NATIONAL CANCER INSTITUTE

The 26th Annual CCR
Fellows and Young Investigators Colloquium

The NIH Advantage:
Where High Risk Meets High Reward

May 14 - 15, 2026

NCI Shady Grove
Rockville, MD

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 26th 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. The Colloquium is one event organized by the CCR-FYI to enable CCR scientists, especially trainees, to come together, gain knowledge, foster collaborations, form connections and hone the skills needed to achieve their career goals. Other opportunities organized by the CCR-FYI include the CCR-FYI Newsletter, the CCR-FYI seminar series, networking, social and outreach events.

We are assisted by the NCI CCR Office of the Director, the Center for Cancer Training (CCT) Office of Training and Education (OTE), and the Office of Cancer Research Capacity Building (CRCB), who work to enhance the intramural training experience. Within NCI and CCR community, the CCR-FYI would like to thank Drs. Anthony Letai, Carol Thiele, Deborah Citrin, James Gulley, Chanelle Case Borden, Shauna Clark, Brandi Carofino, Angela Jones, and Maria Moten for their assistance and continued support. We would also like to acknowledge the work of the CCR-FYI Steering Committee and CCR-FYI Colloquium subcommittee. In particular, we would like to acknowledge the two vice co-chairs, Ms. Olga Drozdovitch (Frederick) and Dr. Lauren Cutmore (Bethesda) and the two CCR-FYI Steering Committee chairs, Dr. Kathryn Muilenburg (Bethesda) and Dr. Theodore Reed (Frederick).

The theme for this year's Colloquium, "***The NIH Advantage: Where High Risk Meets Reward***", highlights the extensive research scope of the CCR. This year, *we have a record-breaking 45 oral presentations by CCR fellows*, showcasing the cutting-edge research being performed in the CCR and the NCI intramural program.

This year, we are excited about keynote presentations from CCR investigators **Dr. Naomi Taylor** and **Dr. Steven Cappell**. We are also delighted to feature a talk from this year's winner of the Outstanding Postdoctoral Fellow Award, **Dr. Kristen Fousek**, and sessions featuring short talks from finalists of the Outstanding Postdoctoral Fellow (OPF) Award and Outstanding Postgraduate Fellow (OPGF) Award. Furthermore, we have a fantastic opportunity to hear from Survivorship Speaker **Ricki Fairley**, who will share her perspective as a triple negative breast cancer survivor/thriver and CEO of TOUCH, an organization founded to help Black women diagnosed with breast cancer to get the resources, help, and support they need.

Your feedback is incredibly important, and we aim to improve the Colloquium each year. We have invited Core Facilities within CCR to engage with the fellows and share what resources they provide. Excitingly, we also have an interesting series of workshops and panels planned spanning across topics, including **the Colloquium's first ever career informational session entitled: *Exploring Scientific Careers, One Conversation at a Time*.** We strive to elevate attendees' engagement with professionals across various fields, learn more about what career path is right for them, and grow their network.

We are absolutely thrilled to have you all here to enjoy and experience the Colloquium! The Colloquium is possible not only by the Colloquium Planning Committee's hard work since September 2025 but also the volunteer work of 100+ judges and countless day-of volunteers. Moreover, your participation and enthusiasm for sharing your science with your peers is what truly makes this Colloquium successful. We encourage you to make the most of these two days by building new connections, participating in the various sessions, and celebrating the high risk-high reward research led by our outstanding CCR fellows.

On behalf of the entire CCR-FYI Colloquium Planning Committee, we sincerely thank you for joining us,

Dr. Ashlie Santaliz Casiano & Dr. Christine S. Brugger-Muli
2026 CCR-FYI Colloquium Co-Chairs

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Agenda

Thursday, May 14th, 2026

8:30 – 8:45 a.m. **Opening Remarks from Colloquium Planning Committee Chairs**
(Joseph F. Fraumeni Jr., M.D. Conf. Rm TE406/408/410)
Ashlie Santaliz Casiano, Ph.D., CCR-FYI Colloquium Co-Chair, Bethesda
Christine S. Brugger-Muli, Ph.D., CCR-FYI Colloquium Co-Chair, Frederick

8:45 – 9:15 a.m. **NCI Director's Address** *(Joseph F. Fraumeni Jr., M.D. Conf. Rm TE406/408/410)*
Anthony Letai, M.D., Ph.D., Director, NCI

9:15 – 10:00 a.m. **Keynote Speaker I** *(Joseph F. Fraumeni Jr., M.D. Conf. Rm TE406/408/410)*
"Harnessing CAR/TCR immunotherapeutic precision: Hinges, Fuzzy Logic, and Metabolic Constraints"
Naomi Taylor, M.D.,Ph.D., Senior Investigator, Pediatric Oncology Branch, NCI

10:00 – 10:15 a.m. **BREAK**

10:15 – 11:45 a.m. **Concurrent Oral Presentations** *(3 sessions)*

I. Drug Discovery: Disease Models, Pharmacology, & Therapeutics
(Joseph F. Fraumeni Jr., M.D. Conf. Rm TE406/408/410)
Kinjal Bhadresha
Smriti Kanangat
Woonghee Lee
Qiaoya Lin
Monessha Nambiar
Ira Phadke

II. Cancer Biology: Initiation, Progression, & Metastasis
(Conf. Rm 2W910/912)
Abdul Ahad
Alice Browne
Sarah Hammoudeh
Pierre Martine
Nisha Pawar
Gisele Rodrigues

III. Genetics, Epigenetics, & Gene Regulation
(Conf. Rm2W908)
Vinutha Balachandra
Shuang Feng
Genevieve Gilson
Karambir Kaur
William Nathan
Caleb Sinclear

11:45 – 12:30 p.m. **LUNCH**

12:30 – 2:00 p.m.

Poster Session I *(Seminar Room TE110)*
12:30 – 1:30 Odd Present
1:00 – 2:00 Even Present
**Odd and even numbered presenters overlap during 1:00 – 1:30 p.m. to allow enough time for poster judging.*

- **Data Science, Bioinformatics, Epidemiology, & Genomics**
- **Immunology & Virology**
- **Genetics, Epigenetics, & Gene Regulation**

2:00 – 3:00 p.m.

Exploring Scientific Careers, One Conversation at a Time *(Career Speed Networking Session) (Joseph F. Fraumeni Jr., M.D. Conf. Rm TE406/408/410)*

Chanelle Case Borden, Ph.D., Branch Director, Office of Training and Education, Center for Cancer Training, NCI

Jane Chisholm, Ph.D., Clinical Project Manager, Novus Vision

Patrick Hanley, Ph.D., Chief & Director, Cellular Therapy Program; Associate Professor of Pediatrics; Children's National Hospital, George Washington University

Kyle Hoban, Ph.D., Scientific Communications Manager, Stryker

Karin Lee, Ph.D., Associate Principal Scientist, AstraZeneca

Sarwat Naz, Ph.D, Licensing Manager, Technology Commercialization Office, George Washington University

Daniel Pham, Ph.D., Director, BD2: Breakthrough Discoveries for Thriving with Biopolar Disorder

Troy Pellom, Ph.D., Senior Staff Fellow, Radiation Therapy Team, Office of Product Evaluation and Quality, Food and Drug Administration

Christina Ross, Ph.D., Medical Science Liaison, Johnson & Johnson Innovative Medicine

Yvette Seger, Ph.D., Experienced Leader in Science Policy and Workforce Development

Joshua Stone, Ph.D., Director of Bioinformatics, Psomagen

Naomi Taylor, M.D., Ph.D., Senior Investigator, Pediatric Oncology Branch, NCI

3:00 – 3:45 p.m.

Survivorship Speaker
(Joseph F. Fraumeni Jr., M.D. Conf. Rm TE406/408/410)
Ricki Fairley, CEO and Co-Founder of TOUCH

3:45 – 4:00 p.m. **BREAK**

4:00 – 5:00 p.m. **Outstanding Postgraduate Fellow (OPGF) Finalists Session**
(Joseph F. Fraumeni Jr., M.D. Conf. Rm TE406/408/410)

"NAMPT inhibitors and pyrimidine agonist antimetabolites floxuridine and 5-FU in combination in rhabdomyosarcoma impair proliferation and survival"
Jamie Gudyka, Postbaccalaureate Fellow, Pediatric Oncology Branch, NCI

"A T-cell Engager Antibody Targeting the Non-Shed Site of Mesothelin in Solid Tumors"
Eber Guzman-Cruz, Postbaccalaureate Fellow, Laboratory of Molecular Biology, NCI

"drGT: Attention-Guided Gene Assessment of Drug Response Utilizing a Drug-Cell-Gene Heterogeneous Network"
Yoshitaka Inoue, PhD Candidate, University of Minnesota; Developmental Therapeutics Branch, NCI

"Anti-tumor Actions of Neutrophils on Bone Metastatic Prostate Cancer"
Sanjana Rajgopal, Visiting Predoctoral Fellow, Cancer Innovation Laboratory, NCI

5:00 p.m. **ADJOURN**

5:30 – 7:00 p.m. **Social Networking at Coastal Flats**
(135 Crown Park Ave, Gaithersburg, MD 20878)

Agenda

Friday, May 15th, 2026

8:30 – 9:00 a.m. **Fireside Chat with Dr. Carol Thiele**
(Joseph F. Fraumeni Jr., M.D. Conf. Rm TE406/408/410)
Carol Thiele, Ph.D., Acting Co-Director of CCR, NCI

9:00 – 10:00 a.m. **Outstanding Postdoctoral Fellow (OPF) Finalists Session**
(Joseph F. Fraumeni Jr., M.D. Conf. Rm TE406/408/410)

"HIV uses non-canonical pathways to escape from integrase inhibitors"
Yuta Hikichi, Ph.D., Postdoctoral Fellow, HIV Dynamics and Replication Program, NCI

"YAP localization mediates mechanical adaptation of human cancer cells during extravasation in vivo"
Woong Young So, Ph.D., Postdoctoral Fellow, Laboratory of Cell Biology, NCI

"Longitudinal immune profiling and biomarkers of clinical response in castration-resistant prostate cancer"
Nicole Toney, Ph.D., Postdoctoral Fellow, Center for Immuno-Oncology, NCI

"Interactions between Hsp90s and J-domain cochaperones are conserved across evolution"
Anushka Wickramaratne, Ph.D., Postdoctoral Fellow, Laboratory of Molecular Biology, NCI

10:00 – 10:15 a.m. **BREAK**

10:15 – 11:45 a.m. **Concurrent Oral Presentations** *(3 sessions)*

I. Data Science, Bioinformatics, Epidemiology, & Genomics
(Conf. Rm 2W910/912)
Charles Breeze
Maximilia Frazao de Souza Degenhardt
Josef Horak
Bandana Kumari
Sushant Patkar
Arashdeep Singh

II. Immunology & Virology
(Joseph F. Fraumeni Jr., M.D. Conf. Rm TE406/408/410)
Subhajit Chatterjee
Dongya Jua
Dan Li
Dipanwita Mitra
Lisa Poppe
Constanza Rodriguez

