.

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CLINICAL AND EPIDEMIOLOGICAL AND TRANSLATIONAL RESEARCH

46. Automatic cell type annotation using SingleR with custom reference for single-nucleus multiome data from frozen breast tumor samples

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Single-nucleus joint ATAC- and RNA-sequencing (snMultiome) can be used to identify cell subpopulations with different transcriptomic and epigenetic profiles within complex samples. Accurate cell type annotation is critical to successful snMultiome data analysis. Several computational methods have been used for automatic annotation. Traditional cell type annotation first clusters cells using unsupervised learning methods based on the gene expression profiles, then labels the clusters using the aggregated cluster-level expression profiles and marker genes. This method relies heavily on the clustering results. As the purity of clusters cannot be guaranteed, false detection of cluster features may lead to wrong annotations. Further, canonical cell surface markers may not always be suitable in single-nucleus RNA-seq studies because single-nucleus RNA-seq generally have lower detected transcript numbers compared to typical single-cell RNA-seq and many cell type marker genes in the snRNA-seq data differed from the scRNA-seq data, reflecting biological differences in the cytoplasmic and nuclear RNA pools. Moreover, malignant cells are best left out of the reference because they are too ill-defined and patient-specific. Reference-based automated algorithms such as SingleR enable quick and unbiased classifications by leveraging a collection of built-in reference data sets for human (e.g. Human Primary Cell Atlas (microarray-based) and a combined Blueprint Epigenomics and Encode data set (RNA-seq-based)). Still, SingleR often returns many erroneous classifications. In our dataset that we generated using the 10x Genomics snMultiome platform, which includes 296,557 nuclei from 82 frozen breast tumors of diverse genetic ancestral background, we sought to improve the accuracy of cell type annotation by SingleR. To achieve this, we first separated malignant and non-malignant cells based on DNA copy number aberrations (aneuploidy) through CopyKAT. To cells determined to be non-malignant, we built the custom reference from snRNA-seq data set, recently available from The Human Breast Cell Atlas, then applied singleR with custom reference where each cell type is represented by single-cells of that type, allowing a well-founded estimate of the confidence with which a cell type call can be made. Using this approach, we successfully identified 11 distinct cell types for non-malignant cells, including fibroblast, adipocyte, pericyte, basal, luminal-secretory, luminal-HR, myeloid, mast, vascular, lymphatic, and T-cells. Furthermore, we checked each cluster using the known canonical markers and transferred the cell type labels to snATAC-seq and was able to link peaks to genes in each cell type. We believe this approach that refines SingleR through application of a custom reference dataset of the normal breast tissue can greatly improve accuracy and minimize misclassification when annotating cell types in breast tumors using snMultiome data.

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47. A HPV16/18 Viral Clearance and Progression to CIN2+ among women aged 18-25 years enrolled in the COSTA RICA HPV Vaccine Trial

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Purpose: NCT03253744 is a phase I trial with the primary objective to identify the maximally tolerated dose (MTD) of salvage stereotactic body radiotherapy (SBRT) in patients with local prostate cancer recurrence after brachytherapy. Additional objectives included biochemical control and imaging response.

Methods and Materials: This trial was initially designed to test three therapeutic dose levels (DLs): 40Gy (DL1), 42.5Gy (DL2), and 45Gy (DL3) in 5 fractions. Intensity modulation was utilized to deliver the prescription dose to the MRI and PSMA-based PET imaging-defined gross tumor volume while simultaneously delivering 30Gy to an elective volume defined by the prostate gland. This phase I trial followed a 3+3 design with a 3-patient expansion at the maximum tolerated dose (MTD). Toxicities were scored until trial completion at two years post-SBRT utilizing CTCAE v5.0 criteria. Escalation was halted if two dose limiting toxicities (DLTs) occurred, defined as any persistent (>4 days) grade (G) 3 toxicity occurring within the first 3 weeks after SBRT or any â‰¥G3 GU or G4 GI toxicity thereafter.

Results: Between 08/2018 and 01/2023, 9 patients underwent salvage SBRT and were observed for a median of 22 months (Q1-Q3,20-43 months). No DLTs were observed. Escalation was halted by amendment given excellent biochemical control at DL1 and DL2 in the setting of a high incidence of late G2 GU toxicity, thus, the MTD was determined to be DL2. No G3-5 toxicities were observed. One- and two-year biochemical progression free survival (bPFS) were 100% and 86% representing a single patient in the trial cohort with biochemical failure (PSA nadir+2.0) at 20 months post-treatment.

Conclusions: The MTD of salvage SBRT for the treatment of intraprostatic radiorecurrence after brachytherapy was 42.5Gy in 5 fractions producing an 86% 2-year bPFS, with one post-study failure at 20 months. The most frequent clinically significant toxicity was late G2 GU toxicity.

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49. Assessment of Geographic and Socioeconomic Factors in Primary Brain Tumor Patient Accrual to a Natural History Study

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Purpose: To better understand neuro-oncology trial access barriers in the United States (US), we assessed the role of geography, population density, and socioeconomic factors in

the accrual of adult primary brain tumor patients in the Natural History Study (NHS, NCT02851706) at the National Institutes of Health (NIH).

Methods: Participant addresses were linked to zip code geography and population data from the US Census and the Neighborhood Atlas Area Deprivation Index (ADI; 1 to 100 from least to most disadvantaged). Locations with population density greater than 1000 inhabitants per square mile were considered urban. Locations were categorized based on their distance from NIH as local (<50 mi), short-distance (50-200mi) and long-distance (>200 mi). T-tests, chi-square tests, and multivariate logistic regression compared socioeconomic and urbanity factors.

Results: 667 NHS participants arrived from 43 states and territories, with the majority (61%) from Maryland and neighboring states, and other states contributing no more than 5% of the participants each. The home locations of the participants were disproportionately urban (60% versus 19% of all US locations, p <0.001) and socioeconomically advantaged (mean ADI 26.94 versus 57.30 across all US locations, p<0.001). Local participants (49%) had greater racial and ethnic diversity (73% White, 11% Black, 9% Asian, 2% other; 11% Hispanic/Latino) compared to short-distance participants (11% total; 96% White, 3% Black, 0% other; 0% Hispanic/Latino) and long-distance participants (39% total; 89% White, 3% Black, 3% Asian, 2% Other; 6% Hispanic/Latino). Local participants lived in the most socioeconomically advantaged locations (mean ADI 16.39) compared to short-distance (mean ADI 40.88, p<0.001) and long-distance (mean ADI 39.69, p<0.001) participants. Short-distance participants were from disproportionally non-urban areas (69%) compared to local (28%, p<0.001) and long-distance (46%, p<0.001) participants. Multivariate analysis also confirmed that local participants were more likely to live in more advantaged areas than short-distance (OR=0.90, p<0.001) and long-distance (OR=0.93, p<0.001) participants, and short-distance participants were less likely to live in urban areas compared to local (OR=0.19, p<0.001) and long-distance (OR=0.44, p=0.008) participants.

Conclusion: The trial enrolled patients across the US, albeit with most participants arriving from neighboring states and socioeconomically advantaged, urban areas. Greater rural patient participation from nearby areas reflects the positive impact of NIH subsidies in reducing the financial challenges linked to participating in clinical trials. Current trends indicate that continued and targeted efforts to enable participation from local disadvantaged communities can ameliorate access to neuro-oncology clinical care and research.

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50. A Phase I Trial of Salvage Stereotactic Body Radiation Therapy for Radiorecurrent Prostate Cancer After Brachytherapy

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51. Bromodomain and Extra-terminal Protein Inhibitors Target NFIC to Suppress Stemness and Growth in Small Cell Lung Cancer

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Objectives: Recent insights regarding mechanisms mediating stemness, heterogeneity, and metastatic potential of lung cancers have yet to be fully translated to effective regimens for the treatment of these malignancies. This study sought to examine complex epigenetic mechanisms underpinning the aggressiveness of SCLC, with a focus on understanding those mediating stemness in these highly lethal cancers.

Methods: Normal small airway epithelial cells (SAECs) were reprogrammed into iPSCs using lentiviral transduction of Yamanaka factors. Reprogramming validation involved various assays, such as spectral karyotyping and teratoma formation. Genome-wide chromatin landscapes of lung iPSCs (Lu-iPSC) and SAECs, as well as NSCLC and SCLC lines were characterized using RNA-seq, DNA methylome, and DNase-hypersensitivity sequencing (DHS). Gene knock-downs were established using siRNA and shRNA techniques, along with qRT-PCR, immunoblotting, proliferation, and tumorigenicity assays. Glycolysis and oxidative phosphorylation were analyzed using Seahorse assays. In-silico analysis of the NFIC promoter was performed using TFmapper. NFIC and related gene expression in human SCLC cells following NFIC or Bromodomain-containing protein (BRD) knockdown or exposure to BET inhibitors (BETi) were evaluated. Stemness scores were calculated using established RNA-seq criteria, and gene set enrichment analyses were conducted. Proliferation assays and xenograft models were used for in-vitro and in-vivo SCLC growth assessment.

Results: RNA-seq and DNA methylome analyses revealed shared characteristics among SAEC, Lu-iPSC, SCLC, and NSCLC. DHS-linked gene expression exposed both known and novel pathways in SCLC, distinct from SCLC-specific functionality, whereas SOXs and POUs showed strong activity in Lu-iPSC but not SCLC. NFIC, with elevated chromatin occupancy in SCLC, exhibited increased expression compared to SAEC. NFIC KD in SCLC significantly curtailed cell growth in-vitro and in-vivo, affecting multiple pathways, including glycolysis and stem cell signaling etc. Seahorse assays confirmed NFIC's role in regulating glycolysis and oxidative phosphorylation in SCLC. Furthermore, we identified occupancy sites for BRD4 in the NFIC promoter, and knockdown of BRD4, but not BRD2 or BRD3, reduced NFIC expression in SCLC cells. BET inhibitor (BETi) treatment dose-dependently decreased NFIC expression in SCLC lines and human SCLC PDXs, irrespective of subtype. BETi treatment also reduced SCLC stemness and showed a significant overlap in gene regulation with NFIC knockdown, affecting pathways related to gluconeogenesis, neuronal differentiation, and embryonic organ morphogenesis. Importantly, BETi treatment effectively reduced SCLC cell

growth in-vitro and in-vivo, suggesting the potential therapeutic value of targeting NFIC via BET inhibition in SCLC.

Conclusions: NFIC, a unique transcription factor in SCLC, emerges as a key downstream target of BRD4. These findings underscore the potential druggability of NFIC in clinical settings, provide compelling rationale for evaluating BETi for SCLC therapy.

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52. A comprehensive algorithm to predict malignant transformation of NF1 nerve sheath tumors from single-cell transcriptomic profiling

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Objective: A life-threatening complication of having NF1 is the development of an aggressive and highly metastatic malignant peripheral nerve sheath tumor (MPNST) at an earlier age compared to the general population. Currently there are no effective treatments for MPNST other than complete surgical resection with wide negative margins. This study aims to depict the alterations in the tumor microenvironment along the trajectory of malignant transformation from benign plexiform neurofibroma (PN) through precancerous atypical neurofibroma (AN) to MPNST.

Methods: To dissect the oncogenic mechanisms of NF1-deficient Schwann cells during the malignant transformation and describe the concurrent changes in the TME, we utilized single-cell RNA sequencing (scRNAseq) to profile the intra-tumoral heterogeneity of clinically annotated NF1 tumors collected from all pathological stages. We built a NF1 tumor single-cell atlas (reference) by performing integrative analysis to correct for batch effects and to compare the transcriptomic profiles of 55 NF1 nerve tumors. Furthermore, we developed an algorithm to predict malignant transformation of NF1 nerve sheath tumors by comparing scRNAseq data from a new cohort of 21 tumors to this reference.

Results: After quality control and filtering, we analyzed 421,377 cells, including 132,446 cells from 25 PN, 214,522 cells from 25 AN, and 74,409 cells from 5 MPNST. A total of 34 transcriptionally distinct clusters were discovered to belong to seven major cellular compartments: fibroblasts, pericytes, myeloid and lymphoid immune cells, endothelial, Schwann, and malignant cells. These cellular compartments changed composition between benign PN, AN, and MPNST, with notable decreases of the fibroblast, myeloid immune cell, and Schwann cell populations over the course of malignant transformation. Conversely, MPNST exhibited increases in the lymphoid immune cell and tumor cell compartments. To further investigate the changes of immune components accompanying the malignant transformation, we performed subclustering analysis using the 199,921 immune cells using the Harmony algorithm. These immune cells were further grouped into 32 transcriptionally

distinct clusters, including functionally distinct cytotoxic T cells, regulatory T cells (Tregs), B cells, NK cells, NKT cells, mast cells, dendritic cells, monocytes, and macrophages. The immune-cellular composition was unchanged in the comparison of PN and ANF. Notably, there was emergence of CTLA4+ Tregs and loss of activated macrophages in MPNST. Finally, we discovered a unique transitioning cell population in some AN, marking them as potentially high risk of malignant transformation. Using the signatures of these transitioning cells and the transcriptional signatures of malignant MPNST cells, we developed an algorithm for the early identification of MPNST.

Conclusions: In summary, we describe the cellular intra-tumoral heterogeneity of NF1 nerve sheath tumors using data from scRNAseq of patient samples. We show that MPNST exhibits an immunosuppressive TME characteristic of diminished activated macrophages and the presence of Tregs, which may play a role in malignant transformation. Importantly, the discovery of a malignant cell signature presents a unique opportunity for early detection of high-risk NF1 nerve sheath tumors that may undergo malignant transformation.

