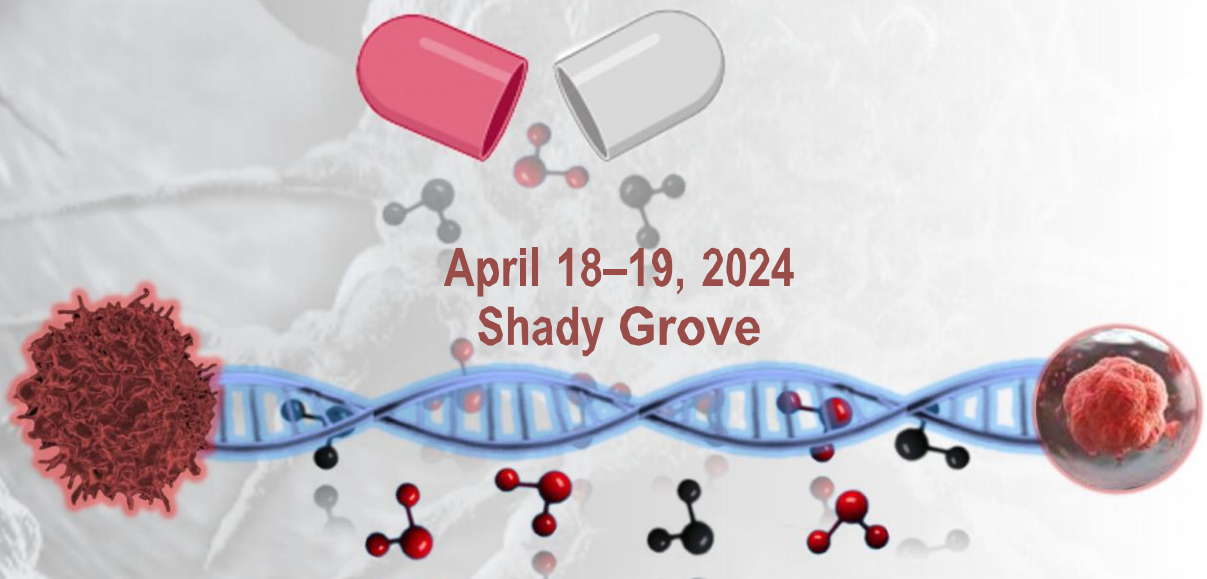
NIH NATIONAL CANCER INSTITUTE

24th

Annual CCR Fellows
& Young Investigators
COLLOQUIUM

BRIDGING THE GAP:
Integrating Basic Science and Biomedical Discoveries in Cancer Research



April 18–19, 2024
Shady Grove

Abstract Book

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

24th Annual

CCR Fellows and Young Investigators Colloquium

April 18-19, 2024

NCI Shady Grove
Rockville, MD

For the full program book including abstracts and speaker biographies, please visit the CCR-FYI Colloquium webpage https://events.cancer.gov/cct/fyi-colloquium

Welcome Letter

Welcome Colloquium participants!

On behalf of the NCI Center for Cancer Research Fellows and Young Investigators (CCR-FYI) Steering Committee and Colloquium Planning Subcommittee, we welcome you to the 24th Annual CCR-FYI Colloquium. The CCR-FYI strives to promote scientific, career, and personal success and growth among postdoctoral fellows, clinical fellows, postbaccalaureate fellows, and graduate students on NIH campuses. To achieve our mission of enabling CCR scientists to come together to share ideas, foster collaborations, gain knowledge, and to hone the skills needed to attain their career goals, the CCR-FYI organizes opportunities including the Annual Colloquium, the CCR-FYI Seminar Series, networking and outreach events, and the CCR-FYI Newsletter. We are kindly assisted by the NCI's Center for Cancer Research (CCR) Office of the Director and the Center for Cancer Training (CCT) Office of Training and Education, who work to enhance the intramural trainee experience. The CCR-FYI would like to thank Drs. Kimryn Rathmell, Douglas Lowy, Tom Misteli, Glenn Merlino, James Gulley, Oliver Bogler, Erika Ginsburg, Angela Jones, and Maria Moten for their continuing assistance.

The theme for this year's Colloquium, "Bridging the Gap: Integrating Basic Science and Biomedical Discoveries in Cancer Research", aims to highlight one of the NCI's many great strengths: the marriage of clinical and fundamental approaches to better understand and treat cancer. We hope to showcase how asking important fundamental questions can advance our understanding of oncogenesis, and how these findings can be leveraged towards the development of new therapies. By supporting and bringing together researchers from basic, translational, and clinical areas of expertise, the NCI promotes amazing breakthroughs in cancer research.

We are pleased to present an exciting agenda for this year's Colloquium. We are looking forward to several incredible keynote presentations: Curtis Harris from the NCI, Kris C. Wood from Duke University, Christine Heske from the NCI, and Ashani Weeraratna from Johns Hopkins University. We are also excited to present talks from this year's winner of the Outstanding Postdoctoral Fellow award, Dr. Dan Li, as well as an inspiring presentation from childhood cancer survivor Naomi Bartley. We have renowned speakers for several panels designed to improve NCI trainee career development: Exploring Careers at the Bench: Academia and Beyond, Navigating Career Transitions into Science Writing, Policy, and More, and Cultivating Inclusion: A Roadmap for Scientists in Training. Furthermore, we also have informative career development workshops: Communicating with Confidence and Clarity, Empowering your Training Journey: Navigating NCI Resources, and Grant Writing Decoded. We are also featuring an information table from the CCR Office of Science and Technology Resources (OSTR). Please stop by to learn how CCR cores can support your research.

We are thrilled to have you all here to enjoy and experience the Colloquium in person. Your participation and enthusiasm for sharing your science with your peers is what truly makes this Colloquium successful, so we sincerely thank you for joining us. We encourage all participants to use this opportunity to attend posters and talks featuring your colleagues' work, learn valuable career development skills in the panels and workshops, and most importantly, network!

Thank you,
CCR-FYI Colloquium Subcommittee Co-Chairs:
Kathleen Reed, Ph.D. & Ramesh Chingle, Ph.D.
& the CCR-FYI Colloquium Planning Committee

Agenda

Thursday, April 18th, 2024

8:30 – 8:40 a.m. **Opening Remarks from Center for Cancer Training Leadership and Colloquium Planning Committee Chairs**
(Joseph F. Fraumeni Jr., M.D. Conf. Rm TE406/408/410)
Erika Ginsburg, M.A., Branch Director, Office of Training and Education, CCT
Kathleen Reed, Ph.D. CCR-FYI Colloquium Co-Chair, Bethesda
Ramesh Chingle, Ph.D. CCR-FYI Colloquium Co-Chair, Frederick

8:40 – 9:20 a.m. **NCI Director's Address** *(Joseph F. Fraumeni Jr., M.D. Conf. Rm TE406/408/410)*
Kimryn Rathmell, M.D., Ph.D., Director, NCI

9:20 – 10:20 a.m. **Keynote Speaker I** *(Joseph F. Fraumeni Jr., M.D. Conf. Rm TE406/408/410)*
"Precision medicine of lung and environmental cancer"
Curtis Harris, M.D., Chief of Laboratory of Human Carcinogenesis,
National Cancer Institute, Center for Cancer Research

10:20 – 10:30 a.m. **BREAK**

10:30 – 11:45 a.m. **Concurrent Oral Presentations** *(3 sessions)*

I. Biophysics, Chemistry, Pharmacology and Structural Biology
(Conf. Rm TE406)
McKenna Crawford
Riley Metcalfe
Emily Xu
Woong Young So

II. Genetics, Genomics, Chromatin, Signal Transduction, and Transcription
(Conf. Rm TE408/410)
Arwa Fallatah
Payel Mondal
Gauri Prasad
Maxine Rubin

III. Cancer Models, Cancer Stem Cells, Carcinogenesis and Metastasis
(Conf. Rm 2W910/912)
Fengchao Lang
Olivia Tuckey
Natalia Yakobian
Srujana Yellapragada

11:45 – 12:30 p.m. **LUNCH**

12:30 – 2:00 p.m. **Poster Session I** *(Seminar Room TE110)*
12:30 – 1:15 Odd Present
1:15 – 2:00 Even Present

- **Bioinformatics, Epidemiology, and Translational Research**
- **Immunology, Virology, and Metabolism**
- **Molecular and Cellular Biology and Microbiology**

2:00 – 2:15 p.m. **BREAK**

2:15 – 3:15 p.m. **Keynote Speaker II** *(Joseph F. Fraumeni Jr., M.D. Conf. Rm TE406/408/410)*
"Evolution of tumor dependencies"
Kris C. Wood, Ph.D., Associate Professor of Pharmacology and Cancer Biology, Duke University

3:15 – 4:00 p.m. **Outstanding Postdoctoral Fellow**
(Joseph F. Fraumeni Jr., M.D. Conf. Rm TE406/408/410)
"Camel nanobody-based B7-H3 CAR-T cells with high efficacy against large solid tumors"
Dan Li, Ph.D., Research Fellow, Laboratory of Molecular Biology, National Cancer Institute

4:00 – 4:15 p.m. **BREAK**

4:15 – 5:30 p.m. **Concurrent Workshops and Panels** *(3 sessions)*

I. Communicating with Confidence and Clarity *(Workshop)*
(Conf. Rm TE408/410)
Tracy Costello, Ph.D., Founder and Career Coach, Coach4Postdocs

II. Empowering your Training Journey: Navigating NCI Resources *(Workshop)* *(Conf. Rm 2W910/912)*
Chanelle Case Borden, Ph.D., Associate Director of Training Programs, Center for Cancer Training

III. Exploring Careers at the Bench: Academia and Beyond *(Panel)*
(Conf. Rm.TE406)
Clara Bodelon, Ph.D., M.S., Senior Principal Scientist, Survivorship Research, American Cancer Society
Jesse Boehm, Ph.D., Chief Scientific Officer, Break Through Cancer, Principal Investigator, MIT Koch Institute for Integrative Cancer Research
Dmitry Gabrilovich, M.D., Ph.D., Chief Scientist, Cancer Immunology, AstraZeneca
Michael La Frano, Ph.D., Director, Metabolomics and Proteomics, University of Illinois Urbana-Champaign
Evagelia Laiakis Ph.D., Associate Professor, Georgetown University

5:30 – 5:45 p.m. **ADJOURN**

5:45 – 7:30 p.m. **Social Networking (Coastal Flats at Crown Gaithersburg)**

Friday, April 19th, 2024

8:30 – 8:45 a.m. **CCR Basic Science Director's Address**
(Joseph F. Fraumeni Jr., M.D. Conf. Rm TE406/408/410)
Glenn Merlino, Ph.D., CCR Scientific Director for Basic Research, National Cancer Institute

8:45 – 9:00 a.m. **CCR Clinical Director's Address**
(Joseph F. Fraumeni Jr., M.D. Conf. Rm TE406/408/410)
James Gulley, M.D., Ph.D., CCR Clinical Director, National Cancer Institute

9:00 – 10:00 a.m. **Keynote Speaker III** *(Joseph F. Fraumeni Jr., M.D. Conf. Rm TE406/408/410)*
"Development and Translation of Strategies to Target Tumor Metabolism for the Treatment of Pediatric Solid Tumors"
Christine Heske, M.D., Investigator, Pediatric Oncology Branch, National Cancer Institute, Center for Cancer Research

10:00 – 10:15 a.m. **BREAK**

10:15 – 11:30 a.m. **Concurrent Oral Presentations** *(3 sessions)*

I. Bioinformatics, Epidemiology, and Translational Research
(Conf. Rm TE406)
Alexandra Dreyzin
Yong Yean Kim
Sounak Sahu
Nai-Yun Sun

II. Immunology, Virology, and Metabolism
(Conf. Rm TE408/410)
Domenico D'Atri
Caitlin Huguely
Dipanwita Mitra
Shweta Tiwary

III. Molecular and Cellular Biology and Microbiology
(Conf. Rm 2W910/912)
Helena Muley-Vilamu
Divya Nambiar
Tara O'Shea
Soumya Sundara Rajan

11:30 – 12:15 p.m. **LUNCH**

12:15 – 1:45 p.m. **Poster Session II** *(Seminar Room TE110)*
12:00 – 12:45 Odd Present
12:45 - 1:30 Even Present
- **Biophysics, Chemistry, Pharmacology and Structural Biology**
- **Genetics, Genomics, Chromatin, Signal Transduction, and Transcription**
- **Cancer Models, Cancer Stem Cells, Carcinogenesis and Metastasis**

1:45 – 2:00 p.m. **BREAK**

2:00 – 3:00 p.m. **Keynote Speaker IV** *(Joseph F. Fraumeni Jr., M.D. Conf. Rm TE406/408/410)*
"The Ravages of TiME: How the aging tumor immune microenvironment drives cancer progression"
Ashani Weeraratna, Ph.D., E.V. McCollum Professor, Chair of Biochemistry and Molecular Biology, Johns Hopkins University

3:00 – 3:45 p.m. **Survivorship Speaker** *(Joseph F. Fraumeni Jr., M.D. Conf. Rm. TE406/408/410)*
"From Survivor to Advocate: Navigating the long-term side effects and championing less toxic therapies for childhood cancer patients"
Naomi Bartley, M.S., Clinical Information Science Director, Biometrics and Information Sciences, AstraZeneca

3:45 – 4:00 p.m. **BREAK**

4:00 – 5:15 p.m. **Concurrent Workshops and Panels**

I. Grant Writing Decoded *(Workshop) (Conf. Rm. TE406)*
David Armstrong, Ph.D., President and Founder, Grant Writing Mentors

II. Navigating Career Transitions into Science Writing, Policy, and More *(Panel)* (*Conf. Rm TE408/410)*
Luz Milbeth Cumba, Ph.D., AAAS Science and Technology Policy Fellow, US Department of State
Claudia Frehe, Ph.D., Senior Patent Agent, Cooley LLP
Paz Vellanki, Ph.D., Medical Oncologist, USDA
Vijay Walia, Ph.D., Senior Director, CDx Program,Quest Diagnostics

III. Cultivating Inclusion: A Roadmap for Scientists in Training *(Panel) (Conf. Rm.2W910/912)*
Ashley Bear, Ph.D., Director of the Committee on Women in Science, Engineering, and Medicine; National Academies of Sciences, Engineering, and Medicine
Danny Dickerson, Ph.D., Director of Diversity and Inclusion Division, National Institutes of Health
Giovanna Guerrero-Medina, Ph.D., Director, Yale Ciencia Program; Director, Diversity, Equity, and Inclusion, Wu Tsai Institute, Yale University; Executive Director, Ciencia Puerto Rico
Tiffany Wallace, Ph.D., Program Director, CRCHD, Office of the Director, National Cancer Institute
Rob Winn, M.D., Director, Massey Comprehensive Cancer Center, Virginia Commonwealth University

5:15 – 6:00 p.m. **Closing Address & Travel Awards**
(Joseph F. Fraumeni Jr., M.D. Conf. Rm TE406/408/410)
Douglas R. Lowy, M.D., Deputy Director, NCI

6:00 – 6:15 p.m. **ADJOURN**

6:15 – 7:30 p.m. **Social Networking** (The Bench at Rio Gaithersburg)

CCR-FYI Leadership

Kathleen Reed, Ph.D.
CCR-FYI Colloquium Co-Chair

Dr. Kathleen Metz Reed is a postdoctoral fellow in the Laboratory of Receptor Biology and Gene Expression at the National Cancer Institute in Bethesda. She is the Bethesda Co-Chair of the Colloquium Planning Committee. A Maryland native, Dr. Reed completed her Bachelor of Science degrees in Biochemistry and Cell Biology & Molecular Genetics at the University of Maryland in College Park in 2016, where she began research in the lab of Dr. Charles Delwiche, using bioinformatics to study the algal tree of life. She then earned her Ph.D. in Genetics and Molecular Biology with a certificate in Bioinformatics and Computational Biology from the University of North Carolina Chapel Hill in 2022. While at UNC she worked in the lab of Dr. Douglas Phanstiel, studying the role of three-dimensional organization of the genome on gene regulation and the functional impact of changes in chromatin structure during immune cell activation. In fall of 2022, Dr. Reed joined the lab of Dr. Tom Misteli as a CRTA fellow, where she continues to explore the significance of genome organization on cancer development. Her current work focuses on understanding the regulatory mechanisms underlying breast cancer progression using cutting edge genomics and high-throughput microscopy techniques. Dr. Reed has a passion for open scientific communication and aims to help the scientific community build an inclusive and welcoming environment that can inspire, encourage, and support the next generation of scientists.

Ramesh Chingle, Ph.D.
CCR-FYI Colloquium Co-Chair

Dr. Ramesh Chingle is a postdoctoral fellow in the Chemical Biology Laboratory at the National Cancer Institute in Frederick. He is currently serving as the Co-Chair of the Colloquium planning committee. Additionally, he has held leadership roles as Co-Chair for the steering committees and for the seminar series and contributed to the NCI Equity and Inclusion Program. Dr. Chingle completed his Master of Science degree with honors in Organic Chemistry from Goa University, in 2006. He subsequently gained extensive industrial experience as a research scientist at the Bristol Myers Squibb-Biocon Research Center within Syngene International Ltd., part of the Biocon group based in Bangalore, India. In 2018 Dr. Chingle earned his doctoral degree in Organic and Medicinal Chemistry from the Université de Montréal under the guidance of Prof. William Lubell. His graduate work was supported by scholarships from the Ministry of Education and the Québec government, Canada. During his Ph.D. work he made significant contributions to the field of azo-peptides and their application to making rigid peptide-turn surrogate peptide mimetics. Dr. Chingle published pioneering work on the synthesis and reactivity of these mimetics, which paved the way for their use in constraining azapeptides to turn conformations.

In the fall of 2018, Dr. Ramesh Chingle joined Dr. Terrence Burke's lab as a Visiting Fellow, embarking on an exploration of biologically-active peptide molecules and studying their protein-protein interactions. His current research is dedicated to designing macrocyclic fluorophore-conjugated peptide mimetics as diagnostic probes for bio-imaging, specifically targeting colorectal cancer cells. Additionally, he leverages solid-phase peptide synthesis with sequential native chemical ligation to synthesize modified histones. The aim of this work is to unravel HIV-1 integration mechanisms at a molecular level. Dr. Chingle is driven by a passion for fostering open scientific dialogue and building an inclusive environment to empower future generations of scientists. Beyond his research, he actively engages in mentoring, peer-review activities, and science communication efforts to promote knowledge sharing and outreach within the scientific community.

Oliver Bogler, Ph.D.
Director of the Center for Cancer Training National Cancer Institute

Oliver studied Natural Sciences at Cambridge University, completed his PhD at the Ludwig Institute for Cancer Research in London and did post-docs at the Salk Institute, and the Ludwig Institute, San Diego. He was on faculty at Virginia Commonwealth University, Henry Ford Hospital and the University of Texas MD Anderson Cancer Center where he also served as director of basic research for the Brain Tumor Center. His work focused on EGFR signaling and novel platinum compounds in glioblastoma. In 2010, he became MD Anderson's Vice President for Global Academic Programs supporting a network of 35 Sister Institutions in 22 countries and fostered cancer research and training across the globe. In 2011, he was also appointed Senior Vice President for Academic Affairs, stewarded MD Anderson's education mission and accreditation, and oversaw 300 people, who supported 1,700 faculty and more than 2,000 trainees and students. In 2018 he became COO at the ECHO Institute at the University of New Mexico and helped democratize scarce expert knowledge to improve services to the underserved in healthcare, education and beyond. In 2020 Oliver joined the National Cancer Institute's Center for Cancer Training which supports the goal of training cancer researchers for the 21st century.

Erika Ginsburg, M.A.
Branch Director, Office of Training and Education,
Center for Cancer Training National Cancer Institute

Erika currently serves as Branch Director of NCI's Center for Cancer Training (CCT) Office of Training and Education (OTE). She uses her passion for training and mentorship to manage career development programs for trainees. Several years ago, Erika revised the Sallie Rosen Kaplan Postdoctoral Fellowship for Women Scientists to better prepare NCI's female postdoctoral fellows to transition to independence and to retain them in science. Fellows who have successfully completed the one-year program and have gone on to their next career stage have all remained in the biomedical workforce. In addition, she has developed, coordinated, and evaluated other career development workshops, courses, and programs in the CCT. Erika leads the NIH Fellows Editorial Board, NCI Explore On-Site program, and the NCI Director's Innovation Award, just to name a few. Her effort in initiating the Responsible Conduct of Research training course for NCI trainees was recognized by an individual NCI's Director's Award in 2016. In 2006, the Association for Women in Science, Bethesda Chapter, awarded her Mentor of the Year. As Branch Director of OTE, Erika will further advocate for trainees, and continue to facilitate and promote training opportunities by working closely with trainees, PIs, and senior leadership.

Before joining CCT, Erika had a long career in NCI's intramural program as a Technical Laboratory Manager in CCR's Mammary Biology and Tumorigenesis Laboratory where she studied prolactin's action on breast cancer. Prolactin is an important hormone responsible for the development of the breast and may be positively associated with breast cancer risk. Together with Dr. Barbara Vonderhaar, she was the first to demonstrate that human breast cancer cells synthesize and secrete significant amounts of biologically active prolactin. She mentored over 150 trainees in the laboratory and has over 50 publications in the fields of drug metabolism, and hormone regulation of the normal and cancerous breast.

Erika received her undergraduate training in Biophysics and Microbiology from the University of Pittsburgh, her master's degree from Johns Hopkins University in Science/Medical Writing, and holds a master's Certificate in Biotechnology Management from the University of Maryland University College.

CCR FYI Colloquium Planning Committee

Colloquium Planning Vice Chairs
Monika Chandravanshi, Ph.D.
Sophia Varriano, Ph.D.

Steering Committee Chairs
Shivalee Duduskar, Ph.D.
Kristen Fousek, Ph.D.

Planning Committee Members
Vinutha Balachandra, Ph.D.
Christine Carney, Ph.D.
McKenna Crawford, B.S.
Priyanka Desai, Ph.D.
Sarah Dremel, Ph.D.
Kelcie Foss, B.S.
Katie Hebron, Ph.D.
Sophie Kang, Ph.D.
Arfa Moshiri, Ph.D.
Anna Newen, Ph.D.
Gauri Prasad, Ph.D.
Chathuranga Rathnamalala, Ph.D.
Josh Rich, Ph.D.
Ashlie Santaliz Casiano, Ph.D.
Shalu Sharma, Ph.D.
Julia Medina Velazquez, Ph.D.
Geraldine, Vilmen, Ph.D.
Kaustubh Wagh, Ph.D.

NCI Leadership

Kimryn Rathmell, M.D., Ph.D.
Director of the National Cancer Institute
National Institutes of Health

W. Kimryn Rathmell, M.D., Ph.D., M.M.H.C., was sworn in as the 17th NCI director on December 18, 2023. She previously led the Vanderbilt University Medical Center as physician-in-chief and chair of the Department of Medicine.

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. She was a leader of The Cancer Genome Atlas's (TCGA) kidney cancer projects and served as a TCGA analysis working group member across the spectrum of cancers, winning 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.

Dr. Rathmell has held leadership positions with the American Society of Clinical Oncology and the American Society for Clinical Investigation, serving as secretary–treasurer and president. As a result of her efforts, Dr. Rathmell has been elected to the Association of American Physicians, the American Academy of Arts and Sciences, and the National Academy of Medicine.

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 biology to develop interventions to improve patients' lives. Dr. Rathmell's research has resulted in more than 250 articles in leading peer-reviewed journals, including *The New England Journal of Medicine, Nature, Proceedings of the National Academy of Sciences, and the Journal of Clinical Investigation.*

Dr. Rathmell earned undergraduate degrees in biology and chemistry from the University of Northern Iowa and her Ph.D. in biophysics and M.D. from Stanford University. She completed an internal medicine internship at the University of Chicago and an internal medicine residency, medical oncology fellowship, and postdoctoral studies at the University of Pennsylvania. In 2022, she completed her Master of Management in Health Care from the Vanderbilt University Owen Graduate School of Management.

Douglas R. Lowy, M.D.
Principal Deputy Director of the National Cancer Institute,
National Institutes of Health

Douglas R. Lowy, M.D., is Chief of the Laboratory of Cellular Oncology and, since 2010, has also served as Principal Deputy Director of the National Cancer Institute (NCI). He has previously served as acting director four times during his tenure, most recently from November 2023 through December 2023. Dr. Lowy received his medical degree from New York University School of Medicine in 1968 and trained in internal medicine at Stanford University and dermatology at Yale University. He has directed a research laboratory at NCI since 1975, after receiving training as a Research Associate in the National Institute of Allergy and Infectious Diseases. Dr. Lowy is a member of the National Academy of Sciences (NAS) and of the Institute of Medicine of the NAS. For his research with John Schiller on technology that enabled the preventive HPV vaccines, they have jointly received numerous honors, including the 2007 Federal Employee of the Year Service to America Medal from the Partnership for Public Service, the 2011 Albert B. Sabin Gold Medal Award, the 2012 National Medal of Technology & Innovation (awarded in 2014), the 2018 Szent-Györgyi prize and the 2017 Lasker-DeBakey Clinical Medical Research Award, the country's most prestigious honor for biomedical research. Dr. Lowy has also received the National Medal of Honor for Basic Research from the American Cancer Society and is an fellow of the AACR Academy.

NCI Leadership

Glenn Merlino, Ph.D.
Acting Co-Director, Center for Cancer Research
CCR Scientific Director for Basic Research, National Cancer Institute

Dr. Merlino obtained his B.A. summa cum laude in 1975, then went on to receive a Ph.D. in biological sciences in 1980 from the Department of Cellular and Molecular Biology at the University of Michigan, Ann Arbor. He joined the NCI in 1988. As a postdoctoral fellow in Dr. Ira Pastan's lab, Dr. Merlino was the first to report the amplification/rearrangement of the epidermal growth factor receptor (EGFR) proto-oncogene in human cancer, and as a young independent investigator, he was among the first to show that growth factors could function in vivo as oncogenes using transgenic mouse models. With long-time George Washington University collaborators, Drs. Frances Noonan and Ed DeFabo, Dr. Merlino's group also developed the first human-like mouse melanoma model and provided the first experimental evidence supporting the notion that childhood sunburn is a critical melanoma risk factor.

Dr. Merlino was Chief of the Laboratory of Cell Regulation and Carcinogenesis from 2004 to 2006. This lab, along with two others, merged into the Laboratory of Cancer Biology and Genetics (LCBG) in 2006. He then served as Co-Chief of the new Laboratory from 2006 to 2017. During this period, Dr. Merlino also served as a CCR Deputy Director (2010-2015). Currently, he is CCR's Scientific Director for Basic Research. At NIH/NCI, Dr. Merlino has served as the NIH Ombudsman for Animal Welfare, on the Steering Committee of the NCI Center of Excellence in Integrative Cancer Biology and Genomics and on the CCR Science Board.

From 2009 to 2012, Dr. Merlino was Executive Editor of *Pigment Cell and Melanoma Research;* he has also served on the Editorial Board of *Cancer Research*. Currently, he is Co-Chair of the Scientific Advisory Council, Melanoma Research Foundation and Adjunct Professor at the University of Maryland Regional College of Veterinary Medicine.

James L. Gulley, M.D., Ph.D.
Acting Co-Director, Center for Cancer Research
CCR Clinical Director, 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 PhD in microbiology in 1994 and an MD 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 (NCI).

Dr. Gulley serves within the Center for Cancer Research (CCR) of the National Cancer Institute as Co-Director of the Center for Immuno-Oncology, and also serves as the Clinical Director, 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.

Keynote Speakers

Thursday, April 18th, 2024

Curtis Harris, M.D.
Chief of Laboratory of Human Carcinogenesis
Center for Cancer Research, National Cancer Institute

Precision medicine of lung and environmental cancer

Receiving his M.D. from Kansas University School of Medicine and his clinical training at both UCLA and the NCI. He has held positions of increasing responsibility at the NCI and is an Adjunct Professor of Oncology at Georgetown University School of Medicine. Receiving numerous honors including, e.g., the Alton Ochsner Award relating Smoking and Health (American College of Physicians), Deichmann Award (International Union of Toxicology), Charles Heidelberger Award (International Society of Gastroenterological Carcinogenesis), Distinguished Service Medal (highest honor of the U.S. Public Health Service), NCI Outstanding Mentor Award in 2007 and 2013, Ph.D. (Honorary) Nippon University School of Medicine in 2013. He was awarded the AACR-Princess Takamatsu Award in 2009, the ILCA Nelson Fausto Award and AACR-American Cancer Society Award for Research Excellence in Cancer Epidemiology and Prevention in 2014. In 2016, he was awarded the Distinguished Medical Alumnus Award from Kansas University School of Medicine and in 2020, was awarded the Environmental Mutagenesis and Genome Society Annual Award. Becoming a Fellow of the AACR Academy class of 2021 and is a Fellow at the American Society of Clinical Investigation and AAAS. Publishing more than 700 journal articles, 100 book chapters, edited 10 books and holds more than 30 patents for the U.S. Government. Served as Editor-in-Chief for the journal, Carcinogenesis for 40 years. He has also held/currently holds elected offices in scholarly societies and non-profit foundations including the AACR, the Keystone Symposia on Molecular and Cellular Biology, which he co-found and also a co-founder and President of the Aspen Cancer Conference. He has a wide range of scientific interests and accomplishments spanning molecular genetics and epigenetics of human cancer to molecular epidemiology of human cancer risk and mechanistic biomarkers of cancer diagnosis, prognosis, and therapeutic outcome. Harris has a long productive history of investigating the mutation and function of p53 and recently, p53 isoforms involvement in cellular reprogramming and senescence. He has trained more than 300 physician-scientists worldwide. In his spare time, he co-wrote an International Spy novel entitled “High Hand”, published in 2016.

Kris C. Wood, Ph.D.
Associate Professor of Pharmacology and Cancer Biology,
Duke University

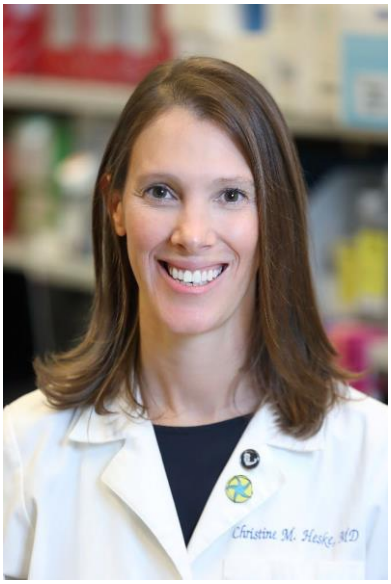
Evolution of tumor dependencies

Kris C. Wood, Ph.D., is an Associate Professor in the Department of Pharmacology and Cancer Biology at Duke University. He received his Ph.D. in Chemical Engineering from the Massachusetts Institute of Technology, where he developed self-assembling polymeric systems for controlled gene and drug delivery under the supervision of Professors Paula Hammond, Ph.D. and Robert Langer, Sc.D. As an NIH and Misrock Fund postdoctoral fellow at the Whitehead Institute for Biomedical Research and the Broad Institute of Harvard and MIT, his work focused on the development of functional genomic tools to study the determinants of anticancer drug sensitivity.

Kris' lab at Duke, founded in 2012, focuses on two related themes: (1) identifying, mechanistically characterizing, and translating new, molecularly targeted therapeutic strategies for biomarker-defined cancer subtypes and (2) defining rational strategies to control long term tumor evolution. To power these studies, Kris' team develops and adapts a range of new functional genomic technologies. The lab's work has been recognized by early career awards from the Ovarian Cancer Research Fund Alliance, the V Foundation, the Stewart Trust, the Forbeck Foundation, the Whitehead Foundation, and the NIH BIRCWH Program, Idea Development Awards from the DoD Lung Cancer Research Program, and Breakthrough Awards from the DoD Breast Cancer Research Program. It has also inspired the design of multiple ongoing clinical trials and the creation of three biotechnology companies: Celldom (Silicon Valley, CA), Tavros Therapeutics (Durham, NC), and Element Genomics, now a wholly owned subsidiary of UCB Pharma (Brussels).

Keynote Speakers

Friday, April 19th, 2024



Christine M. Heske, M.D.
Investigator, Pediatric Oncology Branch,
Center for Cancer Research, National Cancer Institute

Development and Translation of Strategies to Target Tumor Metabolism for the Treatment of Pediatric Solid Tumors

After completing her undergraduate work at Harvard University, Dr. Heske received her M.D. from The George Washington University School of Medicine and Health Sciences. She completed her pediatric internship and residency at Brown University/Hasbro Children's Hospital, followed by her fellowship training at the combined National Cancer Institue~Johns Hopkins University Pediatric Hematology and Oncology program, where she served as Chief Fellow. IN 2016, Dr. Heske began her own group as a Physicial-Scientist Early Investigator in the Pediatric Oncology Branch. She was promoted to Investigator in 2021 and currently leads the Translational Sarcoma Biology Group.

Ashani Weeraratna, Ph.D., E.V.
McCollum Professor, Chair of Biochemistry and Molecular Biology,
Johns Hopkins University

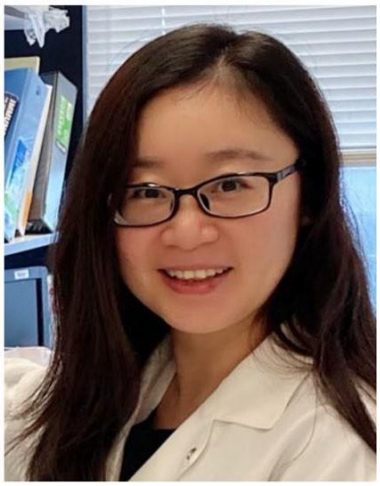
The Ravages of TiME: How the aging tumor immune microenvironment drives cancer progression

Dr. Weeraratna is the Bloomberg Distinguished Professor of Cancer Biology, E.V. McCollum Chair of Biochemistry and Molecular Biology at the Johns Hopkins Bloomberg School of Public Health, as well as the Associate Director for Laboratory Research at the Sidney Kimmel Cancer Center, Johns Hopkins School of Medicine. She is a Past President of the Society for Melanoma Research, and was recently appointed by President Biden as a member of the National Cancer Advisory Board. Prior to joining Johns Hopkins, she was the Ira Brind Professor and Co-Program Leader, Immunology, Microenvironment & Metastasis Program Member at the Wistar Institute. Born in Sri Lanka and raised in Lesotho in Southern Africa, Weeraratna first came to the United States in 1988 to study biology at St. Mary's College of Maryland. She earned a Ph.D. in Molecular and Cellular Oncology at the Department of Pharmacology of George Washington University Medical Center. From 1998 to 2000, she was a post-doctoral fellow at The Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins Oncology Center, before joining the National Human Genome Research Institute as a staff scientist. In 2003, she moved to the National Institute on Aging, where she started her own research program, before joining the Wistar Institute from 2011-2019.

Dr. Weeraratna is an expert in melanoma metastasis, Wnt signaling, and aging, and her research focuses heavily on the effects of the tumor microenvironment on metastasis and therapy resistance. She is one of the first to study how the aging microenvironment guides metastasis and therapy resistance in melanoma. For this innovative work, she was selected by *Nature* to be a part of their "Milestones in Cancer Research" video series and in 2021 the NCI selected her as one of their "Top 5 Cancer Researchers Accelerating Cancer Research Into the Future". Moreover, the quality and impact of Dr. Weeraratna's research is further recognized by the award of numerous peer-reviewed grants and awards.

Finally, Dr. Weeraratna has been a champion of increasing diversity for many years, and this is evident in her writings which call for gender and racial equity (e.g., *Nature*, *Nature Medicine*, *Nature Reviews in Cancer*, *Cancer Cell* and *Cancer Discovery*). She mentors junior faculty all over the world, and is spearheading efforts to increase the diversity among the Hopkins faculty. In her own department she has successfully implemented strategies to increase diversity both through faculty recruitment, and in her student body. She has written a book for the lay public called "Is Cancer Inevitable?" meant to highlight the progress made in the field, and the importance of diversity in cancer research. She is also heavily invested in Public Health, with multiple calls for sun protection and awareness through her social media presence, and community outreach.

Outstanding Postdoctoral Fellow Awardee



Dan Li, Ph.D.
Research Fellow, Laboratory of Molecular Biology,
Center for Cancer Research, National Cancer Institute

Camel nanobody-based B7-H3 CAR-T cells with high efficacy against large solid tumors

Dr. Dan Li received her Ph.D. from East China Normal University in 2019. She then started postdoctoral training in Dr. Mitchell Ho's lab at the National Cancer Institute, where her research aims to develop novel antibody engineering-based immunotherapy for treating solid tumors. She developed chimeric antigen receptor (CAR) T cells targeting glypican-3 for treating liver cancer. Based on her work, a clinical trial of GPC3 CAR-T therapy was approved by the FDA for treating advanced liver cancer patients at the NIH Clinical Center (ClinicalTrials.gov NCT05003895). Dan has received the Outstanding Postdoctoral Fellow Award (2024) and the Federal Technology Transfer Act Award (2021, 2022 & 2023).

Outstanding Postdoctoral Fellow Finalists

Vasty Osei Amponsa, M.S., Ph.D.

Vasty Osei Amponsa is a post-doctoral fellow at Center for Structural Biophysics Laboratory of the Center for Cancer Research at the National Cancer Institute (NCI). In her current position, she leverages her cell and molecular biology background to design and execute cell-based functional studies as complementary approaches to structural studies. Dr. Osei-Amponsa obtained her PhD in Biomedical Sciences and Clinical Translational Sciences at the PennState University.

Christina Fitzsimmons, Ph.D.

Dr. Christina Fitzsimmons received her PhD in Chemistry and Chemical Biology from the University of California-San Francisco, where her doctoral work focused on the enzymology of radical SAM enzymes. Dr. Fitzsimmons is currently an American Cancer Society postdoctoral fellow with Dr. Pedro Batista in the Laboratory of Cell Biology. Her work focuses on the intersection of metabolism and RNA modifications in cancer.

Sounak Sahu, Ph.D.

Dr. Sounak Sahu is a postdoctoral fellow at NCI-Frederick working under the mentorship of Dr. Shyam Sharan on integrating principles from developmental biology into the study of cancer. Following the completion of his doctoral studies from the University of Oxford under the mentorship of Prof. Aziz Aboobaker, Dr. Sahu embarked on a transformative postdoctoral journey, shifting his research field from flatworms to mammalian stem cells. One of his groundbreaking accomplishments was the development of a pioneering technique for differentiating embryonic stem cells into mammary organoids, opening new avenues for understanding normal development and cancer. Beyond working in the lab, Sounak is equally passionate about mentoring, peer-reviewing, science communication, and outreach.

Kun Wang, Ph.D.

Kun Wang is presently a research fellow at the Cancer Data Science Lab at the National Cancer Institute (NCI). He earned his Ph.D. in computational biology from the University of Maryland, College Park.His primary research is focused on developing innovative computational methods to dissect the tumor microenvironment and facilitate advancements in cancer immunotherapy.

Scott Wilkinson, Ph.D.

Scott Wilkinson is a CRTA postdoctoral fellow in the Sowalsky lab, studying the mechanisms underlying prostate cancer response or resistance to androgen deprivation therapy. Dr. Wilkinson has published first author manuscripts in Nature Communications and European Urology, and is a co-author on 18 additional manuscripts, including in Nature and Nature Cancer. Dr. Wilkinson has served as a mentor to 7 junior members of his laboratory and served as the Chair of the trainee leadership committee, COMPASS, for the American Society of Cell Biology from 2019-2023. Dr. Wilkinson is a recipient of the prestigious Prostate Cancer Foundation (PCF) Young Investigator Award, a DoD Early Investigator Research Award, the CCR Excellence in Postdoctoral Research Transition award, and a recent K22 Transition Career Development award.

Woong Young So, Ph.D.

Dr. Woong Young So obtained his PhD in chemistry at Carnegie Mellon University where he specialized in fluorescence microscopy to study the photophysical properties of newly emerging nanoparticles such as conjugated polymer nanoparticles, surface-modified silicon nanoparticles, and gold nanoclusters. Then, he joined NCI CCR as a postdoctoral fellow under the mentorship of Dr. Kandice Tanner to study the role of the immune on tissue mechanics and the metabolism of tumors using zebrafish while also studying the mechanical adaptability of cancer cells at early metastasis.

Speakers: Special Presentation and Workshops

Naomi Bartley, M.S.
Clinical Information Science Director,
Biometrics and Information Services, AstraZeneca

From Survivor to Advocate: Navigating the long-term side effects and championing less toxic therapies for childhood cancer patients

Naomi has overcome significant personal medical challenges and dedicated her life to making a difference in the field of pediatric oncology. As a two-time childhood and young adult cancer survivor, she has a unique perspective on the needs and struggles of cancer patients and their families.

Naomi has long been an advocate for pediatric cancer research and patient/family education. She developed the first iOS app to help cancer patients navigate their cancer journey, served as an executive board member for the American Childhood Cancer Organization (ACCO) for five years, and raised a quarter of a million dollars for ACCO through her fundraiser “Naomi’s Hope for a Cure”.

In 2021, Naomi was appointed as a patient advocate to the Childhood Cancer Data Platform working group for the National Cancer Institute’s ambitious Childhood Cancer Data Initiative (CCDI). The initiative supports maximizing the use and benefit of data from childhood, adolescent, and young adult cancer research for patients and survivors and aims to make it easier for researchers to learn from each of the approximately 16,000 children and adolescents diagnosed with cancer in the United States each year.

Naomi's dedication to developing medicines for unmet needs in pediatrics has had a significant impact at AstraZeneca. She played a key role in establishing a pediatric Center of Excellence within her therapeutic area, with the vision of bringing new therapies to pediatric patients and optimizing the drug development process for this population. Before joining AstraZeneca in 2009, Naomi gained valuable experience in clinical research at Coley Pharmaceuticals and PRA International in Canada, as well as at the Center of Genetic Medicine at Children's National Medical Center and Vanda Pharmaceuticals in the Washington DC area. Her diverse background and expertise in clinical operations, quality assurance, and information practice have contributed to her success in her current role as Clinical Information Science Director at AstraZeneca.

Naomi holds an MS in Biotechnology from Johns Hopkins University. She lives in Maryland and enjoys photography, rock climbing, playing the violin, and spending time with her daughter, friends and family.

David Armstrong, Ph.D.
President and Founder, Grant Writing Mentors

Grant Writing Decoded

Dr. David Armstrong received his Ph.D. from the Ohio State University following studies at the Ohio State University and Cornell Medical College. Dr. Armstrong was a post-doctoral fellow at the Albert Einstein College of Medicine and thereafter appointed Assistant Professor, Albert Einstein College of Medicine and University of California, San Diego; Associate Professor, Georgetown University Medical College; Professor and Associate Director of the Institute of Aging at the MCP-Hahnemann School of Medicine; and Professor and Deputy Director of the Lankenau Institute of Medical Research, Jefferson Health System. Dr. Armstrong was the principal investigator on multiple grants from the NIH and private foundations to study neuronal vulnerability in Alzheimer's disease and stroke. He has authored or coauthored more than 100 peer-reviewed articles and has served on numerous NIH review panels and maintained positions on the editorial boards of many journals. In 2001 Dr. Armstrong joined the Center for Scientific Review, NIH as Chief of Brain Disorders and Clinical Neurosciences, Integrated Review Group and in 2005 accepted the position as Chief of the Scientific Review Branch, National Institutes of Mental Health, NIH. For his dedication to the NIH and public service, Dr. Armstrong has received numerous NIMH and NIH Director's Awards. Currently, he is founder and President of Grant Writing Mentors, LLC and co-director of a graduate course in Grant Writing, Uniformed Services University of the Health Sciences where he also holds the position of Adjunct Professor.