III. Cellular Physiology: Metabolism, Microbiology, & Cellular/Molecular Biology
(Conf. Rm 2W908)
Phuong Doan
Eli-Eelika Esvald
Shaoli Lin
Haizhen Liu
Helena Muley
Taewoo Yang

11:45 – 12:30 p.m. **LUNCH**

12:30 – 2:00 p.m. **Poster Session II** *(Seminar Room TE110)*
12:30 – 1:30 Odd Present
1:00 - 2:00 Even Present
**Odd and even numbered presenters overlap during 1:00 – 1:30 p.m. to allow enough time for poster judging.*

- **Drug Discovery: Disease Models, Pharmacology, & Therapeutics**
- **Cancer Biology: Initiation, Progression, and Metastasis**
- **Cellular Physiology: Metabolism, Microbiology, & Cellular/Molecular Biology**

2:00 – 2:45 p.m. **Outstanding Postdoctoral Fellow Award Winner**
(Joseph F. Fraumeni Jr., M.D. Conf. Rm TE406/408/410)
"Characterization of the anti-tumor efficacy of memory cytokine enriched NK cells against tumors with neuroendocrine features"
Kristen Fousek, Ph.D., Research Fellow, Center for Immuno-Oncology, NCI

2:45 – 3:30 p.m. **Keynote Speaker II** *(Joseph F. Fraumeni Jr., M.D. Conf. Rm TE406/408/410)*
"How cell cycle dynamics shape drug response"
Steven D. Cappell, Ph.D., Stadtman Investigator, Laboratory of Cancer Biology and Genetics, NCI

3:30 – 3:45 p.m. **BREAK**

3:45 – 4:45 p.m. **Concurrent Workshops and Panels**

I. ***How to Use Artificial Intelligence in Science***
(Workshop) (Conf. Rm 2W910/912)

Rama Chellappa, Ph.D. Bloomberg Distinguished Professor in Electrical and Biomedical Engineering, Johns Hopkins University

II. ***The Art of Explanation: Tailoring Science Communication to Different Audiences***
(Workshop) (Conf. Rm 2W908)

Leigh Anne Kelley, M.B.A., Director of Communications, Fralin Biomedical Research Institute at Virginia Tech Carilion (VTC) School of Medicine, Virginia Tech

III. ***Work/Life Balance in Academia and Industry***
(Panel) (Conf. Rm TE408/410)

Kaitlyn Sadtler, Ph.D., Senior Investigator, National Institutes of Biomedical Imaging and Bioengineering, NIH

Allen Su, Ph.D., Assistant Professor and Scientist 2, Henry Jackson Foundation; Adjunct Investigator, NCI

Whitney Do, Ph.D., Bioinformatics Senior Consultant, Deliotte

Sisi He, Ph.D., Senior Scientist, AstraZeneca

4:45 – 5:30 p.m. **Closing Address & Travel Awards**
(Joseph F. Fraumeni Jr., M.D. Conf. Rm TE406/408/410)
Shauna Clark, Ph.D., Associate Director, Office of Cancer Research Capacity Building, NCI

5:30 p.m. **ADJOURN**

6:00 – 7:30 p.m. **Social Networking at Ted's Bulletin**
(220 Ellington Blvd, Gaithersburg, MD 20878)

CCR-FYI Leadership

Christine Brugger-Muli, Ph.D.

CCR-FYI Colloquium Frederick Co-Chair

Dr. Christine S. Brugger-Muli is a postdoctoral fellow in the Center for Structural Biology at the National Cancer Institute in Frederick. She is the Frederick Co-Chair of the Colloquium Planning Committee. Dr. Brugger-Muli completed her B.S. in Biochemistry at Cal Poly in San Luis Obispo, California, where she began synthetic chemistry research in the lab of Dr. Hasan Palandoken. In 2014, she began working at the pharmaceutical company Genentech in South San Francisco, California, supporting several early drug discovery small molecule programs. Dr. Brugger-Muli earned her Ph.D. in Medicinal Chemistry & Molecular Pharmacology at Purdue University in West Lafayette, Indiana, in 2022. While at Purdue in the lab of Dr. Darci Trader as an F31 pre-doctoral fellow, she synthesized and investigated chemical tools to harness and perturb the ubiquitin-proteasome system in hematological cancers.

In 2023, Dr. Brugger-Muli joined the lab of Dr. Kylie Walters as an Intramural Continuing Umbrella of Research Experiences (iCURE) CRTA fellow, where she continues to study tools for the ubiquitin-proteasome system, with a focus on targeted protein degradation therapeutics. In addition to research, Dr. Brugger-Muli actively enjoys mentoringand inspiring younger scientists to grow an appreciation and passion for science. When not engaged in science, she can be found doing creative hobbies, such as drawing, painting, photography, and fiber arts.

Ashlie Santaliz Casiano, Ph.D.
CCR-FYI Colloquium Bethesda Co-Chair

Dr. Ashlie Santaliz Casiano is a postdoctoral fellow in the Laboratory of Human Carcinogenesis at the National Cancer Institute in Bethesda. She is the Bethesda Chair of the CCR Fellows & Young Investigators Colloquium. Dr. Santaliz Casiano completed her B.S. in Biology at the University of Puerto Rico at Mayagüez, where she conducted her first cancer research characterizing anti-cancer bioactive compounds from soursop. She went on to earn her Ph.D. in Nutritional Sciences at the University of Illinois at Urbana-Champaign, supported by an NIH T32 traineeship in the Tissue Microenvironment program. In the lab of Dr. Zeynep Madak-Erdogan, she bridged bench and computational science to uncover metabolic and molecular drivers of ER+ breast cancer disparities and estrogen receptor signaling in cancer metabolism and drug resistance.

In 2023, Dr. Santaliz Casiano joined the lab of Dr. Stefan Ambs as an Intramural Continuing Umbrella of Research Experiences (iCURE) CRTA fellow, where she investigated how chronic stress, neighborhood deprivation, and environmental exposures drive prostate and breast cancer disparities. She continues this work under Dr. Xin Wang, examining how stress reshapes immune cell composition and B cell differentiation in breast cancer patients. To further strengthen her epidemiological training, she is concurrently pursuing a part-time M.P.H. in Epidemiology at Harvard University. Beyond her research, Dr. Santaliz Casiano is deeply committed to mentorship and service through non-profit organizations. Outside the lab, she enjoys playing tennis.

Lauren Cutmore, M.A., Ph.D.

CCR-FYI Colloquium Bethesda Vice Co-Chair

Dr. Lauren Cutmore is a visiting fellow in Surgery Branch, Center for Cancer Research, National Cancer Institute, Bethesda. She is the Bethesda Vice Co-Chair of the Colloquium Planning Committee. Dr. Cutmore completed her Bachelor of Science degree in Biomedical Sciences at Imperial College London and her Master of Research degree in Translational Immunology at the QueenMary, University of London. She completed her Ph.D. in Immunobiology at Barts Cancer Institute, London, UK, where her doctoral research focused on the generation of CAR T cell therapies against pancreatic ductal adenocarcinoma. In 2021 Dr. Cutmore joined the lab of Dr. James Kochenderfer, where she currently works on development of CAR T cell therapies for the treatment of hematological malignancies. In addition to research, Dr. Cutmore is an active member of the CCR fellows community and enjoys doing science outreach. Outside of the lab, she enjoys reading, traveling and trying out new restaurants.

Olga Drozdovitch, B.Sc.
CCR-FYI Colloquium Frederick Vice Co-Chair

Olga Drozdovitch is a postbaccalaureate fellow in the Molecular Targets Program at the National Cancer Institute in Frederick. She is the Frederick Vice Co-Chair of the Colloquium Planning Committee. Olga earned her A.S. in Biological Sciences at Montgomery College and B.Sc. in Biological Sciences at the University of Maryland at the Universities at Shady Grove Campus in Rockville, Maryland. Her journey into research began as a member of the NIH Summer Internship Program, under the mentorship of Dr. Ludmila Prokunina-Olsson, where she assisted in the effort to functionally characterize a novel polymorphic tandem repeat. Following the completion of her degree, she returned to the NCI as an Intramural Continuing Umbrella of Research Experiences (iCURE) CRTA fellow, joining the lab of Dr. Michael Aregger where she currently works on identifying possible kidney cancer therapeutic targets by establishing various CRISPR/CHyMErA stable kidney cell lines for genome-wide screens to validate cancer-specificity in previous Renal Medullary Carcinoma screen hits. This fall, she will begin her Ph.D. studies at the University of Virginia School of Medicine in Charlottesville, VA. When not vigorously pipetting, she engages in voice acting, digital art, and devoting time to mentoring other first-generation undergraduate women in STEM as a project coach at NvolveInc.— a non-profit organization which she is an alumna of.

Shauna A. Clark, Ph.D.
Associate Director
Office of Cancer Research Capacity Building

Shauna A. Clark, Ph.D., serves as the Associate Director of the Office of Cancer Research Capacity Building (CRCB) in the Center for Cancer Research at the National Cancer Institute. In this role she also serves as Training Director and Senior Advisor to the CCR Director. She leads the development and implementation of policies and programs that foster a culture of excellence and strengthen the scientific workforce. She is deeply committed to ensuring that all who aspire to contribute to science have meaningful opportunities to participate, thrive, and feel valued.

Prior to joining CCR, Dr. Clark served as Director of the NIH Academy, a health disparities program for NIH fellows, in the NIH Office of Intramural Training & Education. Under her leadership, the Academy expanded into a dynamic, multi-faceted program that deepened engagement across the scientific community and significantly broadened its reach and impact. Throughout her career, Dr. Clark has been actively engaged in initiatives that educate and mentor future researchers, public health professionals, and clinicians, while advancing efforts to build a strong biomedical research workforce. Her contributions have been recognized with multiple NIH Awards.

Dr. Clark earned her undergraduate degree in biochemistry from Texas A&M University and her Ph.D. in Infectious Diseases and Microbiology from the University of Pittsburgh Graduate School of Public Health. She completed her postdoctoral training in the Liver Diseases Branch at the National Institute of Diabetes and Digestive and Kidney Diseases.

Chanelle Case Borden, Ph.D.

Branch Director, Office of Training and Education
Center for Cancer Training

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 the Branch Director of the Office of Training and Education. 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.

CCR-FYI Colloquium Planning Committee

Nicolas Bertuol, B.S.
Colloquium Secretary

Wan-Ning (Chloe) Li, Ph.D.

Subhash Sethi, Ph.D.

Shikha Joon, Ph.D.

Akshay Deshpande, Ph.D.

Rachel Carter, Ph.D.

Natasha Vinod, Ph.D.

Nandhini Ranganathan, Ph.D.

Kristen Fousek, Ph.D.

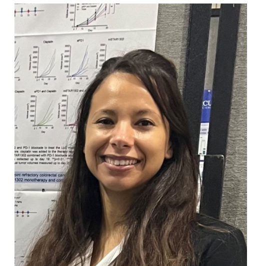
Udochi Azubuike, Ph.D.