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53. Reduced levels of TTDN1 protein in cells from Sabinas brittle hair syndrome form of trichothiodystrophy (TTD) patients with germline mutations in DBR1, a new TTD causing candidate gene

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Background and Hypotheses: Trichothiodystrophy (TTD) is a rare (1 per million) inherited autosomal recessive multisystem developmental disorder. TTD has been reported to be caused by 3 nucleotide excision repair (NER)/ basal transcription (TFIIH) genes: ERCC2/XPD, ERCC3/XPB and GTF2H5/TTDA. In contrast to the increased cancer risk in xeroderma pigmentosum (XP) patients with NER/TFIIH gene mutations, TTD patients have different NER/TFIIH gene mutations and normal cancer risk. TTD patients have also been reported with defects in basal transcription factor GTF2E2/TFIIEÎ², amino acid charging tRNA genes (TARS, MARS1, AARS1, and CARS), and with defects in a gene of unknown function (MPLKIP/TTDN1). In our laboratory, more than 200 patients with features of either XP or TTD and XP/TTD overlaps were diagnosed and have mutations in one of the known XP or

TTD causing genes. However, in some clinically diagnosed TTD patients, mutations in the known TTD or XP causing genes were not identified. We performed the whole exome sequencing (WES) to identify a candidate gene in patients with a mild form of TTD.

Study Design and Methods: We conducted DNA sequencing of the known TTD or XP causing genes in our patients and performed clinical laboratory tests as well as extensive medical evaluations. We performed different tests in the cells of the TTD patients and detected the levels of DBR1 and TTDN1 proteins. We performed WES on the genomic DNA from our unknown TTD patient samples to find out the candidate genes that might be the cause of the TTD disorder in these patients.

Results and Conclusions: We describe 4 non-photosensitive adult patients from 3 unrelated families with Sabinas brittle hair syndrome (named for the Mexican town), a mild form of TTD. They harbor identical homozygous missense mutations in DBR1 (p.D262Y) encoding the RNA lariat debranching enzyme DBR1. Post-UV DNA repair was normal, indicating that DBR1 was not involved in NER. We found reduced levels of DBR1 mRNA and no detectable DBR1 protein in the patients' cells. The TTD patients had short, brittle hair with transverse tiger-tailed banding seen on polarized microscopy. Previous reports described interaction of TTDN1 with DBR1 at the protein level which is crucial for pre-mRNA splicing and transcription. We found markedly reduced levels of TTDN1 protein in cells from patients with DBR1 mutations and no detectable levels of TTDN1 protein in the cells from patients with TTDN1 mutations. This suggests that the stabilization of either of these proteins through their interactions is critical for splicing and transcription. Thus, we identified DBR1 as a candidate gene causing Sabinas brittle hair syndrome form of TTD. DBR1 is involved in RNA splicing and transcription supporting the hypothesis that TTD is caused by transcriptional impairments.

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54. Peripheral Immune Analyses Correlate with Clinical Outcome: A Phase II Trial of Nivolumab, Capecitabine, or the Combination in Patients with Triple Negative Breast Cancer

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Introduction: Patients with triple negative breast cancer (TNBC) with residual disease after neoadjuvant chemotherapy have a higher risk of recurrence and worse outcomes than patients achieving pathologic complete response. Here, we report on immune correlates from the OXEL study (NCT03487666), an open label randomized phase II trial in TNBC patients with residual disease following neoadjuvant chemotherapy, who were treated with post-neoadjuvant nivolumab (360 mg intravenously every 3 weeks, Arm A), capecitabine (1,250 mg/m2 orally twice daily on days 1-14 of each 3-week cycle, Arm B), or the

combination of nivolumab and capecitabine (Arm C). The primary endpoint was to assess the immunologic effects of nivolumab, capecitabine, or the combination.

Methods: Peripheral blood mononuclear cells were evaluated at baseline and after 6 and 12 weeks of therapy in patients from Arm A (n=15), B (n=14), and C (n=13) by flow cytometry to identify 158 immune cell subsets. Peripheral immunoscores reflective of enhanced immune cell function were calculated to evaluate changes in the immune profile induced by each therapy, and the association between the immune profile at baseline and disease recurrence.

Results: At 6 weeks versus baseline, an increase in immune cell function was seen in patients on Arms A+C compared to Arm B. Additional distinct immune subsets showed statistical changes after 6 and 12 weeks of therapy that were specific to each arm. At baseline, a higher peripheral immunoscore was associated with a lack of eventual disease recurrence in patients on Arm A or Arms A+C, but not for patients on Arm B. Patients on Arm A (p=0.0003) or Arms A+C (p=0.0085) with a peripheral immunoscore above the median also had a longer interval of disease-free survival than patients at or below the median; this association was not seen in Arm B. Distinct immune subsets at baseline also associated with development of recurrence in each arm; higher levels of naive CD8+ T cells and HLA-DR+ Tregs and lower levels of NKp30+ NK and PD1+ NKT cells associated with recurrence in Arm A, while in Arm B, greater levels of intermediate and nonclassical monocytes associated with recurrence. In Arm C, higher frequencies of multiple Treg subsets were associated with recurrence.

Conclusions: Patients receiving nivolumab, with or without capecitabine, showed enhanced immune cell function. A peripheral immunoscore based on the immune profile at baseline was associated with disease recurrence only in patients receiving immunotherapy. These data highlight the importance of assessing peripheral blood to reveal important immunologic phenomenon and potential associations with clinical outcome.

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55. Atrazine use and markers of kidney function and nephrotoxicity among male farmers in the Biomarkers of Exposure and Effect in Agriculture Study

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Background: Atrazine is a widely used herbicide that has been associated with malignant and non-malignant kidney disease and reduced kidney function in the Agricultural Health Study (AHS). However, little is known about the association between atrazine use and specific kidney disease biomarkers.

Objective: We evaluated the associations between atrazine use and clinical biomarkers of kidney function and nephrotoxicity among male farmers in the Biomarkers of Exposure and Effect in Agriculture study, a sub-cohort of the AHS.

Methods: We included 264 farmers with: 1) recent atrazine use (use within the last 3 months), nearly all of whom also had past use; 2) former atrazine use (top tertile of lifetime use but no recent use); and 3) no/low atrazine use (no recent use and no or low lifetime use). These groups (n=88 each) were frequency-matched on age and state of residence (Iowa, North Carolina). Markers were measured in serum (creatinine, cystatin C, urea nitrogen, uric acid) and urine (kidney injury molecule-1, albumin, creatinine). The 2021 CKD-EPI creatinine-cystatin C equation was used to calculate estimated glomerular filtration rate (eGFRcr-cys). The percent difference in these markers across groups of atrazine use was estimated using multivariable linear regression.

Results: Recent atrazine users had lower eGFRcr-cys (-6.7%; 95%CI: -11.7%, -1.4%) and higher levels of serum creatinine (7.0%; 95%CI: 1.4%, 12.8%), cystatin C (7.4%; 95%CI: 1.2%, 13.9%), and uric acid (6.1%; 95%CI: -0.4%, 13%) compared to farmers with no/low atrazine use. Comparisons between farmers with former atrazine use and no/low use were null, except for an inverse association with blood urea nitrogen (-6.9%; 95%CI: -12.9%, -5.0%).

Conclusions: Our findings add to the evidence that atrazine use, particularly among recent users, is associated with diminished kidney function. Additional studies are needed to clarify the mechanisms through which atrazine may contribute to kidney damage.

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56. Gene therapy for X-linked Severe Combined Immunodeficiency results in long-term and sustained T, NK, and B cell engraftment with low-dose conditioning

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X-linked Severe Combined Immunodeficiency (X-SCID) is a primary immunodeficiency leading to absence of T and natural killer (NK) cell development, in humans. While B cell development is intact in X-SCID, B cells are functionally impaired. The disorder is caused by mutations in the gene encoding the interleukin-2 receptor gamma chain (IL2RG) leading to nonresponsiveness to multiple IL2RG-dependent cytokines, including IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21. We performed a gene therapy clinical trial using a modified self-inactivating gamma-retrovirus (SIN-gRV) expressing IL2RG under the control of the EFS promoter in 9 infants, with the last patient (XSCID09) receiving low-dose chemotherapy conditioning

before infusion. Autologous CD34+ cells were isolated from bone marrow, prestimulated with cytokines and transduced 3 times with the SIN-gRV vector. Transduced cells were infused fresh without cryopreservation, and transduction efficiency was retrospectively calculated, ranging from 0.35-2.92 average vector copies per diploid genome. Here, we are reporting long-term follow up and immune reconstitution 5-12 years post therapy.

Unlike previous trials using a gRV with intact viral enhancers, no patient has developed insertional oncogenesis after SIN-gRV with >11 years median follow-up. Although most patients developed T cells post therapy, NK and B cell reconstitution was superior in XSCID09 who received conditioning. This patient was the only one to have sustained gene corrected NK cell generation (vector copy number (VCN) of 2.23 at 5 years for XSCID09 versus mean of 0.4 for the rest of the patients). XSCID09 was the only patient with gene corrected B cells (VCN of 0.85 for XSCID09 at 5 years versus mean of 0 for the rest of the patients).

To measure IL2RG-dependent B cell function in these patients, we stimulated their peripheral blood mononuclear cells with IL-21 and CD40L for 6 days and assessed B cell proliferation, differentiation into CD27+CD38+ plasmablasts (PB) and memory B cells, class switching, and IgG secretion. B cell function was suboptimal in all the patients that did not get conditioning as the B cells remained mostly uncorrected. We observed a gradation of B cell function (probably due to partially functional IL2RG mutations) with some patients exhibiting some degree of proliferation, PB differentiation, and memory B cell differentiation. To look deeper, we sorted B cells from a patient who had no gene marking in B cells, and tested T cell-dependent B cell activation by coculturing his naÃ¯ve B cells with healthy control CD4+ T cells. His B cells could differentiate to PB but failed to secrete IgG, indicating a major defect in terminal maturation and function of IL2RG mutated B cells. Only B cells from XSCID09 had all measures of B cell in vitro response including PB differentiation, memory B cell formation, and class switching. Importantly, only B cells from XSCID09 secreted IgG in response to CD40L and IL-21 stimuli ex vivo and he was the only patient that responded to tetanus vaccine in vivo. Thus, low-dose conditioning leads to sustained NK immune marking and functional B cells in XSCID patients treated with a modified gRV vector.

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57. Amplified LZK is a novel therapeutic target in esophageal squamous cell carcinomas

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Background: Esophageal Squamous cell carcinoma (ESCC) is the dominant histological type (90%) of esophageal cancers worldwide. The estimated global frequency is 512,000 new cases/year, with a 5-year survival rate of less than 25%, ranking it as one of the deadliest cancers with an urgent need for new therapies. Distal amplification of chromosome 3 (3q26-3q29, the 3q amplicon) occurs in 35% of ESCC patients. Our lab has previously identified the 3q resident gene, MAP3K13 which encodes for Leucine Zipper-bearing Kinase (LZK), as an oncogenic driver in head and neck squamous cell carcinomas. The focus of our research is to examine whether amplified LZK can also serve as a therapeutic target in ESCCs with the 3q amplicon.

Study Design: To examine whether amplified MAP3K13 is a genetic dependency in ESCCs, we assessed the effect of dox-inducible shRNA-mediated knockdown of LZK on viability of ESCC cells with the 3q amplicon (identified using DepMap and cBioPortal). In collaboration with the Swenson lab, we next moved to developing small molecule inhibitors of LZK and testing these inhibitors through in vitro kinase assays as well as Western blot and ELISA in cultured cells. Effective LZK inhibitors that potently bind to LZK and abolish its kinase activity were then assessed for their ability to reduce ESCC cellular viability. We confirmed the specificity of drug-induced decreases in viability using a rescue system expressing drug-resistant mutant form of LZK (Q240S). We also assessed the effect of catalytic inhibition of LZK in suppressing tumor growth in vivo in ESCC PDX models. We have begun to explore the precise mechanisms through which amplified LZK promotes oncogenic functions in ESCCs with the 3q amplicon. Finally, our LZK inhibitors are being screened by NCATS matrix drug screening program to identify synergistic pairs that could be used as combination therapies.

Results & Conclusions: In this study, we showed that knocking down LZK decreases viability of ESCC cells with the 3q amplicon, consistent with amplified MAP3K13 promoting cell survival and proliferation in this cancer subtype. Through catalytic inhibition experiments, we established that lead LZK inhibitors reduce the tumorigenic phenotypes of ESCC cell lines with the 3q amplicon, compared to those lacking the amplification. Furthermore, the observed decreases in viability were rescued by the expression of the Q240S drug-resistant mutant form of LZK, confirming the specificity of these small molecule inhibitors to LZK. We identified Akt as a downstream target that is regulated by the catalytic activity of LZK in ESCC cells with 3q amplicon. We also demonstrated that the lead LZK inhibitors suppress in vivo tumor growth in PDX models with amplified LZK. Finally, our initial matrix drug screens identified several synergistic pairs that will be investigated as combination therapies with LZK inhibitors in the future studies. In conclusion, this study defines LZK as a therapeutic target in ESCCs where catalytic inhibition reduces cell viability and in vivo tumor growth via Akt, demonstrating the promise of targeting LZK, alone or in combination with synergistic drugs, as a potential new treatment strategy for these patients.

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58. Relationship between Polygenic Risk Score, Lifestyle Factors, and Colorectal Cancer Risk

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Background and Hypotheses: Although heritable factors are estimated to explain up to 35% of colorectal cancers (CRC), most are diagnosed in people without a family history of CRC suggesting that inherited genetic variants, environmental factors, or a combination of both, contribute to CRC risk. We explored the relationship between a polygenic risk score (PRS) for CRC risk and lifestyle risk factors to understand whether individuals with higher genetic risk, who do not develop CRC, engage in healthier lifestyle behaviors, or conversely, whether those with lower genetic risk, who do develop CRC, engage in riskier behaviors in the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial.