Chanelle Case Borden, Ph.D.
Associate Director of Training Programs,
Center for Cancer Training

Empowering your Training Journey: Navigating NCI Resources

Dr. Case Borden received her Ph.D. in Molecular Medicine from George Washington University as a student within NIH Graduate Partnerships Program in 2012. She continued her training at the National Cancer Institute as a postdoc, where she worked to determine the molecular mechanism of transcription factors known to play a role in cancer. Her passion for science education and outreach led her to join the Center for Cancer Training as an administrative postdoc in 2016, where she became a Scientific Program Specialist in 2018. Since then, Dr. Case Borden has served multiple roles within the Center for Cancer Training and is currently Associate Director of Training Programs. In this role, she provides support to trainees/fellows, leads recruitment and science outreach efforts, and works diligently to improve the training experience at NCI.

Tracy Costello, Ph.D.
Founder, Career Coach Coach4PostDocs
Director, Postdoctoral Affairs and Graduate Student Development,
Office of Graduate Studies, University of South Florida

Communicating with Confidence and Clarity

Tracy Costello, PhD, currently serves as the director of postdoctoral affairs and graduate student development at the University of South Florida, as well as consultant and coach for Coach4Postdocs. Since 2012, she has focused on professional and career development for postdoctoral fellows and graduate students with expertise in career exploration, job searches, leadership, mentoring, communication, grant writing, and ethics. Tracy earned her doctorate in biomathematics and biostatistics and human and molecular genetics from The University of Texas MD Anderson UTHealth Graduate School of Biomedical Sciences. During her postdoctoral training at MD Anderson Cancer Center, she was actively involved with postdoc affairs and served on the Board of Directors of the National Postdoctoral Association. She then transitioned to staff positions in academia and industry, ultimately shifting to academic administration. Tracy founded Coach4Postdocs, a consultancy providing professional development training at universities, professional societies, and nonprofits, as well as offering private 1-1 early- and mid-career coaching. She also serves on the STEMPeers Board of Directors, provides executive coaching for the STEMPeers Gurukool program, and serves as a study section reviewer for NIH and previously for NSF.

Panelists: Exploring Careers at the Bench: Academia and Beyond

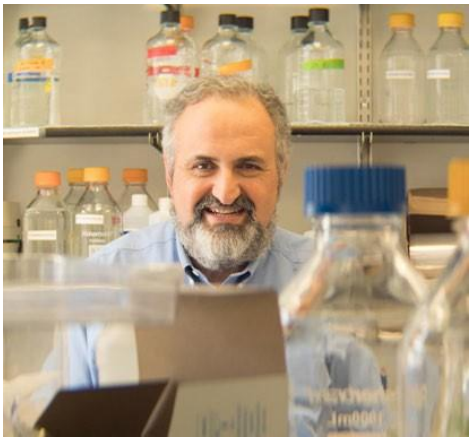
Clara Bodelon, Ph.D., M.S.
Senior Principal Scientist, Survivorship Research,
American Cancer Society

Dr. Clara Bodelon received her Ph.D. in Mathematics from Boston University and then went on to get her M.S. in Epidemiology from the University of Washington. Dr. Bodelon joined the Division of Cancer Epidemiology and Genetics (DCEG) at the National Cancer Institute as a postdoctoral fellow and was awarded the Sallie Rosen Kaplan Cancer Research Training Fellowship. While at DCEG, she conducted research in the Integrative Tumor Epidemiology Branch (ITEB). In 2023, Dr. Bodelon became a Senior Principal Scientist in the intramural program of the American Cancer Society (ACS). There, she conducts integrative analyses of questionnaire, genetic and molecular data to characterize long-term effects among cancer survivors using the Cancer Prevention Studies, some of the largest available cohorts.

Dr. Bodelon has been the recipient of multiple awards, including several NIH Intramural Research Awards, DCEG Fellows Awards for Research Excellence, NIH Fellows Awards for Research Excellence, two AACR Scholar-in-Training Awards, a Division Director's award for the most outstanding research paper and the 2020 Director's Innovation Award.

Jesse Boehm, Ph.D.
Chief Science Officer, Break Through Cancer,
Principal Investigator, MIT Koch Institute for Integrative Cancer Research

Jesse Boehm is the Chief Science Officer of Break Through Cancer, a novel foundation dedicated to harnessing the power of extraordinary partnerships between cancer centers to bring urgency to focusing on overcoming barriers impossible for any single organization to solve. Break Through Cancer currently unites five leading cancer institutions: Dana-Farber Cancer Institute, Johns Hopkins Kimmel Cancer Center, University of Texas MD Anderson Cancer Center, Memorial Sloan Kettering Cancer Center, and MIT's Koch Institute for Integrative Cancer Research. Dr. Boehm also maintains a research lab at MIT's Koch Institute focused on bringing the power of functional genomics to bear on living samples from cancer patients with particular emphasis on rare and underrepresented tumors. Before transitioning to MIT, Jesse previously spent 14 years in the Broad Institute's Cancer Program, most recently as an Institute Scientist and Scientific Director of the Cancer Dependency Map project. As the Director of the Broad's Cancer Model Development Center (part of the National Cancer Institute's Human Cancer Models Initiative), he led his laboratory in developing a scalable capacity to convert patient tumors into organoids and other cell models. Jesse received his BS in biology from MIT and his PhD from Harvard University, Division of Medical Sciences at Dana-Farber Cancer Institute.



Dmitry Gabrilovich, M.D., Ph.D.
Chief Scientist, Cancer Immunology, AstraZeneca

Dmitry Gabrilovich, MD, Ph.D., is an Executive Director and Chief Scientist, Cancer Immunology, AstraZeneca where he performs discovery and translational studies of of tumor immunology with special emphasis on myeloid cells. He studied dendritic cell (DC) biology under Dr. Stella C. Knight at the Imperial College of London in the UK and then was trained in cancer research at U.T. Southwestern Medical School and Vanderbilt University. In mid-1990s his group demonstrated, that DCs in cancer were functionally impaired. They have described the first tumor-derived factor directly implicated in DC defects in cancer and determined that myeloid progenitors were the main targets for this negative effect. His group implicated lipid accumulation as one of the mechanisms negatively regulating function of DCs in cancer. Dr. Gabrilovich was instrumental in the discovery and characterization of myeloid-derived suppressor cells (MDSC). His group described number of molecular mechanisms regulating expansion and function of these cells. His group provided first evidence that MDSC can be therapeutically targeted in patients. Dr. Gabrilovich was involved in number of clinical trials testing the effect of cancer vaccines and small molecules and antibodies that target myeloid cells. Before joining AstraZeneca Dr. Gabrilovich was Robert Rothman Endowed Chair in Cancer Research and Head, Section of Dendritic Cell Biology at H. Lee Moffitt Cancer Center and then Christopher M. Davis Professor in Cancer Research at the Wistar Institute in Philadelphia and Wistar Professor at the Department of Pathology and Laboratory Medicine, Perelman School of Medicine, University of Pennsylvania. Dr. Gabrilovich is American Cancer Society Research Professor and during last 10 years is listed as one of the most highly cited researchers in the field of Immunology.

Michael La Frano, Ph.D.
Director, Metabolomics and Proteomics,
University of Illinois Urbana-Champaign

Dr. La Frano is the Director of Metabolomics & Proteomics at the Roy J. Carver Biotechnology Center, University of Illinois Urbana-Champaign. His work as Director of metabolomics focuses on the development of new untargeted and targeted methods to better capture the diversity of the metabolome, as well as creation of new data processing and analysis programs to improve QC and results interpretation, respectively. His independent research has investigated biomarkers of disease, including cancer, and nutritional metabolomics. His proteomics work centers on optimization of lab operations to increase capability and capacity. He is an Affiliate of the Personalized Nutrition Initiative at University of Illinois Urbana-Champaign and Affiliate Member of the metabolomics Quality Assurance and Quality Control Consortium (mQACC).

Dr. La Frano earned a Ph.D. in Nutrition Biology at the University of California, Davis in 2012. Subsequently, his postdoctoral fellowship role was as a pilot project scientist for West Coast Metabolomics Center (WCMC) NIH Pilot & Feasibility Project studies where he performed targeted metabolomics analysis as part of the Newman Laboratory for Lipid Mediators. These data were combined with WCMC untargeted analyses to support various biomedical studies. In 2016, he joined the faculty at the California Polytechnic State University, San Luis Obispo and was later promoted to Associate Professor with tenure. He also served as Director of the Cal Poly Metabolomics Service Center, which he founded in 2019.

Evagelia Laiakis, Ph.D.
Associate Professor, Georgetown University

Dr. Laiakis received her Ph.D. degree in Human Genetics from the University of Maryland at Baltimore, studying radiation induced genomic instability and the contribution of pro-inflammatory processes. She subsequently completed her postdoctoral fellowship at Georgetown University, in the field of radiation biodosimetry through metabolomics. She is currently an Associate Professor in the Department of Oncology at the Lombardi Comprehensive Cancer Center with a secondary appointment in the Department of Biochemistry and Molecular & Cellular Biology. She is an elected Council Member to the National Council on Radiation Protection and Measurements (NCRP) and has been serving as a member of PAC-1 of NCRP (Basic Criteria, Epidemiology, Radiobiology, and Risk) since 2016. In 2021-2022 she served in a committee organized by the National Academies of Sciences, Engineering, and Medicine on “Developing a long-term strategy for low-dose radiation research in the United States”.

Dr. Laiakis’ lab aims to expand the field of radiation metabolomics and lipidomics through mass spectrometry with untargeted and targeted approaches. Her research focus includes understanding metabolic responses to scenarios involving a wide range of doses (low dose to acute radiation syndrome associated doses), dose rates, normal tissue responses, and radiation quality effects (photons, alpha-particles, neutrons, high energy particles), utilizing biofluids and tissues from rodents to humans. Her work is also encompassing space radiation effects and space biology, in combination with stressors such as microgravity or general spaceflight effects, with emphasis on immune and muscle related changes. Finally, she is an Associate Editor for the International Journal of Radiation Biology (IJRB), the Radiation Research journal, and Space Research Today and the 2019 recipient of the Jack Fowler award from the Radiation Research Society.

Panelists: Navigating Career Transitions into Science Writing, Policy, & More

Luz Milbeth Cumba, Ph.D.
AAS Science and Technology Policy Fellow, USAID

Dr. Luz Cumba Garcia is an immunologist working at the intersection of biomedical science, public policy, and science diplomacy. Currently, Luz is a second-year AAAS Science & Technology Policy Fellow in the Office of Mexican Affairs at the U.S. Department of State. In this role, she leverages her technical expertise to inform and support bilateral policy development between the U.S. and Mexico. In her initial fellowship year, Luz worked with the Middle East Regional Cooperation (MERC) Program at the U.S. Agency for International Development (USAID), contributing to the advancement of Arab-Israeli scientific collaboration and regional development through research grants. Her active role as an Inter-American Institute for Global Change Research (IAI) Science, Technology, and Policy (STeP) Science Diplomacy Fellow underscores her dedication to policy and diplomacy. Dr. Cumba Garcia's influence extends beyond her fellowship roles; she is a founding member of the Science Diplomacy Network in Latin America and the Caribbean and participated in several science diplomacy workshops, including the AAAS Science Diplomacy and Leadership Workshop, the AAAS/TWAS Science Diplomacy Course, and the InnScid SP Science Diplomacy School. Her academic background comprises a Ph.D. and M.S. in Immunology, alongside a B.S. in Cellular and Molecular Biology. Originating from San Juan, PR, she strives to champion advocacy, science communication, and community engagement.

Claudia Frehe, Ph.D.
Senior Patent Agent, Cooley LLP

Dr. Claudia Frehe is a senior patent agent in the intellectual property litigation and patent practice at Cooley. Claudia focuses her practice on the preparation and prosecution of patent applications, strategic patent counseling, and freedom-to-operate in a variety of fields, including immuno-oncology, cell therapy, CAR-T, CRISPR, RNA-editing, gene therapy, antibodies, and organ transplantation.

Prior to joining Cooley, Claudia completed a postdoctoral fellowship in experimental transplantation and immunotherapy at the National Cancer Institute (NCI) where she worked with Dr. James Kochenderfer. Claudia earned her Ph.D. in translational biology and molecular medicine at Baylor College of Medicine.

In addition to her biomedical research, Claudia also has clinical experience having spent over five years conducting pre-operative testing in advance of solid-organ and stem cell transplantation at Houston Methodist J.C. Walter Jr. Transplant Center.

Paz Vellanki, Ph.D.
Medical Oncologist, USDA

Paz Vellanki is a medical oncologist and cross-disciplinary team leader on the thoracic and head and neck cancer team at the U.S. Food and Drug Administration (FDA). In this role, she focuses on the regulation of drugs and drug approvals for patients with lung and head and neck cancers. At the FDA, she is also involved with efforts related to ctDNA and drug development. She continues to care for patients with head and neck cancer as a Clinical Assistant Professor at the University of Maryland Marlene and Stewart Greenebaum Comprehensive Cancer Center. Prior to working at the FDA, she received her MD/PhD at the University of Maryland School of Medicine. Her doctoral work was in Biochemistry and involved studying the structural and molecular biology of MutY Homolog, a DNA repair enzyme mutated in a hereditary colorectal cancer syndrome. She completed her residency in Internal Medicine at the Wake Forest School of Medicine in Winston-Salem, North Carolina and her fellowship in Oncology at the Johns Hopkins Sidney Kimmel Comprehensive Cancer Center in Baltimore, Maryland.

Vijay Walia, Ph.D.
Senior Director, CDx Program,
Quest Diagnostics

Dr. Vijay Walia is a Sr. Program Director for Companion Diagnostics (CDx) in the Quest Diagnostics Pharma Services team. Quest Diagnostics Pharma Services Group develops CDx devices that are essential for the safe and effective use of a corresponding drug or biological product developed by its Pharma clients. Dr. Walia has deep knowledge in the field of In Vitro Diagnostics and multiple technologies used in the development of IVDs, such as ELISA, IHC, NGS, etc., which are required for the marketing of the drug. Dr. Walia has over 18 plus years of experience in molecular biology, in-depth knowledge of regulations, product development, registrations, the lifecycle of drugs, and medical device development with knowledge of FDA review and approval pathways. In his previous roles, Dr. Walia led review of CDx devices, breakthrough devices, PMAs, 510(k), DeNovo, and COVID-19 EUA devices at the US FDA. Dr. Walia was also the FDA Commissioner's fellow (Class of 2016) and was actively involved in the launch of the precisionFDA initiative. Dr. Walia's past research at NCI and NHGRI focused on rare genetic diseases and cancers. Dr. Walia has a Ph.D. in Pharmacology, MBA in general management, and is a lifelong member of Harvard Business School community and FDA Alumni Association.

Panelists: Cultivating Inclusion: A Roadmap for Scientists in Training

Ashley Bear, Ph.D.
Director of the Committee on Women in Science, Engineering, and Medicine,
National Academies of Sciences, Engineering, and Medicine

Dr. Ashley Bear is the Director of the Committee on Women in Science, Engineering, and Medicine. In this capacity, Dr. Bear oversees a portfolio of projects dealing with critical issues relating to increasing the participation and leadership opportunities for women in these fields. In her time at the Academies, Dr. Bear has led committees of experts and a dynamic staff team in carrying out consensus studies, workshop series, and national outreach efforts with the goal of providing a range of stakeholders with advice and guidance on how to support the improved recruitment, retention, and advancement of women in science, engineering, and medicine. Before coming to the Academies, Dr. Bear worked for the National Science Foundation's (NSF) Division of Biological Infrastructure in the Directorate for Biological Sciences, where she managed a portfolio of mid-scale investments in scientific infrastructure and led analyses of the impact of NSF funding on the career trajectories of postdoctoral researchers. Dr. Bear also previously worked as a Science Policy Officer for the State Department's Office of the Science and Technology Adviser to the Secretary of State, where she worked to promote science diplomacy and track emerging scientific trends with implications for foreign policy, managed programs to increase the scientific capacity of State Department, and acted as the liaison to the Bureau of Western Hemisphere Affairs and the Bureau of East Asian and Pacific Affairs. Dr. Bear holds a Sc.B. in Neuroscience from Brown University and a Ph.D. in Ecology and Evolutionary Biology from Yale University.

Danny Dickerson, Ph.D.
Director of Diversity and Inclusion Division,
National Institutes of Health

Mr. Danny Dickerson is the Director, Division of Inclusion and Diversity, for the National Institutes of Health (NIH). He is primarily responsible for overseeing the administration of the Affirmative Employment Programs and managing a robust Diversity and Inclusion Portfolio. Mr. Dickerson has also served as the EDI Senior Policy Advisor, as well as a Reasonable Accommodations consultant. Mr. Dickerson is also a member of the Equity, Diversity, and Inclusion (EDI) training cadre, where he is responsible for conducting training in the areas of Anti-Bullying, Equal Employment Opportunity Compliance, Civil Treatment, and LGBTI training (Safezone). Previously Mr. Dickerson was a Senior Equal Employment Opportunity Specialist (EEO) at the Department of Health and Human Services (HHS). Mr. Dickerson retired from the United States Air Force after twenty years of service.

Giovanna Guerrero- Medina, Ph.D.
Director, Yale Ciencia Program,
Director, Diversity, Equity, and Inclusion, Wu Tsai Institute, Yale University
Executive Director, Ciencia Puerto Rico

Dr. Giovanna Guerrero-Medina is Director of the Yale Ciencia Initiative in the Office of Diversity, Equity and Inclusion of Yale School of Medicine, and Assistant Director for Diversity, Equity and Inclusion of Yale's Wu Tsai Institute. Through these positions, Dr. Guerrero-Medina leads programs that broaden participation and increase inclusion and retention in STEM training pathways and careers. She is Principal Investigator of the Yale Ciencia Academy, an NIH-funded professional development program that has provided >210 biomedical and health science PhD students across the U.S. with training, outreach opportunities, mentoring, and peer support. She is also the Co-Chair of the Intersections Science Fellows Symposium, a platform to accelerate a more diverse professoriate. Dr. Guerrero-Medina is the founding Executive Director of Ciencia Puerto Rico. Under her leadership, CienciaPR has become one of the largest networked communities of Hispanic scientists in the world. The organization has been recognized for their work promoting justice, equity, diversity, and inclusion in the areas of science communication, education, and careers by the Union of Concerned Scientists, the AAAS-Caribbean Division, and the White House. Originally from Puerto Rico, she has a Bachelors in Science from the University of Puerto Rico Rio Piedras and a PhD in Molecular and Cell Biology from the University of California, Berkeley, followed by a science policy fellowship with the National Academies and science policy work at the NIH and the Van Andel Institute. She has served on two committees of the National Academies of Sciences, Engineering and Medicine for her expertise in the development of inclusive scientific communities. Dr. Guerrero-Medina's work is funded by grants from the NIH/NIGMS and the Burroughs Wellcome Fund, among others.

Tiffany Wallace, Ph.D.
Program Director, CRCHD,
Office of the Director, National Cancer Institute

Dr. Wallace is the Program Lead for the Disparities and Equity Program (DEP) at the NCI within the Center to Reduce Cancer Health Disparities (CRCHD). In this role, she coordinates and strengthens NCI's overall cancer disparity research activities, encompassing basic, clinical, translational, and population-based research. In addition to her roles in CRCHD, Dr. Wallace co-chairs the NCI's Cancer Disparities Activities Committee (CDAC) and an NCI Equity and Inclusion Program Working Group, as well contributing to numerous health disparity initiatives across NIH.

Prior to joining the CRCHD, Dr. Wallace was an Oncology Scientist at Human Genome Sciences, where she managed clinically relevant research programs and conducted preclinical development of promising cancer therapeutics. Dr. Wallace received her Ph.D. in biomedical sciences from the University of Florida in Gainesville, FL. She completed her postdoctoral training in the Laboratory of Human Carcinogenesis at NCI, where she conducted basic and translational research to identify biomarkers of aggressive prostate and breast cancer, with a focus on variations between different racial/ethnic groups.

Rob Winn, M.D.
Director, Massey Comprehensive Cancer Center,
Virginia Commonwealth University

As director of VCU Massey Comprehensive Cancer Center, Robert A. Winn, M.D., oversees a cancer center designated by the National Cancer Institute that provides advanced cancer care, conducts groundbreaking research to discover new therapies for cancer, offers high-quality education and training, and engages with the community to make advancements in cancer treatment and prevention equally available to all. He is leading the nation in establishing a 21st-century model of equity for cancer science and care, in which the community is informing and partnering with Massey on its research to best address the cancer burden and disparities of those the cancer center serves, with a local focus but global impact.

His current basic science research, which has been supported by multiple National Institutes of Health and Veterans Affairs Merit awards, focuses on the molecular mechanisms and novel therapeutic approaches for human models of lung cancer. He has authored or co-authored more than 80 published manuscripts in peer-reviewed academic journals.

As a pulmonologist, Winn is committed to community-engaged research centered on eliminating health disparities. He is a principal investigator on several community-based projects funded by the NIH and National Cancer Institute, including the All of Us Research Program, a NIH precision medicine initiative. Winn has nearly 20 years' commitment to Veterans Affairs health services and held appointments at the Denver VA and Jesse Brown VA in Chicago, where he established the first multidisciplinary pulmonary nodule clinic.

Winn is the President of the Association of American Cancer Institutes (AACI); the Chair of the National Cancer Policy Forum of the National Academies of Sciences, Engineering, and Medicine; a Fellow of the American Association for Cancer Research (AACR) Academy; and a member of the Board of Directors for the American Cancer Society and LUNGevity Foundation. The recipient of numerous awards and honors, Winn has received the National Cancer Institute Center to Reduce Cancer Health Disparities CURE Program Lifetime Achievement Award; the AACR-Minorities in Cancer Research Jane Cooke Wright Lectureship; the AACI Cancer Health Equity Award; and the Prevent Cancer Foundation Cancer Prevention and Early Detection Laurel Award for Increasing Health Equity. In 2022, the Bristol Myers Squibb Foundation Diversity in Clinical Trials Career Development Program was renamed the Robert A. Winn Diversity in Clinical Trials Award Program (Winn Award), which is committed to increasing diversity in clinical trials and training the new generation of community-oriented clinical researchers.

Winn holds a B.A. from the University of Notre Dame and an M.D. from the University of Michigan Medical School in Ann Arbor. He completed an internship and residency in internal medicine at Rush-Presbyterian-St. Luke's Medical Center in Chicago and a fellowship in pulmonary and critical care medicine at the University of Colorado Health Sciences Center in Denver.

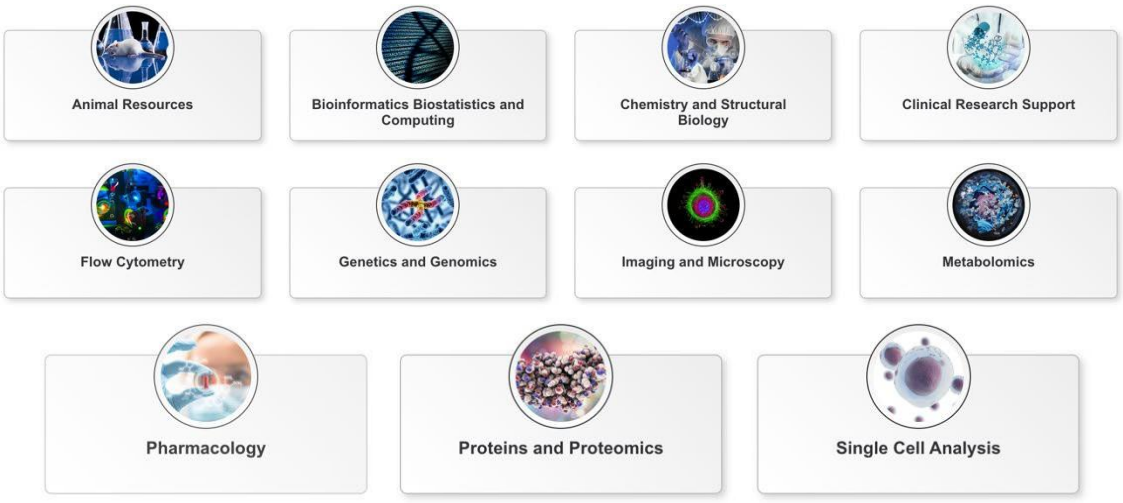
Core Resources Available to CCR Scientists

**Please plan to stop by the OSTR table during breaks and poster sessions to learn how the CCR cores can assist you with your research.
It is located next to the registration desk outside of the conference room.**

The CCR Office of the Director (OD) has invested heavily in technology infrastructure that is made available through **centralized core facilities**. The CCR Office of Science and Technology Resources (OSTR) within the CCR OD serves a central role in planning and implementing new technologies based on the emerging needs of CCR investigators. This includes advanced instrumentation and specialized expertise required for **making innovative technologies broadly available** to CCR investigators. These centralized resources offer established applications and assays but also engage in technology enhancement and development to innovate solutions based on the unique needs of CCR research. While access to most facilities is fully or heavily subsidized, **supplemental funds dedicated for technology access** through the STARS program are available to further reduce costs for expensive technologies or large and complex projects.

OSTR has also created a comprehensive bioinformatics program, the CCR Collaborative Bioinformatics Resource, involving collaborative support for complex genomic analysis. Furthermore, OSTR has developed a training program (Bioinformatics Training and Education Program) designed to educate the CCR scientific community about bioinformatics approaches to empower CCR researchers to perform basic, informed set of analysis on their own behalf. Lastly, OSTR provides the CCR research community access to a variety of centrally funded scientific software tools. These include commercial applications for pathway analysis, as well as genomics and proteomics interpretation of complex datasets.

To browse the various cores open to all CCR scientists, please visit the OSTR Core Resources webpage and select the appropriate discipline:
https://ostr.ccr.cancer.gov/resources/core



Abstracts for Oral Presentations

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5- AVENGERS: Analysis of Variant Effects using Next Generation sequencing to Enhance BRCA2 Stratification
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Abstracts for Poster Presentations

Some abstracts were withheld from publishing by authors' request.
The title and author list are printed in these instances.

Bioinformatics, Epidemiology, and Translational Research

1- Effects of Treatment Delays on Lung Cancer Survival

Guzman, Percy[1,3]; Segura, Sharon[2]; Halpern, Michael[3]

Background: Previous studies have established a link between treatment initiation delays and reduced overall survival in individuals diagnosed with lung cancer. These delays are often influenced by diagnostic complexities, logistical hurdles, and patient-related factors. However, the association between treatment delays and survival outcomes has yet to be extensively investigated using data from a large national cohort. This study aims to fill this research gap and provide unique insights into the relationship between treatment delays and lung cancer survival by utilizing data from a substantial national cohort.

Methods: The study population consisted of 3,723 lung cancer patients participating in the NCI PLCO Cancer Screening Trial. Cox Proportional Hazards (PH) Regression analyses examined the time between cancer diagnosis and treatment initiation using several measures including intervals from tumor doubling/stage progression time models by Spratt et al. (1964) and Detterbeck et al. (2008). Analyses controlled for patient and clinical characteristics including age, sex, race/ethnicity, smoking status, histopathologic type, cancer stage, and treatment modality. Data analysis was conducted using Microsoft Excel, STATA, and IBM SPSS software.

Results: PH Regression analyses demonstrated a clear association between longer time intervals from diagnosis to treatment initiation and increased risk of mortality. Patients with time intervals exceeding 20 days exhibited a hazard ratio (HR) for mortality of 1.21 ($p < 0.001$, 95% confidence interval [CI] 1.1158-1.3176). The Spratt tumor progression model, considering intervals exceeding 88 days, revealed a HR of 1.22 ($p = 0.03$, 95% CI 1.0146-1.4885). On the other hand, the Detterbeck model, which considered intervals exceeding 118 days, did not show a statistically significant association with increased mortality risk. Furthermore, PH analysis identified cigarette smoking status, marital status, and tumor grade as significant predictors of mortality risk.

Conclusions: Data from a large national cohort study indicate that delays in lung cancer treatment initiation have detrimental impacts on survival. Treatment initiation shortly after diagnosis had the most significant differential impact on mortality risk. Timely initiation of treatment is of utmost importance and should be prioritized in lung cancer care. By addressing factors contributing to treatment delays, it is likely possible to improve the survival rates of patients with lung cancer.

2- Targeting DLK1, a Notch ligand, with an antibody-drug conjugate in adrenocortical carcinoma

Nai-Yun Sun[1], Suresh Kumar[1], Amber Weiner[2], Yoo Sun Kim[1], Arnulfo Mendoza[3], Rosa Nguyen[3], Reona Okada[3], Yves Pommier[1], Dan Martinez[4], Jennifer Pogoriler[4], Sharon Diskin[2], John Maris[2], Jaydira Del Rivero[1] and Nitin Roper[1]

Adrenocortical carcinoma (ACC) is an aggressive and rare endocrine malignancy with poor prognosis. First-line therapy (adrenolytic agent mitotane in combination with chemotherapy) for recurrent and metastatic ACC has limited efficacy and there are no approved second-line therapies. Antibody-drug conjugates (ADCs) are an emerging immunotherapeutic class in which a cytotoxic payload is directed to tumor cells via antibodies to cell surface proteins. As Notch ligands, such as DLL3, are ADC targets in neuroendocrine cancers, we screened for expression of Notch ligands in ACC. Among Notch ligands (DLL1, DLL3, DLK1, JAG1, JAG2), DLK1 (delta-like non-canonical Notch ligand 1) was the most highly expressed. Moreover, ACC had the near highest expression of DLK1 across all TCGA tumors likely because this ligand is of adrenal origin (with limited expression in other normal tissues apart from the pituitary gland and ovaries). By immunohistochemistry, we verified DLK1 to be expressed, at variable levels, in 97% of ACC tumors (n=30/31). We then tested a DLK1-directed antibody-drug conjugate (ADCT-701; DLK1-PBD), consisting of a humanized anti-DLK1 monoclonal antibody coupled to DNA damaging pyrrolobenzodiazepine (PBD) dimers (SG3199; drug-to-antibody ratio~1.8) via a cleavable linker in ACC pre-clinical models. ADCT-701 exhibited potent, nanomolar cytotoxicity in DLK1-expressing ACC cell lines and ACC patient-derived short-term organoid cultures. ADCT-701 cytotoxicity was dependent on DLK1 expression as DLK1 knockout and DLK1 overexpression abrogated and facilitated cytotoxicity, respectively. Mechanistically, ADCT-701 induced cytotoxicity through DLK1 dependent receptor-mediated internalization and DNA damage (γH2AX) as well as apoptosis (annexin V/PI positive cells, cleaved PARP, and cleaved caspase-3). In vivo studies showed that ADCT-701 was highly effective against DLK1-positive ACC xenografts and PDX models with durable anti-tumor activity. Our pre-clinical data demonstrate DLK1 as an important therapeutic target in ACC and support an upcoming phase I clinical trial of ADCT-701 in patients with neuroendocrine tumors including ACC (NCT06041516).

3- Survival Dynamics of Transcript Alternations and Differential Splicing in the Evolution of Acquired MAPKi Resistance in Melanoma

Sumit Mukherjee [1], Arashdeep Singh [1], Hyunjeong Joo [2], Sumeet Patiyal [1], Hyungsoo Kim [2], Lipika R. Pal [1], Kun Wang [1], Chi-Ping Day [3], Ze'ev A Ronai [2], Eytan Ruppin [1], Sridhar Hannenhalli [1]

The primary therapy for melanoma with a BRAF mutation involves Mitogen-Activated Protein Kinase inhibitors (MAPKi), targeting the dysregulated MAPK signaling pathway. Despite the initial response, many patients often develop acquired resistance. Resistance to MAPKi could be linked to various altered signaling pathways, including reactivation of the MAPK pathway, activation of alternative survival pathways like PI3K/PTEN/AKT, engagement of receptor tyrosine kinases (RTKs) such as PDGFRβ and EGFR, and developmental pathways. While numerous omics studies have aimed to understand the mechanism of acquired resistance, how these pathways are altered remains elusive. To gain further insights into the acquired MAPKi resistance in melanoma, we explored the role of RNA splicing events in this context by analyzing publicly available datasets of pre- and post-MAPKi treated melanoma from patients and in vitro studies using cell lines. We investigated the differential transcript usage (DTU) to detect the specific splice variants that are altered during the development of resistance. We find that the genes involving DTU are enriched in various pathways related to MAPKi resistance, such as the MAPKi signaling pathway, PI3K/AKT pathway, signaling by RTKs, etc. Furthermore, the DTU-centered analysis provides better insights into MAPKi resistance mechanisms compared to standard differential gene expression analysis. Next, we analyzed the developmental splicing signatures during MAPKi resistance development, revealing developmental reprogramming as a key factor in MAPKi resistance. Further, we identified the top 10 transcription factors as potential upstream regulators of the perturbed transcripts, among which five have been previously reported to be involved in various stages of embryonic development. This finding suggests a link between the reactivation of developmental pathways and MAPKi resistance development. Further, by analyzing MAPKi-treated melanoma cell-line models at different time points, such as no-treatment, on-treatment, and at the resistant stage, we observed significant alterations in transcript isoforms during the development of resistance. These changes involve lipid metabolic reprogramming and PI3K/Akt/mTOR signaling, providing further insights into the mechanisms of MAPKi resistance. Our study also uncovers a global shift in transcript profiles towards shorter 3' and 5' UTRs in response to MAPKi treatment, suggesting the existence of a master regulatory mechanism influencing global splicing changes during therapy resistance. In summary, our study deciphered the post-transcriptional mechanism coordinating the signaling changes, underscoring the complexity of MAPKi resistance in melanoma, as revealed by the joint analysis of both transcript alterations and splicing events.

4- CAR-T cells based on a TCR mimic nanobody targeting HPV16 E6 show antitumor activity against cervical cancer

Zhijian Duan[1], Dan Li[2], Nan Li[2], Shaoli Lin[2], Hua Ren[2], Jessica Hong[2], Christian Hinrich[3], Mitchell Ho[1,2]

E6 and E7 oncoproteins of human papillomavirus (HPV) are considered promising targets for HPV-related cancers. In this study, we evaluated novel T cell receptor mimic (TCRm) nanobodies targeting E629-38 peptide complexed with human leukocyte antigen (HLA)-A*02:01 in the chimeric antigen receptor (CAR) format. We isolated two dromedary camel nanobodies, F5 and G9, by phage display screening. F5 bound to the complex expressed on cells including peptide pulsed T2, overexpressed 293E6, and cervical cancer lines CaSki and SS4050 more efficiently than G9 did. CAR-T cells based on the F5 nanobody showed specific killing of target cells, including 293E6, CaSki, and SS4050 in vitro. Importantly, F5 CAR-T cells inhibited the growth of CaSki and SS4050 tumor xenografts in mice. These findings demonstrate that HPV-16+ cervical cancer can be targeted by F5 nanobody-based CAR-T cells, which offer a valuable alternative strategy for treatment against HPV-16+ malignancies.

5- AVENGERS: Analysis of Variant Effects using Next Generation sequencing to Enhance BRCA2 Stratification

Sounak Sahu[1], Melissa Galloux[2], Eileen Southon[1], Dylan Caylor[1], Teresa Sullivan[1], Matteo Arnaudi[3,4], Josephine Geh[1], Raj Chari[5], Elena Papaleo[3,4], Shyam K. Sharan[1]

Background and Hypotheses: Increased genetic testing with the ease of Next-Generation sequencing (NGS) approaches has led to the identification of genetic variants, of which the majority are DNA repair genes that are frequently mutated in somatic cancer. Due to limited access to epidemiological data to determine their pathogenicity, many of these genetic variants are of unknown clinical significance (VUS). To date, the BReast CAncer gene- BRCA2 has the highest number of VUSs in clinical repositories like ClinVar. We have utilized mouse embryonic stem cells (mESC) as a model system for the functional evaluation of BRCA2 VUSs using a recombineering-based method, leading to the classification of around 400 VUSs reported in ClinVar. While this approach has proven extremely reliable, it is time-consuming with limited potential to multiplex these variants. To overcome this shortcoming, we have developed "AVENGERS" acronym for Analysis of Variant Effects using NGs to Enhance BRCA2 Stratification, utilizing CRISPR-Cas9-based saturation genome editing (SGE) in a humanized-mouse embryonic stem cell line.

Study design and methods: Mammalian haploid cells have been immensely informative in uncovering cellular phenotype. This allows us to perform high-throughput genome editing studies by bypassing the possibility of having an unedited allele or harboring different mutations in the other allele. We have generated a humanized mouse embryonic stem cell line (mESC) expressing a single copy of human BRCA2 that can rescue the lethality of Brca2-deficient mESC. Using SGE, we have changed every nucleotide position to all non-wild type nucleotides along exon 15-26 of BRCA2 that codes for the C-terminal DNA binding domain (CTD).

Results and conclusions: Loss of BRCA2 being cell lethal, we show a significant drop in the frequency for pathogenic variants of BRCA2, whereas functional variants (neutral) are enriched in the pool. We have categorized nearly all possible missense single nucleotide variants (SNVs) encompassing the C-terminal DNA binding domain of BRCA2. We have generated the function scores for 6270 SNVs, covering 95.5% of possible SNVs in exons 15-26 spanning residues 2479-3216, including 1069 unique missense VUS, with 81% functional and 14% found to be nonfunctional. Our classification aligns strongly with pathogenicity data from ClinVar, orthogonal functional assays, and computational meta-predictors. Our statistical classifier exhibits 92.2% sensitivity and 96% specificity in distinguishing clinically benign and pathogenic variants recorded in ClinVar. Furthermore, we offer proactive evidence for 617 SNVs being non-functional and 3396 SNVs being functional demonstrated by impact on cell growth and response to DNA-damaging drugs like cisplatin and olaparib.

Relevance and importance: The clinical interpretation of genetic variants is still a bottleneck for personalized medicine. Only 2% of missense variants have a clinical interpretation which imposes significant challenges for patients and clinicians for genetic counselling and risk assessment. Using this high throughput approach, we have classified more than 6000 SNVs of BRCA2 that include the variants reported in ClinVar along with functional data of variants that are yet to be identified in patients, giving a piece of first-hand information to clinicians for personalized medicine.

6- Novel protein drug alters the tumor microenvironment of pancreatic cancer

Mayrel Palestino Dominguez[1], Philip Homan[2], Xianyu Zhang[1], Sandra Navas Reyes[3], Theresa Guerin[3], Laura Bassel[3], Serguei Kozlov[3], and Christine Alewine[1]

Pancreatic cancer is the third leading cause of cancer-related death in the United States, and the incidence increases by one percent every year. The unique tumor microenvironment (TME) of pancreatic ductal adenocarcinoma (PDAC) contributes to drug resistance, and current data suggests modulation of multiple components of the TME will be required for enhanced therapeutic efficacy. ProAgio is a small protein therapeutic that was specifically designed to bind outside the RGD ligand pocket of the alpha V-beta 3 (αV, β3) integrin heterodimer, then to induce apoptosis in cells expressing this target. ProAgio is currently undergoing testing in a Phase 1 clinical trial with favorable safety profile seen so far, but the effect of ProAgio on cellular components within PDAC TME remains largely unexplored. We hypothesize that ProAgio decreases the stroma density and alters the TME cellular components, leading to decreased tumor-induced immunosuppression. We re-analyzed publicly available single cell RNA-seq datasets of human and mouse PDAC samples, assessed integrin co-expression using flow cytometry, performed immunophenotyping, histopathological analysis and nCounter assay to identify changes in TME cell subsets, structure and gene expression induced by ProAgio. Integrins αV and β3 were both expressed at the RNA level in tumor microarray, myeloid cells, lymphocytes (T, B and NK), endothelial cells and multiple CAF subtypes. In the autochthonous KPC mouse model, we confirmed that myeloid cells, CD4+ and CD8+T cells co-expressed integrin αVβ3 at the protein level. One week of ProAgio treatment retarded tumor growth and increased tumor collagen content. After 2 weeks of ProAgio treatment, no changes in immune components, CAF subsets or endothelial cells were observed, but treated tumors had decreased density and nCounter analysis identified ProAgio-dependent effects on proliferation, angiogenesis, and apoptosis. Concordantly, in vitro studies have shown that ProAgio can alter viability of some cancer cell lines and induce proliferation of macrophages. Future studies will seek to identify the main targets of ProAgio and refine the proposed mechanism of ProAgio action in the PDAC TME.

7- Anti-VEGF treatment enhances immune checkpoint inhibitor antitumor responses by targeting regulatory T cells

Mohamed-Reda Benmebarek[1], Cihan Oguz[2], Benjamin Ruf[1], Yuta Myojin[1], Patrick Huang[1], Kylynda C. Bauer[1], Chi Ma[1], Matthias Seifert[1], Marina Villamor-Payà[3], Marlaine Soliman[1], Rajiv Trehan[1], Cecilia Monge[1], Changqing Xie[1], Justin Lack[2], William Telford[4], Firouzeh Korangy[1], Tim F. Greten[1,5]

Background: Previously we reported early and unexpected results of an open label phase II trial treating patients with histologically confirmed hepatocellular carcinoma (HCC) and cholangiocarcinoma (CCA) treated with anti-CTLA4 + anti-VEGF + anti-PD-L1. We described exceptional clinical responses in 6 out of 7 patients accompanied with moderate to severe immune-related adverse events in the majority of patients (1). Preliminary correlative studies using paired PBMC and serum samples demonstrated a proinflammatory cytokine profile, reinvigoration of precursor exhausted CD8 T cells, and expansion of the Treg and non-classical monocyte subsets. Herein, we aimed to further translate these findings to better understand the mechanisms behind the striking clinical efficacy and treatment-related toxicity.

Methods: We utilized the subcutaneous (s.c.) and intrahepatic SB1, as well as the plasmid-induced YAP-AKT CCA mouse models, previously described by our group (2), to study treatment efficacy, explore changes in the tumor immune microenvironment (TME), and investigate the mechanistic interplay potentiated by the treatment combination.

Results: In three murine CCA models, the aVEGF + aCTLA4 + aPD-L1 combination induced significantly better tumor control over the aCTLA4 + aPD-L1 double therapy or vehicle-treated groups. Cytokine analysis of mouse tumor lysates revealed that additional VEGF-A blockade led to an IFN signature characterized by higher levels of CXCL9, CXCL10, IFN-□ and IFN-□ in mice. Tumor immune profiling revealed an increase of infiltrating B cells, driven by higher levels of monocyte-derived B cell activating factor (BAFF) in the TME. Activated B cells adopted a Be-1 phenotype characterized by enhanced IL-12 production. Furthermore, there was an increase in the frequency of Tregs in the TME of triple-treated mice. These tumor-infiltrating Tregs were rewired towards a ‘fragile’ Th1-like phenotype characterized by higher expression levels of IFN-□□and T-bet. Congruently, the suppressive function of triple-treated tumor-infiltrating Tregs was impaired. IL-12 blockade abrogated the therapeutic effects of the triple-therapy in mice and reversed the fragile Treg phenotype previously observed. B cell depletion and BAFF blockade similarly reversed the therapeutic efficacy of the triple therapy in triple-treated mice.