Katie Lothstein, Ph.D.

Ali Mokhtar Mahmoud, Ph.D.

Dana Vargas, B.S.

Amit Singh, Ph.D.


Shantel Angstadt, Ph.D.

Seketouile Keretsu, Ph.D.

Mahgol Behnia, Ph.D.
Steering Committee Secretary & Planning Committee

Katie Muilenburg, Ph.D.
Steering Committee Co-Chair, Bethesda, & Planning Committee

Theodore Reed, Ph.D.
Steering Committee Co-Chair, Frederick

NCI Leadership

Anthony Letai, M.D., Ph.D.
Director, National Cancer Institute

Dr. Anthony Letai, M.D., Ph.D., joined NCI as the 18th director on September 29, 2025. He previously served as professor of medicine at Harvard Medical School and medical oncologist at the Dana-Farber Cancer Institute.

Dr. Letai received his Bachelor of Arts degree in physics from Princeton University. After receiving his M.D. and Ph.D. from the University of Chicago with Elaine Fuchs, Ph.D., Dr. Letai completed his residency in internal medicine at Brigham and Women's Hospital, and a fellowship in hematology and oncology at the Dana-Farber Cancer Institute. His interest in cancer cell death evolved during his post-doctoral research training at Dana-Farber Cancer Institute in the laboratory of Dr. Stanley Korsmeyer. His career and pioneering work in functional precision oncology helped advance therapies such as BCL-2 inhibitors—drugs that are now standard treatment for many adults with leukemia. In addition, his team developed a technique, called BH3 profiling, that helps predict how cancer cells will respond to chemotherapies. BH3 profiling is now being tested to discover how it can be used in clinical practice to find the right drugs for the right patients.

An advocate for research that can quickly bring new, effective treatments to patients, Dr. Letai served as co-founder and President of the Society for Functional Precision Medicine, which works to improve patient care and outcomes by supporting the use of functional assays in clinical care. Additionally, he is the recipient of the Sidney Kimmel Foundation Scholar Award, the Leukemia and Lymphoma Society Scholar Award, the European Cell Death Organization Career Award, the Smith Family Prize for Outstanding Scientific Contributions, and the NCI Outstanding Investigator Award. He also serves as an elected member of the American Society of Clinical Investigation and Association of American Physicians. In 2019, the Web of Science Group named Dr. Letai in its list of Highly Cited Researchers, whose papers rank in the top 1% by citations for their field and year of publication, showing his significant impact on his area of research. Dr. Letai's work has helped transform cancer treatment and advance functional precision medicine.

Carol J. Thiele, Ph.D.

Acting Co-Director, Center for Cancer Research

Dr. Thiele received her Ph.D. in Microbiology and Immunology from the University of California, Los Angeles. She completed her postdoctoral research as a Cancer Research Institute and a Damon Runyon-Walter Winchell Fellow at the NCI. Dr. Thiele was one of the founding editors of Cell Death & Differentiation, andhas served on the editorial boards of Cell Death & Differentiation, Cancer Research and Molecular Cancer Therapeutics. Dr. Thiele was Chair of the AACR Women in Cancer Research and has a long-standing interest in developing programs so that young scientific investigators can realize their potential. As the Chief of the Cell and Molecular Biology Section in the Pediatric Oncology Branch, Dr. Thiele's scientific interest is in the field of cancer biology with a special emphasis on pediatric neuroectodermal tumors and neuronal development. She has been involved in the organization of the Advances in Neuroblastoma Research Association (ANRA). Her research strives to understand molecular mechanisms involved in the pathogenesis of neuroblastoma tumors and utilizes insights gleaned from these studies to develop novel therapeutic strategies for pediatric tumors.

Keynote Speaker I

Thursday, May 14, 9:15 – 10:00 a.m. in TE406/408/410

Naomi Taylor, Ph.D.
Senior Investigator
Head, Basic to Translational Oncology Section
Head, Immunology-Hematology Section
CCR Deputy Director

"Harnessing CAR/TCR immunotherapeutic precision: Hinges, Fuzzy Logic, and Metabolic Constraints"

Naomi Taylor is a Deputy Director of the Center for Cancer Research and Senior Investigator in the Pediatric Oncology Branch (NCI, NIH). She studied at Princeton University and the Weizmann Institute before earning her MD/PhD at Yale University School of Medicine with Dr. George Miller. Following pediatrics training at Yale and the Children's Hospital of Los Angeles as a Howard Hughes Fellow, Dr. Taylor launched her own lab at the Institut de Génétique Moléculaire in Montpellier, France. She currently holds an adjunct professorship at the Université de Montpellier.

Dr. Taylor leads a research group internationally recognized for its work on T cell-based gene and cell therapies, metabolic regulation of normal and malignant hematopoiesis, and thymus differentiation. Recently, the group has pioneered studies exploring how metabolite transporters and cellular fuel choices shape immune and hematopoietic function. These efforts have identified novel metabolic programs regulating physiological and pathological hematopoietic lineage commitment, including erythropoiesis. Their discoveries have also directly informed the development of more effective T-cell immunotherapy protocols—advancing the design and performance of chimeric antigen receptor (CAR) T-cell therapies.

For Naomi Taylor, scientific research is a powerful platform that has the unique ability to bring together individuals from different backgrounds—it is a field that breaks down walls and fosters international cooperation. In this context, Dr. Taylor has promoted the careers of students, clinicians, and scientists from >30 different countries, spanning 6 continents. She has received numerous awards for her research and training including the French National Inserm Research Award (2010), the 2021 NCI Women in Science Mentoring and Leadership Award, election to the Association of American Physicians (2023), and the NCI Director's Award for Making an Impact-NCI Champions (2024).

Keynote Speaker II

Friday, May 15, 2:45 – 3:30 p.m. in TE406/408/410

Steven Cappell, Ph.D.
Stadtman Investigator
Head, Single-Cell Dynamics Section

"How cell cycle dynamics shape drug responses"

Dr. Cappell received his B.S. in marine science and biology from the University of Miami, FL. He obtained his Ph.D. from the University of North Carolina, Chapel Hill, where he studied G protein signaling in yeast in the lab of Dr. Henrik Dohlman. He completed his postdoctoral training as a Damon Runyon Cancer Research Foundation Fellow in the laboratory of Dr. Tobias Meyer at Stanford University. His postdoctoral research focused on understanding how cells make the decision to divide. He joined the Center for Cancer Research, NCI as an NIH Stadtman Investigator in 2017.

Survivorship Speaker

Thursday, May 14, 3:00 – 3:45 p.m. in TE406/408/410

Ricki Fairley
CEO & Co-Founder of TOUCH

Ricki is an award-winning seasoned marketing veteran that has transformed her strategic acumen into breast cancer advocacy. Ricki co-founded and serves as CEO of **TOUCH, The Black Breast Cancer Alliance**to address Black Breast Cancer as a unique and special disease state, with the overall goal of reducing the mortality rate for Black women. Ricki founded and serves as co-host for "**The Doctor Is In**," a weekly live breast cancer advocacy web series on the BlackDoctor.org Facebook page that reaches over 3 million viewers. Ricki founded **#BlackDataMatters** to spotlight the urgent need for inclusive science, a mission that served as the launchpad for her **When We Tri(al)** movement. Since its inception in January 2021, the movement has revolutionized clinical trial participation by directing over 33,000 Black women to a custom, user-friendly portal designed specifically to improve health outcomes and advance the study of Black breast cancer. In January 2023, she founded the **For The Love of My Gurls** Campaign and **The Pink Table Talk** monthly web series effectively changing perceptions of breast health in over one million young Black women. In 2023, Ricki founded **TOUCH Care**, the first and only Black woman survivor led 24/7 nurse navigation program supporting breast cancer clinical trials. In 2024, Ricki founded **BELONG** (Blessties Empowering Leadership and Opportunity and Nurturing Growth) to provide education, leadership coaching and resources for Black-led grass-roots organizations, support groups, and ministries and community leaders. BELONG has representation in 40 states, the UK, Ghana, Nigeria, South Africa and Kenya. Ricki founded and hosts "**SAMBAI Speaks**," a monthly web series reaching 10,000 viewers monthly in the SAMBAI geography of the US, UK, Ghana, Nigeria, South Africa, Ethiopia and Kenya. In collaboration with AACR, The Triple Negative Breast Cancer Foundation and Nueva Vida, she launched **BlackTNBCSanctuary.org** as the only comprehensive resource hub Black and Afro-Latina patients and their families to help navigate a triple negative breast cancer.

Ricki is a Principal Investigator for the Cancer Grand Challenge Project SAMBAI (Social, Ancestry, Molecular and Biological Analysis of Inequalities) leading a Patient Advocacy team for this global research initiative, led by Dr. Melissa Davis at Morehouse School of Medicine, striving to decode the factors that cause and influence disparate cancer outcomes in unsupported populations of African descent. Ricki curated the first and only Black health hub, **TOUCH Talks Wellness** on Martha's Vineyard addressing critical health issues impacting Black families and empowering the community with vital information and "edutainment" during the annual August gathering of Black families, community leaders, and influencers in Oak Bluffs, MA, reaching over 118,000 with the content.

In January 2026, Ricki expanded her commitment to health equity by founding **Navigating Trials**, a new venture that provides proprietary recruiting and navigation services designed to align clinical research representation with the actual disease burden disproportionately affecting Black patients.

Ricki serves on the Board of Trustees for the Triple Negative Breast Cancer Foundation and Black In Cancer. She is a board member for the Center for Healthcare Innovation and the Comprehensive Cancer Centre of Excellence in Kumasi, Ghana. She is a member of the Boobie Queen Company Advisory Board for Navigation and the FNIH Patient Engagement Council, and serves on numerous patient/advocacy advisory boards.

Ricki has been featured in FiercePharma, OncoDaily, Healthline, Oprah Daily, Shondaland, Platform Q Health, Essence Magazine and Salud and Ebony Magazine, The TODAY Show with Hoda and Jenna, Good Morning Washington, Black Enterprise to name a few. She is a recipient of the Goldman Sachs One Million Black Women Impact Grant. Ricki has been recognized as one of 25 leaders making an impact in patient engagement by 1nHealth. Ricki received the 100 Global Women Icon Award from Women of Wealth Magazine and the Dr. Dorothy I. Height Leadership Award at 34th International Salute to the Life and Legacy of Dr. Martin Luther King, Jr. Rick has been recognized as one of BlackDoctor.org's 2026 Top Blacks in Healthcare.

Ricki has two daughters, Amanda Brown Lierman and Hayley Brown, and four granddaughters, Belle, Leia, Hart, and Jetson who remind her of her purpose every day. Ricki is a graduate of Dartmouth College and holds an MBA from Kellogg Graduate School of Management at Northwestern University.

Outstanding Postdoctoral Fellow

Friday, May 15, 2:00 – 2:45 p.m. in TE406/408/410

Kristen Fousek, M.S., Ph.D.