Study Design and Methods: Using summary statistics obtained from a large independent GWAS meta-analysis of CRC by the GECCO consortium, we developed a genome-wide PRS for CRC using SBayesR and applied it to participants of European ancestry genotyped in the PLCO Trial (n=74,396). For all subjects, we computed an environmental (E) score based on 19 lifestyle risk factors for CRC, a WCRF/AICR score that measured adherence to the 2018 Cancer Prevention recommendations, and a 2015 healthy eating index (HEI); for each score, a higher value indicates a healthier lifestyle profile. We used t-tests to evaluate differences in lifestyle scores between CRC-free participants in the highest quartile of PRS and incident CRC cases in the lowest quartile of PRS. To examine the correlations between the PRS and lifestyle scores, we performed multivariable linear regression models regressing PRS on each lifestyle score. We used multivariable Cox hazards regression models to compute hazard ratios and 95% confidence intervals for CRC risk; multiplicative interactions between PRS and lifestyle scores were evaluated using the Wald test for the interaction terms.

Results: During the 15 years follow-up period, a total of 1,052 CRC cases were diagnosed. Individuals in the highest quartile of PRS who remained cancer-free were more likely to engage in healthier behaviors than those in the lowest quartile of PRS who were diagnosed with incident CRC (E-score: -0.01 vs. -0.18, WCRF/AICR score: -0.05 vs. -0.32, HEI score: 0.06 vs. -0.13); however, differences were not statistically significant (P value: 0.1-0.4). PRS and lifestyle scores were significantly (all P <0.0001), albeit weakly ($\hat{I}^2$: -0.02 to -0.03), associated among participants who remained CRC-free but not among CRC cases (P: 0.1 to 0.3). PRS-lifestyle score interactions were not statistically significant (P: 0.2-0.4).

Conclusions: Although the differences and multiplicative interaction models were not statistically significant, we found that individuals with a higher PRS who remained CRC-free were slightly more likely to engage in healthier behaviors than those at a lower genetic score who developed CRC. Additional studies with larger sample size are warranted to further explore the interplay between genetic risk and lifestyle factors related to CRC risk.

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59. Characterizing the endogenous retroviral envelope protein ERVMER34-1 as a target for a therapeutic cancer vaccine

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Endogenous retroviruses (ERVs) are remnants of retrovirus germline infections that occurred over the course of evolution and constitute between 5-8% of the human genome. While most ERV encoded proteins are epigenetically silenced in normal adult human tissues, ERV expression has been reported in several types of cancers, representing a pool of potential targets for cancer vaccines. This study evaluated for the first time the ERV envelope protein ERVMER34-1 as a target for a therapeutic cancer vaccine. The results of this study demonstrate that the ERVMER34-1 protein, unlike most other ERVs, is over-expressed in several types of human carcinomas while being absent or expressed at low levels in healthy adult tissues. ERVMER34-1 specific T cells could be detected in PBMCs of cancer patients but not from healthy donors following an overnight stimulation; however, reactive T cells could be expanded from both healthy donor and cancer patient PBMCs following a 7-day in vitro stimulation. ERVMER34-1 specific CD8+ T cells could selectively kill human carcinoma cells expressing ERVMER34-1. A rationally modified ERVMER34-1 therapeutic cancer vaccine has now been designed. The vaccine was shown to mediate regression of established syngeneic murine tumors expressing the human ERVMER34-1 protein. This study supports the development of a novel, potentially clinically relevant therapeutic strategy targeting ERVMER34-1.

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62. Chromosome 7 to the rescue: overcoming chromosome 10 loss in gliomas?

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Gliomas, including aggressive forms such as glioblastoma (GBM) and lower-grade gliomas (LGG), present formidable challenges for treatment due to their poor prognosis. The co-occurrence of chromosome 10 loss (10 loss) and chromosome 7 gain (7 gain) in gliomas is the most frequent loss-gain co-aneuploidy pair in human cancers, a phenomenon that has been investigated without resolution since the late 1980s. The reasons for this frequently co-occurring event are not very clear, with some prior studies suggesting a few driver genes as culprits. In this study, we aim to study and understand the phenomenon of 10 loss and 7 gain in gliomas in a systematic, chromosome-wide level. Notably, the computational analysis developed and presented here could advance the study of other frequent co-occurrence alterations in the future.

Our analysis consists of four main analyses, which bring independent sources of evidence to address this fundamental long-standing research question:

1. By analyzing over 39,000 patient tumors in the large-scale Progenetix database and developing rigorous mathematical models, we show that the probability of 7 gain occurring after 10 loss is significantly greater than 10 loss after 7 gain, finding that the less frequent sequence 10 loss after 7 gain can be treated as occurring by random chance.

2. We analyzed hundreds of genomic and transcriptomic samples from brain cancer patients and cell lines. Our aim was to identify a category of clinically significant genetic interactions that functionally compensate for the genes located on the lost arm (synthetic rescues). This step revealed that genes on chromosome 10, when lost, can be best functionally compensated by the upregulation of genes on chromosome 7, compared to genes on other arms. We also show that glioma patients with 10 loss and 7 gain events tend to have worse overall survival, testifying to enhanced underlying tumor fitness.

3. Analyzing large-scale in vitro CRISPR essentiality screens in central nervous system (CNS) cancer cell lines, we show that the fitness effects of different possible sequences of chromosomal arm alterations further support the capacity of 7 gain to compensate for 10 loss.

4. Lastly, we analyzed expression data from thousands of normal non-cancerous samples from human brain tissues testifying that the normal transcriptome state in the cortex or frontal cortex (which are common tissues of origin for GBM) are permissive of 10 loss and 7 gain co-occurrence in cancers arising from these tissues.

The mystery behind the co-occurring loss of chromosome 10 and gain of chromosome 7 in many gliomas has been an open problem for over four decades. While prior studies have pinned hopes for explaining these frequently co-occurring events with a few driver genes as culprits, our comprehensive, genome-wide analysis—encompassing vast patient and cell line genomic and transcriptomic data—offers a more complex and chromosomal-wide understanding. From a future translational standpoint, it additionally points to key rescuer genes on chromosome 7 that may be potentially targeted. The new multi-pronged approach could be applied in future studies of other common co-occurring aneuploidies across many cancer types.

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63. Evaluating clinical and sociodemographic risk for symptom-related functional interference in the primary brain tumor population

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64. Improved immunologic and therapeutic activity of anchored interleukin (IL)-12 immunotherapy in combination with cytotoxic chemotherapy and immune checkpoint inhibitor in a head and neck cancer model

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Background: Head and neck squamous cell carcinoma is a heterogeneous group of cancers associated with high rates of locoregional recurrence and metastatic disease despite chemoradiation therapy. These tumors have exhibited limited responses to immune checkpoint inhibitors. Murine ANK-101 (mANK-101) is a novel anchored form of IL-12 that persists in the tumor microenvironment (TME) for up to 30 days without systemic toxicity. We tested the hypothesis that local IL-12 could potentiate the therapeutic response of chemotherapy or immune checkpoint blockade for head and neck cancers.

Methods: In C57BL/6 mice, we used established murine oral carcinoma (MOC1) tumors as a model for non-HPV-related head and neck carcinoma with the treatments commencing when the tumors were 100-200 mm3. Treatment with cisplatin (5 mg/kg, intraperitoneal (i.p.) injection once weekly for 3 weeks), murine ANK-101 (mANK-101; 5 Âµg intratumoral (i.t.)), anti-PD-1 (200Âµg, i.p. once weekly for 3 weeks), or various combinations were evaluated. Tumors were measured with calipers and tumor size one week after last treatment was used for comparisons of tumor growth. Mice were also followed for survival. A subset of tumors was collected for immunohistochemistry and flow cytometry 5 days after ANK-101 treatment, when the tumors were regressing. Tumor size was compared using student's ŧ-test between groups and multiple comparisons utilized two-way ANOVA testing.

Results: In the MOC1 model, anti-PD-1 and cisplatin alone had no impact on tumor growth. mANK-101 alone, however, was associated with a significant delay in tumor growth. The addition of cisplatin or anti-PD-1 to mANK-101 treatment did not improve the anti-tumor effect when compared to mANK-101 monotherapy. On the other hand, triple combination therapy with mANK-101, cisplatin, and anti-PD-1 further delayed tumor growth and resulted in the highest number of animals cured. The combination of mANK-101, cisplatin, and anti-PD-1 was associated with increased CD8+ T cell recruitment to the tumor microenvironment. Furthermore, mANK-101 monotherapy and triple combination therapy skewed the macrophage population from M2 to M1.

Conclusions: Anchored IL-12 improves therapeutic responses to cisplatin chemotherapy and immune checkpoint blockade in the murine PD-1-refractory MOC1 head and neck cancer

model. These preliminary data demonstrate that combining local IL-12 with cytotoxic chemotherapy and/or immune checkpoint blockade merits investigation in other tumor models.

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65. Therapeutic Targeting of Breast Cancer Brain Metastasis by Blocking CSF1R signaling

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Breast cancer is the most common cancer in women and the second leading cause of cancer-related death in women. Cancer metastasis accounts for more than 90% of cancer deaths including breast cancer. Of the four common sites where breast cancer metastasizes liver, lung, bone, and brain metastasis has the poorest survival outcomes. However, there are currently no effective therapies to prevent or treat brain metastases. Microglia, the major resident macrophages within the brain parenchyma, plays a pivotal role in various facets of brain metastasis, encompassing inflammation, angiogenesis, and immune modulation. The colony-stimulating factor 1 receptor (CSF1R) is a key tyrosine kinase transmembrane receptor that modulates microglial homeostasis. Using animal models of breast cancer brain metastasis, we tested whether targeting microglia in the tumor microenvironment with a CSF1R inhibitor, BLZ945 (provided by Novartis), could inhibit brain metastasis in vivo. Brain metastatic tumor burden was quantified, and tissue sections were used for quantitative immunofluorescence staining of various cell markers. We found that inhibition of microglia by BLZ945 significantly reduced the burden of brain metastases in both prevention and treatment settings. Quantitative analysis of Ki-67 staining revealed a significant reduction in cancer cell proliferation following BLZ945 treatment. In addition, BLZ945 treatment inhibited the activation of astrocytes in the tumor microenvironment. Next, we sought to determine the effects of BLZ945 on microglia and elucidate its mechanism of action in vitro. We found that BLZ945 did not directly affect breast cancer cell proliferation. However, BLZ945 inhibited cell proliferation, activation of the MAPK signaling pathway, and secretion of inflammatory cytokines including IL-6 and TNFÎ± in microglial cells. BLZ945 could reduce cancer cell proliferation in a microglia and cancer cell co-culture assay. Taken together, our findings establish a role for microglia in regulating the development of breast cancer brain metastases and provide a novel therapeutic approach for the prevention and/or treatment of brain metastases.

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66. Phase II Study of Intravenous and Intraperitoneal Paclitaxel and Oral Nilotinib for Peritoneal Carcinomatosis from Colorectal, Appendiceal, Small Bowel, Gastric, Cholangiocarcinoma, Breast, Ovarian, or Other Gynecologic Primary Cancer

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Background: Peritoneal carcinomatosis is an aggressive and frequently encountered manifestation of gynecologic (60 percent), gastrointestinal (GI) (40 percent), and primary peritoneal malignancies. Complete or optimal cytoreduction followed by heated intraperitoneal chemotherapy (HIPEC) has demonstrated survival benefits, but many patients are not candidates for such interventions due to high disease burden. Intraperitoneal (IP) chemotherapy leverages higher AUC (area under the curve) for peritoneal administration compared to plasma levels, thereby accentuating regional treatment effect. Paclitaxel is ideal for iterative IP administration due to its high molecular weight and hydrophobic properties. Bidirectional therapy (intravenous (IV) plus IP chemotherapy) may provide additive benefit. Preclinical studies with oral nilotinib, a tyrosine kinase inhibitor, in combination with IV paclitaxel have exhibited promising preclinical and clinical responses in NCI60 tumor cell line panel, mouse xenograft models, and the ongoing phase 1 clinical trial that was based on the NCI ALMANAC study (NCT02379416). We hypothesized that bidirectional paclitaxel combined with nilotinib could lead to disease reduction and facilitate subsequent cytoreduction (NCT05185947).

Methods: This is a phase II trial in participants aged greater than 17 years with advanced GI, gynecological, or other primary malignancies who are not candidates for cytoreductive surgery as assessed by initial screening laparoscopy number 1 and peritoneal biopsy. Disease evaluation is conducted by RECIST 1.1 (Response Evaluation Criteria in Solid Tumors) and/or PCI (Peritoneal Cancer Index). The primary objective is to assess the rate of downstaging of peritoneal disease burden to become resectable based on PCI.
The treatment plan for eligible participants comprises loading dose of oral nilotinib 300 mg twice daily starting day -4; diagnostic laparoscopy number 2, biopsy, and IP port placement on day 0; IP paclitaxel 60 milligram per meter squared (MGM2) on day 1; and IV paclitaxel 60 MGM2 on day 2 and 80 MGM2 on day 8 and beyond. Participants will undergo up to six 21-day cycles, with repeat laparoscopic assessment after Cycles 3 and 6 (laparoscopy numbers 3 and 4, respectively). At laparoscopy number 3, biopsy and PCI scoring as response assessments will be performed. Participants who demonstrate disease stability or response but who are still deemed unable to undergo complete cytoreduction will undergo Cycles 4 to 6. Patients will come off study for disease progression either by imaging or if PCI increases by greater than 4 points. At laparoscopy number 4, biopsy and PCI scoring will be repeated; the IP port is removed. Participants with stable disease may continue IV paclitaxel and oral nilotinib until progression.

Results: As of data cutoff date (January 23, 2024), 10 participants have been screened, with 3 eligible who started protocol therapy, and 1 evaluable for response. Reasons for screen failures included insufficient disease in 4 patients, and 1 patient each with mucinous ascites,

high-volume ascites, and intercurrent illness. One participant achieved partial response by PCI, another was unevaluable due to withdrawing from treatment due to IP toxicity, and response assessment is pending for the third.