Conclusions: We identify a novel mechanism describing how additional VEGF blockade could induce critical changes in the immune contexture of HCC and CCA, sensitizing tumors to aCTLA4 + aPD-L1 therapy. Specifically, we found the triple combination potentiated an IFN signature leading to BAFF production by the myeloid compartment. BAFF acted on B cells, resulting in enhanced IL12 production, rewiring Tregs towards a less suppressive, proinflammatory ‘fragile’ phenotype.

8- Psychometric analysis of Neuro-QoL Perceived Cognitive Function tool in primary brain tumor patients

Morgan Johnson[1], Elizabeth Vera[1], Kimberly Reinhart[1], Hope Miller[1], Anna Choi[1], Tricia Kunst[1], Bennett McIver[1], Ewa Grajkowska[1], Michelle L. Wright[1], Terri S. Armstrong[1], Tito Mendoza[2]

9- Cell-type specific transcriptional regulation of APOBEC3A in cancers

Dhanusha Yesudhas, Bilal Lone, Kelly Butler, Arup Chakraborty and Rouf Banday

APOBEC3s (A-H) are a family of seven cytosine deaminases that inactivate viruses as part of the innate immune response. However, APOBEC3A and APOBEC3B have been found to mediate mutagenesis in tumor genomes by deaminating ssDNA that transiently arise during transcription and DNA replication. The mechanisms that trigger these enzymes to generate high mutation burdens in some cancers and affect disease progression and clinical outcomes are largely unknown. Here, we explored cell-type-specific transcriptional regulation of APOBEC3s using single-nucleus/cell RNA-seq (sn/scRNA-seq) data (>100 samples and >300,000 cells) from four tumor types – bladder, lung, breast, and kidney – that display different levels of APOBEC-induced mutation burden. Our analyses revealed that among seven members of the APOBEC3 family, APOBEC3A is expressed in a cell-type-specific manner. The expression was predominant in KRT13 and CDH12 epithelial cell-types and inflamed macrophages. The APOBEC3A+ epithelial cell populations were abundant in the bladder, moderate in the lung and breast, and nearly absent in the kidney tumors. APOBEC3A expression correlated with interferon and pro-inflammatory signaling in KRT13+ cells. Transcription factors ELF3 and ATF3 were significantly upregulated in APOBEC3A+ KRT13 cells. Pseudotime analysis of sn/scRNA-seq data revealed that APOBEC3A cells are in epithelial-mesenchymal transition. Finally, several gene markers defining APOBEC3A+ KRT13 cell types were identified. Ongoing analyses include investigations into clinical correlates, cell-type-specific mutation burdens, and validation through in vitro functional approaches. Our results propose that APOBEC3A is turned on in specific epithelial cell types by multiple signaling pathways during tumorigenesis, thereby driving tumor heterogeneity, particularly in high mutation-burden cancers.

10- Pan-viral serological characterization in African Americans and European Americans with Hepatocellular Carcinoma

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Hepatocellular carcinoma (HCC) is often diagnosed at late-stages, particularly among African Americans (AA). Poor screening and diagnosis are key factors that contribute to worse prognoses and disproportionately high mortality rates. We propose investigating viral exposure history as a potential useful screening method. Additionally, by accounting for neighborhood deprivation index (NDI), a metric for differences in socio-economic statuses, we can potentially mitigate this disparity. With NDI and viral exposure history, our study aims to assess HCC prognoses in racially disparate populations. We used a technique called VirScan, which conjugates Phage-Immunoprecipitation (PhIP) and DNA-sequencing, to profile the antibody repertoire against a viral epitope library. Analyzing serum and plasma samples from population controls (PC), individuals at high-risk for developing HCC (HR), and HCC cases, we performed VirScan on 524 AA (272 PC, 205 HR, and 47 HCC) and 342 European Americans (EA) (141 PC, 130 HR, and 71 HCC) participants from the NCI-UMD cohort between 2009-2020 from the greater Baltimore area (NCT00913757; clinicaltrials.gov). We used XGBoost, a machine-learning model, to classify viral features that can discriminate HCC cases from PC group. XGBoost predicted 31 important viral features which distinguish HCC from PC. Of the 31 viral features, 6 viruses were different between AA and EA (FDR < 0.05). To determine the extent to which socio-economic factors contribute to the cancer disparities, we gathered variables from the 1999 census tract as contributors to deprivation. We used Principal Component Analysis (PCA) to determine loadings of these contributor variables; composite of the final variables is derived as NDI. Comparing across groups, we find that average NDI is highest in HR group ($x = 0.33$), followed by HCC group ($x = 0.21$), then PC group ($x = 0.19$). We find that NDI for AA is significantly higher in HCC group ($p = 1.3e-09$), HR group ($p < 2.2e-16$), and PC group ($p = 5.0e-10$) when compared to that of EA.Thus, we demonstrate that both the viral exposure history and NDI of an individual might be useful in prognoses of HCC. This approach could be used to examine the disparities we see in both incidence and mortality of HCC between AA and EA.

11- Long-Term Follow-Up Post-CAR T-Cell Therapy in Children and Young Adults (CAYA) with r/r B-Cell Acute Lymphoblastic Leukemia (B-ALL)

Chloe N. Hoang[2], Bonnie Yates[1], Lauren Little[3], Toni Foley[2], Monica Epstein[4], Haneen Shalabi[1] and Nirali N. Shah[1]

Background/Hypotheses: Chimeric antigen receptor (CAR) T-cell therapies have been used in CAYA with relapsed/refractory (r/r) B-ALL for over 10 years, yet data on long-term follow-up (LTFU) is limited. Given the potential role of CAR T-cells in achieving long-term cure, we hypothesize that this analysis is imperative to build a foundation of the late effects following CAR T-cell therapy in CAYA.

Study Design/Methods: We retrospectively evaluated long-term outcomes in CAYA with r/r B-ALL who received CAR T-cells in one of our 3 phase I studies targeting CD19 and/or CD22, and remained in LTFU (NCT03827343). The primary cohort were those who were > 2 years post-CAR T-cell induced remission, had not received additional disease directed therapy, and included those who proceeded to a consolidative stem cell transplant (SCT). The secondary cohort comprised CAYA who did not achieve a CR or had a confirmed relapse post-CAR T-cells, but currently remain in an ongoing remission. The secondary objective was to describe their clinical course following post-CAR T-cell relapse. Outcomes focused on the LTFU safety parameters per FDA gene therapy guidance, with additional review of current medications and ongoing medical issues. Data was extracted from chart review. Statistical analysis was primarily descriptive. Data cut-off was September 1, 2023.

Results/Conclusions: The primary cohort consisted of 25 patients. 24 (96%) proceeded directly to SCT post-CAR T-cell induced remission. Median age at CAR T-cell infusion was 19 years (range, 9-36). Median follow-up time was 6.7 years (range, 2.1-10.4). Median lines of pre-CAR T-cell therapy were 3, and 4 patients received prior SCT. Two (8%) developed a new primary cancer (papillary thyroid and a fatal cholangiocarcinoma), 4 (16.7%) had a new neurologic disorder (including: trouble focuing, memory and problem-solving difficulties, and neuropathy), 1 (4.2%) had a significant infection within the past year, and 17 (70.8%) had “other” illnesses. This “other” category was comprised of 6 (25%) patients with nonmalignant tumors, 15 (62.5%) with endocrinopathies, and 5 (20.8%) with cGVHD. Seventeen (70.8%) remained on active medications. The secondary cohort was comprised of 10 patients. The median lines of prior therapy excluding SCT was 6 (range, 2-8), with 4 patients having prior SCT. Median age at CAR T-cell infusion was 13.5 years (range, 6-28). Three (12%) did not achieve a CR post-CAR T-cells and 7 (28%) had a confirmed relapse. Median time to relapse was 1.2 years (range, 0.5-3.9). A variety of subsequent treatments were used post-CAR T-cell to induce remission in this cohort, most commonly reflecting a combinatorial approach with additional CAR T-cells, SCT, targeted agents, and/or chemotherapy. The median duration of CR was 3.2 years (range, 0.8-6.6) and median follow-up time post-CAR T-cell therapy was 4.2 years (range, 2.4-8.4). Prospective studies are necessary to compare the long-term toxicities in CAYA with B-ALL who receive CAR T-cells as a bridge to SCT to CAYA directly undergoing SCT.

Relevance/Importance: Retrospective studies of post-CAR T-cell late-effects will serve as a critical foundation to understand what late effects may be experienced, particularly in those who do not require a consolidative SCT for cure.

12- Transcriptional Characterization of Resistance in Early Drug Response

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Therapeutic resistance is a leading cause for treatment failure and cancer death. While resistance can be driven by genetic mutations, mounting evidence points to epigenetic basis of resistance. Much of this epigenetic, or non-genetic, resistance has been attributed to drug resistant transcriptional cell states that are either induced by drug treatment or pre-exist in a fraction of cells and are selected for upon treatment. It is however not well understood to what extent and what aspects of long-term resistance is manifested in the early inherent cellular response to drugs, and what aspects, if any, of this early response to drug-induced transcriptional response are evolutionarily conserved properties of cells. To address these questions, here we integrate multiple datasets of long-term resistance in multiple cell lines and early response data across multiple cell lines and drugs, as well as the data on drug response and resistance in bacteria and yeast. Our findings suggest that cell states in the drug naive population as well as in the population shortly after treatment share transcriptional properties of fully established resistant cell states. Furthermore, the resistance states manifested as early response to drugs are evolutionarily conserved. Finally, we show that early resistant states discriminate responders from non-responders across multiple human cancer trials.

13- The Role of Germline-Somatic Interactions in Breast Cancer Treatment Response

Jonathan Wooten[1,2], Santiago Avila[3], Becca Pollard[4], Padma Sheila Rajagopal[1]

Background and Hypotheses: Testing for germline (inherited or hereditary) and somatic (tumor-specific) genetic variants in cancer patients is a critical step in oncology patient management, as these are independently used as predictive or prognostic biomarkers. However, only ~1% of oncology clinical trials describe any use of germline, hereditary, or inherited data. This is primarily because recommendations for germline testing in cancer patients only began accelerating after 2014 with the FDA approval of olaparib, a PARP inhibitor recommended for patients with germline BRCA1/2 mutations. As such, there has been limited information collected until recently on germline and somatic variants in the same patients. It is unknown how the presence or absence of concurrent germline and somatic variants may affect anticipated treatment response based on only one of these variants. We hypothesize that germline and somatic interactions are associated with variation in treatment response in patients with cancer.

Study Design and Methods: Next-generation sequencing data from clinical trials or datasets identified through dbGaP, GEO, clinicaltrials.gov, and literature searches were extracted and filtered to identify clinical trials in breast cancer in which patients had germline and somatic sequencing. We will utilize the NIH Integrated Data Analysis Platform (NIDAP) to organize and visualize the clinical and variant data in a relational database framework. We will use standardized variant calling pipelines to identify clinical variants in germline (from blood) and somatic (from tumor) sequencing. We will perform association testing between germline-somatic variants, interaction effects, and treatment response in breast cancer patients.

Results and Conclusions: This project is a work in progress. A total of 12 trials/datasets have been identified that total 1,723 patients across at least 3 treatment options. Potential measures of response that can be studied across these trials include pathological complete response (pCR) in early-stage patients, overall survival (OS), progression free survival (PFS), relapse free survival (RFS), >2 year response, (Y/N) response status, and duration of disease response in metastatic patients.

Relevance and Importance: Although germline and somatic data are collected clinically, their implications have been historically siloed, with germline data (outside of BRCA1/2) primarily used for cancer risk and somatic data primarily used for cancer treatment response prediction. This has made it more difficult to leverage all this data on patients' behalf. Despite the large number of clinically identifiable germline variants, oncology clinical trials focus almost entirely on genes related to DNA damage repair. There is a critical opportunity to expand the use of germline data in the oncology context. Associations drawn between germline and somatic variants in this study will potentially support clinicians and patients in improving their decision-making about cancer treatment options.

14- Association between C-reactive protein and cognitive impairment in IDH-mutant gliomas

McKenzie C. Kauss[1], Michelle L. Wright[1], Dhaivat Raval[1], Emory Hsieh[1], Kaitlynn Slattery[1], Terri S. Armstrong[1], Tito R. Mendoza[2], Vivian A. Guedes[1]

15- The impact of neighborhood-level disadvantage on the patient-reported outcomes of primary brain tumor patients

Zuena Karim[1], Kimberly Reinhart[1], Orieta Celiku[1], Yeonju Kim[1], Hope Miller[1], Elizabeth Vera[1], Jacqueline B. Vo[2], Terri S. Armstrong[1], Macy L. Stockdill[1]

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

Bennett Ann McIver[1], Tara S. Davis[1], Kimberly Reinhart[1], Liz Vera[1], Anna Choi[1], Tricia Kunst[1], Morgan Johnson[1], Ewa Grajkowska[1], Hope Miller[1], Terri S. Armstrong[1], Michelle L. Wright[1]

17- Automated Detection and Grading of Extra-prostatic Extension of Prostate Cancer at MRI via Cascaded Deep Learning and Random Forest Classification

Benjamin D Simon[1,2], Katie M Merriman[1], Stephanie A Harmon[1], Bradford J Wood[3,4], Peter L Choyke[1], Peter A Pinto[5], Baris Turkbey[1]

Background and Hypothesis: Extra-prostatic extension (EPE) is a well-established factor that is significantly predictive of prostate cancer severity and recurrence after localized therapy. Accurate EPE assessment prior to radical prostatectomy is crucial and can impact surgical approach. We aim to utilize a deep learning-based AI workflow for automated EPE grading and detection on prostate MRI.

Study Design and Methods: An expert genitourinary radiologist conducted prospective clinical assessments of MRI scans for 634 patients and assigned risk for EPE using an established grading technique prior to radical prostatectomy. Of these patients, 507 were used for training and 127 were reserved as an independent test set. Previously developed deep-learning AI models for prostate organ and lesion segmentation were leveraged to extract spatial features for inputs to random forest classification models. Variation of expert prostate organ segmentations were evaluated to generate confidence intervals in AI-generated boundaries. Features extracted from the index lesion, as identified by volume or distance from organ boundary, included outer volume, variance zone volume, inner volume, 3D distance into variance area, 2D (axial) distance into variance area, and repeated measurements using a reflected prostate mask. Models were evaluated using four different AI-based lesion detection probability thresholds, area and/or distance features with and without reflected prostate measurements, and with and without class weights. Model performance in the independent test set was evaluated using balanced accuracy scores, ROC AUCs for each EPE grade, as well as sensitivity, specificity, and accuracy compared to EPE on histopathology using each grade as a cutoff for EPE.

Results and Conclusions: The best performing model achieved a balanced accuracy score of .390 ± 0.078. This was achieved using a lesion detection probability threshold of 0.45, distance features only, no reflected measurements, and no weights. Using the independent test set, this model achieved ROC AUCs for radiologist-assigned EPE grades 0, 1, 2, and 3 of 0.70, 0.63, 0.72, and 0.58 respectively. When using EPE 3 as the threshold for predicting positive EPE on pathology, the AI model achieved a sensitivity of 0.44, specificity of 0.89, and accuracy of 0.80 compared to radiologist sensitivity of 0.44, specificity of 0.96, and accuracy of 0.85. Lowering the EPE threshold to EPE ≥2, the AI model achieved a sensitivity of 0.56, specificity of 0.76, and accuracy of 0.72 compared to radiologist sensitivity of 0.67, specificity of 0.76, and accuracy of 0.74. Further lowering the EPE threshold to EPE ≥1, the AI model achieved a sensitivity of 0.67, specificity of 0.73, and accuracy of 0.72 compared to radiologist sensitivity of 0.78, specificity of 0.64, and accuracy of 0.67.

Relevance and Importance: Our AI-driven workflow for assigning EPE grades at MRI data has the potential to enhance physician decision-making for prostatectomy patients.

18- Serum IL-10 is associated with neurologic symptoms and interference in primary brain tumor patients

Kaitlynn Slattery[1], Michelle L. Wright[1], Dhaivat Raval[1], Emory Hsieh[1], McKenzie C. Kauss[1], Terri S. Armstrong[1], Tito Mendoza[1], Vivian A. Guedes[1]

19- Structural Variant Dynamics in Melanoma: Unraveling Tumor Heterogeneity and Evolution

Xiwen Cui[1,2], Ayse Gokce Keskus[1], Farid Rashidi Mehrabadi[1], Salem Malikic[1], Mikhail Kolmogorov[1], Chi-Ping Day[2], Glenn Merlino[2], S. Cenk Sahinalp[1]

Melanoma is characterized by significant intratumoral heterogeneity and complex evolutionary dynamics. This diversity in genomic alterations leads to the emergence of various subclonal populations within a single tumor. In our research, we established a new model system composed of 24 single-cell-derived clonal sublines (C1-C24), originating from the M4 melanoma model. This model was developed using a genetically engineered hepatocyte growth factor (HGF)-transgenic mouse. We employed Trisicell (Triple-toolkit for single-cell intratumor heterogeneity inference), a cutting-edge computational tool for scalable analysis of intratumor heterogeneity and evaluation based on single-cell RNA mutations. This enabled us to construct a phylogeny tree, revealing melanoma's intricate branching evolutionary patterns. These patterns show ancestral clones evolving into genetically distinct subclones, which demonstrate varied phenotypic traits such as drug sensitivity or resistance, cellular plasticity, and immunogenicity. In our study, we conducted long-read sequencing on these clonal sublines in the phylogeny and identified structural variants (SVs) using Severus, a tool optimized for phasing in long-read sequencing. The types of SVs we discovered include deletions, insertions, amplifications, translocations, and inversions. We explored their roles in subclonal evolution, particularly focusing on how they disrupt genes and accumulate during melanoma progression. Our initial data from eleven sublines indicated a higher prevalence of ancestral SVs, shared by all sublines, compared to subline-specific SVs, representing later events. Notably, the individual sublines showed a higher rate of gene disruption by SVs, hinting at potential functional selection. Our analysis further revealed that SVs common to all sublines are linked with genes in key cell growth pathways, such as Rap1, Hippo, and calcium signaling pathways. In contrast, subline-specific SVs primarily affect genes involved in neurophysiological pathways, such as glutamatergic synapse and morphine addiction pathways. These findings suggest that different genes, associated with various pathways, are disrupted at distinct stages of melanoma progression, providing insights into the genetic factors that may predispose individual melanocytes to melanomagenesis. This methodology presents a comprehensive tool for characterizing tumor genomes and understanding their relationship with disease progression and therapy resistance. We are continuing our analysis to map SVs across the entire mutation-based phylogeny of all sublines, which will further our understanding of melanoma's genomic landscape.

20- Apheresis Product Characteristics Predict Response to CD22 CAR T-cell Therapy in Pediatric and Young Adult Patients with B-ALL

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Background and Hypotheses: While CAR T-cells have transformed therapy options for relapsed B-cell acute lymphoblastic leukemia (B-ALL), 20-30% of patients have primary non-response.(1,2) Data on predictors of response to CAR T-cells, especially in pediatrics, are limited. Given the ability to modify manufacturing and pre-collection therapies to optimize CAR T-cell functionality, identifying key biomarkers at the time of apheresis is imperative for improving outcomes. We hypothesized that the patients' T-cells, prior to CAR T-cell manufacturing, could distinguish responders from non-responders, based on markers of exhaustion and differentiation.

Study Design and Methods: We retrospectively analyzed CD4/CD8-selected apheresis materials (T-cells) from children and young adults who were treated with CD22 CAR T-cells for B-ALL (NCT02315612). Analysis included flow cytometry for T-cell immunophenotype, activation, and exhaustion markers, Seahorse metabolic profiling, and RNA sequencing. We compared patients who achieved complete response (CR) with those who had partial response, progressive, or stable disease (NR). Additional analysis compared patient T-cells to those of healthy donors. Mann-Whitney tests were used to compare flow cytometry and metabolic data between groups. Differential gene expression analysis identified genes with >2x fold change.

Results and Conclusions: Across 30 patient apheresis samples, 22 (73%) achieved CR and 8 (27%) did not (NR). Demographics, with exception of disease burden, were comparable. As expected, median pre-infusion disease burden was higher in patients with NR: 59% (r: 0.33-97.9) vs 28% (r: 0.04-98.2%) in CR (p=0.24).Analysis of flow cytometry data by FlowSOM distinguished NR from CR by absence of activated, naive CD4 cells, and presence of CD4 effector cells. Manual gating confirmed that patients with NR had significantly lower CD4/CD8 ratios, fewer naïve (CD45RA+) CD4 T-cells, lower expression of CD62L, CD28 and CD127 (IL-7R) (activation markers), and higher CD69. RNA sequencing similarly showed lower IL-7R expression in the NR group.Even among patients with high baseline disease burden (M3 marrow, >25% blasts) (n=18), T-cells from those with NR (n=6) differed significantly from those with CR, with fewer CD127+, CD8+CD28+, and CD4+CD45RA+ cells. Specifically, having <50% of CD4 cells expressing CD127 fully separated NR from CR in this high baseline disease cohort. Compared to healthy donors, patients had higher expression of exhaustion markers, including TIM3, LAG3, CD39. However, those with CR resembled healthy donors in all markers that distinguished them from NR. Additionally, while healthy donors had higher ATP production and respiratory capacity and less glycolysis compared with B-ALL patients, metabolic features did not distinguish CR from NR.

Relevance and Importance: Based on our analysis, starting material characteristics can predict non-response to CAR T-cells, using a flow cytometric assay alone. These assays can be integrated into routine apheresis processing, and the results can provide real-time guidance to patients, families, and providers. Additionally, CD4+CD127+ cells in the starting material appear to be necessary to overcome high disease burden, suggesting the importance of optimized manufacturing to expand this favorable cell subset. Finally, the similarity between healthy donors and patients with CR demonstrates the capacity of some heavily pre-treated patients to produce CAR T-cells equivalent to allogenic cells.

21- Endogenous HiBiT-tagging of PAX3-FOXO1 reveals downregulation of the fusion oncogene by CDK inhibitors and has synergy with vincristine

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BACKGROUND: Oncogenic fusion genes are attractive therapeutic targets due to their tumor-specific expression and driver roles in cancers. PAX3-FOXO1 (P3F) is the dominant oncogenic driver of fusion-positive rhabdomyosarcoma (FP-RMS) with no current targeted therapy. We developed methods to directly measure endogenous P3F protein levels amenable to high-throughput drug screens.

METHOD: HiBiT tag, an 11 amino acid peptide of NanoLuc luciferase, was inserted into the endogenous P3F using CRISPR in FP-RMS cell lines RH4 and SCMC. Western blot was used for HiBiT tag and downstream target validation. RNA-seq and ChIP-seq were used to assess transcriptomics and DNA binding of HiBiT-tagged P3F (P3F-HiBiT). High-throughput drug screen using Nano-Glo luciferase assay was performed using the Mechanism Interrogation PlatE (MIPE 5.0) drug library, a 2,480 drug library with known mechanisms of action. CellTiter-Glo was used to monitor cell viability. Mouse xenograft models of FP-RMS were used to investigate in vivo efficacy.

RESULTS: We validated HiBiT tagging of P3F by Western. Both P3F-HiBiT and unmodified P3F activated the same gene sets in fibroblasts by RNA-seq Gene Set Enrichment Analysis (GSEA). ChIP-seq using HiBiT antibody for P3F-HiBiT matched the genomic locations from ChIP-seq with P3F antibody in RH4 and SCMC. Using a cutoff value of > 90 Area Under the Curve (AUC) of CellTiter-Glo minus AUC of HiBiT (Nano-Glo), in both RH4 and SCMC, we identified 182 compounds which downregulate P3F (Nano-Glo) before cell death (CellTiter-Glo). Filtering for drugs with ≥ 3 hits for the same target identified 14 drug classes that suppressed P3F including HDAC inhibitors (3), BRD4 inhibitors (3), and CDK inhibitors (8). FP-RMS was sensitive to CDK1/2, CDK4/6, CDK9, and multi-CDK inhibitors. A multi-CDK inhibitor TG02, currently in human trials, downregulated P3F and RNA-seq GSEA showed marked suppression of P3F targets. TG02 also significantly delayed tumor progression in mouse xenograft model of FP-RMS without weight loss. Interestingly, the commonly used chemotherapeutic Vincristine (VCR) also downregulated P3F in vitro. TG02 with VCR showed synergy by Loewe analysis in vitro. In vivo, testing showed significant delay in tumor progression by the combination compared to TG02 or VCR alone. Of note, tumor RNA-seq GSEA following treatment with TG02, or VCR alone, and in combination significantly downregulated P3F targets.

CONCLUSION: By HiBiT tagging the fusion oncogene P3F, we identified 182 drugs that suppress P3F levels of which TG02 was a top hit. TG02 showed in vivo efficacy indicating that FP-RMS is susceptible to CDK inhibition. We also found synergy between TG02 and VCR, resulting in a significant delay in FP-RMS tumor progression compared to single agents. Interestingly, we found that VCR alone can downregulate P3F and its targets. Combination therapy of TG02 with VCR shows promise for clinical translation in FP-RMS.

22- Natural selection of castration-resistant prostate cancer (CRPC) clones following neoadjuvant androgen-deprivation therapy (neoADT)

Chennan Li, Anson Ku, Scott Wilkinson, Shana Y. Trostel, William L. Dahut, Fatima Karzai, Adam G. Sowalsky

23- Synthetic lethality mediate cell state interactions among the components of tumor microenvironment in IDH mutant gliomas

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Background: Tumor microenvironment (TME) consists of malignant and various non-malignant cells such as immune and stromal cells. These cell types actively communicate with each other via multiple cell-signaling pathways and sets forth the clinical course of the disease and response to therapeutic interventions. Here we develop a computational pipeline to identify such cell state interactions (CSIs) in the TME.

Methodology: To identify the CSIs in the TME, we devised a methodology based on the idea of synthetic lethality, i.e., CSIs having synergistic or antagonistic effect on the tumor fitness should be detectable in the computational screening of genetic interactions using the patient's clinical data. Firstly, we estimate the genome-wide gene expression profiles of distinct cell types present in the TME IDH mutant gliomas by deconvolving a large cohort of IDH mutant gliomas from TCGA. The resulting cell type specific gene expression profiles were than used to derive the latent factors representing distinct cell states using Independent Component Analysis (ICA). The resulting latent factors or independent components (ICs) of each cell type were compared against the ICs of all other cell types to identify the significantly prognostic combinations using the cox-regression.

Results: Using this pipeline, we detected a total of 160 interactions among 7 distinct cell types at the FDR threshold of 0.20 and 70% internal cross-validation accuracy. The CSIs were significantly reproducible in an independent cohort of IDH mutant gliomas. A majority (70%) of the detected CSIs lead to worse prognosis of glioma patients. The signature genes of distinct ICs corresponding to distinct cell states of malignant cells significantly resembled the markers of astrocytic cells, oligodendrocyte progenitor cells, and cycling stem cells which interacted significantly with multiple distinct cell states of other cell types to worsen the patient prognosis. Interestingly, such interactions were significantly enriched among the patients which didn't respond favorably to immunotherapeutic interventions elucidating the role of tumor microenvironment in therapy resistance. The frequently interacting cell states had a higher number of ligand-receptor pairs among their ICs which indicates a mechanistic basis for inferred cell communications. Overall, this analysis provides a unique pipeline to infer prognostic cellular crosstalk in the tumor microenvironment with potential application in stratifying patients for therapeutic interventions.

24- Deactivation of ligand-receptor interactions enhancing lymphocyte infiltration drives melanoma resistance to Immune Checkpoint Blockade

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25- Multi-Omics Approaches to Tumor Reactive CD8 T Cells

Rajiv S. Trehan[1], Xin Wang[2], Patrick Huang[1], Noemi Kedei[1], Xiao Bin Zhu[1], Marlaine Soliman[1], Mohamed-Reda Benmebarek[1], Firouzeh Korangy[1], Chi Ma[1], and Tim F. Greten[1]

Biophysics, Chemistry, Pharmacology, and Structural Biology

26- Developing Nanobodies for LRRK structural and biochemical analysis

Astrid Alvarez de La Cruz[1] , Riley D. Metcalfe[1], Juliana A. Martinez Fiesco[1], Ping Zhang[1]

27- Interactions between J-domain proteins, Hsp90, and Hsp70 in protein remodeling

Anushka Wickramaratne[1], Shannon Doyle[1], Jui-Yun Liao[1], Gabrielle Puller[1], Joel Hoskins[1], Lisa Jenkins[2], Andrea Kravats[3], and Sue Wickner[1]

28- Progress towards the structure of full-length death-associated protein kinase 1

Riley D. Metcalfe[1], Juliana A. Martinez Fiesco[1], Anthony M Ciancone[1], Francis J. O'Reilly[1], Ping Zhang[1]

Background and Hypothesis: Death-associated protein kinase 1 (DAPK1) is a large (1430 residue, 160 kDa), complex multi-domain kinase which serves to regulate several fundamental cellular processes, most notably cell death and autophagy. In several diseases, notably stroke and other neurological diseases DAPK1 activation is correlated with poor disease outcomes. Conversely, in several cancers, including lung, liver, gastric and colorectal cancers, DAPK1 has a role as a tumor suppressor, with loss of DAPK1 expression correlated with poor prognosis. Despite its importance in normal physiology and disease, little structural information is available on DAPK1, with structural information limited to its 300 residue N-terminal kinase domain. In addition to the kinase domain, DAPK1 contains both a Roc-COR tandem GTPase domain and a death domain in the same polypeptide chain. This domain arrangement is unique and raises intriguing questions about how the two domains regulate each other, and the role of the GTPase domain in regulating both the kinase domain and the other functions of DAPK1. Two other human proteins contain both a kinase and GTPase domain, namely, the two human leucine-rich repeat kinases (LRRKs), LRRK1 and LRRK2, although these differ from DAPK1 in the relative position of the kinase and GTPase domain in the polypeptide chain. Both LRRKs are implicated in disease (notably Parkinson's disease in the case of LRRK2). Despite their biological significance, full-length DAPK1, LRRK1 and LRRK2 have presented challenging structural targets, and only recently have full-length structures become available of the LRRKs.

Study Design and Methods: We have developed methods to express and purify DAPK1. We have collected initial cryo-electron microscopy (cryoEM) data, and cross-linking mass spectrometry (XL-MS) data.

Results and Conclusions: We have undertaken biophysical characterization of DAPK1 using multi-angle light scattering and mass photometry, showing that it is a stable protein which is predominately monomeric in solution. We have collected cryo-EM data on the purified protein, obtaining 2D class averages of the correct size and showing secondary structure features. We have generated a medium resolution (~4-5 Å) 3D reconstruction. The 3D reconstruction, which is not yet of sufficient resolution to allow for atomic model building, shows a 'dumbbell' structure, with the kinase and Roc-COR domains connected by the ankyrin repeats. Our XL-MS data, shows extensive cross-links between the ankyrin repeats and the Roc-COR domains. Our initial structural information shows that the structure of DAPK1 is distinct from the structures of LRRK1 and LRRK2, with the kinase and Roc-COR domains adopting a unique arrangement. Future work on DAPK1 is focused on optimism protein expression and purification methods to allow the production of a homogenous sample, to facilitate high-resolution data collection.

Relevance and Importance: DAPK1 is a regulator of several physiological processes and implicated in several diseases. Understanding the structural biology of DAPK1 will allow a mechanistic understanding of its role in disease, and ultimately allow the development of novel treatment strategies in diseases associated with aberrant DAPK1 signaling. This is addition to the broader interest in understanding the regulation of these unusually large and complex multi-domain kinases.

29- High-throughput Assay Exploiting Disorder-to-Order Conformational Switches: Application to the Proteasomal Rpn10:E6AP Complex

Christine Muli[1], Sergey Tarasov[2], Kylie Walters[1]

Conformational switching is pervasively driven by protein interactions, particularly for intrinsically disordered binding partners. We developed a dually orthogonal fluorescence-based assay to monitor such events, exploiting environmentally sensitive fluorophores. This assay is applied to E3 ligase E6AP, as its AZUL domain induces a disorder-to-order switch in an intrinsically disordered region of the proteasome, the so-named Rpn10 AZUL-binding domain (RAZUL). By testing various fluorophores, we developed an assay appropriate for high-throughput screening of Rpn10:E6AP-disrupting ligands. We found distinct positions in RAZUL for fluorophore labeling with either acrylodan or Atto610, which had disparate spectral responses to E6AP binding. E6AP caused a hypsochromic shift with increased fluorescence of acrylodan-RAZUL while decreasing fluorescence intensity of Atto610-RAZUL. Combining RAZUL labeled with either acrylodan or Atto610 into a common sample achieved robust and orthogonal measurement of the E6AP-induced conformational switch. This approach is generally applicable to disorder-to-order (or vice versa) transitions mediated by molecular interactions.

30-Chemical Synthesis of Histone Lysine Coenzyme A Adducts to Capture P300 Acetyl Transferase

Samanthreddy Kedika, Timothy S. Strutzenberg, Dmitry Lyumkis, and Terrence Burke Jr.

The transcriptional coactivator and histone acetyltransferase p300 is a key regulator of gene expression and plays an important role in cellular processes during development and homeostasis. Dysregulation of p300 has been implicated in numerous diseases, including cancer, neurodegenerative disorders, and cardiovascular diseases. The four histone proteins that form the nucleosome core particle are some of the key targets acetylated by p300, and there are preferences for two lysine residues on H3: H3K18 and H3K27. Currently, there are no structures reporting how the full-length p300 protein engages nucleosomes at these sites. Because p300 acetylates multiple lysine residues on histones, different structures exist in equilibrium, which makes structural analysis challenging.1 Therefore, alternative strategies are needed to capture the p300 protein at key lysine residue on nucleosomes. Previously, lysine acetyl-CoA antagonists have been reported to inhibit p300.2 We propose that appending a CoA adduct onto histone lysine residues may be able to specifically “capture” p300 species by: i) Increasing the histone binding affinity towards p300, since the adduct should be able to interact with both the lysine and CoA substrate-binding pockets and ii) Inhibiting the p300 from catalyzing reactions at remaining lysine residues. We have approached synthesizing designer histones having CoA adduct at specific lysine residues using native chemical ligation. Herein, we report both semi-synthetic and total synthetic approaches towards generating CoA-modified histones. Modified histones will be incorporated into nucleosomes for biochemical and structural biology applications. This approach should facilitate advancing the current structural understanding p300 nucleosome assembly and mechanisms of acetylation.

31- Comparative Analysis of Drug-like EP300/CREBBP Acetyltransferase Inhibitors

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The human acetyltransferase paralogues EP300 and CREBBP are master regulators of lysine acetylation whose activity has been implicated in various cancers. Recently, three unique molecular scaffolds have taken precedent as drug-like EP300/CREBBP acetyltransferase inhibitors: an indane spiro-oxazolidinedione (A-485), a spiro-hydantoin (iP300w), and an aminopyridine (CPI-1612). Despite increasing use of these molecules to study lysine acetylation, the dearth of data regarding their relative biochemical and biological potencies makes their application as chemical probes a challenge. To address this gap, here we present a comparative study of drug-like EP300/CREBBP acetyltransferase inhibitors. First, we determine the biochemical and biological potencies of A-485, iP300w, and CPI-1612, highlighting the increased potency of the latter two compounds at physiological acetyl-CoA concentrations. Cellular evaluation shows that inhibition of histone acetylation and cell growth closely aligns with the biochemical potencies of these molecules, consistent with an on-target mechanism. Finally, we demonstrate the utility of comparative pharmacology by using it to investigate the hypothesis that increased CoA synthesis caused by knockout of PANK4 can competitively antagonize binding of EP300/CREBBP inhibitors and demonstrate proof-of-concept photorelease of a potent inhibitor molecule. Overall, our study demonstrates how knowledge of relative inhibitor potency can guide the study of EP300/CREBBP-dependent mechanisms and suggests new approaches to target delivery, thus broadening the therapeutic window of these preclinical epigenetic drug candidates. This sets the stage for applications as chemical probes and in proximity based therapeutics.

32- YAP localization mediates mechanical adaptation of human cancer cells during extravasation in vivo

Woong Young So, Claudia S. Wong, Udochi F. Azubuike, Colin D. Paul, Paniz Rezvan Sangsari, Patricia B. Gordon, Hyeyeon Gong, Tapan K. Maity, Perry Lim, Zhilin Yang, Christian A. Haryanto, Eric Batchelor, Lisa M. Jenkins, Nicole Y. Morgan, Kandice Tanner

33- The conformational dynamics of the PH domain-dependent Arf GTPase-activating protein ASAP1 are altered by a soluble phosphoinositide analogue

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Arf GTPase-Activating Proteins (GAPs) regulate the transition of Arf GTPase proteins from the GTP- to GDP-bound state. Arf GAPs are critical for Arf function as, unlike other small GTPases, Arf proteins have no detectable intrinsic GTPase activity. The Arf GAP ASAP1 (Arf GAP with SH3 domain, ankyrin repeat and PH domain 1) is being investigated for potential roles in cancer. The Arf GAP catalytic activity of ASAP1 is regulated >10,000-fold by the phospholipid phosphatidylinositol 4,5-bisphosphate (PIP2). The reaction can be recapitulated in vitro using a catalytic fragment of ASAP1 in combination with soluble PIP2 analogues, indicating that the mechanism of activation is independent of PH domain recruitment to PIP2-containing membranes. We hypothesized that PIP2 increases activity, at least in part, by altering the conformational dynamics of the catalytic fragment of ASAP1 (which we refer to as PZA for PH, zinc binding, and ankyrin repeat domains). Fluorescence assays using N- and C-terminally tagged ASAP2 PZA were consistent with a change in protein conformation upon addition of soluble PIP2. Studies of ASAP1 fragments using multiple structural methodologies suggested that the PH domain and ZA construct move independently of one another and adopt compact interdomain conformations (relative to all possible conformations) in the absence of PIP2. The data are consistent with the interpretation that PIP2 binding to the PH domain restricts the number of conformational states of PZA, possibly increasing its affinity for substrate Arf protein. This work extends our understanding of how the activity of PH domain-dependent enzymes is regulated.

34- Targeting human oncogene PKC iota in various cancers through PROTAC targeting degradation

Kelcie Foss, Jennifer Wong-Roushar, Sweta Dash, Chandrasekhar Mushti, Katherine Nyswaner, Rolf Swenson, and John Brognard

The protein kinase C (PKC) family, a subsect of serine/threonine kinases, were thought to be involved in tumor promotion, however in a landmark study by Antal and Colleagues, they found a majority of mutations in novel and conventional PKCs are LOF, indicating PKCs are tumor suppressors. Conventional isoforms of PKCs contain DAG and calcium (C2) binding domains. Novel isoforms contain only the DAG domain, and atypical isoforms lack both a functional DAG domain and the C2 region. Activation of atypical PKC isoforms are dependent on protein interactions which are mediated by the PB1 domain. Studies found that PKCi, an atypical isoform, was an amplified driver kinase in many cancers with amplifications of its gene PRKCI in lung squamous cell carcinoma (SCC) (36%), ovarian serous carcinoma (23%), esophageal adenocarcinoma (19%), and head and neck SCC (14%). Phenotypes often implicated in cancer have been shown to be regulated by PKCi, including tumor growth, cellular development, and cell survival. PKCi has also been shown to have a regulatory role in tumorigenesis via protein-protein interactions with MEK5, zip/p62, and par6. Therefore, a PKCi degrader targeting both the catalytically dependent and independent functions of PKCi could serve as a potential novel therapeutic against cancers of unmet need. In collaboration with the Swenson group, an initial set of degraders were developed using compound 49, a PKCi inhibitor developed by Kwaitkowski, et. al. to tether to a VHL E3 ubiquitin ligase ligand. To screen PROTACs, we developed an overexpression system of PKCi in HEK293T cells via transient transfection and treated with our PROTACS across various doses and time points and used western blots to monitor PKCi degradation. Following initial screening, we will move forward with testing in cancer cell lines with amplified PRKCI to compare the efficacy of PROTACS to traditional catalytic inhibitors. We will also look at downstream effects and compare PROTACs to traditional inhibitors in order to elucidate the mechanisms by which PKCi promotes tumorigenesis. We have generated HEK293T cells overexpressing Flag-tagged PKCi and validated our antibodies via transient siRNA mediated PKCi knockdowns. Initial treatments with compound 49 and our first few PROTACs showed no degradation of PKCi, except for PROTAC 8. We will continue testing PROTAC 8 potency at different time points and screen new PROTACs developed by the Swenson group with modified linkers and E3 binding warheads. PKCi is amplified and overexpressed in many cancer types and has been shown to promote tumorigenesis through both noncatalytic and catalytic functions, which makes it a promising target for PROTAC development. We aim to develop PROTACs that target PKCi, characterize the downstream effects of PKCi inhibition and degradation, and understand the efficacy of PROTACs in in vivo studies.

35- HORNET – Determination of conformational landscape of RNA in solution: a direct visualization approach

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RNA biology is one of most active areas of research and RNA structural biology is one of the last frontiers of structural biology. RNA is versatile molecule that can be a genetic carrier like mRNA, gene regulators like riboswitches, enzymes like ribozymes, protein synthesis machinery such as rRNA, amino acid carriers like tRNAs, among others1. The RNA structure and dynamics are a key to understanding RNA biological functions 2, which is important for engineering new RNA devices and developing of druggable RNA targets3. RNA shows a rugged free-energy landscape, with degenerate secondary structure and energetically equivalency among the 3D folding. Contrary to the commonly rational protein structural landscape, RNA folding may follow a different rule: one-sequence may have multiple conformers/structures, i.e., there is not one native structure but rather an ensemble of native structures. The RNA could be described as a system with a high topological fluctuation, addressed by its large degree of freedom of the secondary structure and weak long-range interaction that permits high degrees of conformational heterogeneity and structural flexibility. This energetic degeneracy and topographic equivalence of RNA limits the application of the classic biophysical techniques, that rely on signal averaging. We have developed a new technology combining atomic force microscopy (AFM) and machine learning that allow us to determine the structure of a single molecule directly visualized in relevant physiological solution with an accuracy of ~5Å. This approach allows the determination of the full-conformational landscape of the RNA, and in principle can be applied to resolve any structured RNA. References 1 Serganov, A. & Patel, D. J. Ribozymes, riboswitches and beyond: regulation of gene expression without proteins. Nature Reviews Genetics 8, 776-790 (2007). https://doi.org:10.1038/nrg21722 Russell, R. et al. Exploring the folding landscape of a structured RNA. Proc Natl Acad Sci U S A 99, 155-160 (2002). https://doi.org:10.1073/pnas.2215935983 Childs-Disney, J. L., Yang, X., Gibaut, Q. M. R., Tong, Y., Batey, R. T. & Disney, M. D. Targeting RNA structures with small molecules. Nat Rev Drug Discov 21, 736-762 (2022). https://doi.org:10.1038/s41573-022-00521-4

36- Identification of small molecule binders of mEAK-7 for use in cancer therapies

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Background and Hypothesis: Previous research has demonstrated that mammalian EAK-7 (mEAK-7) is an alternative binding partner that activates the mammalian target of rapamycin (mTOR) signaling pathway, forming mTOR complex 3 (mTORC3). Activation of mTOR signaling results in cellular differentiation and proliferation and is commonly dysregulated in tumors. Thus, the development of mTOR inhibitors has important implications for cancer therapy. In mouse models of cancer, mEAK-7 knockout improves survivability. As a target, mEAK-7 has remained unexplored, and we believe it has great potential for therapeutic development. We hypothesize that investigating potential mEAK-7 inhibitors will lead to the identification of a small molecule that can be developed into a novel drug for specific mEAK-7-positive cancers.