Research Fellow, Center for Immuno-Oncology

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

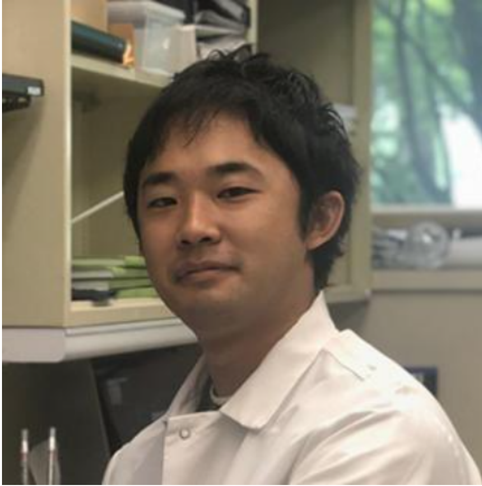
Dr. Kristen Fousek is a Research Fellow in the Center for Immuno-Oncology (CIO) at the National Cancer Institute (NCI). She completed her Bachelor of Science degree in Microbiology at the University of Texas at Austin and her Master of Science degree in Biotechnology at the University of California, Irvine. Dr. Fousek earned her Ph.D. in Translational Biology and Molecular Medicine from Baylor College of Medicine in Houston, TX. Her graduate research focused on targeting resistant B-lineage malignancies using trivalent chimeric antigen receptor (TriCAR) T cells which are now under evaluation in a phase I clinical trial for the treatment of relapsed, refractory acute B-lineage leukemia.

Following graduate school, Dr. Fousek moved to NCI where she has been working in the Tumor Immunoregulation Section of the CIO under the mentorship of Dr. Claudia Palena. Her research has focused on cytokine and natural killer (NK) cell-based therapeutics for the treatment of small cell lung cancer (SCLC) and other neuroendocrine (NE) tumors. Her early work demonstrated that the use of an IL-15 cytokine superagonist (NAI) enables NK cells to have anti-tumor efficacy against all molecular subtypes of SCLC, despite the heterogeneity of these tumors. In her current studies, Dr. Fousek is investigating the potential of memory cytokine enriched NK cells (M-ceNK) for treating neuroendocrine tumors, which prove to be very difficult to treat with standard of care therapies. She is additionally exploring the mechanisms of action by which tumor cells are able to impair the activity of NK cells and how this NK dysfunction may be overcome.

In the future, Dr. Fousek plans to continue to work on the development of immunotherapies including cellular therapies as well as additional modalities such as cytokine, immune checkpoint, or vaccine-based therapeutics. She hopes to continue her career working in the government sector where she can pursue her interests in investigating rare neuroendocrine tumors and other tumors that remain recalcitrant to current standard of care therapies.

Outstanding Postdoctoral Fellow Finalists

Friday, May 15, 9:00 – 10:00 a.m. in TE406/408/410



Yuta Hikichi, Ph.D.

Postdoctoral Fellow, HIV Dynamics and Replication Program

"HIV uses non-canonical pathways to escape from integrase inhibitors"

Yuta Hikichi is a postdoctoral fellow in Dr. Eric Freed's lab in the HIV Dynamics and Replication Program at the National Cancer Institute in Frederick. He earned his Ph.D. from the University of Tokyo under the mentorship of Dr. Tetsuro Matano before joining the NIH in 2019. His research investigates the complex mechanisms of HIV-1 replication and antiretroviral resistance. He has specifically identified "non-canonical" resistance pathways where mutations in non-target proteins facilitate high-level resistance to major drug classes, including second-generation integrase inhibitors.

Woong Young So, Ph.D.

Postdoctoral Fellow, Laboratory of Cell Biology

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

After obtaining PhD in chemistry at Carnegie Mellon University, I came to the National Cancer Institute as a postdoctoral fellow, working in the Laboratory of Cell Biology under the mentorship of Dr. Kandice Tanner. Bridging my background in physical chemistry with cancer biology, I have specialized in quantitative cancer biophysics, investigating the mechanical adaptations of tumor cells during organ-specific metastasis. Furthermore, using a zebrafish xenograft platform to perform high-resolution, real-time screening of therapeutic responses and tumor microenvironment remodeling. I have successfully adapted various advanced biophysical imaging modalities, including optical tweezers, fluorescence lifetime imaging microscopy for metabolic imaging, and Brillouin microscopy to map tissue stiffness and metabolism in vivo. My research has led to significant discoveries, including the role of YAP on cancer cells during early extravasation and the identification of macrophage-mediated remodeling as a critical factor in maintaining brain tissue mechanical homeostasis. In addition to research, I have demonstrated strong leadership and service to the scientific community through editorial roles, scientific society committee roles (American Society for Cell Biology – Committee for Postdocs and Students and Biophysical Society – Early Careers Committee), and outreach.

Nicole Toney, Ph.D.

Postdoctoral Fellow, Center for Immuno-Oncology

"Longitudinal Immune Profiling and Biomarkers of Clinical Response in Castration-Resistant Prostate Cancer"

Dr. Nicole Toney earned her PhD in Biological Sciences from the University of Delaware and is currently a postdoctoral fellow in the Center for Immuno-Oncology at the National Cancer Institute under the mentorship of Dr. Jeffrey Schlom. Her research focuses on characterizing baseline immune profiles and longitudinal changes in the peripheral immunome of cancer patients enrolled in combination immunotherapy trials. Through multiparametric immune monitoring and translational analyses, her work aims to define mechanisms of action of novel immunotherapy agents and identify biomarkers associated with clinical response, with the goal of advancing the optimization of cancer immunotherapies.

Anushka Wickramaratne, Ph.D.

Postdoctoral Fellow, Laboratory of Molecular Biology

"Interactions between Hsp90s and J-domain cochaperones are conserved across evolution"

Dr. Anushka Wickramaratne is a postdoctoral fellow in the laboratory of Dr. Sue Wickner in the National Cancer Institute. She received her Ph.D. in Biomedical Sciences from University of Texas Southwestern Medical Center. Her research focuses on elucidating the biochemical interactions between molecular chaperones, a family of proteins that regulate protein folding, and their roles in protein quality control. By defining how molecular chaperones maintain proteome integrity, Dr. Wickramaratne's work provides mechanistic insight into processes underlying diseases such as cancer and neurodegenerative disorders, including Alzheimer's and Parkinson's disease, with implications for therapeutic targeting of protein quality control pathways.

Outstanding Postgraduate Fellow Finalists

Thursday, May 14, 4:00 – 5:00 p.m. in TE406/408/410

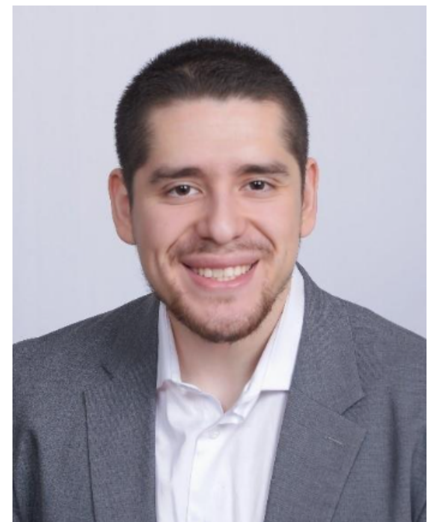
Jamie Gudyka, B.S.
Postbaccalaureate Fellow, Translational Sarcoma Biology Group

"NAMPT inhibitors and pyrimidine agonist antimetabolites floxuridine and 5-FU in combination in rhabdomyosarcoma impair proliferation and survival"

Jamie is a second-year postbaccalaureate fellow at the National Cancer Institute and a graduate of Iona University, where she earned a degree in Biochemistry. As an undergraduate, she conducted research in the Project Symphony group under the mentorship of Dr. Sunghee Lee, investigating the effects of plant polyphenols on the biophysical properties of model cell membranes. Her current work in the Translational Sarcoma Biology section under the mentorship of Dr. Christine Heske focuses on evaluating the mechanism and activity of novel, targeted, clinically relevant drug combinations in models of pediatric rhabdomyosarcoma.

Eber Guzman-Cruz, B.S.
Postbaccalaureate Fellow, Laboratory of Molecular Biology

"A T-cell Engager Antibody Targeting the Non-Shed Site of Mesothelin in Solid Tumors"



Eber received his Bachelor of Science in Molecular Biology from Towson University in 2024, where he conducted research on the development of antibody-based therapeutics to combat factor VIII resistance in individuals affected by Hemophilia A. He currently serves as a Postbaccalaureate Fellow in Dr. Mitchell Ho's lab at the National Cancer Institute, where he is working on the development of a CD3+ bispecific antibody targeting mesothelin-expressing cancers. In 2025, Eber received the Outstanding Oral Presentation Award at the NCI Annual CCR Colloquium. This upcoming Fall, Eber will begin medical school at the University of Maryland School of Medicine.

Yoshitaka Inoue, B.Sc., M.Eng.

Predoctoral Fellow, Developmental Therapeutics Branch

"drGT: Attention-Guided Gene Assessment of Drug Response Utilizing a Drug-Cell-Gene Heterogeneous Network"

Yoshitaka Inoue is a Ph.D. candidate in Computer Science and Engineering at the University of Minnesota and a predoctoral fellow at the National Library of Medicine. His research focuses on developing interpretable machine learning methods for cancer pharmacogenomics, particularly using graph neural networks and large language models to predict drug response and uncover underlying biological mechanisms. His work integrates multi-modal data, including drug structures, gene expression, and cellular responses, to improve both predictive performance and mechanistic insight.

Sanjana Rajgopal, B.Sc.

Predoctoral Fellow, Cancer Innovation Laboratory

"Anti-tumor Actions of Neutrophils on Bone Metastatic Prostate Cancer"

Sanjana Rajgopal was born in India and raised in Kuwait. She earned her Bachelor's degree with honors in Biomedical Sciences, majoring in Human Genetics, from the Sri Ramachandra Institute of Higher Education and Research in Chennai, India. She is now pursuing her Ph.D. in Molecular Genetics and Cell Biology in the Department of Genetics, Cell Biology, and Anatomy at the University of Nebraska Medical Center. Sanjana joined the laboratory of Dr. Leah Cook in 2021, where she became deeply interested in the laboratory's focus on how neutrophils and the tumor microenvironment shape cancer progression and treatment response. After completing her Ph.D. candidacy, she was invited to continue her dissertation work with her mentor at the National Cancer Institute (NCI), NIH in Frederick, MD—a transition that allowed her to broaden her scientific training. In 2024, she transitioned to the Cancer Innovation Laboratory, CCR at NCI under Dr. Leah Cook's continued mentorship. Her research focuses on understanding how neutrophils influence bone metastatic prostate cancer, particularly the mechanisms that drive their cytotoxic activity and potential therapeutic relevance. Using both in vitro systems and mouse models of bone metastasis, she aims to uncover how neutrophils reshape molecular pathways in tumor cells and alter the immune landscape of the bone microenvironment. Her broader interests span tumor immunology, metastasis, and the dynamic biology of the tumor microenvironment.