Conclusions: In the ongoing trial, we anticipate continued enrollment and evaluation of safety and toxicity.

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67. Phase II Study of Intravenous and Intraperitoneal Paclitaxel and Oral Nilotinib for Peritoneal Carcinomatosis from Colorectal, Appendiceal, Small Bowel, Gastric, Cholangiocarcinoma, Breast, Ovarian, or Other Gynecologic Primary Cancer

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68. Project EVOLVE: Evaluation of Lineage Switch (LS), An International Initiative Preliminary Results Reveal Dismal Outcomes in Patients with LS

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Background: Lineage switch (LS) refers to the transformation of acute leukemia from one cell lineage to another (e.g., lymphoid (ALL) to myeloid (AML)). This rare phenomenon, distinct from treatment induced malignancy (i.e., t-AML) and mixed phenotype acute leukemia (MPAL), is increasingly being observed following targeted immunotherapy. Given challenges in the diagnosis and treatment of LS, Project EVOLVE was developed to globally identify cases of LS following immunotherapy.

Methods: A protocol and data collection form for this IRB exempt study was disseminated to capture deidentified LS case information across centers and cancer consortia, including data from previously published reports of LS. LS was defined by the emergence of cell-surface markers warranting reclassification of the original leukemia as a different lineage derivation based on the immunophenotype. Confirmed LS cases included those where retention of original cytogenetic or molecular aberrations and/or the clonal immunoglobulin rearrangement patterns were verified to confirm the clonal relationship. Suspected LS cases included those where clonality could not be confirmed, but the clinical presentation was consistent with LS. The term transition was applied when immunophenotypic shifts were transient. Statistical analysis was primarily descriptive. Data cut-off was July 26, 2023**.**

Results & Conclusions: A total of 58 cases were identified, including 19 cases referenced in prior publications. Collectively, 47 cases of LS (43 confirmed; 4 suspected), spanning across 3 continents and 8 countries, were analyzed.

Cases included transition from B-ALL to AML in 36 (76.6%) and to MPAL/biphenotypic/ambiguous lineage in 11 (23.4%). Two likely had MPAL at diagnosis and a third with chronic myeloid leukemia. The median age at initial diagnosis was 8.4 years (range, 1 day-76.5 years); median age at LS presentation was 11.0 years (range 0.4-77.3 years). Baseline cytogenetics showed KMT2A rearrangement in 27 (57.4%).

The most proximal immunotherapy prior to development of LS was CAR T-cells in 23 (48.9%) and blinatumomab in 24 (51.1%). The median time from the most proximal immunotherapy to development of LS was 1.6 months (range, 7 days-36.5 months). LS presented as isolated marrow relapse in 27 (57.4%), isolated CNS in 2 (4.3%), isolated non-CNS extramedullary disease in 3 (6.4%), combined relapse in 14 (29.8%) and unreported in 1.

First line treatment for LS was chemotherapy induction in 31 (66.0%), palliative care/no therapy in 8 (17.0%), and alternative therapies in the remaining 8 (17.0%). In 35 (74.5%) patients, remission induction was the goal of first line therapy. Fifteen (31.9%) achieved a complete remission (CR) of whom 2 were treated with palliative intent. Eight (17.0%) patients are alive in remission, 2 (4.3%) are alive with active LS and 37 (78.7%) died from LS, their original disease, or complications of treatment of LS. For those alive in CR (n=8), 7 (87.5%) proceeded to a consolidative SCT.

Project EVOLVE provides the largest dataset of patients with LS to date. This international effort reveals substantial complexity and variability in diagnosing and managing LS. Unfortunately, outcomes following LS are grim, underscoring the critical need to identify risk factors of and optimal therapies for this form of immune escape.

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69. Development and implementation of a systematic approach to measure patient-reported outcomes in early-phase cancer trials

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Background: There has been a shift in the clinical trial landscape toward early-phase studies where data from well-designed and pivotal phase I/II trials is leading to accelerated drug approval. Our goal was to develop a standardized approach to the measurement and reporting of patient-reported outcomes (PROs) in early-phase trials to improve assessment of tolerability, address noted barriers to inclusion, and enhance patient-provider communication to inform treatment decisions and ultimately improve outcomes.

Methods: The Office of Patient Centered Outcomes Research (OPCORe) was established in 2022 within the Center for Cancer Research at the National Cancer Institute (NCI) with the mission to advance understanding of the clinical benefit and tolerability of cancer therapies by integrating patient-centered approaches into CCR clinical trials by fostering education and collaboration with stakeholders. Implementation of regulatory/consortia guidelines related to use of core constructs, assessment frequency, and hypothesis-driven objectives and analysis plans through protocol template, development of data capture, email-based alerts and data visualization for clinical providers was developed and standardized templates for protocol language, research staff education, tools for workflow, and tracking implemented.

Results: The OPCORe protocol template includes measurement of 3 constructs, including tolerability, clinical benefit, and disease-specific impact, as well as anchors of meaningful change. Core symptomatic toxicities are measured to inform understanding across trials with agent/class-specific symptomatic toxicities added to assure relevance. Baseline and standardized weekly electronic capture of tolerability during early cycles with alerts to clinical research staff if moderate-severe toxicities reported helps facilitate patient check-ins. Repeat PROs assessments in later cycles are expanded to include disease-specific assessments and align with disease evaluation to further inform clinical impact. To date, we have collaborated with 7 research groups and developed approaches for first-in-human, phase I, and phase II studies with trial start-up and data collection in process.

Conclusion: The value of including PROs in cancer trials is increasingly recognized by regulators, clinicians, patients, and advocates to advance understanding of the clinical benefit & tolerability of emerging therapies. Standardization and incorporation of PROs in earlier phase trials may enable PROs to be optimized, improve later phase trial design, and help inform health policy, support drug approval, and promote shared decision making between providers and patients to inform treatment decisions.

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70. Epigenome-wide association study of lung cancer among never-smokers in two prospective cohorts in Shanghai, China

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Background and Aim: The etiology of lung cancer among individuals who never smoked remains elusive, despite 15% of lung cancer cases in men and 53% in women worldwide being unrelated to smoking. Epigenetic alterations, particularly DNA methylation (DNAm) changes, have emerged as potential drivers. Yet, few prospective epigenome-wide association studies (EWAS), primarily focusing on peripheral blood DNAm with limited representation of never-smokers, have been conducted. However, since most lung cancers develop from epithelial cells lining the airways, DNAm in peripheral blood connective tissue cells may not adequately capture the underlying molecular changes directly related to lung cancer development. Oral rinse samples, which can easily be collected non-invasively and constitute a mixture of epithelial and other upper airway cell types directly exposed to various air pollutants, offer a valuable medium to identify etiologic markers of diseases originating from epithelial tissues, such as lung cancer. The aim of the study was to investigate the associations between oral cell DNAm, epigenetic age acceleration, and incident lung cancer among never-smokers.
Study Design and Methods: This nested case-control study involved 80 never-smoking incident lung cancer cases and 83 comparable never-smoking controls within the prospective Shanghai Women's Health Study and Shanghai Men's Health Study. DNAm was measured in pre-diagnostic oral rinse samples using the Illumina MethylationEPIC array.

Initially, we conducted an EWAS to identify differentially methylated positions (DMPs) associated with lung cancer in the discovery sample of 101 participants using robust linear regression models with adjustments for age, sex, sample plate, and estimated cell-type proportions. The top-50 DMPs were further evaluated in a replication sample of 62 participants using similar models, and results were pooled using fixed-effect meta-analysis. Pathway and functional overlap enrichment analyses were conducted on the top 1000 DMPs using Gorilla and eFORGE (v2.0), respectively. The associations between epigenetic age acceleration and lung cancer were assessed using logistic regression models.

Results and Conclusions: Three DMPs were significantly associated with lung cancer at the epigenome-wide significance level of p-value<8.22e-08. Specifically, hypomethylation of cg09198866 (MYH9, TXN2; p-value=9.68e-09) and cg01411366 (SLC9A10; p-value=1.02e-08), and hypermethylation of cg12787323 (p-value=2.72e-08) were associated with an increased future risk of lung cancer. Additionally, examination of the top 1000 DMPs revealed significant enrichment in epithelial regulatory regions and their involvement in small GTPase-mediated signal transduction pathways, implicated in lung cancer development. Further, GrimAge acceleration was identified as a risk factor for lung cancer (odds ratio=1.19 per year; 95% confidence intervals: 1.06-1.34).
This study marks the first prospective EWAS of lung cancer among never-smokers using oral rinse samples. While replication in a larger sample size is necessary, our findings suggest that DNAm changes in pre-diagnostic oral rinse samples could provide novel insights into the pathogenesis of lung cancer in never-smokers.

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71. Demonstrating the Clinical Benefit of Carboplatin and Bevacizumab for the Treatment of Recurrent Low-grade and Anaplastic Supratentorial, Infratentorial and Spinal Cord Ependymoma in a Multi-Center Phase II Trial Using Patient Reported Outcomes

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Background: Ependymoma is a rare tumor occurring in the brain or spine. Initial treatment and at recurrence often include surgery +/- radiation therapy with limited data supporting the use of chemotherapy. In this multi-center study bevacizumab, a tumor starving, or anti-angiogenic therapy was combined with carboplatin, a cytotoxic chemotherapy, to test their

efficacy in patients with recurrent ependymoma. As a secondary objective, we examined whether there are improvements in symptom severity and symptom interference in patients who responded to the therapy.

Methods: Adult patients (n=22) were enrolled and depending on tumor location, completed either the brain tumor (MDASI-BT) or spine (MDASI-SP) module of the MD Anderson Symptom Inventory at baseline, Cycle 2 and Cycle 4. We categorized patients either as a responder (stable/no disease progression) or non-responder (disease progression) based on MR imaging. We fitted regression lines over time to compare responders with non-responders in those with brain and spine tumors using scale factor scores and symptom interference. Positive slopes indicate worsening while negative slopes denote improvement.

Results: Completion rates were 91%, 91% and 72% at baseline, cycle 2 and cycle 4, respectively. Brain tumor responders showed reduction while non-responders had an increase in both neurologic (slope=-0.11 vs 0.23) and cognitive symptoms (slope=-0.20 vs 0.35), with no differences in slopes noted between the groups for affective and treatment-related symptoms and both showing a reduction in general disease symptoms (slope=-0.12 vs -0.16). Spine tumor responders and non-responders both showed worsening disease-related symptoms (slope=0.39 vs 0.25) with stable constitutional/treatment-related symptoms in responders and worse emotional symptoms for non-responders. Activity-related interference worsened for both groups (brain and spine), whereas mood-related interference was stable in those with brain tumors.

Conclusion: Collection of longitudinal disease was feasible and treatment-related symptom data in a multi-center study of a rare disease demonstrates differential effects in disease and mood-associated measures in brain tumor patients with similar treatment associated symptoms and worsened interference. These findings are consistent with the anti-edema-related symptomatic benefit with bevacizumab in brain tumors but not spine tumors and cumulative effect of symptomatic toxicities on interference.

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72. Adoptive transfer of membrane-restricted IL-12-TCR T cells promotes antigen-spreading and elimination of antigen-negative tumor variants

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74. Serum Proteins are Associated with Neurocognitive Symptoms and Patient-Reported Outcomes in Primary Brain Tumor Patients

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Background and Hypotheses: Primary brain tumors (PBT) are associated with a range of symptoms as a direct result of the tumor or its treatment. IDH-mutant gliomas are associated with longer survival, but with higher incidence of seizures and neurocognitive symptoms. The risks in patients with IDH-mutant gliomas and role of interconnectivity of the tumor with neurons and the tumor microenvironment has recently heightened interest in exploration of biomarkers of impairment to inform care. Here, we investigated peripheral blood levels of cytokines and vascular injury protein markers in a cross-sectional cohort (n=47) of adult, IDH-mutant PBT patients enrolled in a natural history study (NCT02851706, PI: T. Armstrong). We report links between protein levels and severity of neurocognitive symptoms and patient-reported outcomes (PROs).

Study Design and Methods: Neurocognitive symptoms were evaluated using the Montreal Cognitive Assessment (MoCA). MoCA raw scores (range 0-30) were dichotomized (â‰¤ 25 = cognitive dysfunction). PROs and activity-related interference were collected via the MD Anderson Symptom Inventory-Brain Tumor at study entry. Serum samples were stored at -80Â°C for batch processing and used for biomarker analysis. Cytokines (Interleukin-1Î² [IL-1Î²], IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, interferon-Î³ [IFN-Î³], and tumor necrosis factor-Î± [TNF-Î±]) and vascular injury markers (C-reactive protein [CRP], serum amyloid A [SAA], intercellular adhesion molecule-1 [sICAM-1], and vascular cell adhesion molecule-1 [VCAM-1] were quantified in duplicate in a QuickPlex SQ 120 instrument (Meso Scale Discovery [MSD], Rockville, MD). Lab personnel were blinded to diagnosis and patient clinical status. Descriptive statistics, correlation analysis, and logistic regression were conducted using IBM SPSS and SAS Statistics software.