Study Design and Methods: 22,452 total compounds were initially screened via small-molecule microarrays (SMM) for their ability to bind mEAK-7. These compounds were evaluated for their selectivity, structure, and statistical effect size. Compounds (n = 45) that passed the minimum thresholds for binding and selectivity were tested for their phenotypic effects in various cancer lines via proliferation, migration, and cytotoxicity assays. With our collaborators, we generated new analogs with various modifications for further testing. Compounds that inhibit proliferation and migration of cancer cells will be optimized by exploring structure-activity relationships (SAR). The mechanism of action of these compounds will also be further elucidated using phosphoproteomics and bulk RNA sequencing.

Results and Conclusions: We have identified a lead inhibitory compound from the original list of 45 through our screening assays. This compound inhibits the proliferation and migration abilities of multiple cancer cell lines. Modifications to the lead compound have shown to be more effective at inhibiting cell proliferation. Key findings from phosphoproteomics and transcriptomics will be shared at the time of presentation.

Relevance and Importance: The mTOR signaling pathway is responsible for cell proliferation, survival, and metabolism, and dysregulation of mTOR signaling can promote tumor growth and metastasis in many cancers. Thus, mTOR inhibitors may provide a promising therapeutic avenue to treat cancers. Established mTOR inhibitors such as AZD2014 and rapamycin result in widespread toxicity. Therefore, there is a need to develop more specific and effective treatments. Identifying mEAK-7 binders could potentially aid in the development of mTOR inhibitors to improve patient outcomes and further our understanding of cancer biology.

37- Paralogue-specific degradation of the histone acetyltransferase EP300

Joycelyn Williams[1], McKenna Crawford[1], Ying Xiong[1], Xuemin Chen[1], Anver Shaik[2], Nathan Coussens[3], Rolf Swenson[2], Jordan L. Meier[1]

An emerging topic of oncology is the prevalence of lysine acetylation in post-translational modification (PTM). This process could provide a selective route for targeting oncogenic transcription therapeutically. Specifically, EP300 and CREBBP are human enzymes involved in catalyzing acetylation. These multidomain protein paralogues, commonly denoted as EP300/CREBBP, exhibit highly similar acetyltransferase active sites, sharing over 95% identity. Since dual knockout of EP300/CREBBP is lethal in mammals, it has been hypothesized that to be effective a therapeutic it would be optimal to develop paralogue-specific inhibitors of these enzymes. However, a major question is how to differentiate them based on their high similarity. Here we report a targeted protein degradation (TPD) approach to paralogue-specific inhibition of EP300. We describe assays to assess selectivity as well as differentiate inhibition versus degradation across several cancer cell lines. By identifying a new strategy for paralogue-specific inhibition, our studies provide a foundation for the development of less toxic and more effective EP300 targeting in cancer.

Cancer Models, Cancer Stem Cells, Carcinogenesis and Metastasis

38- Pericyte to tumor cell transfer alters disseminated tumor cell fate decisions

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Background and Hypothesis: Success of metastasis is aided by the formation of a pre-metastatic niche. The pre-metastatic niche is a complex environment that refers to a site that is considered favorable to tumor cell seeding, composed of recruited bone marrow, activated stromal cells, and altered extracellular matrix component. Pericytes are implicated in the development of a pre-metastatic microenvironment when they are activated by primary tumor derived factors, and become synthetic, migratory, and proliferative. The microenvironmental changes associated with pericyte activation influence disseminated tumor cell fate decisions, promoting metastasis. We hypothesized that activated pericytes in the early metastatic microenvironment could confer a survival advantage to disseminated tumor cells through transcriptional and metabolic reprogramming.

Study Design and Methods: To investigate the role of pericytes in the early metastatic microenvironment, we developed a protocol to isolate and culture primary mouse lung pericytes that maintains their known phenotypes and functions in normal physiology and metastasis. To evaluate the interactions between lung pericytes and triple negative breast cancer (TNBC) cells in the early metastatic microenvironment live imaging co-culture experiments were performed and tumor cell fate was assessed after co-culture, both in vitro and in vivo.

Results and Conclusions: Co-culture experiments revealed the direct transfer of lipids from lung pericytes to metastatic 4T1 tumor cells that was not observed with non-metastatic 67NR tumor cells. Lipid transfer was dependent on direct cell contact between pericytes and tumor cells and was not observed with transwell assays or co-culture conditioned media, suggesting that tumor cells may take advantage of pericyte coupling mechanisms. RNA-Sequencing of 4T1 cells after direct co-culture with pericytes demonstrated activation of pathways related to syncytium formation and cell fusion, as well as inhibition of cell death pathways. In normal physiology, pericytes respond to mechanical stimuli to rapidly alter blood vessel diameter and maintain local homeostasis. We hypothesized that direct contact with a tumor cell may activate mechanosensitive channels in pericytes to initiate lipid transfer. The use of mechanosensitive calcium channel inhibitor, gadolinium, demonstrated a significant reduction in the transfer of lipids to tumor cells. Lungs from mice that received 4T1 cells from co-culture via intracardiac injection demonstrated an increased colonization efficiency, indicated by enrichment in the number of small lesions (<6 cells) compared to the monoculture group. Immunofluorescence demonstrated significantly less Ki67+ 4T1 cells in lungs of mice that received cells from co-culture vs monoculture. Seahorse metabolic assays also demonstrated an energetic to quiescent shift following co-culture with pericytes potentially suggesting that pericyte interaction could promote tumor cell quiescence in the early metastatic niche. Together our data suggest that tumor cells are reprogrammed by direct pericyte contact in the early metastatic lung to aid persistence and colonization.

Relevance and Importance: Approximately 40% of TNBC patients will experience recurrent metastatic disease in their lifetime, which may be explained by the ability of some TNBC cells to remain dormant for years before. A better understanding of the signals that influence the disseminated tumour cell fate decisions will be critical to improving treatment for patients with recurrent disease.

39- Spatial Localization of Cancer Stem Cell Phenotypes, CD8+ Cytotoxic T cells, Vasculature, and Hypoxia in 4T1 Tumors: A Mouse Model of Triple-Negative Breast Cancer

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Triple-negative breast cancer (TNBC) is an extremely aggressive form of cancer with high likelihood of recurrence following treatment. Moreover, treatment options are limited, because of a lack of molecularly-targeted therapies. Populations of cancer stem cells (CSCs) within tumors lead to this likelihood of recurrence and pose crucial complications for treatment of aggressive forms of cancer, such as triple-negative breast cancer, as these cells allow for renewal, differentiation, drug resistance, and tumorigenicity. A look inside the tumor environments in which these cells reside provides understanding of their function. Regions of hypoxia and inflammation have been known to induce stress responses within tumor environments, it is hypothesized that these stress responses induce cancer stem cells and drive them to migrate up the oxygen gradient towards vasculature, ultimately promoting metastasis. Cell surface markers such as CD44+, CD44v6+, CD49f+, and CD24- can be used to identify cells that maintain cancer stem cell properties within tissues. The spatial localization and distance of cancer stem cells in relation to regions of hypoxia and vasculature informatively reveals aspects of their function. In the 4T1 tumors, a syngeneic mouse model of human triple-negative breast cancer, CSCs were found to have decreased populations in tumors where the active NOS2 protein had been knocked out in the mouse (NOS2KO), compared to wild-type mice. Since the NOS2KO mice also had reduced metastases to the lung, we proposed that CSCs have a role in driving metastases in this model. CSCs were seen to exhibit a layering pattern with NOS2+, CD8+ Cytolytic T cells, and reduced pimonidazole, a hypoxia marker. This presents insight into the role of these combined elements in tumor metastasis and the lack thereof. Cell-to-cell interaction or a proximity of CD8+ Cytolytic T Cells to tumor cells has been shown to increase CSCs in breast cancer cells, including MDA-MB-231 cells, a TNBC cell line. In this study, CD8+ Cytolytic T Cells were found at higher populations in Indomethacin treated samples of 4T1 mice which demonstrates the greater infiltration of immune cells into the tumor core using Indomethacin when compared to control tissues. CSCs were seen in proximity to CD8+ Cytolytic T Cells which provides insight into the possibility of CD8+ Cells bystander effect role in activation of stemness characteristics, which has been reported by others. Further study is needed to understand the spatial relationship between hypoxia and CSCs. This will be done using cultured live cells in the Restricted Exchange Environment Chamber (REECs), which imposes a defined gradient of hypoxia and nutrients across the cell culture. Cancer stem cells, likely the origin of cancer metastasis, may pose a vital target in cancer treatment as they maintain resistance to conventional chemotherapy and treatments of radiation.

40- The role of immune cells in organ specific metastasis in melanoma

Udochi F. Azubuike[1], Patricia, B. Gordon[1], Woong Young So[1], Kevin Bishop[2], Blake Carrington[2], Raman Sood[2], Kandice Tanner[1]

41- Functional redundancy of BRC repeats of BRCA2 for Homologous Recombination and mouse survival

Arun Prakash Mishra, Natasha Oberoi, Shyam Sharan

BRCA2 is an essential gene for survival and is considered to be tumor suppressor. It has multiple domains interacting with different proteins and complexes. One such region has 8 BRC repeats in exon11 that bind to RAD51. These BRC repeats help BRCA2 in loading of RAD51 on the DNA double strand breaks (DSBs) and repair by homologous recombination. BRC repeats are conserved across different species and have differential affinity towards RAD51 binding speculating functional redundancy. We wanted to study what is the minimum number of BRC repeats needed to suffice for BRCA2 functions. ES cells based studies revealed BRC1-4 are enough for fully functional BRCA2 and BRC5-8 are dispensable. Then we concentrated on BRC repeats 1-4 and made BRCA2 with single BRC repeats (1,2,3 and 4 individual). High rate of functional redundancy in the BRC repeats was observed and even one repeat was enough for cell survival and RAD51 loading at radiation-induced DNA DSBs. BRCA2 having just BRC1 and BRC3 were found to be sensitive to DNA damaging drugs and exhibited lower number of radiation induced RAD51 foci positive cells. BRCA2 having just BRC2 and BRC4 performed as good as WT BRCA2. This excited us to check if a single BRC repeat is enough for mouse viability. To our surprise we were able to generate animals with Brca2 having just BRC2 and BRC4 using CRISPR/CAS9 technology. These animals are generated in regular mendelian ratio in both homozygous and hemizygous (where one allele is mutated and the other is knockout) conditions. It should be noted that we also tried to remove all the BRC repeats but did not get any surviving animals. These mice are under study now for phenotypic analyses. BRCA2 is a huge protein (3418aa) and in order to get BRCA2 with single BRC repeat we deleted around 1100aa. It is surprising that removal of about one-third of the protein is not only functional for in vitro assays but also supports mouse survival. Also at least one BRC repeat is needed for ES cell and mouse survival.

42- In vitro restoration of fumarate hydratase activity in HLRCC using modified mRNA

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Hereditary leiomyomatosis and renal cell carcinoma (HLRCC) is an autosomal dominant condition characterized by a mutation in the fumarate hydratase (FH) gene, which leaves the susceptible individuals at risk for developing highly aggressive and metastatic cancer. Loss of FH activity leads to the accumulation of fumarate, a TCA cycle intermediate, which disrupts energy metabolism and activity of cellular enzymes, leading to changes in gene expression, promoting tumorigenesis(1). HLRCC is not responsive to existing treatments, however, expressing functional FH protein from a lentiviral construct has been previously shown to diminish cell invasion capacity and abrogate capacity of cells to form tumor in xenograft experiments(2). As genetic manipulations such as these are difficult to achieve in patients, there is a need to develop alternate ways of treatment. One such strategy is heterologous gene expression from an in vitro transcribed (IVT) mRNA. However, IVT mRNA is at the risk of being recognized as 'non-self' and can trigger innate immune response(3). To overcome this issue, we designed an mRNA synthesis protocol similar to the ones used for mRNA vaccines, complete with a 5'-cap and 3' poly-adenylation, which will be recognized as 'self', and which would also facilitate translation; as well as using a modified nucleotide N1-methyl-pseudouridine, which is known to reduce immunogenicity and enhance ribosome loading on the mRNA(4), thus ensuring maximum translation and minimum immune response.Using this strategy, we first generated an IVT mRNA encoding GFP reporter gene. We were successful in transfecting this mRNA into HeLa cells and were able to observe GFP expression using fluorescence microscopy and flow cytometry. We are currently optimizing GFP mRNA delivery into UOK262 cells, a cell line derived from HLRCC patient(5). We will then use an IVT mRNA to restore FH activity in these cells and investigate its effect on HLRCC related phenotypes such as high glucose dependency and cell invasion capacity. If successful, this strategy could open doors for mRNA-based corrective/restorative therapy for HLRCC patients.(1) Menko, F. H. et al. Hereditary leiomyomatosis and renal cell cancer (HLRCC): renal cancer risk, surveillance and treatment. Fam Cancer 13, 637-644 (2014). https://doi.org:10.1007/s10689-014-9735-2(2) Fitzsimmons, C. M. et al. Rewiring of RNA methylation by the oncometabolite fumarate in renal cell carcinoma. bioRxiv (2023). https://doi.org:10.1101/2023.04.10.536262(3) Linares-Fernandez, S. et al Tailoring mRNA Vaccine to Balance Innate/Adaptive Immune Response. Trends Mol Med 26, 311-323 (2020). https://doi.org:10.1016/j.molmed.2019.10.002(4) Andries, O. et al. N(1)-methylpseudouridine-incorporated mRNA outperforms pseudouridine-incorporated mRNA by providing enhanced protein expression and reduced immunogenicity in mammalian cell lines and mice. J Control Release 217, 337-344 (2015). https://doi.org:10.1016/j.jconrel.2015.08.051(5) Yang, Y. et al. UOK 262 cell line, fumarate hydratase deficient (FH-/FH-) hereditary leiomyomatosis renal cell carcinoma: in vitro and in vivo model of an aberrant energy metabolic pathway in human cancer. Cancer Genet Cytogenet 196, 45-55 (2010). https://doi.org:10.1016/j.cancergencyto.2009.08.018

43- Tumor Necrosis Factor Related Apoptosis Inducing Ligand (TRAIL)- induced cytokine production in TNBC promotes neutrophil chemotaxis and immune suppression.

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Background and Hypothesis: TRAIL induces apoptosis in many preclinical cancer models including breast cancers and has been extensively studied as a potential cancer therapeutic. However, its efficacy in clinical trials is limited, suggesting an unknown modulatory mechanism responsible for lack of TRAIL activity in vivo. Here, we hypothesize that TRAIL treatment elicits transcriptional changes in TNBC cells that alter the immune milieu.

Method: We performed RNAseq of MDA-MB-231 cells treated with TRAIL for different time points, followed by validation with RT-PCR in various TNBC cells. RNAi, silencing key differentially regulated genes, along with RT-PCR, ELISA and CHIP assays, were used to validate RNAseq findings. Elucidation of the functional relevance of the outcome was supported both in vitro and in vivo by chemotaxis assay, cytotoxicity assay, RNAseq analysis of donor isolated neutrophils and intravital microscopy, CODEX analysis in TNBC xenografts from mice treated with the TRAIL respectively. Using TNBC humanized mice model the changes in tumor immune environment caused by TRAIL are currently under investigation.

Results: TRAIL treatment of the TNBC significantly induced expression of several cytokines, such as CXCLs 1, 2, 3, 8,11 and IL6, both in vitro and in vivo which are known to affect neutrophil function. Mechanistically, induction of these cytokines was predominantly mediated by death receptor 5 and caspase-8 protein, but not caspase-8 enzymatic activity. GSEA of the RNAseq data indicated that NFKB pathway was significantly enriched. Concordantly, we confirmed that both canonical NFKB1 and non-canonical NFKB2 pathways were activated by TRAIL in vitro and in vivo. However, the induction of the cytokine mRNAs was primarily dependent on the NFKB2 pathway. Neutrophils isolated from healthy human donors incubated with supernatants from TNBC cells in vitro indicated that TRAIL-induced CXCLs and IL6 significantly increased neutrophil chemotaxis. Additionally, CODEX analysis as well as intravital imaging confirmed that TRAIL treatment increases the number of neutrophils in the tumor. Preincubation of neutrophils with supernatants from TRAIL-treated TNBC significantly inhibited their cytotoxic effect against TNBCs. Further, transcriptome analysis of neutrophils incubated with either TRAIL or supernatant of TRAIL treated TNBC revealed significant enrichment of expression of inflammatory cytokine genes, immune modulating and immune checkpoint genes like PDL1. Functional studies with these neutrophils confirmed their suppressive effect on T cell function as well as the effect of TRAIL on decreased neutrophil apoptosis. Preliminary data from humanized mice along with these results suggested that exogenous TRAIL exerts an immune suppressive role in tumor microenvironment.

Conclusion: Collectively, our study suggests the novel role of TRAIL-induced NFKB2-dependent cytokine production promoting neutrophil chemotaxis and immune suppression. Importance: This study implies that alterations in the innate immune system may modulate the effects of TRAIL on TNBC tumors.

44- Evaluating the role of ΔNp63α in driving growth and metastasis of pancreatic ductal adenocarcinoma

Daniel H. Ryan, Chin-Hsien (Emily) Tai, Xianyu Zhang, Mayrel Palestino Dominguez, Christine Alewine

Pancreatic ductal adenocarcinoma (PDAC) is the third leading cause of cancer-related deaths in the US and is rising in incidence. PDAC tumors may be either classical or basal in transcriptomic subtype, and PDAC patients with basal tumors have worse prognosis and may have increased resistance to chemotherapy. Previous studies have shown that ΔNp63α drives basal program expression. The goal of this project is to investigate whether ΔNp63α expression is sufficient to increase pancreatic cancer cell growth, metastasis and chemoresistance and whether these cells can outcompete ΔNp63α non-expressers in mixed tumors. Non-ΔNp63α expressing human PDAC cell lines AsPC1 and HPAFII were stably transduced to express either ΔNp63α or mCherry (control). For both AsPC1 and HPAFII, ΔNp63α-expressing and control cells had similar proliferation rates and susceptibility to chemotherapy agents oxaliplatin and SN38 when grown in culture. When control or ΔNp63α-expressing AsPC1 cells were implanted orthotopically into the pancreas of athymic nude mice, there was no significant difference in primary tumor mass and pathological analysis of lungs and livers did not reveal any significant difference in metastatic burden. Bulk RNA sequencing of primary tumors confirmed that tumors in mice inoculated with AsPC1-ΔNp63α had significantly upregulated expression of genes associated with the basal subtype. Collectively, these data suggest that while ΔNp63α drives the basal program in PDAC, its expression is insufficient to cause resistance to these chemotherapies or a growth or metastatic advantage in an immunodeficient setting. Further work is needed to elucidate the mechanism by which ΔNp63α contributes to more aggressive PDAC tumors.

45- NAMPT inhibitor OT-82 resistant RMS lines exhibit alterations in their biochemical and metabolic profiles

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Rhabdomyosarcoma (RMS) is the most common childhood and adolescent soft tissue sarcoma. Nicotinamide phosphoribosyltransferase (NAMPT) catalyzes the rate-limiting step of the NAD salvage pathway and is currently the only pharmacologically targetable NAD production enzyme. We have previously shown RMS cell lines are highly sensitive to NAMPT inhibition and that treatment with the clinical NAMPT inhibitor OT-82 causes complete tumor regressions in vivo. However, we have observed the development of resistance in some in vivo models. Accordingly, the aim of this project is to elucidate potential mechanisms of resistance to OT-82 in RMS. Mice with orthotopic RMS xenograft tumors were treated with OT-82 at 50 mg/kg three days/week for 8 weeks. Upon cessation of treatment, mice were observed for recurrence and retreated when tumors regrew to a volume of 900-2,500 mm3. Time between cessation of initial treatment and retreatment was dependent on individual tumor growth kinetics and ranged from 23-70 days. Tumors that progressed on treatment were harvested and converted to cell lines. Incucyte live cell analysis confirmed retention of OT-82 resistance in vitro and downstream assays were performed to compare sensitive parental cell lines to resistant cell lines. Intracellular NAD and ATP levels were analyzed with luciferase-based assays. Protein analysis was performed to quantify expression levels of NAD production enzymes. Biochemical analysis was utilized to compare glucose, lactate, glutamine, and glutamate consumption and/or production. Extracellular flux analysis was used to determine rates of oxidative consumption (OCR) and extracellular acidification (ECAR). Metabolomic analysis was performed to identify differential levels of metabolic intermediates both in the basal conditions and under OT-82 treatment. RNA and whole exome sequencing were performed to analyze the transcriptome of RMS lines, as well as identify mutations in their genome that could contribute to OT-82 resistance. Resistant lines maintained proliferation at doses of OT-82 up to 30 times above the IC50 of parental lines. At multiple time points and OT-82 doses, NAD loss was observed in both parental and resistant lines suggesting the drug activity remained on target, however resistant lines were able to recover NAD levels despite continued exposure to OT-82. Resistant cell lines maintained higher ATP levels than parental lines in the presence of OT-82. We observed cell line dependent changes in enzyme expression levels of the NAD production enzymes NAMPT and quinolinate phosphoribosyltransferase (QPRT), but not nicotinate phosphoribosyltransferase (NAPRT). Biochemical analysis revealed decreased lactate production in resistant cells both at baseline and at OT-82 doses up to 30nM. Extracellular flux analysis showed resistant cells have increased ECAR in treated conditions and increased OCR at basal and treated conditions, suggesting that resistant cell lines undergo increased oxidative phosphorylation and glycolysis, compared to parental cell lines. Metabolomic analysis revealed that at 24 hours post-treatment, sensitive cell lines show changes in levels of key glycolytic intermediates, whereas resistant cell lines do not. Cumulatively, these data indicate that NAMPT inhibitor-resistant RMS lines exhibit differences in NAD-production enzyme expression levels, ATP production, and glucose metabolism. RNA and whole exome sequencing analysis is in process and will be reported.

46- Non-canonical activation of Hedgehog signaling in skin basal cell carcinoma tumor formation

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Background: Skin basal cell carcinoma (BCC) is the most common cancer in the United States. BCC is caused by mutations in the Hedgehog (HH) signaling pathway, leading to the overactivation of the receptor Smoothened (SMO) and GLI transcription factors. Clinical response rates to SMO inhibitors are low, and resistance to these drugs is common, urging additional therapeutic interventions for BCC. While BCC is mainly caused by dysregulated HH signaling, we have previously found a complementary G-alpha-s (Gs) protein regulated pathway vital to coordinating epithelial cell fate and BCC. In mouse skin, inactivating Gs or its downstream target protein kinase A (PKA) triggers BCC formation. While Gs or PKA disruption is sufficient to activate GLI, questions remained on the similarities of Gs/PKA-induced BCC with tumors arising from canonical Hedgehog pathway activation. Understanding how Gs and PKA regulate HH signaling and BCC formation can reveal new targets for the pharmacological intervention of cancer growth.

Methods: We utilized mouse models to compare BCC tumors by bulk and single-cell RNA sequencing. Canonical BCC formation was initiated by expressing a constitutive active form of SMO (SMOM2) in the skin. Non-canonical BCC was triggered by skin specific knockout of Gs (GnasKO) or overexpression of the PKA inhibitor protein (PKI). To investigate the crosstalk between Gs and HH signaling, we knocked out Smo in conjunction with Gs or the HH regulator GPR161 in mouse epithelial cells.

Results: Analysis of RNA expression profiles from tail tumors in BCC mouse models indicated significant similarities in differentially regulated genes. Furthermore, GnasKO and SMOM2 mice showed a 70% overlap in expression profiles and were almost indistinguishable by principal component analysis. Single-cell RNA sequencing of tail skin revealed a similar expansion and profile of tumor cells which clustered together in graph-based analysis, illustrating their remarkably similar gene expression signatures. Our results established that BCC tumors arising from Gαs pathway inactivation are highly similar to those resulting from canonical Hedgehog signaling. Based on these results, we hypothesized that GnasKO BCC could be a consequence of SMO signaling upregulation. However, knockout of Smo did not alter tumor formation or Hedgehog signaling activation in GnasKO mice, indicating that the Gs BCC pathway is independent from SMO. We then tested the contribution of the canonical inactivator of HH signaling GPR161, a Gs-coupled GPCR. We found that epidermal knockout of GPR161 did not recapitulate GnasKO-induced BCC. Overall, these studies establish our models as a unique resource to understand BCC arising from noncanonical activation of Hedgehog signaling and suggest Gs signaling could be used as a therapeutic target for tumors with SMO inhibitor resistance.

Importance: Overall, our results indicate that Gs and PKA inactivation drives BCC formation independent of the canonical HH pathway members SMO and GPR161 and could be an effective therapeutic pathway for BCC treatment. By dissecting the signaling mechanisms that determine the crosstalk between Gs and SMO signaling, we could provide novel druggable targets for skin cancer treatment.

47- Thyroid hormone receptor α1: a novel regulator of thyroid cancer cell differentiation

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48- Treatment of acute myeloid leukemia with DNA Methyltransferase (DNMT1) inhibitor and immune checkpoint inhibitor leads to improved survival in a murine model

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Background and Hypothesis: Acute myeloid leukemia (AML) is a disease characterized by an overproduction of myeloblasts and the inhibition of normal hematopoiesis. Cytidine analogs have been used to treat AML due to their hypomethylation and reactivation of tumor suppressor genes inactivated by methylation. One cytidine analog, 5-Aza-4'-thio-2'-deoxycytidine (AzaTdCyd) has been shown to increase the mutational burden in mammalian cells. These mutations encode mutant proteins, which may be displayed on the tumor cell surface and recognized by T-cells providing immune surveillance. However, malignant cells often express PD-L1, which binds PD-1 on T-cells and inhibits recognition of tumor cells by T-cells. PD-L1 inhibitors prevent this interaction and allow T-cells to recognize cancer cells. We hypothesize that increasing the number of mutant antigens on the cell surface (through treatment with AzaTdCyd) will increase immune recognition of cancer cells, which will be amplified by a PD-L1 inhibitor.

Study Design and Methods: A murine AML cell line (961C-S) was treated with 300 nM of AzaTdCyd for 12 days to generate mutations and single cell clones were expanded. Selected single cell clones were analyzed by whole exome sequencing (WES) to identify acquired mutations and were transplanted into syngeneic immunocompetent wild type mice via tail vein injection. Following leukemic cell engraftment, mice were then treated with Atezolizumab (a PD-L1 inhibitor) or saline once a week for three weeks. Disease progression was monitored through regular peripheral blood analysis. Mice were euthanized upon symptoms of illness. Flow cytometry and histology staining were performed on collected tissues for further analysis.

Results and Conclusions: Whole exome sequencing documented increased mutations in the AzaTdCyd treated 961C cells, and PD-L1 expression was documented by flow cytometry. Pooled results from three different cell lines and two independent experiments indicated that mice which were transplanted with 961C mutagenized clones treated with Atezolizumab survived longer than mice which were transplanted with 961C mutagenized clones and no Atezolizumab. Further analysis suggested that Atezolizumab treatment had a greater effect in mice transplanted with 961C mutagenized clones which had a greater number of mutations. This preliminary data supports the hypothesis that increasing mutation burden in AML cells improves the effectiveness of PD-L1 inhibitors, and the combination of a mutagenic cytidine analog with an immune checkpoint inhibitor may be a potential approach for cancer treatment. Further testing of these clones in larger groups will help confirm these trends.

Relevance and Importance: Over 20,000 people are diagnosed with AML in the US each year, and there is only a 25% three year survival. Due to the limited number of highly effective therapies, identifying novel approaches to therapy, such as the drug combination outlined here, is an important initial step for improved cancer therapy.

49- Fusion-Negative Rhabdomyosarcoma xenografts in murine tongue provide a model for the study of local invasion, intravasation and metastasis in live animals

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PAX3/7 Fusion-negative rhabdomyosarcoma (FN-RMS) is a childhood malignancy of the mesodermal lineage that predominantly occurs head and neck and genitourinary system. While improved for early-stage disease, treatment of metastatic or relapsed FN-RMS remains a challenge. As a step towards achieving a more complete understanding of advanced disease, we developed an orthotopic tongue xenograft model of FN-RMS for the examination of molecular basis of FN-RMS invasion and metastasis. The behavior of human-derived FN-RMS cell lines injected into the tongue of athymic mice was examined with a combination of in vivo bioluminescence imaging, non-invasive two-photon intravital microscopy (IVM), and histopathology and compared to the two prevailing hindlimb intramuscular and subcutaneous xenograft models. FN-RMS cells were retained in the tongue and invaded into muscle mysial spaces and neighboring tissues. While evidence of hematogenous dissemination to the lungs occurred in both tongue and intramuscular xenografts, evidence of local invasion and lymphatic dissemination to the lymph nodes only occurred in tongue xenografts. IVM of tongue xenografts shows dynamic growth and shifts in cell morphology longitudinally, supported by transcriptional enrichment for myogenesis signatures in tongue xenografts but not submandibular metastases. IVM also shows dynamic homing to blood and lymphatic vessels, lymphatic intravasation, and dynamic membrane protrusion formation. Based on these findings, the tongue orthotopic xenograft of FN-RMS is a valuable model for short-term and longitudinal studies of tumor progression at the tissue, cellular and subcellular levels providing insight into kinetics and molecular bases of tumor invasion and metastasis and, hence, new therapeutic avenues for advanced FN-RMS.

50- Development of a High-Throughput Zebrafish Model of Blood-Brain Barrier Disruption

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51- Development of Model Systems to Evaluate the Immunological Effects of Cell-Restricted Delivery of IL-15

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Background & Hypothesis: Chimeric antigen receptor (CAR) T-cell therapy against disialoganglioside (GD2), a tumor-associated antigen in neuroblastoma (NB), has shown therapeutic promise for patients with high-risk NB. Unfortunately, these therapies continue to be limited by poor T-cell persistence and efficacy within the context of an immunosuppressive tumor microenvironment (TME). Armoring CAR T-cells with interleukin (IL)-15 has been associated with increasing longevity and effectiveness in preclinical and early clinical trials, though the mechanism underlying this effect is incompletely understood. Mechanism studies are impeded by a lack of representative models, and the clinical barriers associated with obtaining patient tumor samples. We therefore engineered murine GD2-targeting CAR T-cells with membrane-tethered (mteth) IL-15 (GD2.CAR.IL15) and tested them against a tumor spheroid/immune cell co-culture. We evaluated the cytotoxic capacity of GD2.CAR.IL15 and performed simultaneous cytokine and phenotypic analysis. We then compared our findings to an in-vivo model of orthotropic neuroblastoma. We hypothesized we could recapitulate immunosuppressive TME in-vitro to understand the mechanism underlying GD2.CAR.IL15 cytotoxicity.

Study Design and Methods: Murine single-chain variable fragment 14g2a-based GD2.CAR and GD2.CAR.IL15 were evaluated for their anti-tumor efficacy in-vitro against the murine MYCN-amplified GD2-overexpressing neuroblastoma cell line, 9464D.GD2. Tumor cells were grown in 3-dimensional (3D) spheroids and co-cultured with 1) murine peripheral blood cells (PBs) and hemopoietic progenitor cells (HPCs) or 2) splenocytes to recreate the TME. Complimentary, we orthotopically implanted 9464D.GD2 in C57BL/6 mice and assessed response to GD2.CAR/GD2.CAR.IL15 therapy with ultrasound tumor measurements. In both systems, we performed cytokine profiling and phenotype analysis of effectors and bystander immune cells and applied single-cell (sc) RNA-sequencing (seq) to characterize rare immune populations. Spatial transcriptomics of in-vivo neuroblastoma tumors allowed us to examine topographic differences between GD2.CAR and GD2.CAR.IL15 within the TME.

Results & Conclusions: GD2.CAR.IL15 was more potent than GD2.CAR and untransduced mock T-cells in eliminating neuroblastoma cells in-vitro and in-vivo. The cytotoxicity in-vitro, was significantly suppressed across conditions by tumor-associated macrophages and down-regulation of the CAR. Compared to GD2.CAR and mock T-cells, GD2.CAR.IL15 demonstrated higher proportions of naïve CD62L+CD44- cells with reduced expression of exhaustion markers in both model systems and higher pro-inflammatory macrophages. Preliminary scRNA-seq results corroborated these findings. Expression of mteth IL-15 preserves a naïve phenotype in GD2.CAR T-cells and repolarizes the TME to enrich for pro-inflammatory macrophages. These immunological findings were noted in both models, suggesting that the in-vitro culture system faithfully recapitulates the in-vivo TME. Further analyses of the scRNA-seq data are underway to characterize the immune cell repertoire. Real-time confocal microscopy is applied to resolve the question of how CARs are sequestered and what macrophage-releasing factors induce this process.

Relevance and Importance: This 3D in-vitro tumor spheroid/immune cell co-culture system mirrors TME challenges like hypoxia, nutrient deprivation, and immunosuppressive cells. It serves as a vital tool for assessing new treatments like GD2.CAR.IL15 T-cells in the context of solid tumors. This study not only reveals the enhanced cytotoxicity mechanism of GD2.CAR.IL15 but also promotes continued exploration of its efficacy in future clinical trials.

52- New pre-clinical brain metastasis models to interrogate the role of the microenvironment in immunotherapy efficacy

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Brain metastases (BrM) remain an intractable, deadly complication for advanced melanoma patients. Although immune checkpoint blockade (ICB) therapy have recently shown promising results, they benefit only a small subset of melanoma BrM patients and efficient therapies are still desperately needed. Furthermore, detailed studies addressing the contribution of the BrM tumor microenvironment (TME) to ICB response are lacking due to limited access to patient samples and the scarcity of appropriate preclinical models. Indeed, in most available models the immune system and/or the brain-blood-barrier (BBB) is unnaturally compromised. To address these limitations, here, we developed two novel isogenic immunocompetent melanoma BrM models, that exhibit a high incidence of BrM without artificial BBB disruption and recapitulate the ICB responses observed in the clinic. Single-cell RNA sequencing and high-parametric spectral flow cytometry of BrM TME revealed a high infiltration and diversity of T cell subsets in the ICB-responsive model (BR1) while neutrophils were enriched in the ICB-resistant model (BR3). Importantly, we uncovered distinct microglia populations exclusively present in BR1 that correlated with T cell infiltration and upregulated genes encoding for T cell-attracting chemokines and antigen presentation. Furthermore, we identified different tumor transcriptional programs (metaprograms) between BR1 and BR3. We confirmed the translational relevance of our models and showed that the specific immune populations and tumor metaprograms present in their TME can also be found in the limited publicly available datasets from melanoma BrM patients. Notably, we found a positive correlation between T cell infiltration and the enrichment of BR1-like tumor metaprograms in patients. To the best of our knowledge, this is the first study that highlights key associations between tumor metaprograms, TME immune composition, and BrM response to ICB. Our unique BrM models, mirroring clinical features, provide a robust platform to identify novel predictive and therapeutic leads to help efforts to overcome therapy resistance.

53- Modulation of the tumor microenvironment induced by immune checkpoint inhibition is countered by docetaxel in preclinical models resistant to immunotherapy

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Background and Hypotheses: Immune checkpoint blockade (ICB) therapies targeting the programmed cell death 1 receptor (PD-1) or its ligand PD-L1 have shown remarkable clinical benefit across several cancer types, yet durable clinical responses have been achieved only in a subset of patients. Among various mechanisms of tumor resistance to treatment, the presence of a collagen-rich tumor-associated extracellular matrix (ECM) and gene signatures of TGF-beta activation have both been associated with the lack of responses to ICB. The addition of chemotherapy to ICB is becoming more commonly used for the treatment of various cancer types in an attempt to increase clinical responses. To provide a rational basis for combination therapy, and to potentially develop more effective approaches, in this study, we investigated the impact of docetaxel on the tumor microenvironment (TME) in the context of PD-1/PD-L1 axis blockade. Docetaxel belongs to a group of chemotherapeutics called taxanes that exert their cytotoxic activity by stabilizing microtubules with resulting cell cycle inhibition during the G2/M phase. Taxanes have been previously shown to also attenuate expression of collagens in models of fibrosis via suppression of TGF-beta activity. Here we hypothesized that docetaxel, when combined with anti-PD-L1, might decrease the expression of collagens and modulate TGF-beta activity, potentially inducing a more permissive TME for anti-tumor immune responses.

Study Design and Methods: To investigate this hypothesis, we used two ICB-refractory murine cancer models, the triple negative breast 4T1 model and the Lewis lung carcinoma (LLC) model. Mice bearing tumors were administered anti-PD-L1, docetaxel, or the combination of both agents, and tumors were collected and assessed for early effects on the TME using flow cytometry, RNA expression profiling via RT-PCR, RNA in situ hybridization, and immunohistochemistry for detection of immune infiltrates, cancer-associated fibroblasts, phospho-Smad2/3 (pSmad2/3) signaling, and multiple molecules related to ECM remodeling.

Results and Conclusions: Our results indicate that PD-L1 monotherapy in the ICB resistant 4T1 and LLC models induces changes in the TME that include the expression of the ECM-associated collagens (Col1a1 and Col1a2) and collagen-remodeling matrix metalloproteinases (MMP2, MMP3, MMP8, MMP12 and MMP13). Additionally, tumors treated with PD-L1 monotherapy showed significantly enhanced TGF-beta signaling denoted by increased expression of pSmad2/3 and increased numbers of alpha-smooth muscle actin positive fibroblasts in the TME. The effects observed with anti-PD-L1 as a monotherapy were not observed in tumors treated with docetaxel monotherapy or in those treated with the combination docetaxel plus anti-PD-L1 suggesting that upregulation of immunosuppressive TGF-beta signaling and collagens in the TME could be prevented by the addition of chemotherapy.

Relevance and Importance: Taken together, our findings suggest that combination therapy may improve antitumor responses by remodeling the ECM and attenuating pSmad2/3 signaling. Ongoing and future studies are aimed at understanding the mechanisms involved in the upregulation of phospho-Smad2/3 signaling and collagen production in the TME in response to anti-PD-L1 therapy in non-responsive tumors, the effect of various chemotherapies in this phenomenon, and the study of additional tumor models for optimization of chemotherapy-ICB modalities.

54- Identification of PAEP as an immune activity modulator to mediate the immune resistance in tumor metastasis

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55- Sensory Overload: Exploiting cancer stem cells' super-sensitivity to microenvironmental quality to block metastatic progression

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Background and Hypotheses: Cancer stem cells (CSCs) are a minor subpopulation of tumor cells that drive tumorigenesis, metastasis, and resistance to therapy. Our prior work showed that breast CSCs and non-CSCs have very different population kinetics early in metastatic lung colonization. CSCs initially undergo a few expansive self-renewing divisions before plateauing, while non-CSCs continue to expand. Live-cell imaging of tumor cell cultures in vitro modeled these population dynamics. Single cell fate mapping showed that CSCs switch from expansive self-renewal to a state of balanced self-renewal and differentiation at a critical cell density. This switch seems to be regulated by transcriptional modulators YAP and TAZ. We hypothesize that YAP/TAZ in the CSCs integrate inputs from multiple environmental signals, and that targeting upstream regulators of YAP/TAZ may induce differentiation in the CSCs, thereby reducing metastatic progression.

Study Design and Methods: Using the Incucyte live-cell imaging system and a lentiviral-based fluorescent sensor of stemness, we were able to monitor CSC and non-CSC population dynamics simultaneously in vitro. We compared the effects of pharmacological inhibitors upstream of YAP/TAZ to reduce CSC proliferation across three triple negative breast cancer models: MDAMB231LM2, SUM159M1, and 4T1. Single cell fate-mapping was used to determine the balance of self-renewal and differentiation. To investigate the effect of chemotherapy and pharmacological inhibitors on CSCs in metastases in vivo, we injected MDA-MB231_LM2 cells into the tail vein of nude mice. The mice were treated with Paclitaxel, LPAR1 inhibitor BMS984020, or both. Lungs were harvested after four weeks and analyzed with quantitative imaging.

Results and Conclusions: We identified several input pathways converging on the YAP/TAZ transcriptional co-regulators that affect CSC self-renewal. These pathways, contributing to the CSC plateau include nutrient/growth factor depletion (AMPK and GPCR signaling), cell matrix interaction (integrin signaling through FAK), cell-cell interaction (through the LATS kinases), and inflammation (through the JAK/STAT kinases). The extent to which the individual factors affect YAP, and subsequently CSC dynamics, depended on the breast cancer cell model. In all models, however, CSCs exhibited a more rapid response than non-CSCs to changes in the microenvironment, suggesting that CSCs may serve as sensitive cellular "sensors" of microenvironmental quality for the entire tumor cell population. In the MDA-MB231_LM2 model, CSCs are particularly reliant on bioactive lipid signaling, and blockade of the LPA receptor 1 (LPAR1) inhibits CSC self-renewal. Fate-mapping and immunofluorescent staining showed LPAR1 inhibition deactivated YAP and shifted the CSC fate balance towards differentiation. In vivo, combining LPAR1 antagonist BMS986020 treatment with the chemotherapeutic Paclitaxel reversed the Paclitaxel-induced enrichment of CSCs, resulting in a decrease in both CSCs and non-CSCs.

Relevance and Importance: Since standard-of-care chemotherapy is relatively ineffective against CSCs, additional approaches are needed. Inhibiting CSC self-renewal has the potential to prevent metastatic progression by eliminating the CSC population which is necessary for formation of new tumors and tumor maintenance. Targeting YAP/TAZ activity directly or via inhibition of upstream inputs may be an effective way to limit CSC-driven metastasis in the clinic.

56- Investigation of molecular alterations associated with double resistance to BRAF and MEK inhibition in melanoma

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57- Unveiling the NOS2 landscape in ER-Negative Breast Cancer: A comprehensive Investigation of Predictive Factors, and Cellular Neighborhoods.