Sanjana has contributed to multiple peer-reviewed publications, including her first-author review article in Cancer Medicine (2023), contributions to a publication in Molecular Cancer Research (2025), and has co-authored a scientific book chapter. She has shared her research at national and international conferences through both poster and oral presentations. Her work has been recognized with honors such as a poster award at the CPDR Scientific Retreat and the 2026 Cancer and Bone Society Outstanding Abstract Award.

Invited Speakers, Panelists, and Workshop Leaders

“Exploring Scientific Careers, One Conversation at a Time”

Thursday, May 14, 2:00 – 3:00 p.m. in TE406/408/410

What career path interests you most? We are pleased to be hosting the first career informational session by the CCR-FYI Colloquium. To expose CCR fellows to various careers and to build their professional network, we have invited 12 professionals from different fields including scientific non-profits, communication, policy, regulation, research, and more!

During this hour, we will have 6 rounds of speed networking. Our invited professionals will be at their own table, and after each 8-minute round, CCR fellows will be encouraged to move to a different table, build connections, and learn about a new career.

Chanelle Case Borden, Ph.D.

Branch Director
Office of Training and Education
Center for Cancer Training
National Cancer Institute

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 the Branch Director of the Office of Training and Education. 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.

Jane Chisholm, Ph.D.

Clinical Project Manager, Novus Vision

Dr. Jane Chisholm is the Clinical Project Manager at Novus Vision, a startup company spun out of the Wilmer Eye Institute at Johns Hopkins, that has developed a novel topical eye drop technology for improved ocular comfort and drug delivery. At Novus Vision, Dr. Chisholm has lead five pilot and early-stage clinical trials conducted for anterior segment indications in addition to assisting with formulation development and preclinical activities. Previously, Dr. Chisholm worked at the clinical-stage pharmaceutical company Graybug Vision that was focused on developing long-acting injectables for the treatment of chronic vision-threatening diseases. During her five years at Graybug Vision, Dr. Chisholm rose from Associate Scientist to Associate Director of Pre-Clinical Development where she led preclinical activities for pipeline programs, managed the development of new drug loaded microparticle and implant formulations for intravitreal injection, and provided support to the clinical team. Dr. Chisholm received a Ph.D. in Chemical and Biomolecular engineering from Johns Hopkins University under the mentorship of Dr. Justin Hanes where she studied pulmonary drug delivery.

Patrick Hanley, Ph.D.

Chief & Director, Cellular Therapy Program

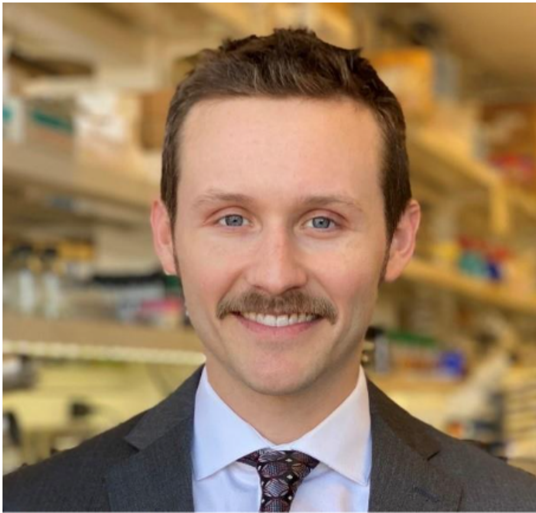
Associate Professor of Pediatrics

Children's National Hospital, George Washington University

Dr. Hanley is the Chief and Director of the Cellular Therapy Program and an associate professor of pediatrics at Children's National Hospital and George Washington University, respectively. He oversees processing for standard of care stem cell transplantation as well as the development, manufacture, quality, and testing of novel cell and gene therapies. Over the past 20 years he has helped to translate more than 600 products on over 25 cell therapy protocols into the clinic, including mesenchymal stromal cells, virus-specific T cells, tumor-associated antigen-specific T cells, and CAR T cells.

In 2020 Dr. Hanley was elected VP-North America of the International Society for Cell and Gene Therapy (ISCT) where he also served on the board of directors, co-founded and served as the inaugural co-chair of the Early Stage Professionals committee which focuses on workforce development, and is the commissioning editor of the society's journal, Cytotherapy. Representing ISCT, he serves on the Regenerative Medicine Forum of the National Academies where he co-leads the workforce working group. He also serves on the board of directors of the Foundation for the Accreditation of Cellular Therapy (FACT) and is a FACT representative at the Cell Therapy Liaison Meeting, serving as a thought leader in a forum with the FDA. Dr Hanley also serves as an advisor for a number of cell and gene therapy biotech companies.

In an attempt to connect the mid-Atlantic cell and gene therapy community, in 2023 Dr Hanley formed the CHARM network which is a regional network that provides quarterly meet ups, regular webinars, and an annual symposium featuring local universities, government, healthcare systems, patient advocacy groups, and biotech. In his free time, he enjoys tweeting with fellow scientists and Bills fans, playing soccer, cycling, cooking, and traveling.



Kyle Hoban, Ph.D.

Scientific Communications Manager, Stryker

Dr. Kyle Hoban is a Scientific Communications Manager at Stryker, a manufacturer of medical devices and equipment. Working in the company's Peripheral Vascular division, he supports the communication of results from Stryker-sponsored studies investigating therapies for patients with arterial and venous diseases. Such communications include manuscripts and conference abstracts and presentations. Prior to Stryker, Dr. Hoban worked as a Scientist at FZata, Inc, a biotechnology company designing live biotherapeutic products to treat patients with gastrointestinal disorders. Dr. Hoban has a PhD in cell and molecular biology from Johns Hopkins University.

Karin Lee, Ph.D.

Associate Principal Scientist, AstraZeneca

Dr. Karin Lee received her Ph.D. in Biomedical Engineering from Case Western Reserve University in 2016, where she developed plant virus-based drug delivery systems as cancer therapeutics. She subsequently joined the National Cancer Institute as a postdoctoral fellow in the Laboratory of Tumor Immunology and Biology (now Center for Immuno-Oncology) under Dr. Jeffrey Schlom. During her fellowship, Dr. Lee investigated neoantigen vaccine immunogenicity and tumor protection in combination with immune modulators using immunocompetent mouse models.

In 2020, Dr. Lee transitioned to industry, joining AstraZeneca as a Senior Scientist at their facility in Gaithersburg, Maryland. At AstraZeneca, she has contributed to diverse projects across the drug development pipeline. Her work has included developing T cell dysfunction assays, conducting high-throughput screens for novel target identification, and supporting IND-enabling activities for Rilvegostomig, a clinical stage PD1-TIGIT bispecific antibody. Currently, as Associate Principal Scientist, Dr. Lee leads a program focused on a next-generation T cell engager designed with enhanced tumor specificity to minimize on-target, off-tumor toxicity.

Sarwat Naz, Ph.D.

Licensing Manager, Technology Commercialization Office, George Washington University

Dr. Sarwat Naz is a Licensing Manager for Life Sciences at the Technology Commercialization Office at George Washington University. She works with faculty, clinicians, and entrepreneurs to manage intellectual property, develop commercialization strategies, and build industry partnerships to advance early-stage technologies. She also supports faculty- and student-led startups by connecting them with funding and strategic resources.

Previously, she served as a Technology Transfer Manager at the National Cancer Institute and held leadership roles including Co-Chair of the CCR-FYI Committee and lead of the PASS seminar series, mentoring postdoctoral fellows in professional development. She holds a Ph.D. in Cancer Biology from Indian Institute of Science, India and completed postdoctoral training at NCI.

Daniel Pham, Ph.D.

Director, BD2: Breakthrough Discoveries for Thriving with Biopolar Disorder

Daniel Pham, PhD, brings a strategic, partnership-driven approach to BD^2's Discovery Research programs, supporting efforts to advance bipolar disorder research. Daniel has played a key role in building the foundation for BD^2 and has co-authored multiple publications related to bipolar disorder research and science philanthropy. He is also the author of the “Giving Smarter Guide, Giving Smarter: Philanthropic Opportunities to Advance Bipolar Disorder Research”. He previously served as a Director at the Milken Institute on the Science Philanthropy Accelerator for Research and Collaboration (SPARC) team.

Troy Pellom, Ph.D.

Senior Staff Fellow, Radiation Therapy Team
Office of Product Evaluation and Quality
Food and Drug Administration

I am from Decatur, GA and I am the oldest of 3 boys. I graduated from Wake Forest Univ. and worked in a microbiology research lab as a lab assistant every year of undergrad studying LCMV infections in pre-clinical mouse models. I then completed a PhD preparation post-bac program at Wake Forest Univ. School of Medicine with the same microbiology preceptor. I went on to Meharry Medical College from 2010-2017 and graduated with a PhD in Biomedical Sciences (concentration: Microbiology/Immunology) and conducted cancer research with the drug Bortezomib in pre-clinical mouse models of breast cancer. I then joined the NCI/NIH as a post-doc with Dr. Jeffrey Schlom in which I conducted HPV-therapeutic vaccine research in pre-clinical mouse models. For the past 4.5 years, I have been working at the FDA as a Senior Staff Fellow in CDRH with the radiation therapy team conducting pre-market submission reviews.

Detailed Description of Scientific Regulatory Experience:
In the Office of Product Evaluation and Quality/Office of Health Technology 8 (OPEQ/OHT8), Digital Health Technologies/Center for Devices and Radiological Health (DHT8C/CDRH), I serve as Lead Reviewer, in which I am responsible for conducting both pre-market and post-market reviews. I work independently, keeping management burden to a minimum, to consistently meet review deadlines.

I have served as a lead reviewer for multiple submissions, including 510(k) notifications, Q-submissions, Investigational Device Exemptions (IDEs), and several 513gs, in which I analyzed and interpreted scientific documentation. I have successfully managed complicated submissions with large review teams for the purpose of reviewing new devices, assessing substantial equivalence, and assessing safety of clinical studies. I have communicated with external stakeholders through teleconferences and written correspondence to provide feedback on specific medical devices and to educate the stakeholders on regulatory procedures. In addition, I have provided my expertise as a biocompatibility, sterility, and animal consultant to other reviewers both within the radiation therapy team and across the office. I have also been involved in the Center's efforts to identify any data integrity issues within biocompatibility test reports. Lastly, I attend trainings, conferences, and experimental learning programs to expand my technical and regulatory knowledge.

Christina Ross, Ph.D.

Medical Science Liaison
Johnson & Johnson Innovative Medicine

Christina Ross, PhD, brings over 16 years of experience in oncology. Born in Manchester, England, she moved to Pennsylvania during junior high. Christina earned a Bachelor of Science in Biochemistry and Molecular Biology from Juniata College, followed by a PhD from the University of Maryland, Baltimore School of Medicine, where her research focused on oncology genetics and drug design. During her PhD, she also completed a science communications internship with the medical school communications department.