Results and Conclusions: The patient sample was mostly male (62%), white (70%), employed (64%), had no prior recurrence (62%), high grade (3-4) tumors (70%), and an average age at diagnosis of 36 years (SD: 9; median: 34; range: 20-53). Dichotomizing MoCA scores showed that 43% of the participants exhibited moderate-severe (â‰¤ 25) cognitive impairment. MoCA binary group was negatively correlated with levels of CRP (r=-0.42, p<.003). The log mean concentration of CRP was higher in participants with cognitive impairment (7.99, SD: 0.47 vs 7.57, SD: 0.45, p<.003) than those without impairment. When controlling for age and sex, CRP remained predictive of cognitive impairment (OR 8.18, 95% CI 1.70,39.23). In addition, IL-10, but not other markers, positively correlated with severity of patient-reported symptoms [pain (r=0.29, p=0.048), numbness/tingling (r=0.30, p=0.039)], and activity-related interference, including work (r=0.32, p=0.027), and walking (r=0.40, p=0.005). Our results suggest an association between inflammatory responses and symptoms in IDH-mutant glioma patients. Further analysis in large and more diverse longitudinal cohorts will be needed to validate these findings in this population and

compare to those with IDH-WT tumors and develop predictive models to inform biomarker development.

Neuro-Oncology Branch, CCR, NCI

75. Cancer mortality and geographic inequalities: a detailed descriptive and spatial analysis across US counties, 2018-2021

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76. Kaposi sarcoma patient-derived xenografts exhibit both an expansion of endothelial cells that actively transcribe viral genes and an amplified gene signature of KS tumors

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Background and Hypothesis: Kaposi sarcoma (KS) is an HIV-associated malignancy defined by hyperangiogenesis, inflammatory infiltrates, and endothelial cells infected with KSHV. KS is a major cause of morbidity and mortality in people living with HIV. Current treatments include antiretroviral therapy, chemotherapy and/or the immunomodulatory therapy pomalidomide, but tumor response is varied, and relapse is frequent. The exploration of novel therapies is hampered by the lack of a patient-derived preclinical animal model. Here, we characterize patient-derived KS xenografts (PDXs) in immunodeficient NOD/SCID/gamma (NSG) mice.

Study Design and Methods: Sixteen cutaneous KS biopsies were subcutaneously implanted into 1-4 NSG or NOG mice transgenic for human IL-6. Variables that were tested included VEGF supplementation, tumor dissociation prior to matrigel embedding, and human IL-6 expression. The PDXs were harvested at end timepoint for 1) immunohistochemistry examination of key cellular markers and viral proteins, 2) Spatial transcriptome analysis and 3) derivation of cell culture.

Results and Conclusions: Immunohistochemistry staining for KSHV LANA in spindled CD34+ endothelial cells revealed that KS-PDXs were maintained in recipient NSG for long periods (103 to 272 days), until the experimental endpoint. KS-PDX tumor sizes did not increase in the first or second passage. Regardless of the tested variables and the clinical history of patient volunteers, there was a consistent mean 2.3-fold increase (range 1.2 to 7.1 fold) in

KSHV LANA+ of human, NUMA-1+ endothelial cells in the KS-PDX compared to the input biopsies. The Ki67 proliferation marker overlapped with LANA+ areas, consistent with latency-driven cell expansion. Spatial transcriptome analysis confirmed the dramatic expansion of endothelial cells and revealed a broad distribution of viral transcripts from latent and lytic classes across the KS-PDX samples. In addition, signatures genes of Kaposi sarcoma tumors and infected primary endothelial cells were conserved and exhibited a more extreme profile in the KSHV+ regions of the KS-PDX. PDX derived cell culture was successfully established with characteristics of tumor associated fibroblast.
The expansion of KSHV-infected endothelial cells and the recapitulation of the KS tumor gene signature supports the application of patient-derived cutaneous KS xenografts as a pre-clinical model to test novel therapies.

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77. Evaluation of Trastuzumab Deruxtecan (T-DXd), a HER2 antibody-drug conjugate, in preclinical models of breast cancer brain metastasis

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78. County-level Ultraviolet Radiation and Kaposi Sarcoma Among People Living with HIV in the US

Jinkyu Jung, Hua Song, Meili Zhang, Nicole Briceno, Wei Zhang, Ukhyun Jo, Yves Pommier, Mark Gilbert, Marta Penas-Prado, Byram H. Ozer

Neuro-Oncology Branch, CCR, NCI, Developmental Therapeutics Branch, CCR, NCI

79. Type 1 Topoisomerase Inhibitor SN38, and the Polyethylene Glycol-Conjugated Investigational Agent PLX-038, Demonstrate Efficacy Against Immortalized, High-Grade Meningioma and Correlate with Expression Levels of TOP1 and the Sensitivity Marker SLFN11

Jinkyu Jung, Hua Song, Meili Zhang, Nicole Briceno, Wei Zhang, Ukhyun Jo, Yves Pommier, Mark Gilbert, Marta Penas-Prado, Byram H. Ozer

Background and Hypotheses: Meningiomas are tumors arising from the lining of the brain and spine and are the most common primary adult neoplasm of the central nervous system. Though the majority are benign and curable by surgery or radiation, nearly 20% are considered high grade, and can recur and be refractory to treatment. With no approved

systemic therapy for these tumors, there is an unmet need to develop treatment strategies. SN38, the active component of the Type 1 topoisomerase (TOP1) inhibitor irinotecan, has demonstrated in vitro and in vivo activity in meningioma preclinical models, but its clinical use was tempered by concerns with toxicity, dosing, and limited tumor accumulation. Recently, in addition to TOP1 expression levels, Schlafen-11 (SLFN11) expression has emerged as a predictor of response to topoisomerase inhibition. New formulations of irinotecan, such as PLX-038, a releasable polyethylene glycol-conjugate of the TOP1 inhibitor SN-38, have significantly improved toxicity and bioavailability profiles. This study aims to evaluate the efficacy of SN38 as PLX-038 to provide preclinical efficacy data and support a forthcoming clinical trial in recurrent high grade meningiomas.

Study Design and Methods: Established (Ben-Men-1, IOMM-Lee, CH-157, MN1-LF, KT21-MG1) and laboratory-generated (GAR, JEN, SAM) meningioma cell lines were characterized by immunoblotting and RNA sequencing for the expression levels of topoisomerase (TOP1, TOP2A, TOP2B) and SLFN11. Cells were subsequently exposed to increasing concentrations of SN38 to determine the IC50. In the animal studies, PLX-038 was administered weekly to mice implanted with either intracranial or subcutaneous IOMM-Lee cells to assess survival and impact on tumor growth.

Results and Conclusions: All tested meningioma cells have TOP1 expression that is at least equivalent or exceeding an established TOP1 inhibitor-sensitive glioblastoma cell line (U251) although there was more variability in TOP2 expression. In vitro cell viability assays using SN38 demonstrated uniform efficacy against all tested meningioma cell lines, with IC50s estimated between 10-100 nM, a concentration range achievable using intravenous administration of PLX-038 in patients. Murine studies demonstrated uniform lethality in control animals by 18 days whereas treatment with single-dose and weekly PLX-038 showed significantly improved median survival. These data suggest that TOP1 inhibition is effective in meningiomas and supports the association of TOP1 and SLFN11 as potential predictive markers of response. These findings support testing PLX-038 in recurrent high-grade meningioma. A prospective trial is planned that will include pretreatment measurement of TOP1 and SLFN11 to evaluate these as predictive markers of response.

Neuro-Oncology Branch, CCR, NCI, Developmental Therapeutics Branch, CCR, NCI

81. Evaluation of the effect of ALFQA adjuvant on immunogenicity and efficacy of the V1-deleted DNA/ALVAC/gp120 anti-HIV vaccine candidate

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Background and Hypotheses: Vaccination with the HIV clades B/A/E recombinant canarypox derived vector (ALVAC) and bivalent clade AE/B gp120-envelope proteins in alum adjuvant significantly reduced the risk of HIV acquisition in the RV144 HIV Phase III vaccine trial in Thailand. A similar vaccination strategy using clade C immunogens and MF59

adjuvant failed to reduce the risk of HIV acquisition in the HVTN702 HIV phase IIb/III vaccine trial in Africa. The non-human primate model of SIVmac251 infection recapitulated the results of the RV144 and predicted the failure of the HVTN702 trial. Both RV144 and pre-clinical studies in macaques identified antibodies to SIV/HIV variable region 2 (V2), CD4 cells, and Antibody Dependent Cell Cytotoxicity (ADCC) as correlates of risk of HIV/SIV acquisition, highlighting this animal models relevance to HIV in humans. In humans and animals, the choice of adjuvant used to formulate the immunogens can greatly impact immunogenicity and vaccine efficacy. The Army Liposome Formulation (ALF) containing QS21 saponin (Q) and adsorbed to aluminum hydroxide (A), also called ALFQA, has shown several advantages in preclinical studies. We hypothesize that substituting the alum with the ALFQA adjuvant to formulate the protein boost of the V1 DNA/ALVAC/gp120 anti-HIV vaccine candidate could favor the induction of protective immune responses and increase vaccine efficacy.

Study Design and Methods: To test this hypothesis, we vaccinated twelve female macaques with our most successful vaccination regimen that provided significant reduction of risk of SIVmac251 acquisition in multiple prior studies. Animals were primed twice with V1-DNA-SIV (weeks 0 and 4), then boosted once with ALVAC-SIV alone (weeks 8), and once with ALVAC-SIV and V1-SIV-gp120 protein (week 12). The protein boost was administered in ALFQA adjuvant [200Âµg 3D-PHADÂ® (ALF), 100Âµg QS-21 (Q) and 850ï -g Al3+ Rehydragel (A)]. Following vaccination, animals were exposed to intravaginal SIVmac251 exposures to assess the vaccine efficacy (VE). Samples collected prior to and following vaccination were analyzed with different canonical (ADCC, flowcytometry and CD14+ efferocytosis of apoptotic neutrophils) and proteomic (Olink) assays. The magnitude of the vaccine-induced immune responses were compared to those obtained in macaques vaccinated with the vaccine regimen using the Alum adjuvant.

Results and Conclusions: The study showed that both regimens, using alum or ALFQA, are efficacious in delaying the onset of SIVmac251. The vaccine efficacy was 65% and 79% in the alum and ALFQA regimens, respectively. Additionally, when compared to ï „V1 DNA/ALVAC/gp120/Alum, the ALFQA regimen induced higher levels of immune responses that correlated with decreased risk of infection in prior studies. Among these responses, the ALFQA increased the antibody response to variable region 2 (V2) of gp120 and efferocytosis. Concomitantly, the use of ALFQA decreased detrimental immune responses associated with an increased risk of virus acquisition, such as mucosal NKG2A-NKp44- Natural Killer cells producing IFN-ï §. Additionally, the principal component analysis of the plasma proteome identified significant differences among vaccine regimens. Thus, the present study showed that adjuvants affect HIV vaccine efficacy, underlying a central role for empiricism in the development of effective HIV vaccine candidates.

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82. HPV-Automated Visual Evaluation (PAVE) Study: Validating a Novel Cervical Screening Strategy

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Background and Hypotheses: Every year, cervical cancer causes 600,000 incident cases and 340,000 deaths globally, mostly in resource-limited settings. Cervical cancer control acceleration will require widespread human papillomavirus (HPV) vaccination and effective screening. Visual inspection with acetic acid used for primary screening or triaging HPV-positives in low-resource settings is subjective and inaccurate, and cytology is impractical. Given its high negative predictive value and reproducibility, HPV-test-based screening is now recognized as the preferred screening test for cervical cancer. However, treating all HPV-positive women leads to substantial overtreatment because most infections self-clear; hence, secondary triaging is required to find the minority of women needing treatment for cervical precancers. We have developed a novel low-cost strategy, HPV-Automated Visual Evaluation (PAVE), to overcome these challenges. It involves 1) HPV screening of self-sampled vaginal specimens; 2) triage of HPV-positive participants with a combination of extended genotyping (n.b., different HPV types have different risks of precancer) and visual evaluation of the cervix assisted by deep-learning-based automated visual evaluation (AVE); and 3) risk stratification permitting immediate treatment of highest risk women with thermoablation or referral for excision. The strategy is undergoing validation and cost-effectiveness analysis in an international, multi-centric study.

Study Design and Methods: Around 50,000 nonpregnant women aged 25-49 years, without prior hysterectomy, and irrespective of HIV status, are being screened at ten sites in low-resource countries. Eligible and consenting participants self-collect vaginal specimens using a FLOQSwab (Copan). Swabs are transported dry for HPV testing using a newly redesigned isothermal DNA amplification test (ScreenFire HPV RS), which provides HPV genotyping by hierarchical risk groups: HPV16, else HPV18/45, else HPV31/33/35/52/58, else HPV39/51/56/59/68. HPV-negative individuals are considered negative for precancer/cancer and do not undergo further testing. HPV-positive individuals undergo a pelvic examination with a collection of cervical images and targeted biopsies of all acetowhite areas or endocervical sampling in the absence of visible lesions. Cervical images refine a deep-learning AVE algorithm that classifies images as normal, indeterminate, or precancer+. The combination of HPV genotype and AVE generates a risk score corresponding to the precancer risk (lower, medium, high, highest).

(Preliminary) Results and Conclusions: As of February 2024, ~17,000 women have been screened. Preliminary analysis is ongoing on 13,435 women recruited by November 2023. The overall HPV positivity rate is 24.6% (3,301) but varies from 16% in Cambodia to 50% in women living with HIV in the Dominican Republic. 1,779 (53.9%) of the HPV-positive women have undergone triage. HPV16 (14.3% of HPV-positives) has the highest risk (12.8%) of precancer+. HPV18/45 and HPV31/33/35/52/58 have a medium risk (3%) of precancer+.

HPV39/51/56/59/68 (35.4% of HPV-positives) has the lowest risk (1.4%) of the precancer+. Histology review, retraining of the AVE algorithm, and validation against a histologic endpoint to assess additional risk stratification to HPV genotyping are underway. This year, we aim to transition to an effectiveness phase. Then, AVE will run in real-time on the point-of-care image capture device. This and the micro-costing of the project components will permit the examination of the PAVE strategy's cost-effectiveness, acceptability, and feasibility in clinical practice.