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Estrogen receptor-negative (ER-) breast cancer is a highly aggressive subtype with limited therapeutic options. In the Wink Lab, we have pioneered a paradigm involving nitric oxide synthase-2 (NOS2) and cyclo-oxygenase-2 (COX2). This model demonstrates upregulated expression of both NOS2 and COX2 in breast tumors, serving as a predictive factor for poor clinical outcomes. In a 21-cohort for breast cancer tissues, a significant association between elevated NOS2 and COX2 levels and deceased patients was revealed, particularly evident at 5-year-post-diagnosis. Our model focused on the co-expression of tumor and stromal NOS2 and COX2 in ER- breast cancer. Human tissues typically exhibit 1-5% NOS2 expression within breast tissues, often forming cellular neighborhoods and clusters. Such clustered areas that play a role in metastasis. Tumor NOS2 emerged as a potent predictor, correlating with reduced survival in ER- breast cancer. There was also a potent relationship between tumor NOS2 and CD8+T cells at the same 5-year post-diagnosis. These findings were validated in a separate cohort using gene expression data, highlighting the strong association of NOS2 to CD8 ratios with poor outcomes in high NOS2 expressing tumors. Multiplex imaging, facilitated by HALO software, identified distinct CD8+T cell phenotypes relative to tumor NOS2 expression in Deceased vs Alive patients. Phenotype layout localization across tissues was achieved by setting thresholds at weak, moderate, and strong levels respectively adding 2,4, or 6 standard deviations to the mean intensity thresholds settings for NOS2 and CD8 phenotypes. This enabled the examination of their distributions and clustering patterns. Specific characterization of CD8+/-NOS2-/+ revealed the infiltration of CD8 into inflamed tumors. In deceased tumors, high NOS2 expression was mostly at the margins, and there was CD8+T cell stroma restriction. Signaling molecules released by NOS2 enzymes were found to activate oncogenic pathways, driving cancer stemness, metastasis, and immune suppression. This comprehensive investigation provides valuable insights into the intricate interplay of NOS2 expression, CD8+T cell phenotypes, and spatial characteristics in ER- breast cancer, offering potential avenues for targeted therapeutic interventions.

58- Inhibition of NOS2 and COX2 Correlates with Increased B cell recruitment to the Tumor Microenvironment during Early TLS Formation

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Our previous work has demonstrated that elevated Nitric Oxide Synthase 2 (NOS2) and Cyclooxygenase-2 (COX2) expression is a strong predictor of poor survival in ER- breast cancer at a 5-year post-diagnosis. COX2 is associated with immunosuppression and Tertiary Lymph Node Structures (TLS) have been associated with immune activation and improved clinical outcomes. This suggests that there may be a potential link between tumor NOS2/COX2 expression and TLS formation. We examined tumor immune landscapes in 4T1 tumors in WT and NOS2 knockout (NOS2KO) mice, treated with or without the COX2 inhibitor Indomethacin. Using specific phenotypes correlating with TLS and lymphocyte activity, spatial analysis demonstrated distinct co-localization of B and T cell aggregates with TLS phenotypes in tumors treated with Indomethacin. Interestingly, we saw a split in the percent cells of B and T cell phenotypes for Indomethacin treated groups only. RNA expression levels also showed a significant increase in the expression of B cell recruitment factor Cxcl13 and T cell receptor development factor LAT for NOS2KO and Indomethacin treated tumors. These data suggest that T cell localization may be important for B cell recruitment for early TLS development and that COX2 may be playing a role with immunosuppressive effects. NOS2-derived NO may also be involved in inhibiting production of B cell recruitment factors and future work will need to be done to determine the effect of COX2 and NOS2 on the cohort split.

59- Extracellular Matrix Biophysical Properties Change During Nevus to Melanoma Progression

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Cutaneous malignant melanoma is one of the most aggressive forms of skin cancer and its major etiological risk factor is ultraviolet (UV) solar radiation. The extracellular matrix (ECM) has been shown to influence many of the hallmark characteristics of tumor development, survival, and progression across several types of cancer, including melanoma. Interactions between the tumor and the ECM often results in rearrangement, cross-linking, and deposit of specific ECM proteins, which can contribute to stiffening of the tissue and tumorigenesis. To simulate tumorigenesis, we have previously used a hepatocyte growth factor (HGF) transgenic mouse model, which develops melanoma nevii when exposed to UVB radiation. This model is highly representative of human melanoma lesions in terms of biological, genetic, and etiologic criteria. In general, microrheology involves injecting beads into a medium, tracking movement of the beads using two-photon microscopy, and using the movement to calculate the viscosity and elasticity of the medium. The physical properties of the ECM during tumorigenesis have not been well studied at the cellular level, and microrheology has rarely been used in vivo. In our in vivo microrheology study, we injected fluorescent beads into the skin of these transgenic mice. Then, we used intra-vital microscopy with particle tracking to image the beads and measure changes in ECM elasticity as nevii grow into melanoma tumors. These results, combined with transcriptomic analysis of ECM regulation and proteomic analysis of ECM signaling, could provide unique insights into components of the ECM that are involved in melanoma tumor development and offer opportunities for therapeutics to target oncogenesis.

60- Surfaceome Analysis of Sensitive and Drug Resistant MCF-7 Lines

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Breast cancer is the most commonly diagnosed cancer, accounting for approximately 30% of all new cancer cases in women each year, and affects 1 in 8 women in the United States. Breast cancer recurrence accounts for up to half of all breast cancer related fatalities. Common drug classes used to treat early breast cancers are taxanes, platins, and anthracyclines in addition to hormonal, targeted, radiation, and immunotherapies. Drug resistant breast cancers are often encountered in clinical settings and present a significant challenge in cancer research. The goal is to characterize the cell surfaceomes of several cell lines and identify targetable surface proteins in resistant breast cancers. It is possible that changes in surface protein localization contributes to drug resistance. A large-scale analysis determining whether paired sensitive and drug resistant lines express different proteins at the cell surface has not been conducted. Surfaceome analysis is the only way to characterize proteins at the cell surface and will help identify targetable proteins. This investigation requires biotin to tag surface proteins and mass spectrometry to identify surface protein candidates in breast cancer lines. Cell surface biotinylation utilizes a thiol-cleavable amine-reactive agent and is later captured on an avidin resin. A series of optimization steps were performed to reduce the time between biotinylation and purification of the sample, reduce intracellular contamination using stringent wash buffers, and normalize the amount of sample loaded onto the mass spectrometer for data analysis. Preliminary analysis of MCF-7 lines resistant to various drug treatments shows unique proteins at the surface, indicating different mechanisms of resistance.

61- Exploring Immune Infiltration in Triple Negative Breast Cancer: Spatial Analysis of 3D Tumor Microenvironment for Therapeutic Insights

Favour O. Nwagugo[1], Valentin Magidson[2], Sally Feng[1], Kristofer E. delas Peñas[3,4], Lisa Ridnour[5], David Wink[5], Robert J. Kinders[6], Jens Rittscher[3,7], Stephen J. Lockett[2], and David A. Scheiblin[2]

62- Deciphering the role of macrophage-derived pericytes in the pre-metastatic perivascular niche

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Metastasis accounts for the majority of breast cancer related deaths annually. Reorganization of distant organs by tumor-derived secreted factors, termed the pre-metastatic niche, facilitates metastasis by promoting disseminated tumor cell survival at secondary sites. Pericytes, perivascular cells that support blood vessel homeostasis, are a vital part of pre-metastatic niche formation and KLF4-dependent pericyte activation is critical for lung metastasis in serval cancer types. Using spatial transcriptomics to examine pericyte phenotypes in the pre-metastatic lung, we discovered a population of NG2+ pericytes that co-expressed the macrophage marker F4/80, termed PeriMacs. With multi-parameter flow cytometry, we revealed PeriMacs were increased in lungs but decreased in bones of tumor-bearing mice compared to controls, suggesting PeriMacs may be recruited from the bone to the lung during pre-metastatic niche formation. Macrophage-derived pericytes have been found in the developing skin and brain, indicating that a proportion of lung pericytes may be hematopoietic in origin and represent an alternate reservoir for pro-tumor pericyte functions in metastatic microenvironments. These observations led us to hypothesize that PeriMacs accumulate in the pre-metastatic niche and promote metastasis. Uncovering the role of PeriMacs in metastasis may inform future pericyte-modulating therapeutic strategies to limit metastasis.To examine pericyte origin and function in metastasis, EO771 mammary tumor cells or HBSS control were orthotopically injected into BL/6 female mice and lungs collected 10 days after tumor injections for spatial transcriptomics analysis to determine pericyte location and expression profile. PeriMacs were generated in vitro by treating murine bone marrow derived cells with M-CSF for 5 days to induce macrophage differentiation, and then treated the cells with TGF-beta for 5 additional days to induce pericyte differentiation. Cells were analyzed by flow cytometry, qPCR, and treated with tumor conditioned media to assess pericyte markers and function. To interrogate PeriMac support of stem cell niches, PeriMacs and bone pericytes were co-cultured with lineage negative, hematopoietic stem cell (HPSC) and mesenchymal stem cell (MSC) enriched populations.At a pre-metastatic time point, PeriMacs were increased in lungs but decreased in bones of tumor-bearing animals compared to controls. TGF-beta treated bone marrow-derived macrophages had significantly increased expression of the pericyte marker protein, NG2 compared to vehicle-treated macrophages, suggesting TGF-beta may induce the differentiation of macrophages into PeriMacs. In contrast to the well-characterized role of pericytes in pre-metastatic lung, PeriMacs were not activated by metastatic tumor conditioned media. Further, when co-cultured with HPSC and MSC enriched populations, PeriMacs were more sufficient at supporting MSC maintenance compared to pericytes, indicating PeriMacs may perform a unique pro-metastatic function from pericytes by sustaining MSCs. Future studies will investigate location of PeriMac accumulation and the mechanism of MSC niche support in the pre-metastatic lung.Survival of metastatic breast cancer patients has not accelerated at the same rate as those diagnosed without metastatic disease. Thus, there is a critical need to investigate the biology of metastasis through the metastatic microenvironment and pericyte involvement. Understanding the association between pericyte origin and role in metastasis is essential for the development of microenvironmental treatment strategies for preventing metastatic breast cancer.

63- Abrogation of the G2/M checkpoint as a chemo sensitization approach for alkylating agents

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BACKGROUND: The cell cycle is tightly regulated by checkpoints, playing a vital role in controlling its progression and timing. Cancer cells exploit the G2/M checkpoint, which serves as a resistance mechanism against genotoxic anti-cancer treatments, allowing for DNA repair prior to cell division. Therefore, manipulating cell cycle timing has emerged as a potential strategy to augment the effectiveness of DNA damage-based therapies such as alkylating agents and radiation.

METHODS: In this study, we conducted a forward genome wide CRISPR/Cas9 screening with repeated exposure to the alkylating agent temozolomide (TMZ) to investigate the mechanisms underlying tumor cell survival under genotoxic stress.

RESULTS: Our findings revealed that canonical DNA repair pathways, including ATM/Fanconi and mismatch repair, determine cell fate under genotoxic stress. Notably, we identified the critical role of the membrane-associated tyrosine- and threonine-specific cdc2-inhibitory kinase, known as Myt1 (encoded by PKMYT1), in ensuring cell survival. Depletion of PKMYT1 led to overwhelming TMZ-induced cytotoxicity in cancer cells. Isobologram analysis demonstrated potent drug synergy between TMZ and other clinically used alkylating agents, such as carmustine, busulfan, and dacarbazine, when combined with a novel Myt1 kinase inhibitor, RP-6306. Mechanistically, inhibiting Myt1 forced G2/M-arrested cells into an unscheduled transition to the mitotic phase without complete resolution of DNA damage, as indicated by a substantial increase in γH2AX-positive cells in mitosis. Moreover, RP-6306 dramatically altered cell cycle timing, reducing the threshold time for mitotic entry. This forced entry into mitosis, along with persistent DNA damage, resulted in severe mitotic abnormalities, including unaligned and partially condensed chromosomes, lagging chromosomes, chromosome bridges, and multipolar spindles. Ultimately, these aberrations led to mitotic exit with substantial apoptosis. Importantly, preclinical animal studies demonstrated that the combination regimen involving TMZ and RP-6306 prolonged the overall survival of glioma-bearing mice. CONCLUSION: Collectively, our findings highlight the potential of targeting cell cycle timing through Myt1 inhibition as an effective strategy to enhance the efficacy of current standard cancer therapies, potentially leading to improved disease outcomes.

IMPORTANCE: Chemotherapeutic strategies for malignancies like Glioblastoma Multiforme (GBM) often employ alkylating agents, including temozolomide (TMZ), which typically lead to only modest enhancements in the disease outcome. The present study, leveraging a comprehensive, unbiased chemogenomic screening approach, identified Myt1 kinase (encoded by PKMYT1) as a critical cell cycle regulator in conferring TMZ resistance and ensuring the survival of cancer cells. Inhibition of Myt1 disrupts the cell cycle timing, establishing a synergistic effect with the DNA damage caused by TMZ. Combination of Myt1 inhibitor with TMZ potentiates chemo sensitization, enhances tumor suppression, and improves the disease outcome in preclinical animal studies.

64- Personalized Therapeutic Screening in Patient Derived Organoids for Gastroenteropancreatic Neuroendocrine tumors

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Introduction: Gastroenteropancreatic Neuroendocrine Neoplasms (GEP-NENs) are a rare subset of cancers which nevertheless are a rising health burden. Development of new therapies suffers from several bottlenecks, including low patient accrual and poor understanding of tumor characteristics. Patient tumor organoids (PTOs) are a novel model capable of improving screening of patient tissue in an accurate, standardized, and high-throughput capacity. In this study, we utilized patient tumors for creation of high-fidelity PTOs from a variety of GEP-NEN primary origins to evaluate therapy responses.

Methods: Tumors from patients undergoing clinically guided surgeries were processed within two hours of resection and dissociated into single-cell suspension. Cells were encapsulated into Matrigel and cultured into two groups. The first group was grown for 10 days to assess viability then treated with a panel of clinically approved therapies and novel treatments recommended by high throughput NEN cell line screening for treatment sensitivity. The second group was grown for long-term expansion and biobanking, followed by characterization using immunohistochemistry and genetic profiling to ensure tumor cell maintenance.

Results: From March 2023-November 2023, 14 patients provided 30 tumors for PTO development. These included small intestine (n=5), pancreatic (n=8), and gastric (n=1) neuroendocrine tumors. Long-term culture (>3 passages) was successful for 23/30 (77%) specimens, with passage timing related to tumor grade. PTOs maintained immunohistochemical characteristics of the parent tumor types and demonstrated similar genetic profiles, including neuroendocrine tumor cell markers synaptophysin, chromogranin A, and matched grade-based Ki67 proliferative index. The early-stage therapeutic screening was performed for 12/14 (86%) patients, demonstrating tumor grade dependent treatment efficacy and showing clinically dose relevant sensitivity towards approved small molecule inhibitor therapies including cabozantinib and sunitinib in a patient and tumor origin-dependent manner. Testing of effective therapy classes recommended by cell line treatment panels suggested high level treatment efficacy for proteosome inhibitors, MEK inhibitors, and topoisomerase inhibitors.

Conclusion: Development of GEP-NEN PTOs is feasible for long term culture and standard of care therapy testing. Further study demonstrated the successful application of novel therapeutic options in PTOs for patients with GEP-NENs.

65- Alteration of Somatostatin Receptor 2 Expression by Cancer Associated Fibroblasts of Pancreatic Neuroendocrine Tumors

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Background and Hypothesis: Pancreatic Neuroendocrine Tumors (PNETs) are rare neoplasms with rising incidence. Current therapeutic strategies remain limited due to a lack of non-invasive treatments. Past studies have demonstrated somatostatin receptor 2 (SSTR2) on the tumor cell surface as a potential prognostic factor. SSTR2 demonstrates inverse relationship between its expression to tumor grade with expression loss in higher grade tumors, hindering its potential as a therapeutic marker. Understanding the loss in expression may provide avenues leading to its restoration, improving detection and treatment options. The role of the tumor microenvironment, in altering tumor cell behavior, including cancer associated fibroblasts (CAFs), has become increasingly important. CAFs have been shown to support tumor cells directly by providing signaling support and indirectly through microenvironment remodeling. This study hypothesizes that CAFs play a role in SSTR2 expression and its loss among higher-grade PNETs.

Study Design and Methods: Three PNET cell lines including grade 3 (BON-1 and NT-18p) and grade 1 (NT-3) were used that possess various levels of SSTR2 expression (BON-1 < NT-18p <NT-3). Fibroblast conditioned media (FCM) was created using CAFs isolated from patients with one grade 2 and one grade 3 pancreatic tumor followed by a 72h incubation in serum-free media. Cell lines were cultured in FCM and a serum-free control medium for two weeks. Assessments including cell proliferation and cell viability assays, colony formation, cell cycle analysis, western blot, and immunofluorescence imaging, were performed during and after incubation.

Results and Conclusion: Cell lines treated with FCM showed morphological changes, a more aggressive mesenchymal phenotype, through daily images recorded during the assay. BON-1 cells treated with FCM presented higher cell proliferation over seven days in comparison to the control, with grade 3 FCM demonstrating the highest growth (+78% for grade 3 FCM and +73% for grade 2 FCM). Similar results were shown among the NT-18p and NT-3 cell lines. Additionally, cell lines cultured in FCM demonstrated increased colony formation (NT-18p grade 3 FCM= 266 colonies, grade 2 FCM=146 colonies) while no measurable colonies formed in the control. BON-1 cells demonstrated an increased number of cells in G2 mitotic state in grade 3 FCM (Grade 2 FCM=12.2% vs Control=1%). Finally, SSTR2 expression was decreased in FCM cultured cells compared to serum free control.

Relevance and Importance: The tumor microenvironment plays a prominent role in affecting tumor cell signaling and behavior. Loss of SSTR2 through CAF-tumor cell interactions may provide a mechanism of acquired resistance to SSTR2 targeting therapies. Further work will analyze this relationship and evaluate tumor progression by bulk RNA sequencing to improve SSTR2 expression for future treatment applications for PNETs.

66- Unveiling the Impact of Nitric Oxide Dynamics in ER- Breast Cancer: Insights into Prognosis and Therapeutic Implications

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Estrogen receptor-negative (ER-) breast cancer is associated with unfavorable clinical outcomes, and the therapeutic options are limited. Nitric Oxide (NO), a gaseous free radical involved in various physiological and pathological processes, has been linked to a poor prognosis in ER- breast cancer, contributing to cancer progression. The impact of NO is influenced by its concentration, temporal dynamics, and spatial distribution. Therefore, exploring its biochemistry is essential to grasp its role in promoting carcinogenesis. Previously, our research indicated that interferon-gamma (IFN-y) is essential for inducing nitric oxide synthase 2 (NOS2), and its effects are amplified by interleukin 1 beta (IL-1b) and tumor necrosis factor-alpha (TNF-a). Our focus has been on replicating this inflammatory microenvironment in vitro and establishing it as a model for examining NO effects on ER- tumors. Our findings revealed that the density of NOS2-expressing cells is pivotal for achieving high NO concentrations, influencing ER- breast tumors. Moreover, in vitro experiments demonstrated that cells highly expressing NOS2 arrange in clusters when exposed to IFN-y, TNF-a, and IL-1b, accompanied by a reduction in the mitochondrial membrane potential marker, TMRE. A similar clustering pattern was observed in tumor tissues from ER- breast cancer patients, where small niches exhibited high, moderate, and low expression of NOS2. In our 21 ER- breast cancer cohort, high NOS2 niches were correlated with adverse clinical outcomes. These findings suggest that the proximity of NOS2-expressing cells plays a significant role in the progression of ER-breast cancer tumors. Further analysis is warranted to gain a deeper understanding of NO concentration in these niches and the mechanisms driving these NO-mediated effects. This will facilitate the exploration and potential development of therapeutic agents targeting NO inhibition.

67- Expression of IMPACT reprograms cancer cell metabolism and immunogenicity to augment NK and CD8 mediated killing

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Background and Hypothesis: To form overt metastases, disseminated tumor cells (DTC) must simultaneously acquire nutrients, circumvent oxidative stress, and evade host innate immune surveillance upon arrival in a foreign microenvironment. Given the propensity for gastrointestinal epithelial cancers to metastasize to the liver and thereby limit patient survival, we hypothesize that an overarching signaling axis governs the ability to circumvent the forementioned obstacles and ultimately drives metastatic colonization.

Study Design and Methods: To systematically identify the metabolic nodes that allow newly arrived DTC to evade the innate immune surveillance and from metastatic niches, we conducted an unbiased in vivo liver-specific metastasis screen using two isogenic pancreatic tumor cell lines harboring KRASG12D and TRP53R273H mutation, that differ in their metastatic capacities to the liver upon splenic injection in nude mice. We used RNAi, CRISPR/Cas9 knockout, FACS, and omics studies to decode the mechanisms of metastatic outgrowth and further validate the results in patient tumors and gene expression datasets.

Results and Conclusions: Our metastasis screen led to the identification of the RNA binding protein general control of amino acid synthesis 1-like 1 (GCN1) as a driver of metastatic outgrowth. RNAi experiments reveal that GCN1 triggers the integrated stress response (ISR) to activate serine, folate, and methionine biosynthetic pathways together with amino acid transporters which act in concert to facilitate acquisition of metabolites critical for cellular functions and restore redox homeostasis. We also found that GCN1 resides in the nucleus where it interacts with another RNA binding protein HNRNPK to suppress the expression of natural killer (NK) ligands, MHC class I, and antigen-presentation pathway molecules to attenuate tumor immunogenicity and escape both innate and adaptive immune surveillance. Intriguingly, we identified that IMPACT, an allosteric inhibitor of GCN1-GCN2 interaction, blocks ISR-dependent metabolic control, disrupts HNRNPK interaction to enhance tumor immunogenicity, and activates NK and CD8 mediated cytotoxicity. Moreover, IMPACT overexpression demonstrated significant abrogation of macrometastases formation, which was augmented with the use of immune checkpoint inhibitors (ICIs). Analysis of patient tumors and metastatic gene expression data demonstrates that elevated expression of GCN1 and HNRNPK with concomitant loss of IMPACT correlates with poor immunogenicity, aggressive metastatic behavior, and poor patient outcomes.

Relevance and Importance: There is an unmet need to identify vulnerabilities in the metastatic cascade to prevent colonization. Our work to do so has unveiled a therapeutic opportunity to increase tumor cell recognition and elimination through the expression of NK ligands for CD8 and NK-dependent killing, which we hypothesize will be applicable to a broad spectrum of patients with overt metastatic disease. Given the low percentage of tumor-reactive T cells in the TME, our identification of IMPACT as an immunometabolic rheostat may be exploited to improve ICI therapy in patients with ostensibly immunotherapy refractory disease.

68- Tumor characterization guides selection of rational immunotherapy combinations to maximize efficacy in resistant tumors

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Despite advances in immune checkpoint blockade (ICB), many cancers remain resistant. Tumors deemed ‘cold’ based on lack of T cell infiltration into tumor stroma show reduced potency for ICB therapy. We show that ‘hot’ tumors can be effectively treated with novel combinations of ICBs including LAG-3, TIGIT, and PD-L1, and anti-TGFbeta Ab; but colder tumors are resistant. Flow cytometric analysis of tumor-infiltrating immune cells from established, untreated tumors, uncovered a paradigm of expression patterns specific to each tumor line analyzed allowed us to predict treatment combinations tailored to each tumor line. The myeloid cells infiltrating MC38 and TC1 tumors express greater CD155, which interacts with the inhibitory receptor TIGIT, than myeloid cells infiltrating CT26, 4T1, and B16-F10 tumors. This, combined with increased PD-L1 expression on the MC38 tumor, led us to try combining anti-TIGIT and anti-PD-L1 to delay MC38 tumor growth, which was successful. However, in TC1 tumors with comparable levels of CD155 to MC38 tumor-infiltrating myeloid cells, the combination of anti-TIGIT and anti-PD-L1 alone was ineffective. TC1 tumors are ‘cold’ and express fewer infiltrating CD8 T cells than the others, so we added a tumor-antigen-specific vaccine to the combination of anti-TIGIT and anti-PD-L1 and observed a triple synergy that was better at delaying tumor growth than any pairwise combinations of vaccine plus either ICB. Further analysis of the tumor lines showed TC1 tumors express the greatest percentage of T regulatory (Treg) cells. Accordingly, depletion of Tregs in TC1 tumors treated with the triple combination caused complete tumor regression in the majority of the mice tested. These data show how baseline tumor characteristics can guide choosing more effective treatment strategy.

69- Investigating the role of stress induced nuclear relocalization of SIPA1 in breast cancer metastasis

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Breast cancer is the most commonly diagnosed cancer among women in the U.S. and metastasis still remains, the leading cause of deaths in breast cancer patients. This disease presents with 86.3% 5-year (2013-2019) survival rate in regional tumors which drops to 31% in metastasized cancers. Investigating the pathways that contribute to metastatic disease will help identify targets and treatment strategies for patients. To gain a better understanding of the factors that can impact metastatic progression, the Hunter lab showed that diverse genetic background has a significant influence on the metastatic nature of this disease. The first metastasis susceptibility gene identified through this approach was SIPA1 or Signal Induced Proliferation-associated protein 1. Previous work from Hunter lab has shown that seven metastasis susceptibility genes were present in the nucleolus, a central stress sensor. During stress, cytoplasmic proteins are sequestered in the nucleolus. In breast cancer cells, the size of the nucleolus increases and sequesters stress response proteins. Therefore, we hypothesized that when breast cancer cells are under stress during metastasis, the relocalization of stress response proteins to the nucleus is essential for metastatic progression. SIPA1 has a GAP activity and is predominantly present in the cytoplasm. However, recent work has shown that SIPA1 is present in the nucleus of breast cancer cells and nuclear SIPA1 levels are associated with poor prognosis in breast cancer patients. In current experiments, we observed that SIPA1 relocalizes to the nucleus in response to stress conditions like heat shock. Though, this relocalization is predominantly in the nuclear speckles and partially in the nucleolus. This response is cell line specific. Additionally, SIPA1 also binds to higher order repeats near the centromeric regions of multiple chromosomes. Our current goal is to understand why SIPA1 relocates to the nucleus to bind to nuclear speckles and the nucleolus under stress, and its probable role in binding to the centromeric regions of the chromosomes. Identifying mechanisms that control the relocation of SIPA1 in the nucleus, will further clarify the pathways responsible for stress response in breast cancer cells during metastasis.

70- Ex vivo pericyte modeling to reveal an organ-specific role for pericytes in metastasis

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Background and Hypotheses: Metastasis is responsible for most breast cancer related deaths, marking it a critical phenomenon for continued study. The metastatic process is significantly aided through establishment of a pre-metastatic niche, where circulating primary tumor-derived factors prepare a distant organ for successful metastasis. This microenvironmental transformation at the distant site includes recruitment of immune cells, alteration of the extracellular matrix, and activation of stromal cells. Within this stromal population are pericytes, a perivascular cell type which wrap around endothelium and mediate homeostatic communications within the vascular network. Specifically in the lung, perivascular cells have an essential role in pre-metastatic niche formation, where activation is characterized by expression of the pluripotency factor Klf4 and increased proliferation, among other phenotypes. While identified in lung, the role of pericyte activation in other organs is less understood, prompting our investigation into pericyte responses to metastatic cues in organs with high frequencies of breast cancer metastasis: bone, brain, liver, and lung. Because of possible heterogeneity in pericyte activation status throughout the body, we hypothesize that organ-specific pericytes have a differential impact upon early metastatic success. Study

Design and Methods: An ex vivo culturing system was established for each organ of interest, capable of retaining pericyte markers, morphology, and vascular stabilizing function. To test activation, pericytes were subjected to tumor cell conditioned media (TCM) from an isogenic breast cancer series with increasing metastatic potential (67NR, 4T07, 4T1), and activation was assessed by increased Klf4 expression and proliferation. Additional TCMs from cell lines with distinct organotropic patterning were also tested to ask if a potential relationship exists between the role of organ-specific pericytes and metastatic organotropism.

Results and Conclusions: Pericytes of the lung recapitulated previous in vivo work and activated only upon stimulation by secreted factors of the most metastatic cell line. When observing proliferation, brain pericytes showed no discrimination between the series of conditioned medias and activated with all three; however, bone and liver pericytes appeared unaffected. Only lung pericytes show a consistent increase in Klf4 expression, indicating that Klf4 kinetics may differ or be unrelated to activation in other organs. Interestingly, assays with organotropic cell lines revealed more distinctions. Lung pericytes demonstrated an increased proliferation with TCM from the bone-tropic cell line, with no response to brain or liver-tropic TCMs. In brain pericytes, we observed a strong increase in proliferation corresponding to the brain-tropic cell line. Lastly, bone pericytes remained unresponsive. Overall, our modeling demonstrates a recapitulation of in vivo pericyte activation in the lung and illustrates heterogeneity between organ-specific pericytes, potentially reflective of expected differences in vivo.

Relevance and Importance: These unique responses to metastatic cues could indicate differential impacts pericytes have in metastatic success throughout the body. These findings continue to identify pericytes as important players in the metastatic microenvironment and potential therapeutic target. Lastly, establishment of our ex vivo culturing system affords a closer evaluation of pericytes in a metastatic context, but also holds strong potential in application toward other spaces of vascular biology and disease.

71- Investigating tumor suppressors as drivers of aggressive lymphoma

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72- In vitro modeling of the Resistance to the Antibody-drug conjugate Enfortumab Vedotin in urothelial carcinoma

Ian Stukes[1], Min-Jung Lee[2], Salah Boudjadi[1], Andrea B. Apolo[1]

73- Investigating the Role of mEAK-7 in Head and Neck Cancer

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Background and Hypothesis: The mechanistic target of rapamycin (mTOR) forms complexes with numerous binding proteins to trigger phosphorylation events and affect processes like cell growth. Notably, mTOR complex 3 (mTORC3), characterized by mTOR binding with the protein mEAK-7, affects cell proliferation, cell migration, and DNA damage repair. 4-Nitroquinoline-1-oxide (4NQO) is a carcinogen used in research studies to induce cancer in laboratory animals. This study aimed to investigate the role of mEAK-7 in head and neck cancer using 4NQO-induced tongue tumors in mice. We hypothesized that C57BL/6 mEAK-7 knock-out mice would have smaller tongue lesions compared to wild-type mice, and heterozygous mice would have an intermediate lesion size between the two genotypes.

Study Design and Methods: Wild-type, heterozygous, and mEAK-7 knock-out C57BL/6 mice were divided into control or 4NQO-treated groups. 4NQO powder was dissolved in propylene glycol at 4 mg/mL. Control mice received propylene glycol in acidified water, while 4NQO-treated mice received 35 ug/mL of dissolved 4NQO in acidified water. Mice received this treatment for four months, followed by both groups receiving acidified water for the remainder of the study. Mouse weights were recorded twice a week and mice were euthanized after losing 15% of their original body weight. Tumors were measured with calipers and tongues were harvested for single-cell RNA sequencing and spatial transcriptomics.

Results and Conclusions: All 4NQO-treated mice expressed tumor growth, with many developing large tumors (3-4 mm in diameter). However, male mEAK-7 knock-out mice exhibited an enhanced survival rate compared to male heterozygous mice, and even higher survivability than male wild-type mice. In contrast, female mice showed no significant differences in survival across genotypes. Additionally, preliminary single-cell RNA sequencing data reveals elevated levels of mEAK-7 in 4NQO-treated wild-type mice. These early results raise questions about sex-specific differences concerning mEAK-7's role in head and neck cancer among male and female mice. Lastly, both partial and total mEAK-7 knock-out seem to have a positive effect on survival in mice with head and neck cancer.

Relevance and Importance: Head and neck cancer is the seventh most common cancer globally and is increasing. There is an association between smoking and risk for developing head and neck cancer. In particular, head/neck cancer covers roughly 4% of all cancers in the United States while also being twice as common in men than women. 4NQO is a tobacco-mimicking carcinogen and provides a model for smoking-associated head and neck cancer. Additionally, mEAK-7 is understudied in the context of cancer and rarely studied among the other mTOR complexes. Given these statistics, being able to study mEAK-7 in the context of head and neck cancer in a reliable animal model is crucial to studying the molecular biology of cancer and generating new therapies.

Genetics, Genomics, Chromatin, Signal Transduction, and Transcription

74- Single-nuclei multiomic analysis of small intestinal neuroendocrine tumors and matched liver metastases reveals gene expression changes associated with disease progression

Stephen G Andrews[1], Shreya Rajhans[2], Thuc Nhi Trungvo[2], James P Madigan[1], Steven D Forsythe[1], Mike Kelly[3], Jaydira del Rivero[4], Jonathan M Hernandez[5], Naris Nilubol[6], H Efsun Arda[2], Samira M Sadowski[1]

75- Cytarabine resistance leads to a unique mutational signature

Dengchao Cao[1], Mianmian Yin[1], Zhenhua Zhang[1], Taimour Baslan[2] Jack Zhu[1], Ryan Bertoli[1], Paul Meltzer[1] and Peter D. Aplan[1]

The prognosis of recurrent T-cell acute lymphoblastic leukemia (T-ALL) is poor, mainly due to chemotherapy resistance. The mechanism(s) leading to chemoresistance at relapse are incompletely understood. We previously characterized a mouse strain that is hypomorphic for the DNA replication factor Mcm2. Mcm2hypo mice develop T-ALL due to acquired DNA copy number variations (CNV) involving tumor suppressor or oncogenes. We hypothesize that treatment of Mcm2hypo cells with specific chemotherapy agents may reveal CNV that lead to chemoresistance.We treated two Mcm2hypo T-ALL cell lines (designated 2883 and 2869) and an Mcm2 WT T-ALL cell line (designated 7298) with cytarabine (ARAC), an active agent often incorporated in T-ALL treatment regimens. After one year of treatment with gradually increasing ARAC concentrations, we obtained ARAC resistant cell lines (designated with the suffix CR) that tolerate drug concentrations up to 10,000X that of parental cell lines. These samples were characterized by sparse WGS (for CNV assessment), RNA-Seq and WES.Both the 2883CR and 2869CR cell lines had bi-allelic mutations involving Dck, which is the rate-limiting enzyme in the cytidine salvage pathway, and is thought to be required for metabolism of ARAC. In both cases, one allele was deleted while the second allele had a splice site/region mutation leading to partial intron retention and resultant frameshift. The 7298CR cell line had homozygous deletion of Dck. Western blotting showed absence of WT Dck protein in all three cell lines.Further analysis of WES data revealed thousands of acquired single nucleotide variants (SNV) in both Dck mutant Mcm2hypo cell lines. These mutations occurred in a specific trinucleotide context (C>T in a GCG context, T>C in a GTC context, T>G mutations in GTC and GTT context, and C>G in a GCC context) generating a mutational signature that is not present in the COSMIC database. In addition, the parental 2883 and 2869 cell lines showed fewer mutations (ie, hundreds vs thousands) in a similar context (C>T in a GCG context, T>C in a GTC context, T>G mutations in GTC and GTT context, but not the C>G in GCC context). A GEMINI (Genotoxic Mutational Signature Identified After Clonal Expansion In Vitro) assay of untreated 2883CR and 2869CR cells demonstrated that ongoing ARAC exposure was not required for the mutational signature. We hypothesize that the basic signature (C>T in a GCG context, T>C in a GTC context, T>G mutations in GTC and GTT context) can be produced by Mcm2 deficiency, and this signature is amplified and modified (to include a C>G in GCC context) by Dck inactivation. In summary, we identified three mechanisms responsible for Dck inactivation associated with ARAC resistance: copy number loss, splice site mutations, and splice region mutations. Moreover, Dck inactivation leads to a specific mutation signature. These findings add to emerging data that exposure to specific chemotherapy agents can lead to specific mutational signatures.

76- Integrative Analysis of Multiplex Interphase FISH Copy Number with Whole Exome Sequencing Identifies Tumor Evolution in Stage II Colon Cancer

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Colon cancer ranks as the second leading cause of cancer-related death worldwide. Although standard therapies are highly effective in stage II lymph-node negative colon cancers, about 10-15% of these patients show disease relapse within the next 5-year period after intended curative surgery. Extensive prior research has highlighted the role of intratumor heterogeneity, arising from genomic instability, as a leading contributor to metastasis in colon cancer. In the current study, we pioneered the integration of Multiplex Interphase FISH (miFISH) and Whole Exome Sequencing (WES) data aiming to depict intratumor heterogeneity and delineate the landscape of genetic aberrations that underlie the early metastatic potential in stage II colon cancer patients. To this aim, we have showcased the genome-wide copy-number alterations and single nucleotide mutations derived from WES analysis of samples obtained from primary stage II colon tumors and their patient-matched liver metastases of nine patients using multi-region sampling. miFISH was employed for the concurrent quantification of copy-numbers for nine genes relevant to colon cancer and a centromeric control probe in intact tumor nuclei derived from archival patient material allowing us to establish ploidy baselines and gain and loss patterns for the genes analyzed. Our study was revealing of several copy number alterations, such as gains of chromosomes 1q, 7, 8q, 13q and 20, and losses affecting chromosomes 17p and 18, which were confirmed by miFISH. Based on our SNV data, most frequently mutated genes were APC, TP53, KRAS. We were able to show several mutations in genes belonging to specific pathways most commonly altered in colorectal cancer, such as APC, TCF7L2, AXIN2, FBXW7 in WNT signaling, SMAD4 in TGF-ß signaling, PIK3CA and PTEN in PI3K signaling and KRAS in RTK-RAS signaling pathways. In nearly all cases, subclones that led to metastasis were already present in the primary cancer, indicating a direct progression from the primary to metastatic disease. However, the divergence from the primary tumor to metastasis enabled us to uncover several potential genetic aberrations present in the metastasis but not observed in the primary tumor. Moreover, in one of our cases, despiteharboring APC and TP53 mutations in both the primary and metastatic tumor, unique mutations for these genes along with several distinct mutations in the metastasis were identified. These observations suggest that the metastasis might not be related to the primary tumor analyzed and may have arisen from an independent lesion. In conclusion, applying miFISH and WES to decipher the mutational and copy number landscape in metastatic stage II colon cancer provided comprehensive insights into the complex and heterogenous nature of primary colon cancer and the clonal evolution leading to subsequent liver metastases.

77- Ptpn1 deficiency collaborates with a NUP98::HOXD13 fusion gene to generate B cell precursor acute lymphoblastic leukemia

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Background: Acute lymphoid leukemia is characterized by inherited or acquired mutations that effect critical differentiation and proliferation pathways. We have shown that Mcm2 deficient mice developed T cell acute lymphoblastic leukemia, due to copy number variations, most commonly interstitial deletions throughout the genome. When crossed with mice that expressed a NUP98::HOXD13 (NHD13) fusion gene, Mcm2-NHD13 mice developed B cell precursor ALL (BCP-ALL). A majority of these BCP-ALL had acquired homozygous deletions of Ptpn1, a protein tyrosine phosphatase, leading to the hypothesis that Ptpn1 deficiency combined with NHD13 expression leads to BCP ALL. Objective: To investigate the role of Ptpn1 deletion in BCP-ALL development using mouse models.

Methods: Mice expressing an NHD13 fusion gene were crossed with Ptpn1 knockout mice, generating 6 possible genotypes. Mice were followed for 18 months. Mice with signs of leukemia were characterized by clinical evaluation, CBC, flow cytometry, and IHC. Primary BCP-ALL and derived BCP-ALL cell lines were also evaluated with RNA-seq and molecular pathway analysis.

Results: NHD13+Ptpn1-/- mice developed BCP-ALL with 65% penetrance, characterized by hyperleukocytosis, anemia, thrombocytopenia, and invasion of non-hematopoietic tissues. Similar to human BCP-ALL, NHD13+Ptpn1-/- BCP-ALL had clonal IGH as well as clonal Tcrd gene rearrangements. Flow cytometry revealed CD19 and/or B220 expression. NHD13+Ptpn1+/- mice with BCP-ALL frequently lost the wild-type (WT) Ptpn1 allele in leukemic cells, reinforcing the hypothesis that Ptpn1 can function as a classic tumor suppressor gene in this context. Whole exome sequencing revealed acquired mutations in B-cell differentiation genes (Pax5 or Bcor) and activating mutations in tyrosine kinase genes (Jak1/3 and Flt3). Transcription signature analysis showed significant upregulation of Hoxa/b gene clusters, RNase12 and LncRNAs subsets.

Conclusion: This study demonstrates that Ptpn1 loss combined with expression of NHD13 fusion gene leads to highly penetrant BCP-ALL in mice, suggesting a role for Ptpn1 in preventing malignant transformation. These findings present a collaborative model for BCP-ALL in which the NHD13 transgene leads to increased stem cell self-renewal, somatic Bcor or Pax5 mutations block normal B cell differentiation, and somatic signaling mutations (Jak1/3,Flt3) lead to hyperproliferation, which is potentiated by Ptpn1 deficiency.

78- Single-nuclei RNA and ATAC Sequencing Uncovers Novel Subtypes In Pancreatic Neuroendocrine Tumors

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Pancreatic neuroendocrine tumors (PNETs) are rare cancers, thought to originate from the endocrine cell lineage in the pancreas, with limited curative options and increasing occurrence. To improve PNET diagnosis and treatments, it's essential to move beyond the current diagnostic approaches that rely on incomplete understanding of PNET markers and subtypes. In this study, we used single-cell multiome assays on cryopreserved primary PNETs of varying grade, stage, and metastasis status to characterize their chromatin and transcriptome profiles at single-cell resolution. Additionally, we performed multiome analysis on normal endocrine and exocrine pancreas samples to identify epigenomic and transcriptomic differences between healthy islet cells and PNETs at the single-cell level.Our multimodal analysis revealed extensive inter-tumor heterogeneity with few shared genes across the PNET cohort. In contrast, we found little to no heterogeneity within each individual tumor, suggesting the tumors might arise from a single founder cell. Using differential gene expression analysis, we further identified several distinct cell populations within PNETs, including tumor, endothelial, and immune cells. This analysis also revealed that the tumors had distinct gene profiles of cell markers (PDX1/ARX), cancer metabolism (VHL and HIF1a/HIF2a), and chromatin regulation (DAXX/ATRX). To identify potential signaling pathway genes that are associated with PNETs, we performed ligand-receptor analysis to find cell-cell communication networks, between tumor and non-tumor cells, within tumor samples, and compared our findings to normal pancreas samples. For a subset of tumor samples, we identified signaling pathways previously understudied in PNETs. Altogether, we observed diverse molecular profiles of PNETs with little overlap between patient samples. This might explain why it has been difficult to find successful treatments for the majority of cases. Our results can help develop personalized medicine approaches by elucidating the molecular markers that vary between PNETs, allowing further classification of PNETs. Moving forward, our group aims to use the data from this study to support the development of an enhanced diagnostic and therapeutic system for PNETs. Overall, our study provides valuable insights into the molecular features of PNETs and their heterogeneity, which can aid in the development of targeted therapies for this rare cancer.

79- Characterization of cell type-specific enhancers in the human pancreas using a massively parallel reporter assay

Meagan Jezek[1], Li Wang[1], Makana Ioh[1], Songjoon Baek[1], and H. Efsun Arda[1]

The pancreas is a vital organ composed of multiple cell types that carry out distinct functions. Accurate control of cell identity is integral to maintaining healthy, functional systems, and dysregulation of the mechanisms underlying cell identity can lead to developmental defects and diseases, including pancreas cancer and diabetes. Therefore, it is crucial to understand the regulatory mechanisms that drive cell type-specific functions. Enhancers are non-coding genetic elements that regulate transcription through interactions with transcription factors and are overwhelmingly responsible for cell type-specific gene expression. Additionally, over 80% of disease-associated genetic variants map to enhancer regions, emphasizing the need to understand their function. While nearly one million candidate enhancer regions have been identified based on enhancer-associated chromatin features, the functional characterization of enhancers has been a daunting challenge, particularly at genome-scale. Our lab has identified over 100,000 lineage-specific candidate enhancer regions unique to the five major human pancreas cell types, and digital footprint analysis of the chromatin accessibility landscape has revealed that these regions are likely bound by cell type-specific transcription factors, supporting their potential role as cell-type specific enhancers. To systematically characterize the drivers of cell identity among the distinct cell types of the pancreas, we are using massively parallel reporter assays to quantify enhancer activity in pancreas cells. We have synthesized thousands of candidate enhancer sequences centered around lineage-specific transcription factor motifs and generated lentiviral reporter libraries that are being used to assess candidate enhancer activity in both primary pancreas cell obtained from healthy donors, and the human pancreas cancer cell line PANC-1. We hypothesize that lineage-specific transcription factors drive cell type-specific enhancer activity, so to test this hypothesis and gain more insight into the regulatory pathways involving enhancer-mediated cell identity, we also plan to alter transcription factor activities in our MPRA experiments through targeted knockdown or overexpression of specific transcription factors. These results will yield the first functional enhancer map of human pancreas cells and advance our understanding of how these enhancers orchestrate cell type-specific gene expression programs.