After her PhD, Christina joined the National Cancer Institute's Laboratory of Cancer Biology and Genetics as a Cancer Research Training Awardee, specializing in the genetic predisposition to metastatic disease. During her fellowship, she also completed NIH courses in translational science and clinical trial design, further strengthening her expertise in bridging research and clinical application. As a Staff Scientist at NCI, Christina was awarded for excellence in mentoring and contributed to the development of the NCI Deaf Scientist Training Program and the Trainee Empowerment Program. After completing her research at the NCI Christina joined the Johnson and Johnson Oncology Medical Science Liaison team. Since starting there in October 2024 she has put her knowledge of translational sciences to good use, as she helped to launch a new bladder cancer drug and became the Early Development MSL Lead for Bladder Cancer.

Outside of her professional career, Christina is a certified personal trainer and teaches spin classes at her local gym. She lives in Bethesda, Maryland, with her husband and son and enjoys exploring Washington, D.C.

Yvette Seger, Ph.D.

Experienced Leader in Science Policy and Workforce Development

Yvette Seger, PhD,was most recently the Chief Science Policy and Workforce Development Officer for the Federation of American Societies for Experimental Biology (FASEB), a coalition of 20 scientific societies collectively representing over 100,000 individual biological and biomedical researchers. In addition to overseeing FASEB's science policy portfolio, Dr. Seger led initiatives to foster a diverse and representative biomedical research workforce for which she received funding from both the National Science Foundation and National Institutes of Health (NIH). Dr. Seger launched her policy career at the National Academies of Science, Engineering, and Medicine as a Christine Mirzayan Science & Technology Policy Fellow where she worked on a report examining processes for identifying and appointing scientists to key federal advisory positions. After leaving the Academies, Dr. Seger held senior policy analyst positions at the research advocacy organization FasterCures, the NIH Office of Science Policy, and Thomson Reuters before joining FASEB in 2013. Dr. Seger holds a PhD in Genetics from Stony Brook University and received a BA in Zoology (Genetics Concentration) and Politics & Government from Ohio Wesleyan University.

Joshua Stone, Ph.D.

Director of Bioinformatics, Psomagen

Dr. Joshua Stone earned his Bachelor of Science degree in General Biology from James Madison University before continuing his training at Northern Arizona University, where he completed a master's degree in General Microbiology and later worked as a research specialist. He went on to earn a PhD in Oncology and Cancer Biology from the Whiddon College of Medicine at the University of South Alabama in 2018. Following his doctoral training, Dr. Stone joined the NIH as a postdoctoral fellow, completing his work there in 2024. Later that year, he joined Psomagen as a Solution Development Scientist and currently serves as the Director of Bioinformatics Operations, where he oversees bioinformatics pipelines and data analysis for the company's genomic and multi-omics services, including recent benchmarking studies for platforms like the Ultima Genomics UG 100 and NovaSeq X Plus.

Naomi Taylor, M.D., Ph.D.

Senior Investigator
Pediatric Oncology Branch, NCI

Naomi Taylor is a Deputy Director of the Center for Cancer Research and Senior Investigator in the Pediatric Oncology Branch (NCI, NIH). She studied at Princeton University and the Weizmann Institute before earning her MD/PhD at Yale University School of Medicine with Dr. George Miller. Following pediatrics training at Yale and the Children's Hospital of Los Angeles as a Howard Hughes Fellow, Dr. Taylor launched her own lab at the Institut de Génétique Moléculaire in Montpellier, France. She currently holds an adjunct professorship at the Université de Montpellier.

Dr. Taylor leads a research group internationally recognized for its work on T cell-based gene and cell therapies, metabolic regulation of normal and malignant hematopoiesis, and thymus differentiation. Recently, the group has pioneered studies exploring how metabolite transporters and cellular fuel choices shape immune and hematopoietic function. These efforts have identified novel metabolic programs regulating physiological and pathological hematopoietic lineage commitment, including erythropoiesis. Their discoveries have also directly informed the development of more effective T-cell immunotherapy protocols—advancing the design and performance of chimeric antigen receptor (CAR) T-cell therapies.

For Naomi Taylor, scientific research is a powerful platform that has the unique ability to bring together individuals from different backgrounds—it is a field that breaks down walls and fosters international cooperation. In this context, Dr. Taylor has promoted the careers of students, clinicians, and scientists from >30 different countries, spanning 6 continents. She has received numerous awards for her research and training including the French National Inserm Research Award (2010), the 2021 NCI Women in Science Mentoring and Leadership Award, election to the Association of American Physicians (2023), and the NCI Director's Award for Making an Impact-NCI Champions (2024).

Concurrent Friday Workshops and Panel

Workshop: "How to Use Artificial Intelligence in Science"

Friday, May 15, 3:45 – 4:45 p.m., in 2W910/912

Rama Chellappa, Ph.D.

Bloomberg Distinguished Professor in Electrical and Biomedical Engineering

Johns Hopkins University

Rama Chellappa is a Bloomberg Distinguished Professor in the Departments of Electrical and Computer Engineering and Biomedical Engineering at Johns Hopkins University. His research interests span artificial intelligence, computer vision, machine learning, signal, image, and video processing. Professor Chellappa's scholarship has been recognized with numerous prestigious honors. Recent awards include the 2025 Azriel Rosenfeld Lifetime Achievement Award and the 2023 Distinguished Researcher Award from the IEEE Computer Society's PAMI Technical Committee, the 2024 Edwin H. Land Medal from Optica and the 2020 IEEE Jack S. Kilby Medal for Signal Processing. He is also the recipient of the 2012 K. S. Fu Prize from the International Association of Pattern Recognition; the Society, Technical Achievement, and Meritorious Service Awards from the IEEE Signal Processing Society; the Technical Achievement and Meritorious Service Awards from the IEEE Computer Society; and the Leadership Award from the IEEE Biometrics Council. He is an elected member of the U.S. National Academy of Engineering and a Foreign Fellow of the Indian National Academy of Engineering. He is also a Fellow of AAAI, AAAS, ACM, AIMBE, IAPR, IEEE, NAI, Optica, and the Washington Academy of Sciences. Professor Chellappa holds nine U.S. patents.

Workshop:
"The Art of Explanation: Tailoring Science Communication to Different Audiences"

Friday, May 15, 3:45 – 4:45 p.m., in 2W908

Leigh Anne Kelley, M.B.A.

Director of Communications, Fralin Biomedical Research Institute at Virginia Tech Carilion (VTC) School of Medicine, Virginia Tech

Leigh Anne Kelley is director of communications for Virginia Tech's Fralin Biomedical Research Institute at VTC, where she leads integrated, cross-platform strategies to elevate research in human health and disease for the institute's labs in Roanoke, Virginia, and Washington, D.C. She has more than 20 years of experience in journalism and strategic communications, including roles as a writer, editor, designer, and managing editor for newspapers, magazines, and digital platforms. Her work focuses on translating complex biomedical research into compelling narratives for broad audiences, supporting institutional visibility, and advancing public understanding of research, with a focus on neuroscience, cancer, and cardiometabolic health.

Panelists: Work/Life Balance in Academia and Industry

Friday, May 15, 10:00 a.m. – 3:00 p.m., in TE406/408/410

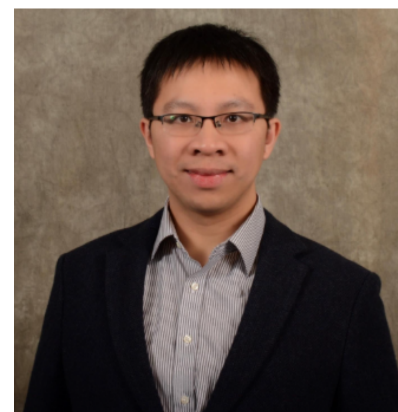
Kaitlyn Sadtler, Ph.D.

Senior Investigator
National Institutes of Biomedical Imaging and Bioengineering, NIH

Dr. Kaitlyn Sadtler is a Senior Investigator and Chief of the Section on Immuno-Engineering at the National Institutes of Health. She began her lab at the National Institute of Biomedical Imaging and Bioengineering after a postdoctoral fellowship at the Massachusetts Institute of Technology in the Department of Chemical Engineering working on the molecular mechanisms of immune activation in the foreign body response. She completed her Ph.D. at the Johns Hopkins University School of Medicine where she showed a role for immune cells in biomaterial-mediated muscle regeneration. She has led research that has been published in journals such as Science, Nature Communications, Nature Materials, and Science Translational Medicine. She was recognized as a TED Fellow and delivered a TED talk that was listed as one of the top-viewed talks of 2018. Dr. Sadtler was selected for the Forbes 30 Under 30 List in Science, the MIT Technology Review 35 Innovators Under 35, the World Economic Forum Young Global Leaders, the TIME 100 Next List, and the National Academies of Science Engineering and Medicine New Voices Program. She also received the 2021 Outstanding Recent Graduate Award from Johns Hopkins University and an honorary doctorate from her undergraduate university, UMBC. At NIH, Dr. Sadtler has lent her lab's expertise to the fight against COVID-19, leading a study that detected 16.8 million undiagnosed SARS-CoV-2 infections in the US after the first pandemic wave in the US. She continues her work on immunoengineering in the context of traumatic injury focusing on the balance of tolerance and autoimmunity during tissue reconstruction, recently implicating a new immune cell type in self-tolerance after volumetric muscle loss.

Allen Xiaofeng Su, Ph.D.

Assistant Professor and Scientist 2, Henry Jackson Foundation; Adjunct Investigator, NCI



Allen Xiaofeng Su received his Ph.D. from Tufts University and completed his postdoctoral training at the Massachusetts Institute of Technology. He is an Assistant Professor and PI at the Center for Prostate Disease Research of the Henry Jackson Foundation, with affiliations to the Uniformed Services University and the National Cancer Institute. His work is supported by multiple national awards, including the Prostate Cancer Foundation Young Investigator Award and the CDMRP Prostate Cancer Research Program Idea Development Award. His lab research focuses on genomic instability and aneuploidy in cancer, integrating single-cell and spatial multi-omics approaches with translational science to understand how chromosomal alterations, DNA damage repair, and replication stress drive prostate cancer progression and reveal new therapeutic vulnerabilities.

Whitney Do, Ph.D.

Bioinformatics Senior Consultant, Deloitte

Whitney Do is a bioinformatics senior consultant at Deloitte, contracting with NIAID as a research specialist. In this capacity, she supports NIAID in developing AI and GenAI solutions with a focus on translating innovative technologies into practical tools that benefit both clinician and research scientists. Before joining Deloitte, she spent three years as a postdoctoral fellow at the Center for Cancer Research, within the Laboratory of Human Carcinogenesis. Her research focused on the molecular epidemiology of liver cancer and identifying early molecular biomarkers to improve detection and intervention strategies. She earned a PhD in Nutrition and Health Sciences from Emory University, where her dissertation focused on the epigenetics of diabetes and cancer.

Sisi He, Ph.D.

Senior Scientist, AstraZeneca

Sisi is a Senior Scientist at AstraZeneca in Gaithersburg, Maryland, where she works in oncology drug discovery with a focus on cancer immunology. She earned her PhD from the University of Illinois Urbana-Champaign in 2020. Prior to joining AstraZeneca, she contributed to preclinical drug development, IND-enabling programs, and clinical biomarker research at a biotech company, and later supported flow cytometry operations at Sanofi. Outside of work, Sisi enjoys rock climbing, running, sailing and loves spending time with her husband and their fluffy Goldendoodle, Noodle.