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83. Oral C. albicans infection promotes upper GI tract tumor progression via neutrophil dysfunction and EGFR activation

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The mycobiome has emerged as an important player in a wide range of human cancers. However, comprehensive investigations interrogating the link between mycobiome and cancer remains very few and how mycobiome leads to cancer still remains largely unknown. In this study, we investigated the role of oral C. albicans infection in squamous cell carcinoma (SCC) development in upper GI tract of APECED-like IKKalpha kinase-dead (KA/KA) mice. We found that oral infection of C. albicans promotes SCC development in the upper GI tract of KA/KA mice. Although lack of Il17a increased C. albicans colonization in KA/KA mice, Il17a is dispensable for SCC development in upper GI tract of C. albicans-infected KA/KA mice. Instead, we found EGFR pathway plays an important role in C. albicans infection-mediated tumorigenesis in KA/KA mice, as evidenced by significantly lower SCC incidence in the upper GI tract of KA/KA mice infected with either Als3-deficient C. albicans or Ece1-deficient C. albicans, each of which was not able to activate EGFR in the epithelial layer of upper GI tract of KA/KA mice. Meanwhile, EGFR inhibition dramatically reduced WT C. albicans colonization in the stomach of KA/KA mice. Interestingly, we found that aberrant infiltrations of neutrophils and macrophages with inflammation signatures were present in the upper GI tract SCCs in KA/KA mice. Infiltrated KA/KA neutrophils expressed significantly higher CXCL2 level in C. albicans-infected KA/KA mice compared to that in C. albicans-infected WT mice. On the other hand, KA/KA neutrophils exhibited inability for C. albicans killing due to functional defects in ROS production and NETs formation, leading to C. albicans overgrowth in KA/KA mice. CXCL2 neutralization or neutrophil depletion significantly reduced esophageal SCC incidence in C. albicans-infected KA/KA mice. Taken

together, our data suggest that KA/KA neutrophil dysfunction orchestrates with C. albicans overgrowth and EGFR activation, ultimately leading to tumor progression in KA/KA mice.

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84. Characterization and Prognostic Significance of Tertiary Lymphoid Structures in the Canine Osteosarcoma Model

Jessica Beck[1], Sushant Patkar[2], Christina Mazcko[1], and Amy LeBlanc[1]

Background and Hypotheses: Osteosarcoma is a rare bone tumor of children and adolescents for which significant advances in care have not occurred in over 40 years. Although metastatic progression remains the most significant limiting factor for survival, a strong immune response has been associated with improved outcomes. Lymphoid activity against tumor antigens can be bolstered by the development of tertiary lymphoid structures (TLS). TLS are organized lymphoid aggregates that form in nonlymphoid tissues. They develop postnatally within regions of chronic inflammation including cancer. The development of TLS is prognostic and predictive of immunotherapy response in multiple cancer types. Pet dogs develop osteosarcoma at a higher frequency than human patients and within the context of an intact, educated immune system making them a promising candidate for immunotherapy trials. As such, under the NCI's Decoding the Osteosarcoma Genome of the Dog (DOG2) initiative, TLS are being examined in outcome-linked tissue samples collected through the efforts of the Comparative Oncology Trials Consortium (COTC). By evaluating clinical trial biospecimens, this study identifies TLS in canine osteosarcoma patient samples and correlates the presence of TLS with immune enrichment of primary osteosarcoma tissues and improved prognosis.

Study Design and Methods: Through the COTC, 324 pet dogs were enrolled in an NCI led, multi-institution, osteosarcoma clinical trial. Following diagnosis of osteosarcoma, enrolled canine patients underwent surgical amputation at which point treatment naive primary osteosarcoma samples, non-tumor tissue, and regional lymph node samples were collected. Formalin-fixed, paraffin-embedded osteosarcoma samples were evaluated histologically for the presence of lymphocyte aggregates by a board-certified veterinary pathologist. Immunohistochemical labeling of T cells (CD3), B cells (PAX5), macrophages (CD204), and high endothelial venules (MECA-79/PNAd) was completed to confirm the presence of organized TLS. Additional canine osteosarcoma samples were submitted for RNA extraction

and sequencing. After QA/QC, primary osteosarcoma samples from 186 dogs were analyzed. A TLS signature, derived from human patients, was applied to determine any association to disease-free interval (DFI), a clinical outcome measure assessed prospectively through serial physical examinations and radiography and defined as number of days from surgical amputation until the detection of metastases. Immune cell enrichment was assessed using bulk transcriptomic deconvolution and confirmed using immunohistochemical labeling of immune cells within tumor tissues.

Results and Conclusions: Canine osteosarcoma patients develop tertiary lymphoid structures similar to those described in human patients including aggregation of T cells and B cells around high endothelial venules expressing peripheral node addressin. Dogs with high expression of TLS genes have increased immune infiltration of their primary tumors and an improved prognosis highlighting a role for TLS in tumor immunity and response to treatment. Our ongoing work aims to use spatial biology techniques to interrogate gene expression in immune cells within the TLS, infiltrating the primary tumor, and within the matched regional lymph node. This study underscores the value of the dog as a preclinical model for osteosarcoma research.

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85. Interdisciplinary and interdivision collaboration for a bench to bed translation: genomic findings to a pilot clinical trial of a cell free DNA (cfDNA) platform for early detection and monitoring of deficient mismatch repair (dMMR) cancers

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Background and Hypotheses: Deficient mismatch repair (dMMR) cancers can be caused by either somatic or germline pathogenic or likely pathogenic (P/LP) variants in genes associated mismatch repair such as MLH1, MSH2, MSH6, PMS2 or EPCAM. Lynch syndrome (LS) is a hereditary cancer syndrome most frequently associated with dMMR colorectal cancer (CRC) and endometrial cancer (EC). dMMR status leads to cancers with innumerable genomic variations. The most characteristic genomic features of dMMR cancers include microsatellite instability (MSI-H) and frameshift mutations (FSM) at coding mononucleotide repeats (cMNRs) in driver and non-driver genes. These changes are thought to be critical in the development and progression of premalignant lesions to invasive cancer.
In LS, counseling and surveillance is important in attempts to decrease cancer-related mortality. However, the guidelines are often based on weaker evidences except for screening of CRCs. To satisfy this unmet need, a liquid biopsy platform that can aid or warn high risk individuals with LS is warranted. In instances of somatic dMMR cases, there is a prominent demand for measures capable of providing precise data regarding the status of cancer throughout the clinical course, thereby facilitating informed decision-making for both patients and their care teams. As somatic dMMR cancers share its genomic characteristics with those of LS-associated cancer, the same platform may be useful.

Study Design and Methods: An FSM-based gene panel of 122 targets was developed and characterized by using targeted next generation sequencing (NGS) using cfDNA from patients with dMMR cancer and healthy individuals with LS. For controls, healthy donor was used. Preliminary results support high sensitivity and specificity for this biomarker panel. As part of an interdivision collaboration, a pilot clinical trial is under development. The goal is to help validate the platform for early detection and monitoring of dMMR cancer. The primary endpoint of the study is the feasibility of the platform, focused on biospecimen logistics and sensitivity/specificity. Additionally, association between findings of the biomarker and clinical events e.g. (pre)malignant lesions will be explored. The study will include a total of approximately 120 healthy volunteers with no history or active cancer, healthy LS carriers, and individuals with history of or active somatic or germline dMMR cancer. They will be followed at the NIH Clinical Center with each cohort's risk-based surveillance in cooperation with the participants primary doctors. Study participants will take NIH ASA24 Dietary Questionnaire. Biospecimen will be curated for batch testing. Plasma will be tested using the cfDNA gene panel. Stool will be assessed for microbiome and fecal DNA. The goal is to explore a further developmental potential of the FSM-based gene panel as a validated guide in clinical decision making.

Results and Conclusions: Ongoing effort to develop a clinical trial in the CCR aimed at translating the biomarker platform developed by NCI DCP is an example of a successful interdisciplinary and interdivisional collaboration. Such a biomarker platform could be extremely valuable in guiding informed decision making and best practices in both preventive and treatment settings.

TECHNOLOGIES AND METHODOLOGIES DEVELOPMENT

86. Rapid Processing of High-Throughput Thermal Shift Assay Data

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Background and Hypotheses: Thermal Shift Assays (TSA), also known as Differential Scanning Fluorimetry (DSF), have been used to determine the melting temperature of a protein. The protein is incubated with a fluorescent dye, which binds to the hydrophobic regions as they are exposed during the denaturation process. The level of fluorescence is monitored over a temperature range and the inflection point of the resultant curve is used to estimate the melting temperature of the protein. Addition of a pure compound or ligand to the mix, may influence the melting temperature of a protein by either stabilizing or destabilizing the native structure, resulting in the characteristic shift of the melting temperature. TSA can therefore be used as an initial exploratory assay to detect potentially novel protein-compound interactions. When implementing this method in a 384-well plate setup, each plate generates around 100,000 data points. A single run can involve hundreds of plates, resulting in tens of millions of data points. Analyzing this substantial volume of data becomes even more challenging when using natural product extracts, instead of pure compounds, as the data tends to be not only noisier but often deviates from the expected sigmoidal distribution. Finally, this process needs to be fast and presented in a user-friendly graphical interface so that users can rapidly and confidently identify compound hits from the data.

Study Design and Methods: To achieve rapid analysis while maintaining user friendliness, a Python script was deployed behind a SpotfireTM interface with the following steps: Raw fluorescence data were acquired, normalized, smoothed, and associated metadata from an in-house Oracle database were assigned. Due to confounding factors (e.g. high initial fluorescence), a model cannot be fitted directly to the smoothed data. Instead, the data for each well was cleaned by extracting only the sigmoidal regions using local minima and maxima as a guide. In the case of multiple inflections, the curve was sliced to generate two or more clean sigmoidal regions per well. Each sigmoidal region was then fitted against a Boltzmann sigmoid model and the melting temperature(s) were calculated. Curves stemming from control wells were assessed first and any wells that exhibited a bad model fit or were outliers in terms of average amplitude or inflection point were removed from the dataset. Experimental curves underwent a similar form of quality control and were then assessed relative to the control curves (per plate) to determine the magnitude of thermal shift. All results were used to automatically generate interactive graphs in a SpotfireTM interface.

Results and Conclusions: The described pipeline has been successfully used in the screening of 461 plates, a total of approximately 45 million data points. When using the multiprocessing approach with 6 CPUs on a standard MacBook pro, 10 wells were processed per second which means that 100 plates (384-well) can be analyzed in a little over an hour. Identified hits have been progressed to further testing.

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87. Tumor Suppression by Anti-Fibroblast Activation Protein Near-Infrared Photoimmunotherapy Targeting Cancer-Associated Fibroblasts

Noriko Sato[1], Raisa Glabman[1,2], Colleen Olkowski[1], Hannah Minor[1], Laura Bassel[3], Noemi Kedei[4], and Peter Choyke[1]

Background and Hypotheses: Cancer-associated fibroblasts (CAFs) constitute a prominent cellular component of the tumor stroma, with various pro-tumorigenic roles. Numerous attempts to target fibroblast activation protein (FAP), a highly expressed marker in immunosuppressive CAFs, have failed to demonstrate anti-tumor efficacy in human clinical trials. We hypothesized that CAF depletion using near-infrared photoimmunotherapy (NIR-PIT), a novel and highly selective tumor therapy that utilizes an antibody-photo-absorber conjugate activated by near-infrared light, would effectively deplete CAFs and suppress the tumor growth in murine models of cancers.

Study Design and Methods: Using CAF-rich syngeneic lung tumors (LL/2) and spontaneous mammary tumors (mouse mammary tumor virus-polyomavirus middle T antigen genetically engineered mouse model [MMTV-PyMT GEMM]), NIR-PIT against FAP or podoplanin was performed in mice. Effect of the therapy to deplete CAFs, as well as tumor growth and activation of tumor-infiltrating lymphocytes, were examined. Further, to examine the effect of depletion of specific type of FAP+ cells, bone marrow chimeras were generated using combinations of wild type mice and FAP-TK transgenic mice, in which FAP+ cells expressed thymidine kinase and could be depleted by injection of ganciclovir.

Results and Conclusions: Anti-FAP NIR-PIT depleted FAP+ CAFs, as well as FAP+ myeloid cells, and successfully suppressed tumor growth. However, anti-podoplanin NIR-PIT was ineffective. Interferon-gamma production by tumor-infiltrating CD8 T cells and natural killer cells was induced within hours after anti-FAP NIR-PIT. Additionally, lung metastases were reduced in the treated spontaneous mammary cancer model. Depletion of FAP+ stromal cells alone, as well as depletion of FAP+ myeloid cells alone, effectively suppressed tumor growth in bone marrow chimeras generated using FAP-TK mice. These results suggest that the depletion of FAP+ CAFs and FAP+ myeloid cells in one treatment, which can be achieved by anti-FAP-PIT, is a unique and effective therapeutic approach. These findings highlight a promising therapy for selectively eliminating immunosuppressive FAP+ cells within the tumor microenvironment.

1. Molecular Imaging Branch, CCR, NCI; 2. Michigan State University, East Lansing; 3. Frederick National Laboratory for Cancer Research, NCI; 4. Collaborative Protein Technology Resources, CCR, NCI

88. Raman-based machine learning platform reveals unique metabolic differences between IDHmut and IDHwt glioma

Adrian Lita[1], Joel Sjandberg[2], David Päfcioianu[3], Nicoleta Siminea[3,4], Orieta Celiku[1], Tyrone Dowdy[1], Andrei Päfun3[,4], Mark R. Gilbert[1], Houtan Noushmehr[5], Ion Petre[2,4], and Mioara Larion[1]

Background and Hypotheses: Raman spectroscopy is routinely used in pathology however, it is currently used for fresh or frozen tissue and not in (formalin-fixed, paraffin-embedded) FFPE tissue due to the high background coming from the embedding media. Spontaneous Raman spectroscopy is a vibrational technique that can capture the molecular fingerprints of tissue in situ without the need for sample preparation. By relying on endogenous chemical signatures, spontaneous Raman spectroscopy can determine the quantitative composition in situ at a spatial resolution of less than 500 nm. We hypothesized that spontaneous Raman spectroscopy could be used in FFPE tissue slides and the background could be removed in the downstream processing steps. Our second hypothesis was that Raman spectroscopy together with machine learning can classify tumors based on their subtype and that unique biological information could be extracted this way. Herein, we developed APOLLO (rAman-based PathOlogy of malignant gliOma), a Raman-based machine learning platform for glioma classification.