80- Investigating the Role of RNA Post-Transcriptional Regulation in Cancer Immune Evasion

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Natural killer (NK) cells are effector lymphocytes of the innate immune system that kill physiologically stressed cells and play a key role in immune surveillance and the destruction of cancer cells. NK cells, despite their ability to infiltrate in many cancers, are unable to completely destroy the tumor and tumors still manage to grow and metastasize. Identifying and characterizing cancer immune evasion mechanisms can improve current and future immunotherapies. RNA modifications and regulatory proteins associated with them have roles in immune cell function and the anti-tumor immune response. This project aims to investigate how RNA post-transcriptional regulation modulates the interaction between NK cells and cancer cells. We have shown, through a CRISPR knockout screen, that knocking out the highly conserved RNA helicase ZNFX1 (zinc finger NFX1-type containing 1) increased UOK262 sensitivity to NK cells. We have also shown that ZNFX1 is a non-essential gene. ZNFX1 is known to be involved in the RNA interference (RNAi) pathway in Caenorhabditis elegans and acts as a cytosolic double-strand RNA (dsRNA) sensor that activates the antiviral response through the MAVS signaling pathway. Individuals with inherited deficiency of ZNFX1 exhibit multisystem inflammation and susceptibility to viral infections and mycobacteria disease. However, no previous reports have described a role for ZNFX1 in tumor biology or NK cell cytotoxicity. Therefore, we will dissect the role of ZNFX1 as a novel regulator of tumor immune evasion to NK cell-mediated killing. Leveraging single cell RNA sequencing, we are investigating how gene expression changes in both target cancer cells and NK cells during a co-culture experiment. We have included both wild type and ZNFX1 KO cells, allowing us to delineate which pathways are under ZNFX1 regulation. Understanding how ZNFX1 modulates NK killing will allow us identify targets to enhance NK based immunotherapy approaches.

81- Investigation of breast tumor biology and microenvironment in women of African descent using a single cell multiomic approach

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Women of African descent are at an increased risk of developing and dying from aggressive subtypes of breast cancer. A connection between aggressive disease and Western Sub-Saharan African ancestry has been postulated, but it remains largely unknown to what extent breast cancer in Africa is reminiscent of breast cancer in U.S. African American (AA) women who experience disproportionately high mortality rates. We performed ATAC- and RNA-sequencing on 9 human triple-negative breast cancer cell lines of U.S. origin and discovered that African ancestry influences the chromatin landscape, leading to disparate transcription factor (TF) activity and downstream gene expression patterns indicative of an aggressive tumor biology. Here, we describe an ambitious study that employs single-nucleus (sn) ATAC- and RNA-sequencing (snMultiome) of frozen breast tumors to characterize chromatin accessibility and gene expression patterns with single-cell resolution in AA (n=33), Kenyan (n=25), and European American (EA, n=24) women in relation to genetic ancestry, risk factor exposures, clinical characteristics, and 5-year survival. To achieve this, we successfully isolated intact, high-quality single nuclei from archival frozen breast tumor tissue through an optimized combination of enzymatic digestion and automated tissue homogenization. We performed snMultiome sequencing of 82 tumors using the 10x Genomics platform. Following filtering, normalization (SCT for snRNA; LSI for snATAC), peak calling (MACS2), and integration (Harmony), our dataset includes a total of 296,557 nuclei. Cancerous (163,419 nuclei) and non-cancerous (133,138 nuclei) cells were distinguished based on DNA copy number (CopyKat). Within the microenvironment, 11 major immune, epithelial, and stromal cell types were successfully annotated, exhibiting distinct patterns by population group (e.g. AA tumors showed markedly increased abundance of myeloid and T-cells, while Kenyan tumors showed increased abundance of pericytes and fibroblasts). A large number of enriched TFs within each cell type varied significantly by population group, suggesting distinct chromatin accessibility patterns related to genetic ancestry. Within cancerous cells, striking intra- and inter-tumoral heterogeneity was observed by genetic ancestry even within molecular subtype groups. Current efforts focus on in-depth molecular characterization of ancestry- and risk factor-related differences in the tumor epithelium and microenvironment and distinct signatures present in lethal disease. This project holds the potential to yield crucial insights into how ancestry or other factors may influence the etiology of different breast cancer subtypes, as well as produce clinically actionable biomarkers and therapeutic targets to enhance precision medicine within patient populations at high risk for aggressive disease.

82- Functional interplay between super enhancer-bearing genes RUNX2 and ZMIZ1 in osteosarcoma

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83- Cell type-specific effects of common germline variation on gene expression and pancreatic cancer risk

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Background and Hypotheses: The human pancreas is a complex organ composed of multiple unique cell types with distinct functions. Exocrine pancreas (90% of pancreas) is the major lineage which is composed of acinar and duct cells and is involved in one of the most aggressive forms of pancreas cancer – Pancreatic ductal adenocarcinoma (PDAC). Pinpointing genes affecting PDAC risk in these cells will aid in early detection, prevention, and effective therapies. High-risk PDAC mutations exist in protein coding regions, but common variants from Genome Wide Association Studies (GWAS) often reside in non-coding regions, complicating the identification of their target genes. Previous studies on noncoding variations used bulk tissue samples, potentially hiding cell-specific genetic effects. Understanding gene expression in distinct exocrine pancreas cells can deepen our grasp of pancreatic cancer's molecular roots.

Study Design and Methods: We used a flow cytometry-based method to purify human acinar and duct cell populations obtained from human organ donors. To identify potential genes influenced by non-coding risk variants, we employ cell type-resolved expression, chromatin accessibility and methylation quantitative trait loci (eQTL, caQTL and meQTL, respectively) analyses in purified pancreatic acinar and ductal cells. Thus far, we performed expression quantitative trait locus (eQTL) analyses using RNA-Seq data and imputed genotypes (using the TOPMED reference panel) from 96 acinar and 94 duct samples. After quality control, we tested associations between ~6M imputed SNPs and expression of ~19,185 genes in acinar and 22,115 genes in duct cells. A permutation procedure was used to control the false discovery rate below 10%.

Results and Conclusions: We identified 66,471 cis-eQTLs (2,288 eGenes) in acinar and 114,723 cis-eQTL (4,010 eGenes) in duct samples (FDR<0.1), of which ~20% were specific to each cell type (P>0.05 in the other cell type). Nearly 49% of acinar eQTLs were shared with duct samples and 28% of duct eQTLs were shared with acinar samples. A common pancreatic cancer risk locus on 9q34.2 in the ABO gene (rs687289) was associated with ABO expression in both acinar (P= 1.47x10-8) and duct (P= 5.81x10-8) samples with similar effect sizes. Interestingly, 30 eGenes each in the acinar and duct sets, showed an effect in the opposite direction in the other set (at P<0.05) indicating reverse regulation of gene expression driven by same genetic variant in two different exocrine cell types suggesting a complex interplay of genetic variants in shaping cellular fate. In conclusion, we performed a comprehensive analysis of the genetic architecture of gene expression and identified genetic variants that influence gene expression in exocrine pancreas cell types.

Relevance and Importance: Our study discovers cell type-specific novel genes and putative regulatory mechanisms underlying PDAC risk with enhanced precision compared to bulk tissue QTL studies. Furthermore, these datasets will serve as valuable resources for annotating non-coding risk loci associated with other pancreatic diseases, including pancreatitis and diabetes.

84- ETS1, a target gene of the EWSR1::FLI1 fusion oncoprotein, regulates the expression of the focal adhesion protein TENSIN3

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Background: Individuals with metastatic Ewing sarcoma (EWS) have five-year survival rates of less than 30%. The advancement of therapies targeting metastatic EWS will require an enhanced understanding of the biological basis for the dissemination of primary EWS tumor cells. However, the mechanistic basis for EWS metastasis remains poorly understood as these tumors harbor few mutations beyond the chromosomal translocation that initiates the disease. Instead, the epigenome of EWS cells reflect the regulatory state of genes associated with the DNA binding activity of the fusion oncoproteins EWSR1::FLI1 or EWSR1::ERG. Collectively, EWSR1::FLI1/ERG fusion proteins' reprogramming of the transcriptome suppresses cell differentiation and promotes tumor growth. Recent studies have highlighted the potential for the expression of genes repressed by EWSR1::FLI1/ERG fusion proteins either because of heterogenous fusion protein levels, or the effect of external stimuli, as a contributing factor to metastatic EWS. In this study, we evaluated the hypothesis that the EWSR1::FLI1/ERG proteins inhibit the expression of transcription factors that, if de-repressed, could contribute to EWS metastasis.

Methods: To examine EWSR1::FLI1/ERG's regulation of gene expression, we assayed DNA binding using ChIP-seq or CUT&RUN, RNA using RNA-sequencing (RNA-seq) or qRT-PCR, and protein using immunoblotting or immunofluorescence (IF). We depleted the expression of EWSR1::FLI1/ERG proteins using RNAi and overexpressed ETS1 using a full-length cDNA or CRISPR activation.

Results and Conclusions: An integrated analysis of the transcriptome of multiple EWS cell lines (control and EWSR1::FLI1 or EWSR1::ERG-depleted), and the assessment of EWSR1::FLI1 binding and epigenetic marks (H3K27Ac, H3K9Me3, H3K27Me3, and H3K4Me3) assayed by CUT&RUN and/or ChIP-seq, along with the expression profiles of primary EWS tumors, highlighted EWSR1::FLI1's repression of multiple genes encoding transcription factors that regulate cell differentiation, including ETS1. Focusing on ETS1, we detected EWSR1::FLI1 binding and a H3K27me3 repressive mark at this locus. Analysis of ETS1 binding following depletion of EWSR1::FLI1 in TC-32 cells defined 2,635 ETS1 binding sites, of which 84% map to promoter regions. This finding contrasts with that of EWSR1::FLI1, which predominately binds distal regions. Ectopic expression of ETS1 in TC-32 cells at levels comparable to that observed following silencing of EWSR1:FLI1 identified 5555 genes as exhibiting altered expression, including 522 that ChIP-seq analysis indicate ETS1 regulates directly. One of these ETS1-regulated genes encodes TENSIN3 (TSN3), a focal adhesion protein. EWS cell lines expressing ETS1 (CRISPRa) exhibited increased TNS3 expression and enhanced movement compared to control cells. The cytoskeleton of control cells and ETS1-activated EWS cell lines also differed. Specifically, control cells exhibited a distributed vinculin signal and a network-like organization of F-actin. In contrast, ETS1-activated EWS cells showed an accumulation of vinculin and F-actin towards the plasma membrane. Interestingly, the phenotype of ETS1-activated EWS cell lines depleted of TNS3 resembled the phenotype of the control cells. Critically, these findings have clinical relevance as TNS3 expression in EWS tumors positively correlates with that of ETS1.

Relevance and Importance: ETS1's transcriptional regulation of the gene encoding the focal adhesion protein TENSIN3 in Ewing sarcoma cells promotes cell movement, a critical step in the evolution of metastasis.

85- Functional Characterization of Enhancers in the Human Pancreas

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The human pancreas is a complex organ consisting of several distinct cell types that function together to regulate glucose metabolism (islet hormone cells) and digestion (acinar and duct cells). Noncoding regulatory genomic regions, also known as enhancer elements, are largely responsible for generating these diverse cell types and maintaining their function. Enhancer dysfunction has been implicated in disease, including pancreas cancer. Due to the importance of cell-type specific enhancers in gene expression, there has been considerable focus on identifying critical enhancers in the human pancreas. Using donor tissue and state-of-the-art genomics assays, our group has identified thousands of putative enhancer elements specific to each human pancreas cell types. We hypothesize that these cell-type specific chromatin regions harbor lineage-specific TF motifs, have regulatory transcriptional activity, and can drive cell-type specific gene expression programs. To test this hypothesis, we performed transcription factor (TF) motif analysis and indeed found the motifs of several pancreas specific lineage regulators enriched in our putative enhancer sequences. As a pilot experiment, we are modulating the expression of exocrine cell TFs in a pancreatic ductal cell line, PANC1. We have generated overexpression lines for five pancreas TFs — HNF1B, BHLHA15, PTF1A, RBPJL, and XBP-1. If our predictions are correct, we expect these TFs to bind their cognate motifs and alter gene expression or chromatin accessibility in PANC1 cells. We will perform ChIP-seq, ATAC-seq, and RNA-seq on these cell lines to reveal the specific binding sequences of the TFs, correlation with open-chromatin regions and the resulting gene expression profiles. Ultimately, our goal is to perform these experiments in primary human pancreas tissue. The results from this study will provide crucial insights into cell-type specific enhancer regulation mechanisms of the human pancreas.

86- HNRNPH1 Promotes the exclusion of EWSR1-Exon 8 in a subset of Ewing sarcoma cells: An appropriated function

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87- Exploring the Oncogenic Role of ACK1/TNK2 in Cancers with the 3q Amplicon

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Background and Hypothesis: Head and neck squamous cell carcinoma (HNSCC) is the 8th most common cancer worldwide and high grade serous ovarian carcinoma (HGSOC) ranks 8th in cancer related mortality in women. Both cancer types are driven by frequent genomic alterations, the most common of which is amplification of the distal region (26-29) of the q-arm of chromosome 3. This amplicon harbors multiple genes encoding for known and potential novel oncogenic kinases, including an unexplored nonreceptor tyrosine kinase referred to as Activated cdc42 associated kinase 1 (ACK1), also known as TNK2. This study aims to investigate the role of TNK2 as potential oncogenic driver in HNSCC and HGSOC.

Study design and Methods: A known small molecule TNK2 inhibitor, AIM100, was used to test the effects of TNK2 catalytic inhibition on 7 HNSCC and 7 HGSOC cell lines. Short term proliferation assays using MTS and long- term colony forming assays (CFA) using crystal violet were performed to test the effects of different concentrations of AIM100 on cell proliferation. Levels of activated TNK2 and its substrates were determined using immunoblotting. Since small molecule kinase inhibitors are promiscuous and often inhibit the kinase activity of additional kinases, siRNA mediated transient knockdown of TNK2 was also performed to validate that effects were due to targeting TNK2 as opposed to off-target effects.

Results and Conclusions: AIM100 treatment did not have any significant effect on the short- term and long- term viability assays in any of the 14 cell lines, however transient TNK2 knockdown showed a significant reduction in cell viability compared to control in 12 cell lines, suggesting that TNK2 promotes carcinogenesis in kinase independent manner.

Relevance and Importance: TNK2 has emerged as an oncogenic kinase in prostate cancer and triple negative breast cancer. It has been shown to act as an intermediatory kinase that bridges multiple receptor tyrosine kinases to their effector pathways. Additionally, it can bind to the activated cdc42 protein in a kinase-independent manner and prevent its GTPase activity thereby maintaining the activated state of cdc42 and its effectors. TCGA pan cancer analysis revealed that 12 to 15 percent of HNSCC and HGSOC patients have TNK2 amplification, yet it remains an understudied kinase in these cancer types. The results from this study suggest that TNK2 could be a potential therapeutic target in HNSCC and HGSOC. Future directions include identifying mechanisms by which TNK2 drives oncogenesis in HNSCC and HGSOC by performing rescue experiments with kinase dead mutants, cdc42 binding domain mutants, and RTK binding domain mutants. In addition, we will develop PROTACs that can inhibit both kinase dependent and kinase independent activities of TNK2.

88- Oncohistone H3 Mutations Facilitate Cse4/CENP-A Mislocalization in Saccharomyces cerevisiae

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Background & Hypothesis: Chromosomal instability (CIN) and aneuploidy are hallmarks of cancer cells. Centromeric (CEN) DNA and associated proteins referred to as kinetochore are key determinants to prevent CIN and aneuploidy. The kinetochore provides an attachment site for microtubules for segregation of sister chromatids during mitosis. Specific recruitment of evolutionarily conserved histone H3 variant CENP-A (Cse4 in Saccharomyces cerevisiae, Cnp1 in Schizosaccharomyces pombe, CID in Drosophila melanogaster) at centromeric chromatin is essential to prevent chromosomal instability (CIN). However, mislocalization of overexpressed CENP-A to non-centromeric chromatin contributes to chromosomal instability (CIN) in yeasts, flies, xenograft mouse model, and human cells. Mislocalization of CENP-A is observed in many cancers and this correlates with poor prognosis. Our 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., NAR 2022). Our recent studies have shown that the interaction of Cse4 with histone H4 facilitates a conformational state of Cse4 in vivo from a “closed” to an “open” state and this contributes to Cse4 mislocalization (Ohkuni et al., NAR, in press). 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 mutations in the C-terminus of histone H3, which are frequently seen (hotspots) in many cancers, affects the interaction of Cse4 with histone H4, and if this leads to mislocalization of Cse4.

Study Design & Methods:We used histone H3 deletion (hht1Δ and hht2Δ), structurally defective (hht1 Y99A), and oncohistone (hht1 E97A) 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 (hht1Δ and hht2Δ) 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.

Relevance & Importance: Given the evolutionary conservation of Cse4/CENP-A, histone H3, and histone H4, it is of interest to examine if oncohistone H3 mutations contribute to mislocalization of CENP-A to non-centromeric chromatin in human cells. These studies are clinically relevant as mislocalization of overexpressed Cse4/CENP-A contributes to CIN in budding yeast and human cells.

89- Targeting SRC kinase family (SFK) members using a novel conformation selective inhibitor, NXP900

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Background and Hypothesis: NXP900 is a novel potent and selective SRC family kinase (SFK) inhibitor that locks its target in “closed” conformation, thereby inhibiting both kinase activity and kinase-independent scaffolding activity via complex formation with protein partners. In contrast, multi-kinase inhibitors currently approved for targeting SRC, including Dasatinib and Bosutinib, block SRC in the active “open” conformation, leading to reduced catalytic activity but having no significant effect on scaffolding functions. In this study, we demonstrate that esophageal squamous cell carcinomas (ESCC) and head and neck squamous cell carcinomas (HNSCC) are exquisitely sensitive to NXP900 treatment, in cell culture and in vivo, and identify a patient population that could benefit from treatment with NXP900.

Study design and Methods: Short term proliferation assays using MTS and long term colony forming assays (CFA) using crystal violet were performed to test the effects of different concentrations of NXP900 on cell viability in eight ESCC and eight HNSCC cell lines. Total expression and activation levels of SFK members were checked using immunoblotting. Xenograft tumors were generated by subcutaneous implantation of ESCC and HNSCC cell lines on the right lower flank of the thigh of CD1 nude mice followed by treatment with vehicle control or 40 mg/kg NXP900 for 28 days.

Results and Conclusions: NXP900 treatment significantly reduced cell viability in ESCC and HNSCC cell lines in both short term (72 h treatment) and long term (14 days) assays. Oral administration of NXP900 in xenograft mouse models resulted in an average decrease of tumor volume of 71% while in the vehicle control group tumor volume increased by 472% ($p \leq 0.001$), demonstrating significant tumor regression. Additionally, immunoblot analysis demonstrated that NXP900 significantly inhibited the activating phosphorylation levels of SFK members at 10 nM, while having no significant effect on other kinases such as CSK at concentrations up to 250 nM.

Relevance and Importance: Targeting SRC using multi-kinase small molecule inhibitors, such as Dasatinib and Bosutinib, has emerged as a promising anti-tumor therapy in hematological malignancies. However, in solid tumors, there is frequent occurrence of drug resistance and off-target toxicity. NXP900 is a novel potent and selective SFK inhibitor with nanomolar IC50 against SFK members. NXP900 has marked antitumor efficacy in ESCC and HNSCC models in vitro and in vivo providing substantial proof of concept for targeting solid tumors. An IND for NXP900 has been cleared by the FDA and phase 1 clinical studies have been initiated.

90- Beta-TrCP-mediated proteolysis of Mis18 beta prevents CENP-A mislocalization and chromosomal instability

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91- Molecular characterization of EBV-positive T/NK-cell lymphoproliferative disorders of childhood uncover distinct changes from their adult counterparts

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Background and Hypotheses: EBV-positive T/NK-cell lymphoproliferative disorders (EBV-TNK-LPD) are rare diseases classified according to the age of onset and clinicopathological features. Cases presenting in adults include Aggressive NK-cell Leukemia and Primary nodal EBV-positive T/NK-cell lymphoma. Most cases presenting in childhood have no known immune deficiency and include Hydroa vacciniforme lymphoproliferative disorder, Chronic active EBV disease (CAEBV), and Systemic EBV-positive T-cell lymphoma of childhood (SEBVTCL). In comparison to their adult counterparts, little is known about their genetic landscape, with previous studies identifying mutations in DDX3X and epigenetic modifiers. We hypothesize that similar molecular alterations occur across the spectrum of EBV-TNK-LPD.

Study Design and Methods: Files were examined for cases of EBV-TNK-LPD across all age groups in 2 academic institutions with subsequent pathological review. Clinical data was collected from reports and clinical records. Two next-generation sequencing targeted panels, both with candidate genes relevant to T/NK-cell lymphomas, were used according to the amount of DNA available. Statistical significance for two-tailed p-value was established as 0.05.

Results and Conclusions: 28 cases of EBV-TNK-LPD were identified. 16 cases were included in the childhood group and 12 cases in the adult group based on current International Consensus Classification criteria. Mean age at diagnosis in the childhood cohort was 14 years old, while in the adult cohort it was 53 years old (unpaired t-test, two-tailed $p<0.001$). Mean number of mutations per case was 0.33 in the childhood group and 1.27 in the adult group (Mann-Whitney, two-tailed $p=0.034$). Cases in the childhood group had pathogenic/likely pathogenic variants in STAT3 (n=2), JAK1 (n=1), DDX3X (n=1), PLCG1 (n=1), and frequent FYN p. R96Q VUS (n=3). Cases in the adult group had pathogenic/likely pathogenic variants in STAT3 (n=2), PLCG1 (n=1), NOTCH1 (n=1), B2M (n=1), JAK3 (n=1), TET2 (n=3), DNMT3A (n=1), SMARCB1 (n=1), ASXL2 (n=1), BCOR (n=1), FYN p.G255E (n=1), and TP53 (n=1). In the whole cohort, no pathogenic/likely pathogenic variants were identified in 15/26 (57.7%) samples, mostly SEBVTCL (8 wild-type cases). In summary, adult-type cases carry a higher number of mutations and are enriched for alterations in epigenetic modifiers, while CAEBV and SEBVTCL carry somatic mutations in STAT3 and other genes in the JAK-STAT pathway, DDX3X, and PLCG1. Still, a large proportion of SEBVTCL is wild-type with current targeted panel strategies. In these diseases, FYN p.R96Q VUS may be a candidate gene to be explored in germline molecular studies.

Relevance and Importance: This study sheds light on the molecular landscape of EBV-TNK-LPD in childhood and may provide a rationale for therapeutic inhibitors of the JAK-STAT pathway, while adult-type EBV-TNK-LPD might benefit from targeting epigenetic changes. We also highlight the gap in understanding the role of VUS that might be related to disease pathogenesis, paving the way for additional studies in these rare diseases.

92- Identification of novel regulators of TLK1-ASF1-HIRA pathway

Simran Khurana[1], Justin W Leung[2] and Travis Stracker[1]

93- Genome wide screening reveals the CST complex as a vulnerability of ARID1A deficient colorectal cancers

Shalu Sharma[1], JordannSmak[1], UmaShankavaram[1],Isabel Garcia-Cao[2], Miguel M. Álvarez[2], Josep Biayna[2],Marina Salvadores[2], Jose Espinosa-Carrasco[2], Fran Supek[2,3,*] and Travis H. Stracker[1,2*]

94- Molecular chaperone DNAJC9 prevents CENP-A mislocalization and chromosomal instability by maintaining H3-H4 supply

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95- Combined MEK and RET inhibition overcomes resistance to single agent RET inhibition in RET-mutant Medullary Thyroid Cancer

Arwa Fallatah[1],[2], Abdelrahman Rahmy[1], Sophia Khan[2], Hsien-chao Chou[1], Kerstin Heselmeyer-Haddad[1], Darawalee Wangsa[1], David Milewski[1], Yong Kim[1], Robert Hawley[1], Young Song[1], Xiaohu Zhang[4], Craig Thomas[4], Jun S. Wei[1], Jong-In Park[3], John Glod[2], Javed Khan[1],[2]

96- Interplay between Cohesion and BRCA2 in establishment of BRCA2 deficient tumors

Satheesh K Sengodan, Kajal Biswas, Dillon Dierman, Sandra Burkett and Shyam K Sharan

97- Developing LZK targeting inhibitors as a novel strategy to treat 3q amplicon positive esophageal and head and neck squamous cell carcinomas

Maxine Rubin[1], Meghri Katerji[1], Amy Funk[1], Katherine Nyswaner[1], Carolyn C. Woodroofe[2], Eric Lindberg[2], Rolf Swenson[2], John Brognard[1]

The 3q amplicon (3q26-2q29) occurs in 35% of Esophageal Squamous Cell Carcinoma (ESCC) and 20% of Head and Neck Squamous Cell Carcinoma (HNSCC) patients. Our lab has previously identified 3q resident gene, MAP3K13, which codes for Leucine Zipper-bearing Kinase (LZK), as an oncogenic driver in these SCCs. The objective of this study is to explore whether LZK serves as a potential therapeutic target using small molecule inhibitors in these cancer subtypes.To test whether LZK can be pharmacologically targeted, we repurposed GNE-3511, an inhibitor that targets a similar kinase. We tested GNE-3511's activity towards LZK using in vitro kinase assays as well as Western blot and ELISA. We then developed and tested novel, more potent and selective LZK inhibitors in collaboration with the Swenson lab. The effects of these LZK inhibitors were tested on the viability of ESCC and HNSCC cells. The specificity of the inhibitors to LZK was confirmed with a drug resistant mutant form of LZK (Q240S) through rescue experiments. The inhibitors were then licensed to startup company, Eureka Bioscience, which optimized the drug-like properties to generate an even more potent inhibitor, UER-292. The effects of these inhibitors on tumor growth in vivo have also been tested. Finally, we have begun to explore the mechanism through which LZK acts as an oncogenic driver. In this study, we showed that GNE-3511 binds to LZK in vitro and inhibits LZK activity in cells by downregulating the downstream targets of LZK. Furthermore, treatment of ESCC and HNSCC 3q+ cell lines with GNE-3511 caused a significant decrease in cell viability compared to amplicon negative cells, supporting LZK's ability to be pharmacologically targeted. These decreases in viability were rescued through expression of the drug-resistant mutant form of LZK (Q240S), confirming the toxicity of GNE-3511 is due to the specificity to LZK. Novel LZK inhibitors developed in-house also caused similar if not better inhibition of LZK activity and subsequent reduction of cell viability. Furthermore, In vivo cell line xenograft and patient-derived xenograft models harboring the 3q amplicon showed suppression of tumor growth when treated with LZK-targeting inhibitors, with UER-292 even causing regression in HNSCC CAL33 xenograft tumor growth. Lastly, we have identified AKT as a downstream target of LZK, specifically regulated by LZK's catalytic function in HNSCC and ESCCs containing the 3q amplicon. Overall, our data shows the potential of pharmacologically targeting LZK in HNSCC and ESCC patients that contain the 3q amplicon. Current treatment options for HNSCC and ESCC patients are limited with low efficacy and adverse effects. Our lab has established LZK as a therapeutic target in squamous cell carcinomas containing the 3q amplicon. LZK-targeting inhibitors have the potential to yield targeted therapies for a large percentage of ESCC and HNSCC patients that do not respond to current treatment options. We have also shown LZK's role in regulating known oncogene Akt, in which catalytic inhibition of LZK causes decreased expression of pAkt in 3q+ HNSCCs and ESCCs. Our leading compound, UER-292, holds significant potential as a novel treatment for these patients.

98- A cytosolic complex between exportin-7 and Ste-20 like kinase signals via PI3K-AKT-mTOR pathway in cholangiocarcinoma

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Cholangiocarcinoma (CCA) is a rare and deadly malignancy with an evolving therapeutic landscape. Although nucleic acid-based evaluations have unveiled some druggable targets, limited options are available for most patients with this disease. The proteomics study of biliary small extracellular vesicles (sEVs) from 16 patients with CCA revealed a 17-fold enhancement of the nuclear exportin-7 (XPO7) compared to non-cancerous sEVs. Hence, we performed IHC analysis of XPO7 expression in CCA tumors from 170 patients which unexpectedly demonstrated intense cytoplasmic staining in 30% of the samples, which correlated with abbreviated survival. Within the cytosol, we demonstrate that XPO7 exists in a molecular complex with the serine/threonine kinase, Ste-20 like kinase (SLK). shRNA-mediated knockdown of either XPO7 or SLK abrogated tumor organoid formation in vitro, and reduced orthotopic tumor formation and its morphology in athymic mice. A kinome screen of FDA-approved drugs revealed tivozanib inhibits SLK (IC50= 81.8 nM), which we confirmed using crystallography. Tivozanib treatment reduced tumor organoid formation in vitro and induced tumor regression in vivo using murine xenografts with resulting morphology similar to XPO7 and SLK knockdown tumors. Importantly, site-directed mutagenesis of SLK was used to confirm the observed phenotypic alterations secondary to SLK inhibition. Signal transduction studies with SLK knockdown in CCA cell lines demonstrated downregulation of PI3K-AKT-mTOR signaling with further effects on DNA damage resulting in G2 cell cycle arrest. Using ex vivo system, we evaluated tumor slice cultures expression high and low XPO7/SLK by treating them with tivozanib. Further evaluation of these tivozanib-treated XPO7/SLK-expressing tumors (N=3) and without this expression pattern (N=6) demonstrated a reduction in the expression of phosphorylated mTOR (S2448), AKT (T308), and S6 (S235/236) in the high XPO7/SLK-expressing tumors but not in tumors without this expression pattern. Lastly, tivozanib monotherapy demonstrated in vivo efficacy with arrest of tumor progression (RECIST stable disease) in an ongoing clinical trial (NCT04645160).

99- Investigating the lineage-specific roles of the Forkhead box (FOX) family in cancer

Allison V. Mitchell[1] and Michael M. Gottesman[1]

Background and Hypotheses: While significant progress has been made in uncovering the universal biology of cancer, tumor phenotypes are closely associated with the epigenetic state of the cells of origin and developmental lineages. The Forkhead box (FOX) superfamily is the largest transcription factor (TF) family in the human genome whose members perform critical functions in development, adult tissue homeostasis, and cancer. Some members drive oncogenesis, offering potential therapeutic targets, while others act as tumor suppressors. Understanding the varied effects of the FOX family across cancer types requires insight into the associated epigenetic mechanisms. Therefore, we hypothesize that comprehensive genomic analysis of cancer cell lines and patient tumor samples can elucidate the contexts in which individual members of the FOX family elicit pro- or anti-tumorigenic effects.

Study design and methods: In this work, we analyzed data from genome-wide cellular fitness screens (CRISPR-Cas9, RNAi) across human cancer cell lines to identify cellular lineages selectively dependent upon FOX genes. In addition, we analyzed TFs that display a highly correlated pattern of cell dependency with each of the FOX genes to uncover a co-dependency network. These results were aligned with gene expression profiles to interrogate the associated functions. Finally, we analyzed patient datasets to determine the relationship between FOX gene expression and patient outcomes.

Results and conclusions: We identified ten FOX genes that were selectively essential within a specific cell-type lineage. These lineage-selective FOX genes were found to have co-dependencies, which included other lineage-selective TFs, suggesting a lineage-specific transcriptional network. We also identified several cancer lineages dependent upon multiple members of the FOX family. In these cases, these FOX genes displayed an overlapping co-dependency network of lineage-specific TFs. Patient data validated these findings, linking FOX gene expression to worse overall survival. In lineages with dependency upon multiple FOX genes, including sarcoma and lung, elevated expression of both FOX members was associated with the worst survival outcomes. These findings suggest potential cooperation among FOX members in regulating pro-tumorigenic functions.

Relevance and importance: These findings highlight the utility of genomic and functional screening data for disentangling complex epigenetic and functional relationships within the FOX family. This work will lay the foundation for prioritizing the contexts where individual FOX family members may be most effectively studied and targeted to improve patient outcomes.

Immunology, Virology, and Metabolism

100- Investigating the immunological role of MAIT cells in Glioblastoma

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101- In vivo detection of hiv-1 antisense transcripts in donors before and during art

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Background: Antisense transcripts (AST) was demonstrated to promote HIV latency through epigenetic modification of histones in the 5' LTR by the Polycomb Repressor Complex 2 (PRC2). Here, we asked whether HIV AST is expressed in infected PBMCs collected from untreated and ART-treated donors.

Methods: AST levels were measured by cell-associated antisense RNA single-genome sequencing (SGS) of a 1.7kb fragment in the opposite orientation of the env coding region. An endpoint digital PCR approach with tagged-cDNA and donor-specific primers was also used to quantify AST copies in the samples.

Results: We detected HIV AST in 11/12 donors with a median of 14 [IQR 5-34] copies/100 infected PBMCs. Antisense SGS revealed that about 5% of infected PBMCs collected from donors on ART contained AST at any given time. Digital PCR showed similar levels of AST expression in untreated donors with varying levels of plasma viremia. Further, in the donors on ART, we observed no statistical difference between the levels of sense and antisense transcripts.

Conclusions: HIV antisense transcripts are expressed at low levels in both ART-treated and untreated individuals. The in vivo expression of AST irrespective of treatment status warrants further investigation into its potential role as a long non-coding RNA capable of regulating HIV-1 sense gene expression and inducing HIV latency. Understanding the role of HIV AST in vivo may inform future strategies for controlling HIV replication without ART.

102- NOTCH1 reverses immune suppression and drives anti-tumor immunity in small cell lung cancer through STING

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103- Sublethal doses of genotoxic chemotherapeutics and antivirals promote polyomavirus replication

Subhajit Chatterjee[1], Gabriel J. Starrett[1]

Transplant recipients are thought to be at an increased risk of virus-mediated cancers due to weakened immune control. Some chemotherapeutics, antivirals, and immunosuppressive treatments can damage DNA, and could also affect persistent, subclinical DNA virus infections. Polyomaviruses (PyV) are ubiquitous pathogens that depend on host DNA damage responses for replication and are linked to cancer in immunosuppressed patients. These viruses can integrate into tumor genomes, sustaining oncogene expression. Our starting hypothesis was that DNA damaging drugs in transplant recipients might promote viral integration, leading to cancer. To investigate this hypothesis, we conducted experiments using immortalized bladder epithelial cells (HBLAKs) infected with BK polyomavirus (BKPyV) and exposed them to chemotherapeutic agents (Etoposide, Cisplatin, 5-Fluorouracil), immune-conditioning drugs (Cyclophosphamide, Fludarabine, Busulfan) and antiviral drugs (Cidofovir, Ganciclovir, Acyclovir), all known to be genotoxic. Surprisingly, our preliminary findings indicate that under mild genome instability (at IC10 concentrations for 48 hours) following infection, BKPyV replication increases four- to fivefold compared to untreated cells. Prolonged exposure for five days further amplifies BKPyV replication, resulting in a nine- to twelve-fold increase over untreated cells. Intriguingly, a six-hour pre-treatment before infection also enhances viral replication by eleven- to fifteen-fold compared to untreated cells, without continued drug exposure. Additionally, we observed increased susceptibility to BKPyV infection following these pre-treatments. Further, we conducted validation experiments under HBLAKs organotypic culture conditions. We are currently investigating alterations in viral genome replication intermediates, and integration rates to decipher the mechanisms underlying these observations. Additionally, we are scrutinizing metagenomic data from skin swabs taken shortly after busulfan and fludarabine treatment in patients to determine if these drugs acutely influence viral replication and recombination prior to the onset of immunosuppressive effects. These findings suggest that even low-level DNA damage resulting from therapeutic interventions can promote PyV replication, contributing to the diseases observed in immunosuppressed patients, including cancer.

104- Peripheral immune analyses from phase I trial of HPV vaccine PRGN-2009 in combination with bintrafusp alfa in patients with HPV-associated cancers

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105- Peptide vaccine delivery using synthetic bacterial spores

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Background and hypothesis: Nanoparticles represent a potent antigen presentation and delivery system to elicit an optimal immune response. Many types of nanoparticles have been developed and tested and all of them showed promising results. Together with the use of nanoparticles the peptides that can elicit humoral and cellular immune response is growing. To develop a clinical safe and effective vaccine, several issues need to be addressed like the selection of the optimal antigen, adjuvant, and delivery system. In this project, we discuss the use of synthetic bacteria spore nanoparticles for the delivery of peptide vaccines.

Study design and methods: To design an effective peptide vaccine delivery system, we fabricated 500 nm synthetic bacterial spores (termed “SSHELs”) in which we encapsulated a highly immunogenic model peptide derived from ovalbumin with the sequence: CSMLVLLPDEVSGLEQLESIINFEKLTEWTS. The resulting particle was termed SSHELsEncPEP. the peptide sequence SIINFEKL embedded in this sequence, was chosen for its capability to induce specific and well-characterized immune responses and because of its ability to be detected by a previously described antibody (H-2Kb 25-D1.16) when the peptide is cross-presented in the context of MHC-I. MutuDC1940 dendritic cells were used in vitro to assess the capability of SSHELsEncPEP to promote cross-presentation of the SIINFEKL peptide. In vivo, we vaccinated C57Bl/6 mice using two FDA-approved routes (IV and IM). Peripheral blood was analyzed to assess the vaccine capabilities of SSHELsEncPEP to stimulate CD8+ T cells response. All experimental data underwent statistical analysis to ensure the reliability and validity of our findings.

Results and Conclusion: SSHELsEncPEP demonstrated increased efficacy in promoting the cross-presentation of the SIINFEKL peptide in MHC-I in vitro compared to free peptide. Furthermore, we demonstrated that SSHELsEncPEP efficiently delivered the peptide in vivo and successfully stimulated activation of CD8+ cytotoxic T lymphocytes (CTLs) compared to free peptide. Our trials showed that mice vaccinated with SSHELsEncPEP produce active CTLs that, when in the presence of the antigen, released the cytokines (TNFα, IL2, IFNγ) necessary to induce an efficient immune response. Our studies therefore demonstrated that SSHELs -mediated delivery of peptide vaccines can result in increased protective and therapeutic efficacy of the vaccine.

Relevance and importance: SSHELs represent an innovative strategy to delivery peptide vaccines without the use of adjuvants. We demonstrated that SSHELs provide superior antigen delivery, both in vitro and in vivo, thereby increasing the efficacy of vaccination protocols. We are now testing, with promising results, the efficacy of SSHEL-mediated vaccination in a murine melanoma tumor model. Beyond melanoma, the preliminary results obtained using SSHEL technology may open a new road for the use of nanotechnologies in the treatment of other cancers. Thus, in addition to their potential in drug delivering drugs to specific tissues (as demonstrated in previous reports), SSHELs may be modified as potent vaccine delivery vehicles for use in cancer immunotherapy.

106- Utilizing RNA devices to control PD-1 gene expression in mammalian cells for cancer immunotherapy

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Immune checkpoint blockade, a major medical breakthrough in recent years, has become one of the most effective approaches in immunotherapy against a broad spectrum of cancers, including those in late metastatic stages. The agents against either the PD-1 receptor or the PD-L1 receptor-ligand have high efficacy and long durability. However, one of the problems associated with the current immunotherapy approach is the exhaustion of T lymphocyte cells. PD1 is an important gene to maintain peripheral tolerance and cellular homeostasis and hence, treatment procedures mediated by checkpoint inhibition are reported to impose deleterious effects. We developed a different approach. Instead of checkpoint blockade, we specifically control expression of genes key to checkpoint inhibition by using RNA devices to control the expression of PD-1 directly at the levels of transcription, splicing and translation through bypassing the host cell regulatory networks and pathway altogether. We have tested our approach using tetracycline regulated RNA devices to control PD1 gene expression by CRISPR knock-in (in EL4 cells) and established the proof of concept that a ribozyme and a couple of functional RNA devices work efficiently in mammalian cells and demonstrated their reversibility and re-inducibility to regulate the gene expression. To further improve the performance of the RNA devices, we have also made a lentiviral library of devices by randomizing the sequences in communication module with the help of our in-house script and we are in the process of performing high throughput screening to identify RNA devices with high efficiency and test the applicability of the RNA devices in animal models before possible clinical application. In summary, our system clearly indicates the robustness, efficiency, and reversibility of the functional riboswitch devices to control PD1 gene expression for the development of a proper treatment regimen for cancer and viral infections with higher success rate relative to the currently available approaches.

107- Type I interferon and IL27 modulate regulatory T cells in a Lupus-prone mouse model

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Systemic lupus erythematosus (SLE) is an autoimmune disease that features deposition of autoantibodies and systemic inflammation, which leads to kidney damage. The heterogeneity of clinical manifestations in SLE prevents a complete response in patients to current treatments, necessitating innovation of novel avenues of therapy. In modeling SLE, our lab has generated a novel mouse strain (the ARE mouse) that demonstrates a lupus-like pathology due to chronic inflammation caused by IFN□. SLE induces an array of lymphocyte and cytokine aberrations. Specifically, elevated type I interferon (IFN) signature has been broadly implicated in SLE. Similarly, IL27, a cytokine in the IL6/IL12 superfamily that signals via the STAT1 pathway, and which parallels IFNy activation, emerges as a promising target of investigation. Thus, we seek to interrogate the dynamics of T cell subsets and how abrogation of type I IFN and IL27 signaling impact their phenotypes. Using flow cytometry analysis of type I interferon receptor (IFNAR)-deficient and IL27Ra-null (WSX1-/-) ARE mice, we first confirmed that kidneys of ARE-/- mice experienced significantly increased leukocyte infiltration and CD8+ T cells. In addition, responder T cells also demonstrated a pattern of systemic activation through a shift to central memory phenotype as well as upregulation of activation markers. Furthermore, the proportion of CD4+ FOXP3+ regulatory T cells expressing CD25 (IL2Ra) decreased in both the spleen and kidneys of ARE-/-. This loss of bona fide Treg population (FOXP3+ CD25+) may be attributed to disease progression, as Tregs' dysfunctional ability to uptake IL2 will lead to an inability to suppress effector T cell responses. Importantly, FOXP3+ CD25+ T cell population remained low in IFNAR-/-/ARE-/-, but not in IFNAR-/- or IFNyR-/-, indicating that CD25 expression is downregulated in chronic IFNy signaling but is independent of type I interferon signaling. Most compellingly, CD25 expression and recruitment of Tregs to kidneys in IL27Ra-/-/ARE-/- improved to WT level.The inflammatory environment created by activated, infiltrating responder CD4+ and CD8+ T cells emphasizes the importance of harnessing the regulatory T cell's ability to regain immunosuppression and dampen renal damage and disease progression. Therefore, cytokines with IFN-like function, such as IL27, offer viable immunomodulatory targets for further investigation to recover Treg suppressive function via alternative blockade.