CCR Core Facilities Exposition

Friday, May 15, 10:00 a.m. – 3:00 p.m. in TE110 and TE406/408/410

CCR Building 41 Flow Cytometry Core

The NCI CCR Building 41 Flow Cytometry Core is a full-service facility within the Center for Cancer Research that supports over 150 users representing 26 laboratories. The Core Facility provides instrument and software training, and technical expertise, including assay development and experimental design for all users. The Core provides sample preparation protocols and reagent recommendations for all users including fluorochrome panel setups for each flow cytometer and reagent cross-reactivity among species. The Core Manager provides assistance with data analysis and figure preparation for presentation of flow cytometry data in publications. While the core staff are experienced in all areas of flow cytometry, they also have extensive expertise in 20+ color flow cytometry, non-human primate flow cytometry, mouse flow cytometry, and immune monitoring by flow cytometry.

Location: Bethesda
Contact: Kathy McKinnon (mckinnonkm@mail.nih.gov)

CCR OSTR Genomics & Spatial Imaging Cores

The CCR Genomics Core and the Spatial Imaging Technology Resource (SpITR), under the Office of Science and Technology Resources (OSTR) at the National Cancer Institute, provide complementary platforms and expertise to enable integrated genomic and spatial biology research. Together, these cores support investigators from nucleic acid profiling to high-dimensional, spatially resolved tissue analysis.

The CCR Genomics Core offers access to advanced molecular technologies, including Illumina next-generation sequencing (MiSeq, NextSeq 2000), Oxford Nanopore long-read sequencing (MinION, GridION, PromethION), NanoString nCounter, and droplet digital PCR (Bio-Rad QX200). SpITR extends these capabilities into spatial biology with cutting-edge platforms such as NanoString GeoMx Digital Spatial Profiling, CosMx Spatial Molecular Imager, and PhenoCycler (CODEX), along with protein analysis tools including Luminex multiplex assays. Across both cores, services include sample quality control, experimental design consultation, assay optimization, and analysis guidance, supporting flexible, investigator-driven projects from concept to interpretation.

Together, these OSTR cores enable integrated multi-omic and spatial workflows, allowing researchers to investigate gene expression, protein dynamics, and tissue architecture within their native context. By combining advanced instrumentation with expert consultation and training, the CCR Genomics Core and SpITR accelerate high-impact, reproducible research across the NIH intramural community.

Location: Bethesda
Contact: Edmund Cauley, Maria Hernandez, Xiaoling Luo, Carl McIntosh, Steve Shema, Qin Wei, Desiree Tillo, Madeline Wong and Elizabeth A. Conner (ncilecdnacore@mail.nih.gov & ccrspitr@mail.nih.gov)

CCR Optical Microscopy and Analysis Core (OMAC)

Our research is focused on understanding how chemical gradients in solid tumors drive disease progression via cellular reorganization of the tumor microenvironment. We collaborate with several CCR labs (Dr David Wink, Dr. Lalage Wakefield, Dr. Anish Thomas), labs in the Frederick National Laboratory (Dr. Kedar Narayan, Dr. George Zaki, Dr. Andrew Weisman, The Advanced Biomedical Computational Science) and other institutions (Dr. Jens Rittscher, Oxford University, UK). We utilize three approaches: (1) Multi-cycle immunofluorescence labeling to investigate the expression of scores of proteins in thin tissue sections. Recently our spatial analysis of ER- breast cancer patient tumors revealed distinct cellular niches associated with immune desert regions and cancer cell stemness and suggestive of metastatic hotspots (DOI: 10.1158/2767-9764.CRC-24-0235). (2) Fluorescence labeling and 3D imaging of thick tissues to understand the 3D context of each cell with application to investigating the interaction of CD8 T cells and DNA damaged tumor cells. (3) Utilization of a restricted exchange environment chamber where cell metabolism generates spatial gradients of oxygen and nutrients across the live cell culture that in turn drive directional cell migration. Applications include dissecting the mechanisms of epithelial to mesenchymal transition, induction of cancer cell stemness and metabolic exchange between host cells and tumor cells.

We provide the Center for Cancer Research (CCR) with cutting-edge expertise in fluorescence microscopy (confocal and super-resolution) and atomic force microscopy. This expertise includes sample labeling, handling of live samples, the provision of microscopes for image acquisition and image analysis.

Location: Frederick
Contact: Stephen Lockett (locketts@mail.nih.gov)

Protein and Metabolite Characterization Core (PMCC)

The Protein and Metabolite Characterization Core (PMCC), located at the ATRF in Frederick, MD, is a dedicated NCI-CCR facility focused on the characterization of a wide range of molecules, from small compounds and metabolites to proteins and entire proteomes. The laboratory is equipped with cutting-edge mass spectrometry and liquid chromatography technologies for both proteomic and metabolomic analyses, in addition it has expertise in variety of affinity purification, chromatographic separation, and broad assay development capabilities. The PMCC develops and applies state-of-the-art analytical approaches to advance understanding of cellular function at the protein and metabolite levels, supporting both short- and long-term collaborative projects tailored to the needs of CCR investigators.

Location: Frederick
Contact: Thorkell Andresson (andressont@nih.gov)

Single Cell and Spatial Core (SCSC)

The Single Cell and Spatial Core (SCSC), which was formerly called the Single Cell Analysis Facility (SCAF) is a NCI Center for Cancer Reseach (CCR)-dedicated resource providing support for projects requiring analysis at single cell resolution. We aim to provide end-to-end support from initial experimental design, advice on cell isolation and handling, expertise on existing and emerging single cell technologies, and some support on bioinformatic analysis. Current technological expertise includes gene expression profiling of single cells and single cell epigenomics, and feature barcoding strategies. SCAF also supports the Visium spatial transcriptomics assay and Xenium in situ sequencing spatial platforms. We also aim to support emerging single cell technologies, methods, and informatics analysis to provide the most current and relevant support resources to the community

Location: Bethesda
Contact: Michael Kelly (michael.kelly3@nih.gov)

Imaging Mass Cytometry (IMC) - Mass Cytometry Core (MCC)

Imaging Mass Cytometry (IMC) is an advanced multiplexed imaging technology that combines principles of mass spectrometry with immunohistochemistry to enable high-dimensional, spatially resolved analysis of biological tissues. In IMC, antibodies are conjugated to rare earth metal isotopes rather than fluorophores, allowing the simultaneous detection of 30–50 or more protein markers within a single tissue section without issues of spectral overlap or autofluorescence. Tissue regions of interest (ROIs) are ablated with 1 μm resolution using a laser, and the resulting plumes are analyzed by time-of-flight mass spectrometry to quantify metal-tagged antibodies, generating highly multiplexed images. This technology provides detailed insights into cellular phenotypes, functional states, and spatial organization within complex microenvironments, such as tumors and immune tissues. IMC is particularly powerful for studying tissue heterogeneity, cell–cell interactions, and immune infiltration in cancer, as well as for applications in immunology, pathology, and drug development. Its ability to preserve spatial context while delivering single-cell resolution distinguishes it from traditional flow cytometry and bulk omics approaches.

At the National Cancer Institute / Center for Cancer Research-supported Mass Cytometry Core (MCC) facility, we collaborate with investigators to advance spatial biology research focused on the tumor microenvironment (TME) and its role in patient prognosis. We have validated over 100 metal-labeled antibodies targeting cancer and immune cell populations and have developed custom panels tailored to specific cancer types. Our work encompasses formalin-fixed paraffin-embedded (FFPE), fresh-frozen, fixed-frozen, and smeared cell samples from both human and mouse origins and includes contributions to the identification and characterization of circulating tumor cells (CTCs). We have developed a robust data analysis pipeline in which raw IMC data are first processed to generate single-cell segmentation masks and to quantify metal-tag intensities at the single-cell or pixel level. Downstream analyses include quality control, normalization, and clustering, followed by identification of cellular phenotypes and their interactions, as well as neighborhood analysis to characterize spatial tissue architecture comprehensively.

We continue to expand our capabilities and currently offer the CyTOF-XT platform for suspension cell analysis. In addition, we are developing three-dimensional (3D) IMC imaging approaches using serially sectioned ultra-thin and thick FFPE tissues. We are also integrating Xenium with IMC to enable combined multiplex transcriptomic and proteomic analysis on the same tissue section and incorporating RNAscope with IMC to target soluble protein markers such as chemokines and cytokines. In our latest efforts, we are profiling FFPE

and immunocytochemistry (ICC) preparations of organoids using panels of over 30 antibody markers to achieve comprehensive spatial proteomic characterization.

Location: Frederick
Contact: Milind Pore (milind.pore@nih.gov)

Advanced Biomedical Computational Science (ABCS)

The Advanced Biomedical Computational Science (ABCS) group supports investigators at the National Cancer Institute (NCI) and National Institutes of Health (NIH) by applying bioinformatics, data science, and artificial intelligence to complex challenges in cancer, infectious diseases, rare diseases, and other biomedical areas. ABCS provides consultation, collaboration, and end-to-end project support, from study design and data generation to analysis, interpretation, and dissemination. We specialize in next-generation sequencing analysis, computational chemistry, protein and nucleotide modeling, drug design, image analysis, scientific web development and multimodal data integration. Our multidisciplinary team, composed of bioinformaticians, statisticians, computational chemists, software engineers, and data scientists, works across basic, translational, and clinical research domains to deliver scalable and flexible solutions tailored to diverse scientific needs.

Using advanced approaches such as deep learning, large language models, and molecular dynamics simulations, we help uncover disease mechanisms, differentiate disease subtypes, identify diagnostic and predictive biomarkers, and advance therapeutic development. We also develop tools, workflows, and infrastructure, and share software, data, and knowledge through publications, open resources, and training programs. By partnering closely with investigators, ABCS accelerates discovery and transforms complex data into meaningful insights.

Location: Frederick
Contact: Uma Mudunuri (uma.mudunuri@nih.gov)

Animal Research Technical Support (ARTS) and Gnotobiotics Core Facility

The Laboratory Animal Sciences Program (LASP) of the Frederick National Laboratory operates the Animal Research Technical Support (ARTS) including a Gnotobiotic Facility (GF) Core facility. Our team has extensive experience working with rodent animal models in basic and translational research, particularly in the fields of immunology and cancer research. ARTS/GF facilitates research projects by working closely with Investigators throughout all stages of the project, providing assistance with animal study proposals, coordinating and carrying out all experimental aspects of studies, providing regular progress reports, and delivering detailed results. The Core collaborates closely with other LASP facilities such as Animal Diagnostics, Small Animal Imaging, Mouse Modeling, Histology, and Pathology to coordinate workflows and services.