Study Design and Methods: FFPE samples from 46 patients whose tumors had been profiled and classified into different methylation subtypes according to the Cecarelli et. al., 2016 classification6 were provided by the Hermelin Brain Tumor Center (Detroit) and are part of The Cancer Genome Atlas Program (TCGA). For each sample, we used parallel sections to confirm the presence of tumor cells using immunohistochemistry. Unstained FFPE tissue slides were then used to record the Raman spectra from areas of approximately 300 x 300 Î¼m2 that contained tumors. Between 2116 to 14945 spectra were recorded for each of the 59 selected regions, with 5 scans, each averaging a 2-second accumulation time, resulting in 300 506 Raman spectra. These regions were then exported as pseudo-3D matrices Xâ‹…Yâ‹…1738 (with (X,Y) ranging from a minimum of (46,46) to maximum of (245,61). Each spot of the X â‹… Y tumor slice was characterized through a numerical vector fingerprint of size 1738, encoding the Raman wavelengths 50âE'3399 cmâE'1. The data were then subjected to pre-processing steps followed by machine learning methods.

Results and Conclusions: APOLLO accurately discriminates tumor versus nontumor regions of the tissue and achieves high receiver operating characteristic curve or area under the precision-recall curve (ROC/AUPR) values when discriminating between IDH1mut and IDHWT, and within IDHmut subtypes, G-CIMP-high versus G-CIMP-low. APOLLO discovered novel frequencies corresponding to DNA and protein levels which are higher in the tumor when compared with paraffin, which were not considered before in the stimulated Raman histology methods. Frequencies that discriminate IDH1mut from IDHWT gliomas are enriched for metabolites from the cholesterol pathway, pointing to the distinct biological differences between the two glioma types, unrecognized before. Overall, these results showcase the potential of APOLLO to predict the methylation subtype of gliomas and to discover novel biology in the most common form of tissue

existent in biobanks. While we demonstrated the feasibility of this approach in glioma FFPE tissues, our workflow is general and can be applied to any other cancer type thus opening the possibility of using these archived tissues in the future without the need for deparaffinization.

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89. The CCR/LGI Flow Cytometry Core

Ferenc Livák

The CCR/LGI Flow Cytometry Core in BG37 is the largest flow cytometry service provider at NCI. Our mission is to offer up-to-date instrumentation and technical expertise to CCR investigators to assist cancer research. The Core provides full-scale, state-of-the-art flow cytometry services including analytical sample acquisition, data analysis, fluorescent cell sorting and experimental planning and consultation and training for users on analytical flow cytometry instruments.

NCI CCR Flow Cytometry Core Laboratory

90. Characterization of Cancer Cell Line Models using SKY and miFISH

Kerstin Heselmeyer-Haddad[1], Danny Wangsa[1], Arwa Fallatah[1], Subhra Dash[2], Dara Wangsa[1], Abdelrahman Rahmy[1], David Milewski[1], Young Song[1], Yong Kim[1], Hsien-Chao Chou[1], Jun Wei[1], Robert Hawley[1], Marielle Yohe[2], Anna Roschke[1], Paul Meltzer[1], John Glod[3], Javed Khan[1]

Background: Cancer cell lines are frequently used to model drug resistance. Detailed characterization, including bulk whole genome/exome sequencing (WGS/WES) and whole transcriptome sequencing (WTS), are standard methods to investigate mechanisms of resistance. They provide mutation and transcriptome data but are average representations. Therefore, single-cell methods are crucial for a comprehensive analysis, including chromosomal instability, tumor evolution, and heterogeneity. Spectral karyotyping (SKY) and multiplex interphase Fluorescence in situ Hybridization (miFISH) are sophisticated cytogenetic single-cell techniques. SKY allows for simultaneous visualization of all chromosomes in different colors enabling the investigation of chromosomal abnormalities. miFISH allows for simultaneous assessment of copy numbers within thousands of cells for up to 35 loci tailored to specific research questions. We offer both methods within the Genetics Branch (GB) OMICS Technology facility.

Study Design and Methods: Study 1: The Medullary Thyroid Cancer (MTC) cell line TT is driven by RET (p.Cys634Trp) and other mutations and copy number alterations. Vandetanib is an FDA approved, RET and multi-kinase inhibitor used for MTC, but patients frequently develop therapy resistance. The ATCC's TT cell line contained two subclones (diploid and tetraploid), from which parental and vandetanib-resistant lines were derived and subjected to WGS, WES, WTS, SKY, and miFISH to determine the mechanism of resistance. Study 2: A MEK inhibitor resistant neuroblastoma cell line is currently karyotyped using SKY. Tailored miFISH panels investigate copy numbers for all RAS, MYCN and other genes.

Results and Conclusions: The TT cell line exhibited a major diploid clone harboring a duplication of the RET gene on one allele resulting in three RET copies per cell. The RET mutation variant allele frequency (VAF) was 0.659, suggestive of the duplicated allele harboring the mutation. The minor tetraploid clone showed exact duplication of the RET alleles resulting in four mutated and two wild type RET copies. There was an additional RET copy on the duplicated allele for the parental diploid cell line resulting in four RET copies, which was maintained in the resistant diploid clone. VAF analysis indicated that the additional copy contained the parental p.Cys634Trp mutation. Of note, the resistant diploid clone developed a new RET mutation (p.Gly810Ser) in one of the already mutated copies. Surprisingly, the resistant tetraploid cell line showed a chromosome 10 loss. Nonetheless, RET copy numbers remained at six with three chromosomes 10 exhibiting two copies of RET each. VAF for the RET mutation in the parental tetraploid line indicated mutation of four RET copies, while in the resistant line all six RET copies were mutated, suggesting loss of the two parental wild type alleles and duplication of the allele with two mutated RET copies. Our results indicate that MTC cell lines increase mutant copy numbers and/or develop additional mutations as mechanisms of resistance to RET inhibitors.Characterization of the neuroblastoma cell line revealed a hypotetraploid karyotype with several translocations. miFISH pinpointed MYCN in a t (2;5) translocation, resulting in a gain of MYCN. In summary, SKY and miFISH analyses provide important karyotype and copy number information, contributing to a better understanding of resistance mechanisms.

1. Genetics Branch, Center for Cancer Research, NCI, NIH, Bethesda, MD; 2. Laboratory of Cell and Developmental Signaling, National Cancer Institute, NIH, Frederick, MD; 3. Pediatric Oncology Branch, CCR, NCI, NIH, Bethesda, MD

Core Abstracts

NCI/CCR/LRBGE Optical Microscopy Core
D. A. Ball, M. Fazel, T. S. Karpova

NCI/CCR/LRBGE Optical Microscopy Core (OMC) is open to everyone within the NCI, and we also welcome collaborations with investigators from other NIH institutes or beyond. We operate state-of-the-art fluorescence microscopes, confocal, wide-field, and super and nano-resolution, and custom-built HILO microscopes, that enable a broad spectrum of fluorescence imaging experiments. We provide expert guidance and troubleshooting in the design, execution, interpretation, and publication of these experiments.

Fluorescence microscopy permits *in situ* imaging of proteins, lipids, DNA and RNA in tissues, cells, and intracellular organelles. The latest technological advances allow imaging of individual molecules. Computerized image analysis is applied to single-molecule biophysics and to building of molecular maps of intracellular organelles. Modern fluorescence markers include fluorescent antibodies, fluorochrome-labeled oligonucleotides, GFP and other fluorescent protein fusions, such as fusions to ligand-bound Halo Tag, Snap Tag, CLIP tag etc., and fluorescent stains for lipids, DNA and RNA. Specific markers are suitable for live cells and for biophysical quantification of diffusion, of binding rates, and of protein dimerization by FRAP, FCS, N&B. Biosensors allow observation of protein interactions and enzymatic activity dynamics by FRET. Resolution in X, Y, and Z may be improved by deconvolution and by super resolution: SIM (Structural Illumination Microscopy), PALM (Photoactivation Localization Microscopy) or STED (Stimulated Emission Depletion Microscopy). The newcomer, MINFLUX, provides nano-resolution, bringing the resolution up to the level of electron microscopy. Image quality may be enhanced by AI.

These exciting technologies are available to NCI and NIH from our Optical Microscopy Core which specializes in single molecule biophysics and nano-resolution, but also provides conventional microscopy imaging. We offer the conventional methods of fluorescence microscopy, including time lapse imaging of cells in 2D or 3D, with computational methods for removal of out of focus light (deconvolution), as well as sophisticated quantitative microscopy: FRAP, FRET, superresolution (SIM and PALM), nano-resolution (MINFLUX), and single molecule tracking (SMT). The staff participates in the development of quantification microscopy technologies.

First-time users should contact Dr. Tatiana Karpova (karpovat@mail.nih.gov, 240-760-6637) to discuss the proposed work.

Optical Microscopy and Related Technologies at NCI-Frederick
Stephen Lockett, Valentin Magidson, David Scheiblin, Favour Nwagugo, Rebecca Moffat, Giana Vitale, William Heinz

The Optical Microscopy and Analysis Laboratory (OMAL) provides the Center for Cancer Research (CCR) with cutting-edge fluorescence microscopy (confocal and super-resolution) and atomic force microscopy for answering cancer and HIV biology research questions. Microscope capabilities are wide-field fluorescence, live cell 3D (confocal), super-resolution of live and fixed samples, plate reading microscope for high throughput, and atomic force microscope. Sample preparation expertise is provided in the areas of live cell preparation and handling, unique restricted exchange environment chamber (REEC) for studying oxygen and nutrient gradients on live cells, multiplex

antibody labeling, and tissue clearing and expansion. The following image analysis capabilities are offered: 3D / 4D visualization, deconvolution, 2D / 3D cell segmentation, cell and molecule tracking, co-localization analysis and high content image analysis. We collaborate with several CCR labs to understand the role of nitric oxide synthase 2 (NOS2) in cancer progression and metastasis and the benefits of NOS2 inhibitors; the role of oxygen and chemical gradients in solid tumor progression, and the development of multiplex immunofluorescence assay for thick tissue sections for understanding the CD8 T cell activity, epithelial-to-mesenchymal transition, and cancer stem cellness. Further information is at: https://confocal.ccr.cancer.gov/nci-microscopy-core-labs/frederick/omal/

Optical Microscopy and Analysis Laboratory

Biophysics Resource (BR) in the Center for Structural Biology
S.G. Tarasov

The Biophysics Resource (BR) in the Center for Structural Biology operates as an open, shared-use facility; in general, BR users learn to operate the instruments and conduct their own experiments. BR staff members train all first-time users and are also available to consult on experimental design/analysis or collaborate with them on more complex studies. The BR offers cutting-edge biophysics technology in the following areas:

- Circular dichroism (CD) spectroscopy to study the optical activity and conformation of biomacromolecules.
- Steady-state and time-resolved fluorescence spectroscopy to study the structure and environment of biomacromolecules and the mechanism(s) of these interactions.
- Isothermal titration calorimetry (ITC) for thermodynamic characterization of biomacromolecular interactions.
- Differential scanning fluorimetry (nano-DSF) for determining thermal stability and transitions in biomacromolecular systems.
- Liquid chromatography with mass spectrometry detection (LC-MS and LC-MS-MS) for macromolecular mass characterization.
- Dynamic light scattering to determine macromolecular particles size and degree of aggregation.
- Microscale thermophoresis (MST) for macromolecular binding studies.
- UV-Vis spectrophotometry with thermal scanning option.
- switchSENSE molecular dynamics technology for binding, kinetic and sizing studies.
- Mass photometry for mass measurement of single molecules in native state, in solution and without labeling.

Center for Structural Biology, CCR, NCI Frederick

Microscopy and Digital Imaging in the CCR Microscopy Core
Michael Kruhlak, Ph.D., Langston Lim, M.Sc. and Andy Tran, Ph.D.

The CCR Microscopy Core provides NCI investigators access to state-of-the-art imaging tools and techniques, including high-resolution confocal, multi-photon, and super-resolution microscopy. The primary mission of the Core is to support the microscopy and digital imaging needs of investigators studying the biological structures and cellular processes involved in the cell biology of cancer. This involves developing specialized microscopy-based assays and imaging techniques needed by Core users, such as photoswitching of specialized fluorescent proteins to monitor the dynamics of sub-cellular structural components by live cell super- resolution microscopy. Confocal microscopy is also valuable for imaging fluorescently labeled specimens and permitting accurate optical sectioning for volumetric studies, such as large extended field of view tile imaging of tumor samples. Techniques available in the Core include:
1) high resolution confocal microscopy, including live cell imaging methods of FRAP, FRET, FLIM, and photoactivation, 2) multi-photon imaging, including second harmonic generation, 3) super-resolution imaging by either structured illumination microscopy (SIM) or Airyscan detection, and 4) super-resolution imaging by Stimulated Emission Depletion (STED) imaging. Instruments available include a Leica Stellaris STED super-resolution microscope, a Nikon SoRa super-resolution spinning disk microscope, a Zeiss LSM 880 Airyscan super-resolution and confocal microscope, a Zeiss ELYRA SIM super-resolution microscope that provides images with twice the lateral resolution of standard confocal imaging, and a Leica Stellaris 8 FLIM confocal microscope with HyD spectral detectors and FALCON FLIM imaging. Advanced image processing and analysis workstations are also available with Zeiss Zen, Nikon Elements, Leica LAS X, Imaris volume reconstruction software, including object-in-object analysis using ImarisCell, and Arivis volume reconstruction software with virtual reality augmentation of multi-dimensional image datasets. Numerous AI-based image analysis modules are also available, such as machine learning and convolution neural network (CNN) based algorithms. The instrumentation and services of the CCR Microscopy Core are open and accessible to all NCI and NIH researchers.