108- Camel nanobody-based B7-H3 CAR-T cells with high efficacy against large solid tumors

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Background and Hypotheses: Chimeric antigen receptor (CAR)-T cell therapy, while promising in treating hematological malignancies, has limited success in long-term disease control, with only 30% to 50% of patients achieving it. The application of this modality in solid tumors is further complicated by the paucity of targetable antigens and suboptimal CAR-T cell performance. The rational design of CAR-T is limited by a lack of knowledge regarding the particular antigenic epitopes capable of evoking the most potent CAR activation. The checkpoint molecule B7-H3 (CD276), encompassing two distinct epitope motifs in its ectodomain - IgC and IgV, emerges as a potential immunotherapeutic target. Consequently, we hypothesize that identification of specific B7-H3 epitopes could be a viable strategy for developing potent CAR-T cells in solid tumor treatment.

Study Design and Methods: Commencing our investigation, we assessed the expression profile of B7-H3 isoforms in tumor cells and cancer patients at both RNA and protein levels. Subsequent endeavors involved the construction of large dromedary camel VHH nanobody libraries to target unique binding sites of B7-H3, followed by a meticulous phage display screening process. A peptide library based on B7-H3 protein sequence was synthesized to facilitate the mapping of epitopes for the selected VHHs. To evaluate the therapeutic potential of these nanobody-based CAR-T cells, we established various tumor models, including pancreatic cancer and neuroblastoma, in immunodeficient mice. A comprehensive analysis involving single-cell transcriptome RNA sequencing and T-cell proteomics analysis was employed to uncover critical genes in sustaining polyfunctional CAR-T cell activity.

Results and Conclusions: Our investigation identified 4IgB7-H3 as the predominantly expressed isoform in diverse tumor types and cancer patients. We successfully isolated three anti-B7-H3 VHHs (B12, C4, and G8) with high affinity and distinct binding epitopes. Notably, CAR-T cells equipped with nanobodies targeting the IgC, but not the IgV domain, demonstrated pronounced antitumor activity, evidenced by rigorous T cell signaling and significant tumor infiltration. Integrative analyses of single-cell transcriptomics and T-cell proteomics revealed a cohort of upregulated genes within a subset of polyfunctional CAR-T cells, likely critical to their enduring presence in the tumor microenvironment.

Relevance and Importance: Our findings highlight the importance of the particular target antigen epitope in governing optimal CAR-T activity. This study introduces an innovative nanobody-based B7-H3 CAR-T construct, poised to advance the treatment paradigm for solid tumors.

109- Characterization of the anti-tumor activity of memory cytokine enriched NK cells against tumors with neuroendocrine features

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Background and Hypotheses: Small cell lung cancer (SCLC) is an aggressive neuroendocrine (NE) carcinoma with few treatment options. Although immune checkpoint blockade (ICB) is approved in combination with chemotherapy in extensive stage disease, only a subset of patients experience an improvement in overall survival. Research suggests that a lack of response to ICB is partially attributable to low expression of MHC-class I. In a recent publication, our group demonstrated that the lack of MHC-class I can be utilized to enable targeting by NK cells stimulated with an immunostimulatory IL-15 cytokine superagonist (N-803). These findings led us to hypothesize that cytokine stimulated memory-like NK cells (M-ceNK) may be effective in targeting SCLC.

Study Design and Methods: M-ceNK are expanded from a healthy donor apheresis product via culture in the presence of cytokines including N-803, IL-12, and IL-18; resulting M-ceNK were characterized by flow cytometry for expression of NK activating and inhibitory receptors and intracellular cytolytic mediators. Evaluation of the functional killing capacity of M-ceNK was assessed via 6-hour in vitro immune cytotoxicity assays against SCLC cell lines representative of each of the four molecular subtypes of the disease (ASCL1, NEUROD1, POU2F3, YAP1).

Results and Conclusions: Flow cytometry demonstrated that M-ceNK express elevated IFN-γ and Granzyme B production, high levels of activating receptors NKp30, NKp44, NKp46, and low levels of inhibitory receptors KLRG1 and TIGIT compared to healthy donor NK cells. Functional evaluation indicated that all SCLC models are highly susceptible to M-ceNK targeting, particularly at low effector to target ratios (E:T). M-ceNK demonstrated a median of 69% lysis (range 35-89%) at an E:T ratio of 5:1 across 6 tumor models (DMS79, H69, H446, H1048, DMS114, H841) as compared to 2% lysis (range 0-58%) with NK cells isolated from peripheral blood of healthy donors. Analogously, NE models of prostate cancer (H660) and carcinoid lung cancer (H720, H727) exhibited high susceptibility to targeting by M-ceNK exhibiting 66%, 42%, and 44% lysis, respectively.

Relevance and Importance: Patients with NE tumors have few good treatment options; these data demonstrate the potential for M-ceNK based approaches for the treatment of NE tumors, including all molecular subtypes of SCLC. It’s conceivable that these findings may extend to additional tumor types that are refractory to ICB. Future studies will expand to evaluate these settings as well.

110- Intact Proviruses Persist in Expressed Genes in People with Non-Suppressible HIV on Long-term ART

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The persistence of replication-competent proviruses during ART is a key barrier to an HIV cure. A previous study identified 3 cell clones carrying replication-competent proviruses that caused non-suppressible viremia (NSV). Here, we asked if additional clones carrying intact proviruses were present in these 3 individuals. Three donors on ART for 10-20 years who recently developed NSV were sampled over 1-4 years. PBMCs were subjected to endpoint-diluted multiple displacement amplification (MDA). MDA wells were screened for HIV LTR, psi (Ψ), and RRE. Proviruses containing LTR, Ψ, and RRE underwent full-length HIV sequencing and integration sites analysis. A custom pipeline mapped both discrete and non-discrete (e.g., centromeric) integration sites. Gene expression levels in memory CD4+ T cells were determined using the Human Protein Atlas Database (www.proteinatlas.org).We identified 8 additional clones carrying sequence-intact HIV proviruses (11 total). 10 of 11 integration sites mapped to expressed genes and 4 were in the same orientation as the gene. Five were in KRAB-ZNF genes (50%), which was significantly enriched compared to the 2.37% of total proviruses in KRAB-ZNF genes across all study donors ($p<10^{-5}$). Reported gene expression levels were not significantly different between genes with intact vs defective proviruses (median=35.7 vs 61.0 TPM; p=0.5). Predicted-intact proviruses (Ψ+RRE) comprised 9-10% of the total population using the IPDA approach and 2-4% using LTR as the denominator. Proviruses that were confirmed intact by full-length sequencing comprised 0.07-2% of the LTR+ MDA wells. In 3 donors with NSV on ART, clones carrying sequence-intact proviruses comprised <2% of the total proviral population. 10 of 11 intact proviruses were integrated in genes that are normally expressed in memory CD4+ T cells, including the 5 in KRAB-ZNF genes. Only 1/10 genic clones declined in size on long-term ART. These results indicate that a stable pool of intact proviruses integrated in expressed genes can persist on long-term ART.

111- The peritoneal tissue resident macrophage niche is dynamic and is supported by both stromal and circulating immune cells.

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Background and Hypothesis: The neuronal metabolite N-acetyl aspartate (NAA) is generated from aspartate and acetate by N-acetyltransferase 8 like (Nat8l), and metabolized to its constitutive parts by aspartoacylase (Aspa), deficiency of which causes the neurological disorder Canavan disease. Our lab has identified NAA as elevated in the mouse peritoneal cavity (PC) compared to serum. The PC is home to peritoneal resident macrophages (resmacs), whose hallmark transcription factor Gata6 is required for Aspa expression. However, our bioinformatic analysis has revealed that the NAA metabolic loop may be present in other resident macrophage populations. Based on these findings, we sought to determine the role of the NAA metabolic loop in the function and maintenance of PC resmac.

Study Design and Methods: To assess the PC resmacs in the most representative state possible, we performed assessments on ex vivo cells. We performed single cell RNA sequencing (scRNAseq) from digested peritoneal tissue. From PC lavage cells, we performed metabolomic analysis, including 13C tracing. We also utilized an in vivo system in which we expanded PC resmac populations by treatment with an IL4- anti-IL4 antibody complex (IL4C).

Results and Conclusions: First, scRNAseq of peritoneal cells confirmed Aspa expressed in resmac, but also identified novel expression of Nat8l in the resmac and the monocytic cells of the PC, further extending the NAA metabolic loop. When we expanded PC resmac populations with IL4C or infection with the large extracellular pathogen Nippostrongylus brasiliensis, NAA levels decreased, corresponding with resmac expansion. Utilizing knockout (KO) mice for either Nat8l or Aspa, we found no differences between genotypes at baseline, but that Aspa KO resmac expand less following IL4C administration compared to wild-type (WT) or Nat8l deficient resmac although flow cytometry showed few differences in cell surface phenotype between groups at either baseline or following IL4C-mediated expansion. However, scRNAseq profiling of these populations reveals substantial differences, particularly in lipid utilization pathways. Metabolomic analysis of both the PC lavage fluid and peritoneal exudate cell (PEC) populations confirms the dynamic nature of NAA within the PC. Following IL4C administration, NAA levels decrease in both the PC lavage fluid and the PEC, and the PEC show a corresponding increase in aspartate levels. However, 13C NAA tracing indicates that NAA metabolism within PEC may be non-canonical, as the labelled NAA is taken up by the cells without detection of abundant labelled aspartate.

Relevance and Importance: Together this work identifies a nascent NAA metabolic loop that includes not only the resmac within the PC, but also the monocytes and stromal cells of this cavity. While this metabolism does not appear to occur in a canonically Aspa-mediated fashion, there is evidence that NAA levels are dynamic within the PC following either experimental expansion or infection, and that modulating these levels through either genetic knockout or administration of supplemental NAA can have profound impacts on resmac numbers, while effects on phenotype are more muted. We conclude, therefore, it is possible NAA serves more as a regulator of resmac homeostasis within the PC, rather than a direct metabolic fuel.

112- Solo LTR formation promotes elimination of proviruses in persons living with HIV

Feng Li, Guanhan Li, Francesco R. Simonetti, Shawn Hill, Robert Gorelick, Chuen-Yen Lau, Frank Maldarelli

113- The development and evolution of ch14.18 utilizing a mammalian display system

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Glycan-binding monoclonal antibodies are useful for basic research and clinical applications. Unituxin (dinutuximab, 14.18) is an FDA-approved anti-glycan monoclonal antibody used as a second-line treatment for children with high-risk neuroblastoma. 14.18 is a chimeric monoclonal antibody that binds to GD2, a disialoganglioside that is overexpressed on neuroblastoma cells. There is a need to investigate and improve the binding specificity and avidity of 14.18 to GD2. Current strategies for anti-glycan antibody discovery such as phage display and yeast display methods lack multivalency which is critical for determining selectivity. A mammalian display approach yields the benefit of a full IgG multivalent display. Utilizing a mammalian display method, we can generate a library of anti-glycan antibodies and elucidate mutations with improved binding affinity and selectivity for GD2. We established and validated a platform to successfully develop monoclonal antibodies using a mammalian display approach. We designed a plasmid incorporating both the heavy chain and light chain using different promoters and a PDGFR transmembrane domain to ensure the antibody is bound to the cell membrane. Flow cytometry validated the presence of monoclonal antibodies on the cells' surface, as well as the activity of the monoclonal antibodies binding to the target capture antigen. Furthermore, 14.18 was expressed and characterized on the glycan microarray to determine its binding capabilities. Dead, weak, and strong binding mutants were developed and screened to verify the function and set selection parameters for the library. The library was generated using site-saturation mutagenesis with four sites altered in each complementarity determining region, with a theoretical library size of 9.6x10^5. Utilizing the mammalian display method and incorporating the 14.18 site saturation mutagenesis library, we can elucidate hits for improved binding affinity and selectivity to GD2.

114- Transcriptional changes induced by the G Protein-Coupled Receptor of the oncogenic KSHV reveal regulation of multiple aspects of B cell biology

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Background and Hypotheses. Chemokines play a critical role in guiding immune cells from the circulation to the microenvironment where anti-tumor immunity can be initiated. However, dysregulation of chemokine and chemokine receptor expression can favor tumor progression and dissemination. Kaposi sarcoma herpesvirus (KSHV) has pirated a homolog of the CXCR2 chemokine receptor (vGPCR/ORF74) that has been implicated in KSHV-driven oncogenesis for over two decades. However, the field is lacking an in-depth analysis of vGPCR-dependent signaling and transcriptional changes in B cells and endothelial cells, the predominant target cells of KSHV infection and cells of origin of KSHV-driven cancers. Furthermore, the role of the host chemokine system in vGPCR-driven tumor formation remains undefined. Here, I report the transcriptomic profile of BJAB B cells that are inducible for vGPCR-expression to characterize the functional role of the viral CXCR2 homolog in B cell infection and pathology.

Study Design and Methods. BJAB cell pools were induced to express the KSHV vGPCR, stimulated with CXC chemokines and transcriptional changes were analyzed by total RNA sequencing. The comparison between constitutively active, wild type and an inactive KSHV vGPCR mutant defines the baseline signaling activity of vGPCR. To pinpoint the effects of chemokine stimulation on vGPCR signaling, we compared chemokine-treated wild type vGPCR expressing cells with cells expressing a chemokine-nonresponsive vGPCR mutant lacking the N-terminal 22 amino acids.

Results and Conclusions. The baseline and chemokine-responsive transcriptome of vGPCR-expressing B cells was analyzed by bulk RNA sequencing. The top upregulated candidates in constitutively active vGPCR expressing B cells include genes associated with B cell activation (e.g. CD83, members of the CCL3 and CCL4 chemokine gene families) and differentiation (e.g. CD72, TRIB1). Gene Set Enrichment Analyses (GSEA) for regulatory target genes, oncogenic signatures and immunological signatures are used to identify regulatory networks and key nodes. Prominent hits include regulation of chemotaxis, GPCR activity and MHC signaling, as well as the STK33/ERK cascade. The functional impact of candidate pathways on gene expression, proliferation, and chemotaxis will be analyzed using knock-out cells and chemical inhibitors of core components.

Relevance. Cancer is one of the most significant global health burdens and novel intervention strategies are needed. 10% of all human cancers are linked to virus infection, with a higher burden in those with immune compromise. Defining cellular pathways regulated by the oncogenic vGPCR in response to host chemokines secreted by KSHV-infected cells or the tumor microenvironment is critical to define chemokines that drive or block the vGPCR-dependent oncogenic processes that, in turn, will identify novel targets for immunomodulatory therapy.

115- The Great Divide: The immune system drives selection and segregation within the tumor microenvironment

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While immunotherapies are promising cancer treatments, response variation across patients poses a significant challenge to treatment. Intratumor heterogeneity, the genetic and non-genetic diversity between individual cells in a tumor, is strongly implicated in this variable response. To understand how cellular heterogeneity affects the immune control of tumors, we studied how the immune system interacted with two different cancer cell lines: Interferon-gamma (IFNg) receptor-wild type (WT) and IFNg receptor-knockout (KO) B16F10 melanoma cells. Tumor sensing of IFNg drives positive feedback loops within the immune system and plays an important role in both immunity and tolerance; therefore, loss of the IFNg receptor on tumor cells is a common mechanism of immune escape. Mutations in the IFNg signaling pathway are frequently found in tumors that fail to respond to immunotherapies like checkpoint blockade and adoptive cell therapy.In isolation, both WT and KO cell lines were equally controlled by the immune system; however, when admixed, WT tumors were selectively killed over the KO. By fitting our data to Lotka-Voterra equations, we determined that differential priming of CD8+ T cells by WT and KO tumors drove selective killing within admixed tumors. T cell infiltration also caused spatial segregation between the WT and KO cells in admixed tumors. Using both an experimental spheroid culture system and an agent-based model, we determined that selective one-to-one combat between immune and tumor cells could directly cause clonal segregation within the tumor microenvironment. By repeating this work with other model systems, we have demonstrated a generalizable principle: immune-driven selective pressure can drive both numerical shifts and spatial reorganization within the tumor microenvironment. By quantifying these processes, we aim to better understand the way immunotherapies shape the tumor-immune microenvironment and, in turn, develop more successful cancer treatments.

116- Genome-wide CRISPR screenings identify essential genes for CAR-T immunotherapy against neuroblastoma

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BACKGROUND AND HYPOTHESIS: Chimeric antigen receptor (CAR) T-cell therapies have shown promising success in the treatment of liquid tumors but are not yet curative against solid tumors, including neuroblastoma (NB), the most commonly occurring pediatric extracranial solid tumor. Glypican 2 (GPC2) or CD276 (B7-H3) are both highly and heterogeneously expressed in NB. GPC2 and CD276 dual-targeting bicistronic (BiCis) CAR-T cells performed better than CAR-T cells targeting a single antigen but, under stress conditions, had limited efficacy in the treatment of NB. Here, we attempted to investigate mechanisms of resistance to CAR-T cell therapies by solid tumors.

STUDY DESIGN & METHODS: We performed genome-wide CRISPR knockout (CRISPRKO) and CRISPR calabrese activation (CRISPRa) screenings in MYCN-A and MYCN-NA NB cell lines treated with BiCisCAR-T cells to identify essential genes for cell-mediated cytotoxicity. We further validated the roles of these identified genes in CAR-T cell-mediated tumor killing in vitro and in vivo using gain-of-function or loss-of-function gene perturbations.

RESULTS AND CONCLUSIONS: We found that the loss of genes in the interferon-γ receptor (IFNγR) signaling pathway (including IFNGR1, IFNGR2, JAK1, JAK2) rendered MYCN-A NB cells more resistant to killing by BiCis CAR-T cells. Furthermore, the loss of suppressor of cytokine signaling 1 (SOCS1), an IFNγR negative regulator, sensitized NB to BiCis CAR-T cell killing. In addition to the IFNγR pathway, the induction of cell-adhesion molecules (including ICAM1, ALCAM, CD164) also made NB more sensitive to BiCis CAR-T cell killing. However, in MYCN-NA NB cells, loss of genes in the death receptor signaling pathway (e.g., FAS, FADD) endowed the cells resistant to CAR-T cell-mediated killing.

RELEVANCE AND IMPORTANCE: These results demonstrate that the IFNγR pathway and adhesion molecules are critical for CAR-T cell-mediated killing of MYCN-A NB, whereas death receptor signaling is central in MYCN-NA NB. Furthermore, given the key roles of the SOCS1 gene in negatively regulating IFN-γ and IL-2 signaling in T cells, we hypothesized that the inactivation of SOCS1 in CAR-T cells will enhance their anti-tumor activity in NB through increasing the local release and activity of these cytokines, which will be tested in future experiments. Therefore, our genome-wide loss and gain of function screening has led to key insights into CAR T-cell killing of neuroblastoma, potentially improving adoptive cell therapies for this lethal disease.

117- High Omega-3 levels improve the efficacy of dendritic cell-based vaccines in murine tumor models

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Background and Hypothesis: The interaction between dendritic cells (DCs) and T cells is crucial in activating and regulating T-cell responses and is essential for fighting infections and cancer. DCs have the unique ability to capture, process, and present antigens to T-cells, making them attractive candidates for developing vaccines. However, most DC vaccine treatments have not been effective in the clinic. Despite advances in the field, tumor-derived immune suppression remains a challenge to cancer therapy, resulting in the sub-optimal efficacy of DC vaccines. Research shows that cancer cells produce specific lipids that interfere with the function of DCs, hindering the effectiveness of DC cancer vaccine therapy. Here we propose that omega-3 and specialized pro-resolving lipid mediators (SPMs) derived from them can significantly improve DC function and efficacy of DC-based cancer vaccines.

Methods:We utilized C57Bl/6 mice expressing the C. elegans FAT-1 gene encoding a desaturase enzyme that converts omega 6(n6) to omega 3(n3) fatty acids. Hence, they have high n-3 fatty acids. Bone marrow (BM) cells derived either from WT (wild-type) or FAT-1 mice were cultured in cRPMI media in the presence of GM-CSF and/or DHA (docosahexaenoic acid-an omega-3 fatty acid)/SPMs for 7 days. DCs were matured with LPS and pulsed with tumor-specific antigen overnight followed by their intradermal injection in tumor-bearing WT mice. We utilized the B16F10 and TC-1 models to assess the effect of the DC vaccines derived from FAT-1 and WT animals on reducing tumor growth in WT animals.

Results and conclusion: Our data showed that antigen-pulsed DCs derived from the BM cells of FAT-1 mice significantly reduced tumor growth compared to those derived from the BM of WT mice in tumor-bearing WT mice. We observed increased vaccine-specific responses in both tumor-bearing and naïve mice when they received the FAT-1 DC vaccine. In vitro, antigen presentation assay showed an increased IFN production by the antigen-specific CD8+ T cells when co-cultured with antigen-pulsed FAT-1 DCs compared to WT DCs. Although FAT-1 DCs showed better antigen presentation ability than WT DCs, there were no significant differences in the expression pattern of activation and co-stimulatory molecules on their surfaces. RNA sequencing of FAT-1 and WT DCs revealed several differentially expressed genes with potential novel roles in antigen presentation and DC-T cell interactions. Time-lapse imaging of antigen-pulsed DCs and antigen-specific CD8 T cell co-culture showed significantly longer T-cell-DC interaction time with FAT-1 DCs compared to WT DCs. WT DCs generated in the presence of DHA and SPMs mimicked the phenotype of FAT-1 DCs in vitro. Overall, our data suggest that the increased omega-3 and SPM levels in the DCs are the reason for their superior cross-presentation ability and may have translational potential in immune modulation and enhancing cancer vaccine therapy.

Relevance and importance: Understanding lipid changes in cancer can reveal new therapies. Our study shows that Omega-3-derived SPMs have immunomodulatory effects in preclinical models and can improve DC vaccine activity and potentially enhance cancer vaccine efficacy in the clinic.

118- The impact of post-transplantation cyclophosphamide on the gut microbiota: implications for graft-versus-host disease prevention

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Allogeneic hematopoietic cell transplantation (HCT) is the only potentially curative therapy for many severe malignant and non-malignant hematologic diseases. The administration of post-transplantation cyclophosphamide (PTCy) has recently revolutionized the HCT field by preventing severe forms of acute and chronic graft-versus-host disease (GVHD). Additionally, it has been recently made clear that the microbiome plays a critical role in the outcomes of patients undergoing HCT. Therefore, we aim to clarify the impact of PTCy on the gut microbiota in our murine MHC-haploidentical allogeneic HCT model of acute GVHD. Here we found that the use of the antibiotic levofloxacin from days 0 to +14 does not affect PTCy's efficacy in preventing GVHD. Even so, assessing serial microbiota changes in singly housed mice, PTCy treatment resulted in increased frequencies of bacterial taxa that are known to be protective against GVHD, such as ruminococcacae, clostridiales, lachnospiraceae, and blautia; many of these effects were seen even in levofloxacin-treated recipients. In fact, at day +21, when vehicle-treated mice begin dying of GVHD and PTCy has resulted in preferential regulatory T-cell recovery contributing to protection from GVHD, the bacterial taxa that most separated the two PTCy-treated from the two vehicle-treated groups was blautia. Also at day +21, we observed increased levels of butyric acid, a metabolite known to be produced by blautia and to protect against GVHD, in PTCy-treated recipients treated with or without levofloxacin compared with vehicle-treated recipients. Interestingly, further depletion of the gut microbiota via administration of a triple antibiotic cocktail (imipenem, vancomycin, and neomycin) in drinking water did not affect PTCy prevention of GVHD. By contrast, when analyzing bacterial viability over time, we observed a faster numerical recovery of bacteria in PTCy-treated recipients regardless of antibiotic administration. To assess the mechanistic importance of PTCy modulation of the gut microbiota on GVHD prevention, we performed fecal microbiota transplant in our model wherein B6D2F1 mice received triple antibiotics for two weeks and then daily oral gavage for five days of stool from mice transplanted in our MHC-haploidentical model and receiving vehicle or PTCy. After the fecal microbiota transfer, the recipient mice were subjected to radiation and transplantation without any post-transplant treatment. Pooling results across four independent experiments, mice receiving PTCy-treated stool had significantly better weights and clinical scores at early post-transplant time points and a trend towards better overall survival, suggesting that the modification of the microbiome by PTCy may contribute mechanistically to PTCy's activity in preventing GVHD. We are currently working to repeat these experiments in a germ-free environment to more definitively isolate the mechanistic role of PTCy-modified microbiota on GVHD prevention.

119-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

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Background and Hypotheses: Glioblastoma and gliosarcoma, both Grade IV primary CNS malignancies, are nearly always fatal. Current standard treatment (surgical resection, radiation, and chemotherapy) only provides modest survival benefit. Despite success in other cancers, an immunotherapy called immune checkpoint inhibition (ICI) has also failed to demonstrate efficacy against malignant gliomas. Due to the immunosuppressive microenvironment of these tumors, the infiltration of peripherally activated immune cells is likely required to achieve an effective anti-tumor immune response. We therefore hypothesized that a clinical response to ICI therapy necessitates a detectable immune response in the periphery. To test this question, we are actively enrolling patients with newly diagnosed glioblastoma or gliosarcoma on a Phase 2 clinical trial where all patients receive combinatorial ICI immunotherapy (NCI-21-C-0015). Extensive monitoring of systemic, longitudinal immune response will allow 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: Forty-eight patients will receive ICI (ipilimumab and nivolumab) with adjuvant chemotherapy. Peripheral blood samples will be regularly collected for peripheral immunological analysis. To accurately reflect interactions between the tumor microenvironment and immune cells (lymphocytes), we created a bead-based T cell stimulation assay to evaluate T cell response in the presence of co-inhibitory signals. In addition to anti-CD3 antibody for T cell receptor (TCR) stimulation, beads are also conjugated with recombinant human CD80 (B7-1)-Fc chimera capable of binding to CD28 (providing a co-stimulatory signal) or CTLA-4 (providing a co-inhibitory signal) on T cells. We postulate that the ability of a patient's pre-treatment T cells to respond to ipilimumab-mediated blockade of CTLA-4 within this assay will be predictive of clinical response to ICI. Patients' peripheral immune response will also be assessed through immunophenotyping, cytokine and chemokine profiling, single cell RNA- and TCR-sequencing, and functionality measurements. Conducting such analysis at multiple timepoints will highlight associations between immune profile markers and clinical trends and outcomes.

Results and Conclusions: As accrual (34 or planned 48) is ongoing, a full analysis of clinical findings is not yet available. Interim efficacy analysis was conducted and passed. Preliminary patient data analysis has confirmed feasibility of proposed methods including that anti-CD3/CD80-Fc microbeads successfully stimulate T cells to proliferate in vitro. Such results highlight the physiological relevance and predictive potential of this assay as it pertains to ICI response. Cytokine profiling has been conducted on preliminary samples, confirming unique patterns between patients, and differing from healthy donors. Our initial data confirm feasibility of patient accrual and sample acquisition and analysis in a prospective clinical trial with extensive correlative studies.

Relevance and Importance: The biological correlates of ICI response identified by this trial can better inform population enrichment of future clinical trials, potentially improving the treatment effect size and therefore establish ICI treatment efficacy. As a result, our novel in vitro assay and others could be used to screen for ICI responders, nonresponders, or those at risk for immune toxicities. The treatment of many difficult-to-treat cancers could therefore become more patient-personalized.

120-Vaccination with Replication-Dead Murine Gammaherpesvirus Protects against Wild-Type Infection and Reactivation in Mice

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Background and Hypotheses: Gammaherpesviruses are oncogenic pathogens that establish lifelong infections. There are no FDA-approved vaccines against Epstein-Barr virus (EBV) or Kaposi sarcoma-associated herpesvirus (KSHV). KSHV infection causes lymphoma and Kaposi sarcoma (KS) on the skin and visceral organs. KS is a prevalent cancer among HIV patients worldwide and is the major cause of cancer deaths in sub-Saharan Africa. Antivirals like nucleoside analogs reduce lytic infection, but these do not impact the latent, chronic infection. An effective vaccine strategy is needed to prevent infection and virus-associated cancers. Since KSHV does not infect mice, we used murine gammaherpesvirus 68 (MHV68) infection of mice as a system for investigation of gammaherpesvirus pathogenesis and vaccine strategies. We previously generated a replication-dead virus (RDV) that does not express the essential replication and transactivator protein (RTA) encoded by ORF50, leading to limited viral gene expression upon de novo infection. To generate a vaccine that more closely model KSHV vaccine design, we removed the unique M gene locus which are major determinants of latency and reactivation in MHV68 in vivo. To test our hypothesis that the removal of the unique M gene locus of MHV68 would not impact vaccine efficacy, we compared protection elicited by vaccination with delta M MHV68-RDV-RTA or with MHV68 RDV-RTA.

Study design and Methods: We generated a second generation RDV vaccine candidate lacking the non-coding tRNA-miRNA-encoded RNAs (TMERs) 6, 7, and 8 and the unique M1-M4 genes using BAC recombineering, followed by virus production in the codon-swapped ORF50 producer cell line. WT C57BL/6 mice were prime-boost intraperitoneally (IP) vaccinated with either MHV68 RDV-RTA (ORF50.Stop) or delta M MHV68-RDV-RTA (ORF50.StopDelM1-M4) and intranasally (IN) challenged with WT MHV68 at 28 days post-boost.

Results and Conclusion: Prime-boost vaccination of WT C57BL/6 mice with ORF50.Stop elicited virus-specific immune responses and led to a near complete block in virus replication and reactivation from latency. Prime-boost intraperitoneal vaccination of mice with ORF50.StopDelM1-M4 stimulated effector T cell responses in lungs and spleens, respective sites of acute replication and latency, that were comparable to ORF50.Stop vaccination. Vaccinated mice exhibited no splenomegaly and no virus reactivation at 28 days post-boost. When challenged intranasally with WT MHV68, vaccinated mice exhibited near-complete abolishment of virus reactivation from the spleens at 17 days post-challenge. These results suggest that major determinants of latency and replication are not required components of an effective gammaherpesvirus vaccine.

Relevance and importance: The virus lifecycle of KSHV is complex and the immune correlates of protection are not well-defined in patients, which necessitates a broadly immunogenic vaccine. We anticipate that our novel technology will propel the KSHV field towards both an effective vaccine design and the identification of key immune components for virus control. This in turn, will enhance our understanding of the vaccine-induced immune correlates of protection. Advancements from this new technology will be translated to humanized mice and non-human primate studies of KSHV, leading to clinical trials aimed to reduce the gammaherpesvirus-associated cancer burden.

121-Interferon-induced transmembrane protein 3 (IFITM3) promotes Akt activation and signaling by altering mTORC2 localization

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Molecular and Cellular Biology and Microbiology

122-Heparan Sulfate Chain Modulation: Impact on the Distribution and Dynamics of Glypican-3 on Liver Cancer Cells

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Glypican-3 (GPC3) is a cancer surface target, notably expressed in hepatocellular carcinoma (HCC), a major liver cancer, and anchored to the cell membrane through a glycosyl phosphatidylinositol linkage. The GPC3 core protein forms complexes with membrane components via two heparan sulfate chains located at Ser495 and Ser509. Both the GPC3 core protein and its heparan sulfate chains associate with Wnt3a, fostering cancer cell progression through Wnt3a-β-Catenin signaling. Despite its significance, the spatial distribution and dynamics of GPC3 on liver cancer cell surfaces have remained elusive. The goal of the present study aims to reveal the roles the two heparan sulfate chains of GPC3 in GPC3 dynamics as well as the Wnt3a signaling transduction.To identify the distribution pattern of GPC3, Nano-Resolution (NR) MINFLUX Microscopy was applied with HCC cell models. Fluorescence Recovery After Photobleaching (FRAP) assays and Single Molecule Tracking (SMT) were performed to characterize the mobile nature of GPC3. Wnt3a reporter assay was used to verify the signaling transduction by GPC3. To assess the functional implications of the two heparan sulfate chains in GPC3, we individually mutated each of the two GPC3 heparan sulfate chain binding sites, as well as both simultaneously. Subsequently, we employed the methods mentioned above to conduct a comprehensive analysis of these mutations.In this study, NR MINFLUX revealed that GPC3 pre-clusters on the HCC (HepG2) cell membrane with a mean radius of 26 nm. The fast mobile nature of GPC3 on the cell membrane was demonstrated by FRAP assay. Further, SMT delineated a mixed diffusion pattern of GPC3, transitioning between free diffusion and confined diffusion on the cell membrane. Treatment with Wnt3a triggered the β-Catenin signaling and induced a less mobile diffusion state for GPC3, while the lack of S509 or both heparan sulfate chains reduced Wnt3a-β-Catenin signal. Notably, the deletion of heparan sulfate chains, especially S509, led to decreased GPC3 cluster density and increased GPC3 mobility, as shown by MINFLUX and SMT analysis.In summary, our findings highlight that GPC3 pre-clusters on the cell surface, exhibiting dynamic diffusion motion. The S509 heparan sulfate chain takes precedence over S495 in Wnt3a-β-Catenin signaling, correlating positively with the clustering of membrane-bound GPC3 and GPC3 immobility. The findings enhance our understanding of spatial dynamics of GPC3 on the cell surface, establishing a potential link between the molecular functionality of GPC3 and its dynamic behavior.

123-mTOR-mediated transient APC/C inactivation ensures mammalian cell cycle entry

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124-Genome-Wide CRISPR Screening Identifies SLC47A1 (MATE1) As a Gene that Confers Resistance to Prexasertib, a CHK1 Inhibitor, in Ovarian Cancer Cells

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Background and Hypotheses: High-grade serous ovarian cancer (HGSOC) is the most lethal gynecologic malignancy in the United States, with limited treatment options. Common mutations in the tumor suppressor p53 characterize HGSOC, resulting in defects in the DNA repair pathway and genomic instability by disrupting the G1/S checkpoint. In such instances, transformed cells heavily rely on the G2/M checkpoint to mend damaged DNA and stabilize stalled replication forks. The ATR-CHK1-WEE1 pathway plays a pivotal role in orchestrating origin firing alongside CDK activity, activating the G2/M checkpoint to mitigate DNA replication stress during the S phase. At the heart of this regulatory network is cell cycle checkpoint kinase 1 (CHK1), which phosphorylates and deactivates CDC25C phosphatases, ultimately triggering G2/M checkpoint activation. The ATP-competitive small molecule inhibitor of CHK1, prexasertib, emerged as a promising anti-cancer drug for the clinical treatment of BRCA wild-type HGSOC. However, despite its potential, resistance to prexasertib monotherapy is swiftly emerging. In a phase 2 clinical trial, only half of the heavily pretreated patients experienced clinical benefit, underscoring the urgent need for a deeper understanding of resistance mechanisms and the development of more effective therapeutic strategies. We identified, from a whole genomic CRISPR-cas9 screen, the Solute Carrier Family 47 Member 1 (SLC47A1, also known as MATE1), an organic cation-proton exchanger and lipid flippase, as a mediator of resistance to prexasertib. In this work we demonstrate a direct correlation between expression of SLC47A1 and resistance to prexasertib with reduced accumulation of prexasertib in the SLC47A1 over-expressing cells.

Study Design and Methods: Multiple stable cell lines were prepared, each either overexpressing SLC47A1 or with reduced expression of SLC47A1. The resistance properties of these engineered cell lines were systematically assessed under varying conditions.

Results and Conclusions: In the CRISPR screen, we uncovered CHK1 overexpression, the primary target of prexasertib, as a resistance mechanism. Additionally, our investigation confirmed the involvement of several other known components (CDC25A, CDK2, CCNE1/2, etc.) in the CHK1 pathway, elucidating their roles in determining the sensitivity or resistance of ovarian cancer cells. Notably, the activation screen identified ABCB1, ABCG2, and SLC47A1 as top hits. The overexpression of ABCB1 and ABCG2 in the resistant cells, known membrane-associated ATP-dependent efflux transporters, confirmed that prexasertib is a substrate for these transporters. Further validation revealed that the gene SLC47A1/MATE1 is also independently linked to prexasertib efflux transport. Our findings underscore that heightened expression of SLC47A1 is associated with increased resistance to prexasertib, especially in the absence of P-glycoprotein (Pgp). Employing MALDI-mass spectrophotometry, we observed a diminished accumulation of prexasertib in cells overexpressing SLC47A1 compared to normal cells. Conversely, the depletion of SLC47A1 heightened the sensitivity of cells to prexasertib.

Relevance and Importance: These findings provide a new understanding of a potential mechanism of resistance of ovarian cancer and other cancers to prexasertib, a CHK1 inhibitor, and if validated clinically, offer a new approach to therapy of ovarian cancer.

125-Isolation and Characterization of an Anti-CD147 Nanobody from a Llama-Derived Phage Display Library

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Hepatocellular carcinoma (HCC) accounts for ~90% of primary liver cancer , and despite advances in treatments, outcomes for most patients remain poor. Curiously, despite the advent of precision oncology, no HCC-selective agents are clinically approved for the diagnosis or treatment of HCC in the US. Basigin (BSG) is a transmembrane glycoprotein that is expressed in 60% of HCC tumors, and is closely associated with tumor invasion, metastasis, and angiogenesis. It has limited expression on normal tissue, making it a promising HCC-specific target. Accordingly, radiopharmaceutical agents specific to BSG could allow for HCC-selective imaging and therapy. While full-length antibodies can be engineered for use as scaffolds to build radiopharmaceutical imaging or therapeutic agents, their long blood half-life makes them suboptimal because of poor tumor: blood at early time points or hematopoietic toxicity, respectively. Llamas produce a subclass of IgGs that have an unpaired heavy-chain variable domain, which function like a conventional IgG. These heavy-chain domains can be expressed as a single domain called a VHH region, or nanobody. Nanobodies are an attractive option, compared to a full-length antibody, for targeting BSG because their smaller size leads to superior kinetic properties, clearance rate, and tissue penetration. Here, we aimed to design a phage display library to isolate specific, high affinity nanobodies against BSG.Using standard methods, a young, naïve llama was immunized five times with recombinant human BSG. Nanobody cDNA sequences were isolated from the B cells of the llama, amplified by PCR, cloned into phagemids, and transformed into E. Coli cells to create a nanobody library with over 10^6 clones. Phage, displaying the nanobody protein, were then produced and used for immunopanning to enrich for specific nanobodies with affinity against BSG. Using antigen-coated high binding tubes, phage particles displaying a specific nanobody bound to BSG and were eluted following stringent wash conditions, while non-specific phage particles were discarded. The eluted phage was then amplified, and a repeat round of phage display was performed. Enriched clones were then screened by ELISA to identify high affinity binders. Lead candidate nanobodies were sequenced and unique clones expressed to determine yield, stability, and purity. Once specific nanobodies were identified, additional biophysical characterization was performed.After multiple rounds of immunopanning, two nanobodies, DMN1 and DMN2 were successfully isolated, specific to BSG. The sequencing results showed that these two nanobodies have unique sequences, and competition assays demonstrated that they have unique binding sites as well. Specific binding was confirmed with ELISA, flow cytometry, and biolayer interferometry, displaying a nanomolar affinity for both nanobodies against BSG. Radiolabeling of these nanobodies are currently underway, and if successful, in vivo target binding will be assessed by positron emission tomography (PET) imaging and biodistribution studies.Unlike many cancer types, liver cancer cannot reliably be detected by 18F-fluorodeoxyglucose PET (18F-FDG-PET) due to its limited tumor uptake. Conjugating a positron-emitting radionuclide to a nanobody specific to BSG will allow for selective imaging of HCC, allowing for tumor-selective imaging, which could be used to inform the design of an HCC-selective therapeutic agent.

126-Resolving the human hepatic proteome at a single-cell resolution

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127-The phosphatase SHP2 regulates tumor cell output of multiple angiogenic regulators

Yuyi Wang[1], Hidetaka Ohnuki[1], Andy D Tran[2], Dunrui Wang[1], Taekyu Ha[1], Jing-Xin Feng[1], Minji, Sim[1], Michael DiPrima[1] and Giovanna Tosato[1]

128-Visualization of the nucleic acid binding protein EWSR1 reveals endogenous EWSR1 exists in two visual modalities that reflect its associations with nucleic acids and concentration at sites of active transcription

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Background and Hypothesis: EWSR1, a member of the FET protein family, is a nucleic acid binding protein that contains multiple low complexity domains. The structure of EWSR1 reflects its multiple functions in regulating gene expression that includes binding of the C-terminal domain of RNA polymerase II (RNA pol II). However, determination of EWSR1's functions have predominately employed biochemical approaches or model systems that do not reflect endogenous protein levels. Here, we have generated reporter cell lines for the nanoscale visualization of endogenous EWSR1 and its localization in the context of DNA, RNA, and proteins that regulate genome organization and transcription.

Methods: For this study, we used two Ewing sarcoma cell lines, A673 and TC-32, that harbor one non-rearranged EWSR1 allele, and genome editing to insert a mNeonGreen (mNG) fluorescence reporter gene 5' of the first exon of EWSR1 to generate cell lines that express only endogenously tagged EWSR1 protein. Following validation of successfully modified clones, we confirmed EWSR1's localization to the nucleoplasm and used fluorescence recovery after photobleaching (FRAP) to assess the kinetics of full length endogenous EWSR1 in our reporter cell lines. We next used structured illumination (SIM) and stimulated emission depletion (STED) super-resolution microscopy techniques to visualize EWSR1 and its associations at a high resolution (~60-120 nm).

Results and conclusions: FRAP analysis (Zeiss Airyscan) showed that in cells, EWSR1 exists in two kinetic fractions, one that rapidly exchanges (A673 T½: 9.47 secs; TC-32 T½: 6.13 secs) and one that remains bound for a longer duration (A673 T½: 92.15 secs; TC-32 T½: 52.63 secs). We determined that EWSR1's nucleoplasmic organization is regular and non-random. Based on mNG fluorescence intensities, we also defined that EWSR1 exists in two visual states, one distributed and one as foci. EWSR1 in both states colocalizes with nascent RNA (visualized using Click-IT chemistry). EWSR1 foci are associated with euchromatin and excluded from condensed regions of the DNA marked by excess DAPI staining. Transcriptional coactivators BRD4 and MED1are present adjacent to EWSR1 foci suggesting specific localization of endogenous EWSR1 relative to these exemplar proteins within the transcriptional cascade. Critically, we observe EWSR1-foci almost wholly associated with phosphorylated RNA polymerase II in states of both transcriptional initiation and transcriptional elongation. Interestingly, the addition of a dual CDC7/CDK9 inhibitor results in substantial shift of the mNG-EWSR1 signal, particularly foci, from the nucleoplasm to nucleoli. We also observed that EWSR1 and FUS, another FET protein, exhibit distinct spatial organizations.