Laboratory of Cancer Biology and Genetics, CCR, NCI

Proteomic and Small Molecule Analysis within the Mass Spectrometry Resource
Lisa M. Jenkins, Connor Jewell, Tapan Maity, Weiming Yang

The CCR Mass Spectrometry Resource aims to bring cutting-edge mass spectrometry methodologies to the NCI community to facilitate basic and translational research. Although mass spectrometry is a powerful technique, experimental design and sample preparation are critical for a successful outcome. Our experience in mass spectrometry has been successfully combined with the biological and biochemical expertise of our collaborators to identify proteins in complexes or organelles and perform quantitative proteomic analyses. Determination of the composition of protein complexes and organelles is critical for understanding their biological functions. Additionally, determination of the relative quantitation of protein levels following treatment, after protein knock-down, or in different

disease states add critical functional information.

Mass spectrometry is also one of the leading methods for determining sites and types of modifications on proteins. These modifications are central to regulation of signaling pathways and protein function. Proteomics approaches can further be used to study conformational changes in proteins upon complex formation and sites of complex interfaces. In addition to protein-based methodologies, we also can perform targeted small molecule and protein quantitation and quantitation of metals in biological samples. By making available our expertise in mass spectrometry, we seek to help researchers with their proteomics experiments, beginning with initial experimental design and sample preparation and continuing through data interpretation.

Laboratory of Cell Biology, CCR, NCI

Empowering your research with services from the CCR Genomics Core

Steve Shema, Qin Wei, Desiree Tillo, Madeline Wong, and Elizabeth A. Conner

CCR Genomics Core under the Office of Science Technology Resource (OSTR) at the National Cancer Institute provides investigators with access to genomic technologies and Next-Generation Sequencing (NGS) with rapid turnaround on smaller-scale projects or projects that are not ready for production. The Core has an open-door policy operating more like a "boutique" and is unique in granting user-accessible instrumentation. Additional resources include training, consultation services, bioinformatics support, and secure data delivery/management. The Core supports 7 different platforms with 17 instruments supporting most of the CCR laboratories at the NIH Bethesda campus and multiple NIH institutes. Core instrumentation and services include [Sanger Sequencing (2 ABI 3500xL Genetic Analyzers), Illumina Next-Generation Sequencing (1-iSeq, 1-MiSeq, 4- NextSeq2000), Nanopore Sequencing (Oxford Nanopore Mk1C, PromethION 2 Solo, GridION), Digital Gene Expression (Nanostring nCounter Analysis System), Digital Droplet PCR (Bio-Rad QX200 Droplet Digital PCR System), DNA/RNA & Library QC (Agilent TapeStation 4150 & 4200, Fragment Analyzer, QuantStudio RT-PCR system, Pippin HT) and Digital Spatial Profiling (NanoString GeoMx DSP). The DSP combines spatial and molecular profiling technologies by generating digital whole transcriptomes and profiling data. This technology is offered in collaboration with the Spatial Imaging Technology Resource (SpITR). In addition, the CCR Genomics Core functions as a support lab, containing instruments for various applications for use by CCR investigators. Analytical software for the various technologies is also made available. All these services function through a charge-back system to ensure that costs are covered and that the services are available as demand grows. This core has proven to be a valuable resource to NCI and NIH investigators with broad usage leading to high impact journals and becoming an integral part of the research community. To learn more about our services visit us in Bldg. 41/Room D310 or our website at https://ostr.ccr.cancer.gov/resources/ccr-genomics-core/

CCR Genomics Core, Office of Science Technology Resource, Bethesda, Maryland

CCR dedicated mass spectrometry core, focusing on protein, proteomics, and metabolite
Ronald Holewinski, Sudipto Das, Maura O'Neill, Gerard Duncan, Josie Schultz, Kiall Suazo, King Chan, Xia Xu, Thorkell Andresson

The Mass Spectrometry Center (Protein Characterization Laboratory – PCL), located at the ATRF in Frederick, MD, is a dedicated NCI-CCR protein and metabolite characterization facility, focused on analyzing a diverse range of molecules spanning single metabolite(s)/protein(s) to entire metabolomes/proteomes. The laboratory is equipped with cutting-edge mass spectrometers for both proteomic and metabolomic analysis. In addition, we have expertise in cell culture, a variety of affinity purifications, chromatographic separation of different biological molecules as well as assay development. We engage in both short and long-term projects, all depending on the needs of individual PIs.

Protein Characterization Laboratory, Cancer Research Technology Program, Frederick National Laboratory for Cancer Research, Frederick, MD

Advanced Biomedical Computational Science
Uma Mudunuri

The Advanced Biomedical Computational Science (ABCS) team supports scientific research at the Frederick National Laboratory for Cancer Research, NCI at Frederick, NCI in Bethesda, NIH, and other federal agencies. ABCS focuses on applications of bioinformatics, computational and data science, and artificial intelligence to problems in cancer, infectious disease, immunology, rare disease, HIV and other specialized areas in biomedical research. The ABCS team has domains of expertise within bioinformatics and next-generation sequencing (NGS), biomedical image analysis and visualization, computational chemistry, protein modeling, statistics and mathematical analysis, data mining and integration, scientific computing and high-performance computing (HPC), scientific web development, and scientific infrastructure. ABCS provides support through a variety of mechanisms ranging from provisioning of bioinformatics applications and databases to help with experimental design, data and results interpretation through consultation, collaboration and training, and technology development and enhancement.

Advanced Biomedical Computational Science, FNLCR, Frederick, MD

CCR Collaborative Bioinformatics Resource (CCBR)
Thomas Joshua Meyer[1], Vishal Koparde[1], Parthav Jailwala[1], and Margaret Cam[2]

The CCR Collaborative Bioinformatics Resource (CCBR) provides a broad range of bioinformatics support to CCR researchers in furthering their research goals. The group offers on-site collaborative bioinformatics services to researchers at both Bethesda and Frederick campuses. In addition to consultation on experimental design, CCBR analysts help with the downstream processing, analysis, and interpretation of the biological data produced by a wide variety of large-scale -omics technologies including NGS (exome- and whole-genome module called Pipeliner, which sets up a user's environment and provides a GUI interface for

automatic execution of these NGS pipelines on the Biowulf cluster. These pipelines are also constantly updated by CCBR to improve performance, ease-of-use and to include cutting-edge data analysis and visualization methods available. For interactive secondary analyses, CCBR has built user-driven workflows in NIDAP (the NIH Integrated Data Analysis Platform) that are available without cost to NCI Investigators. NIDAP is an innovative, cloud-based, collaborative data aggregation and analysis platform that hosts user-friendly bioinformatics workflows and visualization tools. These have been developed by the NCI developer community based on open-source tools and are made immediately available to biologist end-users across the Institutes.

ABCS, Frederick National Laboratory for Cancer Research; 2. OSTR, OD, CCR, NCI

Pathworks: A Suite of Biological Pathway Tools
Richard Finney and Margaret Cam

When analyzing the transcript expression results from an experiment, an important step is understanding the various interactions of the genes. There are many tools that provide methods to help make sense of it all, including commercial ones. Mostly, they are not as easy to run and take some degree of experience. To address the need of making this easier, we have developed Pathworks. This suite of tools includes programs for pathway overrepresentation analysis with List2Pathways (L2P), marking up pathway images with Wikimark, and denovo network generation with Pathact. These tools can be run on the command line, in the R programming language as an installable package and as a webpage using webassembly. All tools are implemented to be small and fast. The website is accessible inside the NIH firewall (VPN) at
http://ccbrweb.nci.nih.gov/helix/apps/richtools/ccbrwebtools.html

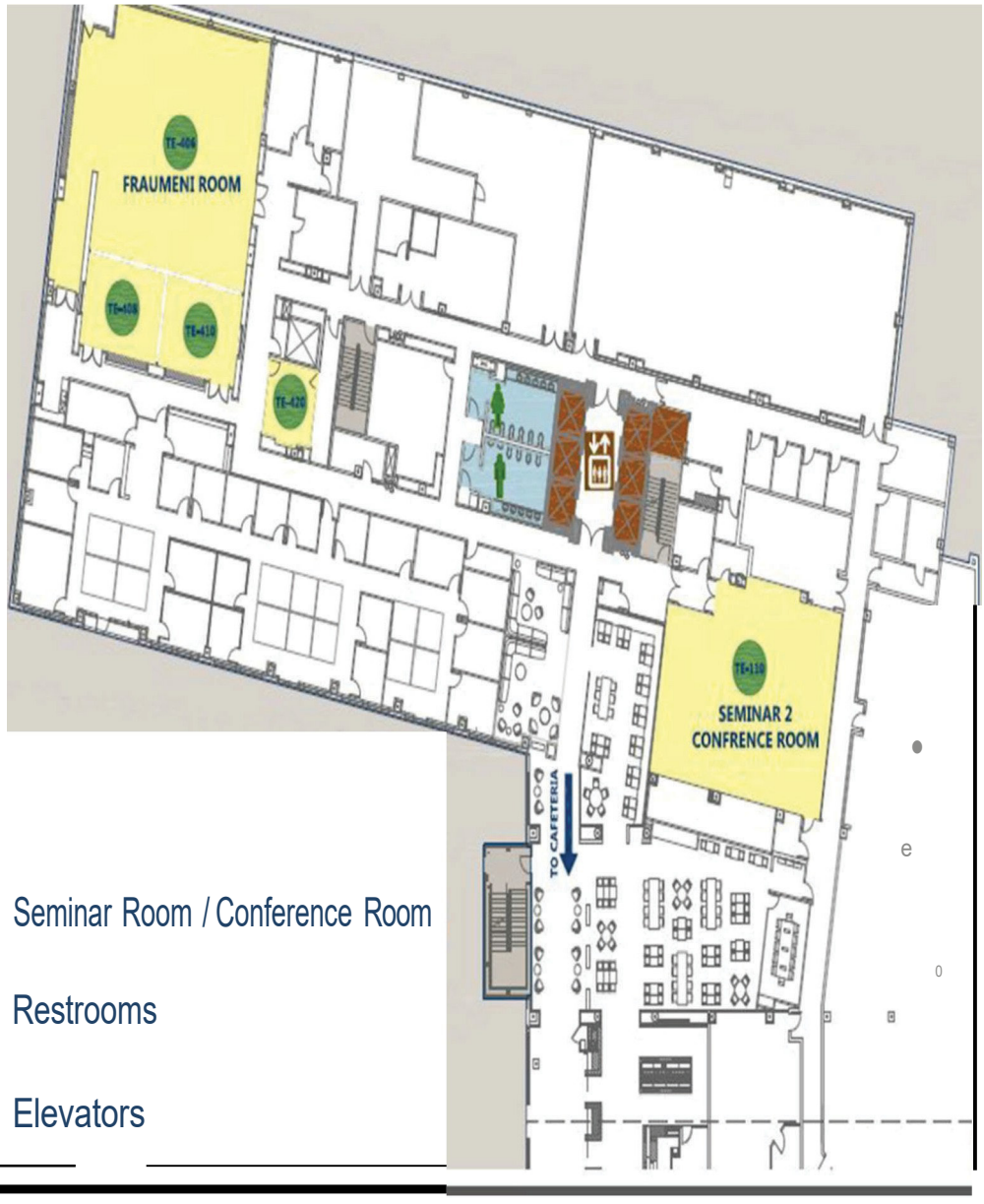
CCR Collaborative Bioinformatics Resource (CCBR), Office of the Director,
Center for Cancer Research, NCI, NIH

CCR Volume Electron Microscopy
Melissa Mikolaj, Adam Harned, Chris Dell, Abhishek Bhardwaj, Madeline Barry, and Kedar Narayan

CCR Volume Electron Microscopy (CVEM) aims to develop new techniques in volume electron microscopy (vEM) and, in collaboration with CCR Investigators, apply these techniques to research cell biological questions well-suited for study by high-resolution 3D electron microscopy. CVEM, headed by Kedar Narayan, uses focused ion beam scanning electron microscopy (FIB-SEM), array tomography (AT), electron tomography (ET) and other complementary methods to visualize ultrastructural features of cells and tissues, including cell membranes, cellular organelles, and virus particles. CVEM is a CCR-dedicated laboratory, but projects outside of CCR can be considered on a case-by-case basis. CVEM is operated by Leidos Biomedical Research Inc. on behalf of NCI as part of the Frederick National Laboratory.

CCR Volume Electron Microscopy, Center for Cancer Research, NCI, Bethesda, MD and
Cancer Research Technology Program, FNLCR, Frederick, MD

TERRACE EAST LEVEL



Seminar Room / Conference Room

Restrooms

Elevators

Food Options Inside the Shady Grove NCI Building:

First Floor

Cafeteria Services and Avanti Self-Service convenience market with entrees, hot and cold beverages, and snacks

Food Options Outside (directly across from) the Shady Grove NCI Building:

Passion Bakery Café

Deli sandwiches, pastries, snacks, coffee, hot/cold tea
6:00am – 7:00pm

Thai Cottage Bistro

Soups, salads, sandwiches, traditional Thai food
11:00am – 9:00pm

Subway

Sandwiches, Salads
8:00am – 8:00pm

20th Annual CCR and DCEG StaGG Scientist and StaGG Clinician Retreat

April 26, 2024

NIH NATIONAL CANCER INSTITUTE