Relevance and Importance: Our results contribute to bridging the gap between our understanding of the biophysical and biochemical properties of FET proteins, including EWSR1, their functions as transcriptional regulators, and the participation of these proteins in tumorigenesis and neurodegenerative disease. Our studies benchmark the organized configuration of EWSR1 within the nucleoplasm and will aid analysis of changes in EWSR1's localization within the nucleus and relative to proteins that regulate gene expression following inhibition of transcription and other external stimuli providing functional insight

129-Targeting IDH1-Mutated Oligodendroglioma with Acid Ceramidase Inhibitors

Helena Muley-Vilamu, Tyrone Dowdy, Faris Zaibaq, George Karadimov, Aiguo Li and Mioara Larion

130-Investigating the molecular basis of oxaliplatin resistance in colorectal cancer (CRC) via genome-scale CRISPR screens

Nisha R Pawar, Heidi M Wade, Robert W Robey, Michael M Gottesman

Background and hypotheses: Colorectal cancer (CRC) is the second leading cause of cancer deaths, with a five-year survival rate of 65%. Oxaliplatin is used as an adjuvant therapy for advanced CRC in combination with fluorouracil, yet 90% of metastatic cancers develop resistance. Oxaliplatin was developed as a third-generation derivative of cisplatin, a DNA damaging agent, but recent evidence points to alternative modes of action. Several mechanisms of oxaliplatin resistance have been proposed, but many are inconsistent, and few are widely accepted. Our objective is to utilize an unbiased genome-scale CRISPR approach to identify multifactorial mechanisms of oxaliplatin resistance in CRC.

Study Design and Methods: Genome-wide CRISPR activation and knockout screens were conducted to identify genetic changes that confer resistance to oxaliplatin in two CRC cell lines with distinct molecular backgrounds (SW620 and RKO). Pooled libraries of guide RNAs covering ~18,000 human genes were utilized to ensure an unbiased approach and identify several potential mechanisms of resistance.

Results and Conclusions: Top hits from activation and knockout screens were identified in major pathways such as amino acid transport, ribosomal and RNA binding proteins, and translational regulation. Guide RNAs corresponding to SLC43A1 (LAT3) were the most significantly enriched in knockout screens and depleted in activation screens in both cell lines, suggesting a potential role for LAT3 in oxaliplatin resistance or mechanism of action. CRISPR knockout and overexpression of LAT3 in SW620 cell lines confirm increased resistance or sensitivity to oxaliplatin, respectively, in in vitro cytotoxicity, colony formation, and proliferation assays. Further studies investigating the role of this amino acid transporter and its family members (LAT1, 2, 4) in oxaliplatin mechanism of action in CRC are ongoing.

Relevance and Importance: We believe this global approach can clarify inconsistencies regarding the molecular basis of oxaliplatin resistance and uncover potential targeted and personalized therapeutic strategies for advanced CRC. Investigation of the role of LAT3 in oxaliplatin resistance can provide new insights into the interplay between essential nutrient uptake and drug transport.

131-Designing an improved YAP1/TAZ-TEAD interaction inhibitor

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Background: In the skin and other tissues, Hippo signaling regulates stem cell fate and tumor growth. YAP1 and its paralog TAZ are the main effectors downstream of Hippo signaling mediating their regulatory and oncogenic functions by interacting with TEAD transcription factors. Studying TEAD inhibition in cells and tissues presents some challenges. Since YAP1/TAZ are paralogs, most of the time, both genes are required to be knocked out in mouse models to see effects. Additionally, YAP1/TAZ do not solely interact with TEAD, but also with countless effectors, transcription factors meaning that knocking out YAP1/TAZ leads to numerous transcriptional and signaling outcomes which are difficult to dissect. To circumvent some of the limitations in studying TEAD inhibition, our group developed TEADi, which is a genetically encoded dominant-negative protein that blocks nuclear interaction of TEAD with YAP1 and TAZ. Our group has proven the specificity and usefulness of TEADi to study YAP1/TAZ-TEAD transcriptional events (PMID: 32193376). However, we have observed that this inhibitor is not well expressed or otherwise degraded in certain cancer cell lines prompting us to improve its design. We hypothesized that post-transcriptional modifications (PTMs) in the TEAD binding domain (TBD) of the original proteins that compose TEADi (TEADiv1 from now on) could affect the stability of the inhibitor. We further hypothesized that modifying these PTM sites would result in a more stable TEAD inhibitor.

Study Design and Methods: To improve the expression and stability of TEADi, we mutated known PTMs in the YAP1 binding domain of TEADiv1 and developed TEADiv2. We then performed Western blot analysis to test TEADiv2 in HEK293 and Cal27 cell lines. Following confirmation of expression, we performed luciferase assays with TEAD reporters to compare TEADiv1 and TEADiv2. We also used immunoprecipitation experiments to assess differences in ubiquitination or sumoylation and checked for differences in phosphorylation between TEADiv1 and TEADiv2 by phospho-tag Western blot.

Results and Conclusions: We find that TEADiv2 is well expressed HEK293 cells as well as Cal27 cells. Additionally, when using a luciferase reporter assay to compare the inhibitory effects of TEADiv1 and TEADiv2, we observed that TEADiv2 provides the greatest inhibition of YAP1 and TAZ dependent TEAD transcription. Preliminary experiments have shown that while there is no difference in ubiquitination or sumoylation in our inhibitor, TEADiv2 appears to be more dephosphorylated compared to TEADv1 in Cal27 cancer cells. We are currently validating the phosphorylation experiments and studying the differences in stability between the inhibitors to confirm the usability of TEADiv2.

Relevance and Importance: Disruption of the YAP1/TAZ-TEAD transcriptional complexes has become a significant target for cancer treatment. However, a detailed analysis of the effectiveness and networks affected by YAP1/TAZ transcriptional targeting is limited. Design of a more stable dominant negative protein to study TEAD inhibition will help validate TEAD as a potential therapeutic drug target in a variety of cancer cells.

132-Characterization of a ciliopathy spectrum disorder caused by variants in WDR44

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133-Revealing Rab11-Rab8 cascade membrane trafficking dynamics

Ipsita Saha[1], Christine Insinna[1], Christopher J. Westlake[1]

134-Developing RNA Modifications To Enable Neo-Antigen Discovery

Manini Penikalapati[1], Supuni Gamage[1], Maria Parkhurst[2], Steven A. Rosenberg[2], Jordan L. Meier[1]

Synthetic messenger RNA (mRNA) is an emerging therapeutic platform with important applications in oncology and infectious disease. Effective mRNA medicines must be translated by the ribosome but not trigger a strong immune response. To expand the medicinal chemistry toolbox for these agents, we have investigated the properties of the naturally occurring nucleobase N4-acetylcytidine (ac4C) in synthetic mRNAs. Replacement of cytidine with ac4C diminishes inflammatory gene expression in immune cells. Synthetic mRNA can also be used as a platform for HLA-based neoantigen discovery and screening without relying on MHC binding algorithms. We are investigating the effects of RNA modifications, such as addition of a 5' cap and homogenous incorporation of ac4c and N1-methyl pseudouridine, on the production of neoantigens capable of stimulating the T-cell response. Our studies hope to improve the general use of RNA modifications as a component of next-generation neo-antigen discovery platforms.

135-Biosynthesis of GalNAC-tyrosine and tools to identify the GalNAC-tyrosine glycosylation sites on glycoproteins

Pavan Patel and Jeffrey C. Gildersleeve

Glycosylation is a vital post-translational modification (PTM) associated in a myriad of biological processes including protein folding and localization, signaling pathways, and cell-cell interactions. Altered glycosylation profiles have been associated with disease progression and are a hallmark of cancer. Apart from Serine/Threonine O-glycosylation, in 2011, a new type of O-linked glycosylation incorporating GalNAc residue on the side chain oxygen of tyrosine was discovered. At present, very little is known about GalNAc-Tyr prevalence, function, or biosynthesis. To aid in our study of GalNAc-Tyr, our lab developed G10C, a GalNAc-Tyr selective monoclonal antibody that is completely selective for GalNAc-Tyr. Using this antibody, we discovered that GalNAc-Tyr is widely expressed in most human tissues, indicating that it is a ubiquitous and underappreciated post-translational modification. Localization to specific cell types and organ substructures within those tissues indicates that GalNAc-Tyr is likely regulated in a cell-specific manner. GalNAc-Tyr was also observed in a variety of cell lines and primary cells but was only present on the external cell surface in certain cancer cell lines, suggesting that GalNAc-Tyr localization may be altered in cancer cells. To better understand the significance of GalNAc-Tyr, we need to understand the biosynthesis of GalNAc-Tyr and develop a better MS proteomics condition to identify proteins containing GalNAc-Tyr modifications. To identify Polypeptide GalNAc-transferases (ppGalNAc-Ts) involved in the biosynthesis of GalNAc-Tyr, we carried out overexpression and knockdown of various ppGalNAc-Ts in multiple cell lines. Our initial efforts identified a couple of ppGalNAc-Ts that may be involved in synthesis of GalNAc-Tyr. We have also identified GalNAc-Y glycosites and glycan composition of key proteins such as DKK1 (Dickkopf WNT Signaling Pathway Inhibitor 1) and GALNT10 (Polypeptide N-Acetylgalactosaminyltransferase 10). Collectively, our initial results and a better MS proteomics tool will enable us to dive deep in the significance of GalNAc-Tyr glycosylation.

136- Complementary exopolysaccharide-targeting antibodies reveal unique biofilm features and synergize to improve antibacterial efficacy

J. Sebastian Temme[1], Zibin Tan[2], Mi Li[3], Alexander Wlodawer[3], Xuefei Huang[2], and Jeffrey C. Gildersleeve[1]

Biofilms are a major human health problem, but limited information about their biosynthesis, composition, and architecture hinders the development of new therapeutics. A key component of many pathogenic biofilms is the exopolysaccharide polysaccharide intercellular adhesin (PIA), a partially deacetylated polymer of poly-N-acetyl glucosamine (PNAG). While deacetylation is known to be required for biofilm formation, the nature, extent, and location of deacetylation in natively expressed PIA is not well understood in part due to a lack of suitable research tools. As part of a broader effort to address the lack of high-quality anti-glycan antibodies (mAbs), we used a glycan microarray with over 800 different components to screen a panel of 516 human mAbs randomly cloned from different B-cell subsets. From this panel, we identified 24 novel anti-glycan mAbs, with most originating from IgG memory B cells and many highly selective for microbial glycans. TG10, one of the mAbs pulled from the screen, was highly selective for highly deacetylated PNAG (dPNAG), a key virulence factor and exopolysaccharide expressed in a number of biofilm-forming pathogens, e.g. Staphylococcus aureus, Staphylococcus epidermidis, and Acinetobacter baumannii. Using the newly discovered TG10, we demonstrate that Staphylococcal biofilms contain distinct regions of high and low N-acetylation, rather than polysaccharides with intermittent deacetylation throughout the biofilm. In additional to revealing important features of biofilm architecture, the discovery suggested that PNAG specific antibodies such as F598, a monoclonal antibody in phase II clinical trials, will not opsonize the entire cell population in the biofilm. We hypothesized that combining the dPNAG-specific antibody TG10 with the PNAG-specific antibody F598 would enhance efficacy. TG10 and F598 synergistically increased in vitro and in vivo activity, providing 90% survival in a lethal S. aureus challenge murine model. The results reveal unique features of biofilms and provide an improved clinical strategy for targeting exopolysaccharides.

137- Pacritinib Inhibits Proliferation of Primary Effusion Lymphoma Cells and Production of Viral Interleukin-6 induced Cytokines

Yiquan Wu, Victoria Wang, and Robert Yarchoan

138- Characterization of Zebrafish homologs of human P-gp and ABCG2 multidrug efflux transporters

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Background and Hypotheses: The ATP binding cassette (ABC) transporters P-gp (P-glycoprotein, encoded by ABCB1) and ABCG2 (ABCG2) were identified due to their ability to confer resistance to multiple different cytotoxic drugs in cancer cells. P-gp and ABCG2 are two of the most important multidrug efflux transporters, due to their wide and overlapping substrate specificities and diverse expression patterns. P-gp and ABCG2 are primarily expressed in excretory organs (e.g., liver, kidney, intestines) and at barriers to protected sites (e.g., blood-brain and placental barriers), and are therefore key drivers of substrate drug pharmacokinetics and distribution. Zebrafish are an ideal model to study drug toxicity and tissue distribution, but these efforts require a detailed characterization comparing the zebrafish and human homologs for translational relevance.

Study Design and Methods: A combination of RNAscope in situ hybridization probes and immunohistochemistry were used to detect ABC transporter tissue localization. HEK-293 cells were stably transfected with zebrafish ABC transporters to assess their individual functions in vitro. Transporter substrate status was determined using cytotoxicity and efflux assays, where transporters confer resistance to cytotoxic drugs, or efflux fluorescent drugs, respectively, if the drug is a substrate.

Results and Conclusions: Previously, we characterized the 2 zebrafish homologs of P-gp (Abcb4, Abcb5) and found both had similar tissue expression patterns to human P-gp, but Abcb4 was the only homolog at the blood-brain barrier (BBB). A high-throughput analysis of substrate transport demonstrated a high correlation between Abcb4 and P-gp (r=0.94) with near perfect substrate overlap. Abcb5 shared fewer substrates (r=0.67) and is expressed at high levels in the zebrafish gills, skin and ovarian follicles.Currently, we are characterizing the 4 zebrafish ABCG2 homologs (Abcg2a, -b, -c, -d). abcg2a, -b and -d shared similar expression patterns to human ABCG2, with all being detected in the liver, kidney, intestines and ovarian follicles. abcg2c was only detected in the kidney and ovarian follicles. abcg2a was most highly expressed in the liver and intestine and Abcg2a was the only paralog at the BBB. abcg2a and abcg2d were the only paralogs expressed in the gills. Abcg2a had the greatest substrate overlap with ABCG2 whereas Abcg2d shared the fewest. This result was surprising as Abcg2d has the second highest amino acid identity with ABCG2. Computational modelling of predicted structures of Abcg2a-d aligned to ABCG2 demonstrated Abcg2d has a more divergent substrate binding pocket than Abcg2a, which may affect substrate recognition or transport.In conclusion, we have identified Abcb4 and Abcg2a as the most phenotypically similar zebrafish homologs of P-gp and ABCG2, respectively.

Relevance and Importance: The identification and characterization of two phenotypically similar homologs of P-gp and ABCG2 with conserved tissue expression patterns, means that zebrafish can be considered a translationally relevant model for studying these transporters. The similarity of substrate profiles and tissue distribution lends credence to the use of zebrafish for drug toxicology and PK/PD studies. Expression of these conserved transporters at the BBB paves the way for the use of zebrafish to study methods of increasing drug delivery to the brain.

139- Optimization of Surfaceome Analysis Techniques for Comparison of Wild-Type and Drug-Resistant Cancer Cell Lines

Rachel Carter, Puneet Mann, Senthil Muthuswamy

Drug resistance is the main cause of mortality in cancer patients, accounting for up to 90% of patient mortality. Despite recent innovations and the development of new classes of therapeutics, drug resistance continues to be an obstacle to successful treatment. Therefore, it is vital to better understand different mechanisms of resistance and how they can be targeted in various cancer types in order to improve patient outcomes. The vast majority of drug targets are cell surface proteins, given both their accessibility for drug delivery and their roles in regulating important biological processes such as interactions between cells and with the microenvironment. Therefore, characterizing the cell surface proteome, or the "surfaceome", of drug-resistant cancer cells would be greatly beneficial for identifying drug targets. However, the surfaceome can be difficult to study given that cell surface proteins are usually poorly soluble and are present in low abundances compared to intracellular proteins. To overcome this problem, several surfaceome analysis techniques have emerged that allow for specific enrichment of cell surface proteins prior to downstream proteomic analyses, including traditional cell surface biotinylation (CSB) and a newer cell surface capture (CSC) approach, which employ different types of biotin reagents to covalently label cell surface proteins on living cells, allowing for subsequent affinity purification with streptavidin. The aim of this work is to utilize both techniques to offset the pitfalls of each method when used individually, and to apply them to analyze the surfaceomes of wild-type and drug-resistant pairs of cancer cell lines to identify druggable targets for treating drug-resistant cancers. Both approaches have been optimized in-house and have been applied to study the surfaceomes of pancreatic and breast cancer cell lines. Future directions include expanding our study to other organs and clinically relevant drug classes to determine the differences and similarities in the mechanisms of resistance across cancer types.

140- A CD3xMSLN Bispecific Antibody Induces T Cell-Mediated Tumor Killing and is Not Blocked by Shed Mesothelin

Tara O'Shea[1], Anirban Chakraborty[1], Masanori Onda[1], Xiu-fen Liu[1], and Ira Pastan[1]

141- Fixing a Broken Gene: Repairing a Newly Characterized Poison-Like Cryptic Exon in Hereditary Kidney Cancer

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142- Peptide antagonist of β-catenin restricts in vitro WNT signaling in Liver Cancer

Julia Sheehan-Klenk[1], Divya Nambiar[2], Joon-Yong Chung[3], Woonghee Lee[4], Jim Rotolo[5], Freddy E. Escorcia[6]

Liver cancer is the third leading cause of cancer-related deaths worldwide. Hepatocellular carcinoma (HCC) is the most common type of primary liver cancer, accounting for nearly all observed cases. WNT/β-catenin signaling is a key pathway in essential cellular processes like proliferation, homeostasis, and stem cell renewal. Activating mutations in β-catenin occur in ~35% of human HCC contributing to oncogenesis. ST316, a novel peptide antagonist (Sapience Therapeutics, NY), inhibits N-terminal binding of beta-catenin to its co-activator, BCL9, an interaction that is essential for nuclear translocation of the overall transcriptional complex. Antagonism of β-catenin/BCL9 complex formation functionally blocks WNT signaling and related oncogenic signaling. ST316 is currently being tested in several solid tumors in a Phase I clinical trial (NCT05848739). Here, we tested the inhibitory function of ST316 in two primary liver cancer types, Hep3B, a hepatocellular carcinoma, and HepG2, a hepatoblastoma. We hypothesized that ST316 would inhibit nuclear transport of beta-catenin, increase beta-catenin cytosolic accumulation and its subsequent degradation, and therefore decrease cell viability. In vivo, we hypothesized that ST316 would reduce tumor volume and decrease tumor viability in mice inoculated with HepG2 and Hep3B tumors. In vitro cell viability was measured in Hep3B and HepG2 cells using flow cytometry after incubation for 48-hour in media containing 0, 0.6, 1.25, 2.5, and 5 μM concentrations of ST316 and subsequent staining for apoptosis/necrosis with Annexin V and proprium iodide. Overall beta-catenin expression was measured by Western Blot analysis of cell lysates in Hep3B and HepG2 cells after 24-hour incubation with 0, 2.5, and 5 μM of ST316. ST316 impact on nuclear and cytoplasmic beta-catenin fractions in Hep3B cells was measured by Western Blot analysis after 24-hour incubation with 0, 1.25, 2.5, 5 and 10 μM doses of ST316. For in vivo models, Hep3B-LUC+ and HepG2-LUC+ tumors were subcutaneously inoculated into the right flank of 24 nu/nu mice. For each group of 6 mice, 100 μL of 2, 5, or 10 mg/kg ST316 in a trehalose vehicle (or the trehalose vehicle alone as control) was delivered by inguinal injection 1X per week for HepG2-LUC+ tumors and 3X per week for Hep3B-LUC+ tumors with an average starting tumor volume of 200 mm3. Tumors were measured and imaged for viability using In Vivo Imaging System software (IVIS imaging) every other day, and mice were weighed on dosage days.

We observed a dose-dependent cytotoxic effect in Hep3B cells treated with increasing doses of ST316. Total β-catenin expression significantly decreased in HepG2 cells treated with ST316, but not Hep3B. Nuclear and cytoplasmic fractions of beta-catenin decreased following ST316 in a dose-dependent manner for Hep3B cells. While ST316 demonstrated efficacy in inhibiting HepG2 and Hep3B in vitro, we did not observe any tumor growth delay, or survival advantage at any dose in vivo. Overall, we show that inhibition of WNT/β-catenin signaling using ST316 demonstrated dose-dependent inhibition of proliferation in both Hep3B and HepG2 cells. However, when tested in vivo, we did not observe an effect of ST316 on local control, tumor viability as measured by IVIS, or survival.

143- Angiopoietin-2 (Ang2) competes with Fibroblast Growth Factors (FGFs) for activation of FGF receptor-2 (FGFR2)

Minji Sim[1], Hidetaka Ohnuki[1], Giovanna Tosato[1]

144-Validation of Synthetic Lethality Candidates from a Genome Wide CRISPR/Cas9 Screen of mTOR

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Background and Hypotheses: Although cancer treatments have progressed in scope and efficacy, one major challenge that remains is treatment resistance, where cancer that previously responded to treatment no longer does. Strategies to mitigate treatment resistance in cancer often involve combining several different treatments, but this is not always successful. A similar, more precise methodology of approaching treatment resistance has emerged: synthetic lethality. Synthetic lethality occurs when the knockdown of two genes (synthetic lethal partners) causes a lethal phenotype, whereas cells can survive the individual knockdown of either gene. Finding synthetic lethal partners allows researchers to target the specific compensatory pathways that cancers use to develop drug resistance. CRISPR screens are a popular way to determine candidates for synthetic lethality, but these results need validation to ascertain true synthetic lethal partners. In this experiment, results from CRISPR screens of synthetic lethal partners for three mTOR complexes will be further validated.

Study Design and Methods A set of 4 isogenic H1299 cell lines (an intact control line, and 3 knockout lines for Raptor, Rictor, and mEAK7) all stably expressing Cas9 were created and used to complete three genome wide CRISPR screens to find synthetic lethal partners for mTOR complexes. The number of single guide RNAs (sgRNAs) in the cell pellets at the end of the screen were compared to the number of sgRNAs in the library to find genes that caused synthetic lethality in these cell lines; then, the genes with the largest log fold change from each screen were merged to find genes which were significant in at least two of the three screens. These merged results were validated by transducing the same four H1299 cell lines with lentiviral particles containing sgRNAs of the gene targets, Puromycin resistance, and mClover3 fluorescence. After successful transduction was verified by treating transduced cells with Puromycin, the growth of cells was monitored using a live-cell imaging device and compared to cells transduced with control sgRNAs.

Results and Conclusions The three initial CRISPR screens found a total of 2,351 possible synthetic lethal partners. 878 of these potential partners were partners with Raptor, 768 were with Rictor, and 705 with mEAK7. Of the 2,351 possible synthetic lethal partners across the three screens, only 48 genes showed a significant difference in more than one screen. These 48 genes, of which 17 were possible Raptor synthetic lethal partners, 23 were possible Rictor partners, and 8 were possible mEAK7 partners, were validated via lentiviral transduction of sgRNAs of each gene and analysis of cell proliferation. Analysis revealed significant deviation between expected synthetic lethal phenotypes and observed cell proliferation and growth; while many targets either displayed general lethality, and others showed no lethality, several targets were identified as candidates of interest for further exploration.

Relevance and Importance This study will not only validate synthetic lethal partners of mTOR complexes, it may also elucidate novel pathways associated with these complexes, provide a more advanced understanding of the mTOR signaling pathway and possibly uncovering novel treatment options for cancers with aberrant mTOR signaling.

145-Glycome Profiling with a DNA-Barcoded Antibody Library

Aneesa Bhakta, Samantha Marglous, Kara Gillmann, and Jeffrey Gildersleeve

Surface glycans are known to differ between cancer and noncancer cells, as well as between different types of cancer cells, but the limitations of current methods for studying surface glycosylation have led to a gap in this area of research. Methods commonly used for studying proteins cannot be applied to carbohydrates and existing technology for studying glycans such as mass spectrometry or lectins are often low throughput or less specific. Anti-glycan antibodies provide a potential alternative with their standardized structure, diverse targets, and greater potential specificity. One promising application for anti-glycan antibodies is the creation of a DNA-barcoded antibody library for use in a variety of sequencing techniques. DNA-barcoded antibodies allow the sequencing of surface glycans on both a bulk and single cell level, and have the potential to be used simultaneously with RNA sequencing. This library of antibodies is created using a standardized expression system and their targets are determined using glycan microarrays containing diverse potential glycan targets. Following production and classification, the anti-glycan antibodies are conjugated with DNA barcodes. These barcoded antibodies can then be used during sequencing to detect the presence and prevalence of specific glycans on the surface of cells. Preliminary sequencing done with SW480 (derived from a primary tumor in a patient with colorectal cancer) and SW620 (derived from a lymph node metastasis in the same cancer patient) cell lines have yielded promising results. Barcoded antibody replicates with the same target but different barcodes display strong linear correlations, suggesting that the barcoded antibodies associate with glycans as predicted and are sequenced reliably. Comparisons between the two cell lines also suggests that sequencing with the barcoded antibodies can detect differences in the surface glycomes of different cell types. Furthermore, UMAP clustering analysis suggests the presence of subpopulations within these cell lines that may have different surface glycosylation patterns which can be detected by the barcoded antibodies. Experiments are currently being done to verify the findings of these sequencing experiments using flow cytometry. This DNA-barcoded anti-glycan antibody library provides a novel method of studying the surface glycosylation of cells with the potential for eventual applications in cancer diagnostics.

146-Engineering CAR-T cells targeting GPC1 for the treatment of pancreatic cancer

Hsi-En Tsao[1], Nan Li[1], Alex Quan[1], Mitchell Ho[1]

GPC1 is an oncofetal glycoprotein that facilitates multiple signaling pathways to regulate cell proliferation and differentiation during development. Evidence has shown that it has elevated expression levels in pancreatic cancer tissues which correlate with poor prognosis, making it a potential target for chimeric antigen receptor (CAR) T cell therapy. GPC1, however, can also be detected in many other adult tissues, raising the risk of on-target-off-tumor toxicity in cancer treatment. Our lab has previously generated a camel nanobody D4 and a monoclonal antibody HM2 to target both the membrane-distal and membrane-proximal epitopes of GPC1. CAR-T cells based on HM2 and D4 successfully eradicate tumor cells in mice. We have recently identified two GPC1 antibodies targeting epitopes that could potentially lead to higher tumor-specificity using mouse hybridoma technology. These antibodies were characterized by ELISA, biolayer interferometry, and mass photometry. Our data showed that Ab-1 likely binds to a conformational epitope with strong binding affinity, whereas Ab-2 binds to a sequence-specific epitope with moderate binding affinity. Variable fragment heavy chain and light chain sequences of these antibodies were determined by 5’RACE and next generation sequencing, and the single chain variable fragment sequence was sub-cloned into different CAR constructs and transduced in donor PBMCs. Our preliminary data showed that CAR-T cells based on Ab-1 can specifically lyse pancreatic cancer cells in vitro in the luciferase-based cytolytic assay. Immunohistochemistry studies are underway to analyze the tissue specificity for these antibodies, and the potency of the CAR-T cells will be further validated in mouse models. This project demonstrates the strategy and potential of engineering CAR-T cells targeting different epitopes of GPC1 to enhance the specificity of immunotherapy in treating pancreatic cancer.

147- Exploring the Potential of LZK and PI3K Inhibitors in Combination Therapy Treatments for HNSCC

Nick Brill-Edwards, Lilly Klapper, and Meghri Katerji

148- A chemical genetic approach to identify cyclin D-CDK4/6 targets

Eli-Eelika Esvald[1], Gretchen Heidenbrink[1], Benjamin Topacio[1] , Mardo Kõivomägi[1]

The G1-phase cyclin-dependent kinases CDK4 and CDK6 form complexes with D-type cyclins to drive cell proliferation. The best-known target of cyclin D-CDK4/6 is the retinoblastoma protein Rb, which inhibits cell-cycle progression until its inactivation by phosphorylation. In addition to the pocket family proteins, such as Rb, only a few other well-established targets are known. It is still unclear how CDK4/6 promote a number of processes in G1 with only a small number of substrates. Notably, in silico predictions have suggested the existence of numerous substrates for CDK4 and CDK6. However, the lack of suitable tools has hindered a comprehensive identification of CDK4/6 substrates.To determine the targets of cyclin D-CDK4/6 complexes in a high-throughput manner, we have developed novel analog-sensitive mutant versions of CDK4 and CDK6. We show that these novel analog-sensitive CDKs can use bulky ATP analogs and can be inhibited by bulky ATP inhibitors. Furthermore, we have developed cell lines allowing inducible expression of analog-sensitive CDK4 or CDK6. We have used these cell lines to label CDK4/6 substrates and determined the substrates of these kinases using mass-spectrometry. Identifying novel targets of cyclin-CDK complexes extends our understanding of how cyclin D-CDK4/6 complexes function and participate in cell-cycle progression and provide novel means for cancer therapeutics.

149-Characterizing Transcriptionally Active Biomolecular Condensates

Emma Davis, David Levens, Brian Lewis

Background and Hypothesis: Biomolecular condensates (BMCs) are dense, membrane-less organelles found within the nucleus and cytoplasm of cells. In vivo BMCs are ill-defined, but BMCs can be formed in vitro with high concentrations of recombinant proteins and a volume excluder to force phase transition. A functional BMC in vitro is desirable, as it can be used as a model to study transcription and the assembly of the preinitiation complex (PIC). Recently, we showed that combining a HeLa nuclear extract with CMV promoter DNA, forms transcriptionally active BMCs that contain many protein complexes necessary for transcription, such as RNA Pol II, NONO, FUS, PARP-1, and OGT. Experiments showed that disruption of the BMC equated with loss of transcription. We continue to explore the hypothesis that any disruption of transcription will disrupt condensate formation and vice versa, as well as address the assembly and composition of BMCs

Study Design and Methods: BMCs were formed in vitro using promoter DNA and nuclear extract. The combination of the PIC with promoter DNA forms the condensate, with the promoter DNA acting as a scaffold for PIC formation and recruitment of additional machinery and factors. Various factors and catalytic inhibitors were added to the nuclear extract before condensate formation to create a factor depleted or transcriptionally inhibited nuclear extract. Using Alexa Fluor 594 labeled promoter DNA, we visualized the condensates under a laser scanning confocal microscope. The addition of Alexa Fluor labeled antibodies allowed us to identify factors present within the condensates. The in vitro transcription assays assisted in the generation and isolation of early elongation complexes using radiolabeled transcripts, allowing us to evaluate the transcription activity of altered nuclear extract.

Results and Conclusions: The addition of catalytic enzyme inhibitors of OGT, PRMT1, and PARP, caused changes in condensate formation as the condensates got larger and clumped together. The addition of poly(G) RNA before PIC formation inhibited RNA binding proteins in the extract and resulted in no transcription or condensate formation. The addition of rFUS and rNONO, which we previously showed were components of the BMC, resulted in a decrease in the number of condensates formed, an increase in the size of formed condensates, and disrupted transcription. Our results show that BMCs require certain factors and complexes to be present to properly form and function. The addition of FUS to the nuclear extract removes any FUS interacting proteins, which are needed for condensate formation and active transcription. The addition of Poly RNAs to the nuclear extract removes any RNA Binding proteins, which are relevant to condensate formation and transcription. These data show that, unexpectedly, NONO, FUS, and RNA binding proteins are essential in transcription.

Relevance and Importance: BMCs require certain factors and complexes to form and to be transcriptionally active. Any removal or inhibition of a factor results in abnormal formation of BMCs as well as disrupted transcription. A better understanding of BMC formation will generate a better understanding of gene regulation and how oncogenes such as MYC alter this regulation.

150- Promoter-specific regulation of RNA polymerase II by cell cycle cyclin-Cdk1 complexes in budding yeast

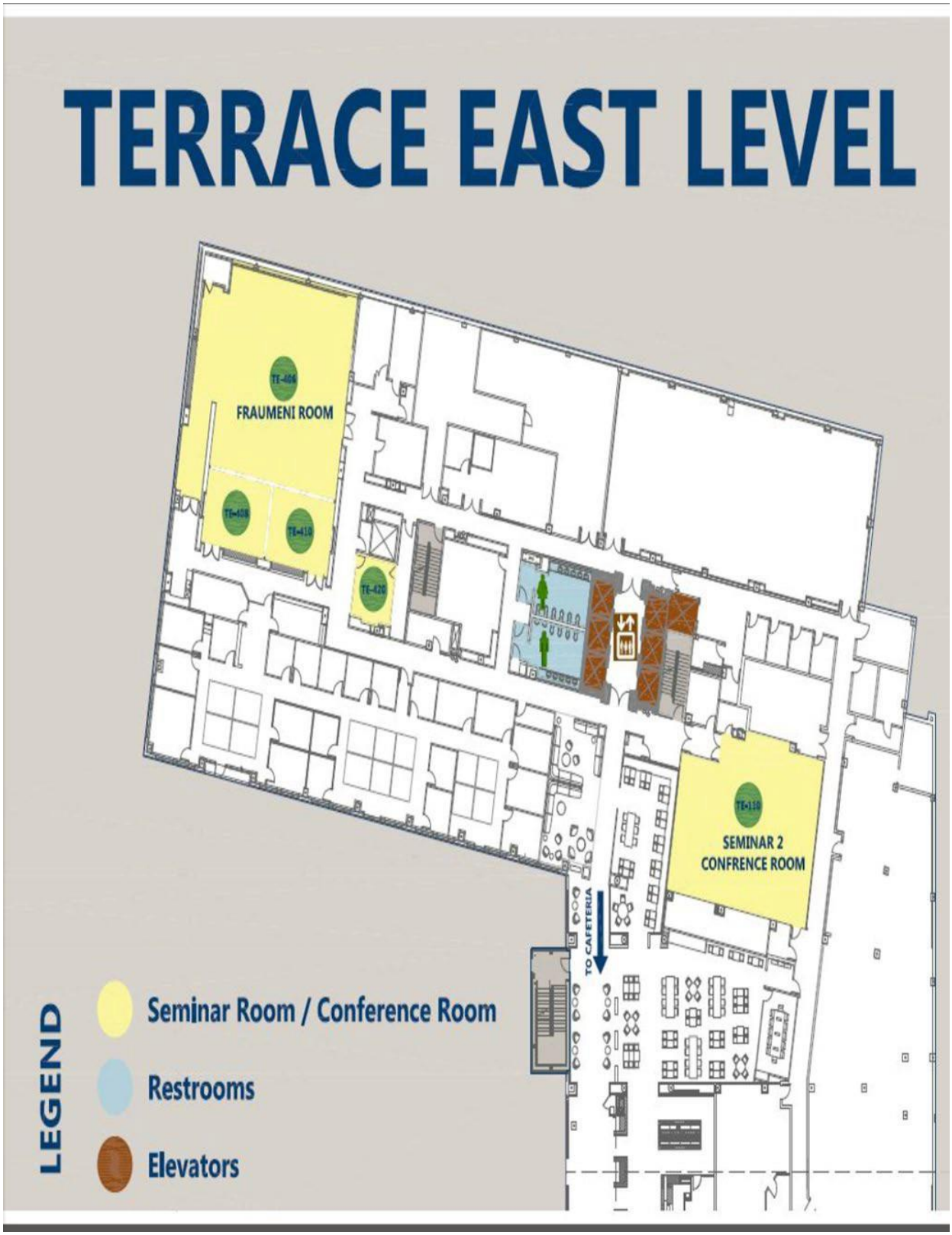
Jürgen Tuvikene[1], Mardo Kõivomägi[1]

Cyclin-dependent kinases (CDKs) are a family of evolutionarily related protein kinases with specific roles based on their interactions with regulatory proteins called cyclins. During evolution, CDKs have undergone notable divergence and specialization. The cell cycle CDKs are the key regulators of proper cell growth and division. In contrast, transcriptional CDKs are specialized in modulating gene expression by directly targeting the transcriptional machinery through the carboxyterminal domain (CTD) of RNA polymerase II (RNAPII). Cell cycle is a tightly coordinated process, involving a series of events that ultimately lead to cell division. The progression through the cell cycle is regulated by various mechanisms, including the synthesis and degradation of cyclins, and post-translational modifications. Depending on the specific cell cycle phase, different cyclin-CDK complexes phosphorylate their target proteins involved in various cellular processes, such as replication, chromosome segregation, and cell division. The conventional understanding has strictly separated the cell cycle CDKs, linked to cell division, from transcriptional CDKs, associated with direct regulation of RNAPII-dependent transcription. Recent studies in budding yeast have identified a novel mechanism in the transcriptional regulation by cell cycle-dependent kinases. According to this novel model, the cyclin-Cdk1 complexes are recruited to specific promoters by interacting with transcription factors and subsequently phosphorylate RNAPII CTD to regulate transcription. In particular, it was reported that cell cycle-dependent G1 phase cyclin-CDK (Cln3/Cdk1) complex binds to promoters regulated by G1-specific transcription factor (SBF) and directly phosphorylates the RNAPII CTD at its serine 5 residue(s), leading to transcriptional changes required for proper G1 to S phase transition (Kõivomägi et al., 2021). Kõivomägi et al. further noted that in addition to Cln3, other cell cycle-dependent cyclin-Cdk1 complexes in yeast could also phosphorylate RNAPII CTD in vitro, although at lower efficiency than the Cln3-Cdk1 complex. However, it is currently unknown whether this also happens in living cells, and what role this phosphorylation could play in the regulation of transcription and cell cycle progression. Here, we hypothesized that other cell cycle cyclins in the budding yeast in addition to Cln3 also regulate transcription by being recruited to specific promoters via sequence-specific transcription factors, and subsequently phosphorylate the RNAPII CTD. To investigate this, we determined the genome-wide occupancy of various cell cycle-associated cyclins using ChIP-sequencing. We further screened for potential novel docking interactions between the different cell cycle cyclins and transcription factors and transcriptional co-regulatory proteins using Alphafold2 protein complex structure prediction.Our study reveals novel mechanistic insights into how transcriptional changes are regulated throughout the cell cycle in the budding yeast, and pave way to understanding the universal principles underlying the cell cycle and transcriptional regulation across eukaryotes.

151- Effects of Concentration, Timing, and Pattern of Hormone Stimulation on Gene Regulation in vivo Mediated by Estrogen Receptors

Diana Stavreva[1], Kaustabh Wagh[2], Le Hoang[3], Hannah LaPoint[4], Apita Upadhyaya[5], Louis Schiltz[6], Gordon Hager[7]

Previous studies have established a strong relationship between circadian rhythms and endocrine homeostasis. However, the secretion of many hormones follows both circadian and ultradian patterns. Utilizing the glucocorticoid receptor (GR) as a model system, the impacts of the stimulation patterns of hormones on GR dynamics and downstream gene regulation have been thoroughly examined (Flynn et al. (2018), Stavreva et al. (2019), Stafford et al. (2020)).Building upon the existing knowledge of GR, we employ techniques, including genomics, single molecule tracking (SMT), and high throughput microscopy, to investigate the effects of ultradian and constant hormone stimulation on the mobility of the estrogen receptor (ER) and regulation of ER target genes. We have successfully tagged endogenous ER in MCF7 cells, allowing us to study the spatiotemporal dynamics of ER under estrogen (E2) treatment. Furthermore, we visualized RNA synthesis at both the single-cell and single-promoter levels by using a previously established cell line with 24xMS2 repeats integrated into the 3’ UTR of the ER-responsive TFF1gene.Our data revealed that in contrast to GR, E2 hormone fluctuations have much weaker impacts on ER dynamics and no effect on ER-mediated gene responses. This is likely due to the strong ER affinity to E2 and the preservation of the ER-E2 complex even after the depletion of E2 from the growth media. Understanding ER dynamics and its role in gene regulation has the potential to aid in the development of advanced treatment strategies targeting cancers that arise from the dysregulation of ER.


TERRACE EAST LEVEL
FRAUMENI ROOM
SEMINAR 2
CONFRENCE ROOM
TO CAFETERIA
LEGEND
Seminar Room / Conference Room
Restrooms
Elevators

Food Options Inside the Shady Grove NCI Building:

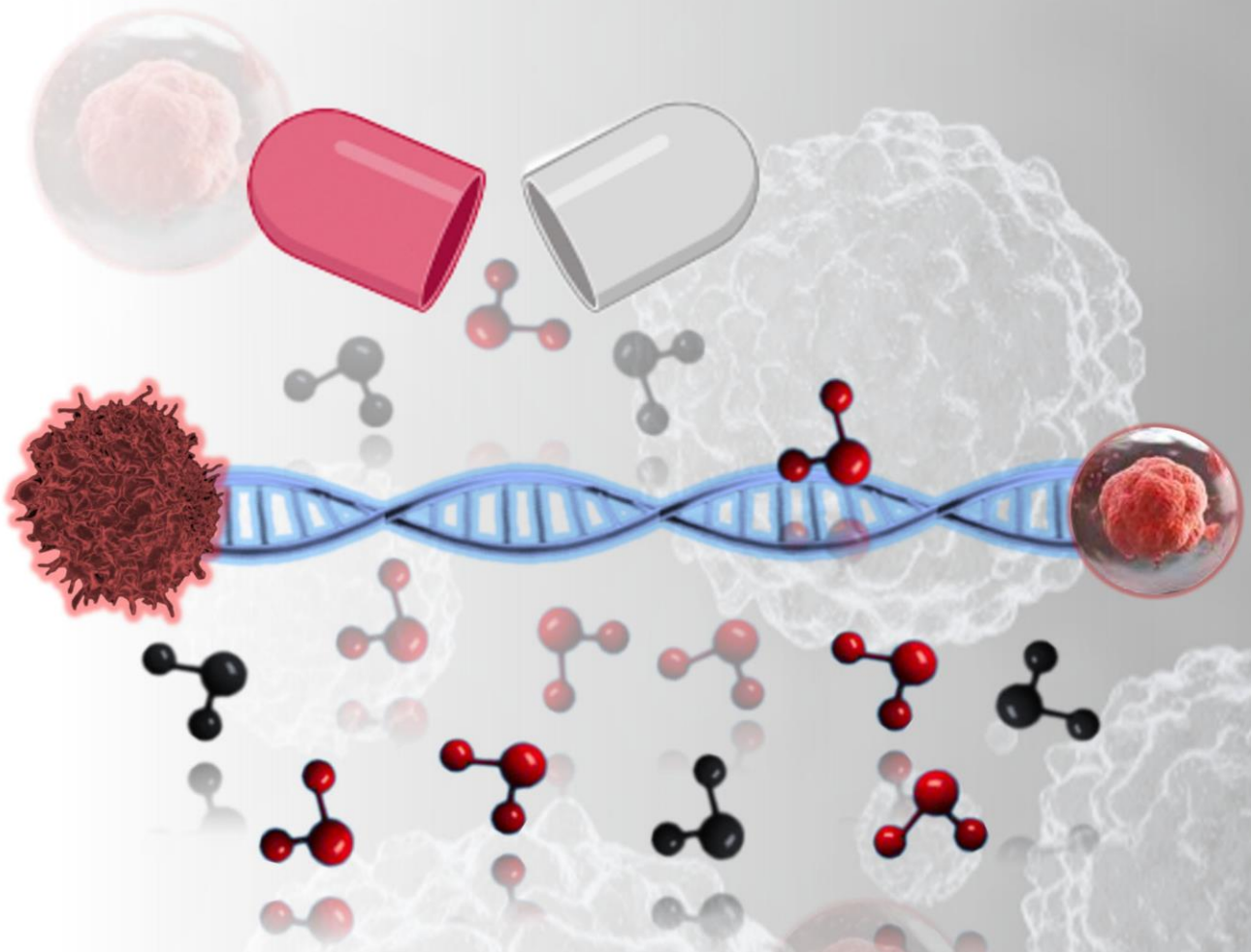
First Floor
Cafeteria Services and Avanti Self-Service convenience market with entrees, hot and cold beverages, and snacks

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



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