Location: Frederick
Contact: Simone Difilippantonio (difilips@mail.nih.gov)

NCI LASP Genome Modification Core (GMC)

Summary of services offered:

- Experimental design consultation
- Reagents/protocols for:
- Single locus editing in cultured cells (human, mouse, and other model organisms)
- Transgenic mouse models
- DNA encoded libraries for genetic screens

Location: Frederick
Contact: Raj Chari (raj.chari@nih.gov)

Microscopy and Digital Imaging in the CCR Microscopy Core

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

Location: Bethesda
Contact: Michael Kruhlak (kruhlakm@mail.nih.gov)

Center for Structural Biology: Biophysics Resource

Biophysics Resource (BR) operates as an open, shared-use facility; in general, BR users learn to operate the instruments and conduct their own experiments. BR staff train first-time users and are also available to consult on experimental design/analysis or collaborate with them on more complex studies. User access fee (less than $1000 per lab per fiscal year) gives all members an unlimited opportunity to use all BR technologies.

BR offers cutting-edge biophysics technologies in the following areas:

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

Location: Frederick
Contact: Sergey G. Tarasov (tarasovs@mail.nih.gov)

NCI CCR Bioinformatics Training and Education Program (BTEP)

The NCI CCR Bioinformatics Training and Education Program (BTEP) helps lab-based scientists learn to analyze their own computational data. To do this, we offer training in the broad categories of programming, artificial intelligence, data and databases, software (both open source and vendor-provided), microbiomes, next-generation sequencing and ‘omics data analysis, statistics, and new technologies.

You can find details on all this training on our website (https://bioinformatics.ccr.cancer.gov/btep) which functions as both a class registration tool and an information resource. Every class we teach has extensive documentation available, including recordings of all lessons. We maintain resource pages on bioinformatics topics of interest including GenAI, data downloads, high performance computing systems like Biowulf, and additional training resources.

Contact: Amy Stonelake (amy.stonelake@nih.gov) & ncibtep@nih.gov

Molecular Histopathology Laboratory (MHL)

The Molecular Histopathology Laboratory (MHL) is a core facility that provides comprehensive research support to NIH investigators and their collaborators. The laboratory offers integrated pathology services, including study consultation and planning, necropsy, full-service histology, phenotyping of genetically engineered mouse models, immunopathology, anatomic and toxicologic pathology, and specimen classification and selection for tissue microarray construction and laser capture microdissection. MHL pathologists have extensive expertise across a broad range of model systems, including rodents, zebrafish, human and nonhuman primates, woodchucks, and other species, as well as organoids and other in vitro model platforms.

The Histology Laboratory provides support that includes necropsy assistance, organ weight collection, and complex tissue sampling such as early embryos and targeted brain regions, along with hematology and clinical chemistry testing. Additional services include fixed and frozen tissue processing, microtomy and cryotomy, and a wide range of routine and special histochemical stains.

The molecular pathology team maintains more than 500 validated IHC assays for use on chromogenic and immunofluorescent platforms in human and rodent tissues. The laboratory optimizes both commercial and investigator-developed antibodies and maintains a bank of human and murine control tissues and reagents to support assay development, validation, and quality assurance. Advanced capabilities include in situ hybridization, RNAscope, and multiplexed assays. The laboratory also performs nucleic acid isolation from frozen and fixed animal and human tissues, blocks, cores, and slides, with associated yield and quality assessments.

MHL maintains a digital pathology infrastructure that provides whole-slide imaging for brightfield and fluorescence applications. Digital slides are archived and managed through HALO, enabling secure storage, remote access, and sharing with collaborators. In addition to diagnostic interpretation, MHL pathologists perform digital image analysis for quantitative biomarker assessment and AI-assisted pathologic analysis. The laboratory also supports spatial transcriptomic and proteomic studies using both fixed and frozen tissues.

Collectively, MHL delivers end-to-end pathology, histotechnology, and digital pathology support for studies of human disease and translational research.

This project has been funded in whole or in part with Federal funds from the National Cancer Institute, National Institute of Health, under Contract No. HHSN26120150003I.
Location: Frederick
Contact: Laura Bassel (tarasovs@mail.nih.gov)

Lab Manager Working Group (LMWG)

The Lab Manager Working Group (LMWG) is made up of NIH staff (in the lab and admin, in all institutes and positions) who volunteer to support many initiatives for the NIH and help inform and provide support for scientific staff. The LMWG is a conduit for sharing information between NIH management and the research community to understand laboratory operations and needs. One of the LMWG committees manages the NIH FreeStuff website located at http://stuff.nih.gov/ . This is a site that can be accessed by anyone who is on the NIH VPN and has a PIV card. It is a place where staff can post or request items for free and keeps government purchased items used in government funded projects, preventing waste and boosting our tight budgets. It is one of the many ways the LMWG supports the NIH community. Stop by our table to learn more about how this group can help you and how to sign up for our informative listserv emails.

Contact: Helen Cawley (helen.cawley@nih.gov)

Abstracts for Oral Presentations

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

Outstanding Postdoctoral Fellow Awardee

1. Characterization of the anti-tumor efficacy of memory cytokine enriched NK cells against tumors with neuroendocrine features

Kristen Fousek[1], Lucas Horn[1], Haiyan Qin[1], Nika Rajabian[1], Miriam Marlene Medina Enriquez[1], Shantel Angstadt[1], Manju Saxena[2], Lennie Sender[2], Patrick Soon-Shiong[2] and Claudia Palena[1]
1 Center for Immuno-Oncology, Center for Cancer Research, National Cancer Institute, Bethesda, MD
2 ImmunityBio, Culver City, CA

Neuroendocrine neoplasms (NEN) consist of slow growing neuroendocrine tumors and highly proliferative neuroendocrine carcinomas. The incidence of NEN continues to rise, yet there remains a lack of effective treatments for this disease. Immune checkpoint blockade (ICB) in combination with chemotherapy is approved in extensive stage small cell lung cancer (SCLC), a very aggressive tumor classically known as neuroendocrine (NE), but only a subset of patients experiences improved survival. Lack of response to ICB is often attributable to low expression of MHC-class I. Our group recently published that the lack of MHC-class I can instead be utilized to facilitate targeting by NK cells. We found that NK cells stimulated with an IL-15 cytokine superagonist (N-803) effectively targeted SCLC of all phenotypes. This led us to hypothesize that cytokine stimulated memory-like NK cells may be successful in targeting SCLC and other types of NE tumors. Memory cytokine enriched NK cells (M-ceNK) are derived from an apheresis product and exposed to a cocktail of cytokines including N-803, IL-12, and IL-18 until a highly purified CD3negCD56high cell population results; 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 in vitro immune cytotoxicity assays against human NE cell line models. Characterization across many donors indicates that M-ceNK are highly activated NK cells exhibiting increased natural cytotoxicity receptors (NKp30, NKp44, NKp46), minimal inhibitory markers (KLRG1, TIGIT), elevated IFN-gamma and Granzyme B production, and increased reliance on glycolysis for their metabolic activity compared to healthy donor NK cells. In immune cytotoxicity assays, M-ceNK demonstrated a median of 69% lysis (range 35-89%) at an effector to target ratio of 5:1 across 5 SCLC models (DMS79, H69, H446, H1048, H841) and 66% and 42% lysis respectively in NE prostate cancer (H660) and lung cancer (H720, H727) models as compared to 6% lysis (range 0-58%) with healthy donor NK cells. Furthermore, M-ceNK provided significant anti-tumor efficacy in two xenograft models of SCLC (H69, DMS79) when administered with N-803 in vivo. To better understand the efficacy of M-ceNK and their role upon encountering tumor cells, we co-cultured M-ceNK and NE tumors together and subsequently performed single cell RNA-sequencing on pairs of matched donor M-ceNK pre- and post- tumor exposure. We observed rapid upregulation of a gene signature indicative of tumor-infiltrating NK cells and increases in the expression of chemokines that play a key role within the tumor microenvironment. Further studies will evaluate the functional importance of these changes in gene expression and how they affect the ability of M-ceNK to target tumor cells, secrete cytokines, and proliferate. 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. Future studies will not only evaluate further immunotherapy combinatorial approaches to treat NE tumors but will also evaluate M-ceNK in the settings of other ICB-refractory tumors.

Outstanding Postdoctoral Fellow Finalists

2. HIV uses non-canonical pathways to escape from integrase inhibitors

Yuta Hikichi[1], Kerri J. Penrose[2], Ryan C. Burdick[3], Sherimay D. Ablan[1], John W. Mellors[2], Vinay K. Pathak[3], Urvi M. Parikh[2], Eric O. Freed[1]
1 Virus-Cell Interaction Section, HIV Dynamics and Replication Program, Center for Cancer Research, National Cancer Institute, Frederick, MD
2 University of Pittsburgh, Pittsburgh, Pennsylvania
3 Viral Mutation Section, HIV Dynamics and Replication Program, Center for Cancer Research, National Cancer Institute, Frederick, MD

People living with HIV (PWH) receiving HIV integrase strand transfer inhibitors (INSTIs) have been reported to experience virological failure (VF) in the absence of resistance mutations in integrase (IN). To elucidate INSTI resistance mechanisms and pathways, we performed long-term (1-2 years) passaging of lab-adapted and primary HIV-1 isolates in human T-cell lines and primary peripheral blood mononuclear cells with an escalating concentration of the INSTI dolutegravir (DTG). Independent of viral strain and cell type, HIV-1 acquired resistance to DTG through the sequential acquisition of mutations in Env and nucleocapsid (NC), with the occasional appearance of IN mutations. The Env mutations confer resistance to INSTIs by increasing virus replication capacity through enhanced cell-cell transfer. In contrast, the NC mutations provide escape from INSTIs by accelerating the kinetics of viral DNA integration. The shortened time frame between the completion of reverse transcription and integration correlates with reduced sensitivity to DTG, suggesting that NC mutations limit the window of opportunity for INSTIs to bind intasomes and block integration. To assess the clinical relevance of results from our cell-culture selections, we analyzed samples from PWH experiencing VF on a tenofovir-lamivudine-dolutegravir (TLD) regimen. Notably, plasma HIV RNA sequences from some individuals at VF showed NC mutations similar to those observed in vitro, with a subset also carrying IN mutations such as IN-R263K. Phenotypic analysis demonstrated that mutations in NC and IN act in concert to increase resistance to DTG. These results provide insights into the mechanism by which HIV-1 escapes INSTIs and underscore the importance of genotypic analysis outside IN in individuals experiencing VF to INSTI-containing regimens.

3. 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

Biophysical profiling of primary tumors has revealed that individual tumor cells fall along a highly heterogeneous continuum of mechanical phenotypes. One idea is that a subset of tumor cells is “softer” to facilitate detachment and escape from the primary site, a step required to initiate metastasis. However, it has also been postulated that cells must be able to deform and generate sufficient force to exit into distant sites. Here, we aimed to dissect the mechanical changes that occur during extravasation and organ colonization. Using multiplexed methods of intravital microscopy and optical tweezer based active microrheology, we obtained longitudinal images and mechanical profiles of cells during organ colonization in vivo. We determined that cells were softer, more liquid-like upon exit of the vasculature, but stiffened and became more solid-like once in the new organ microenvironment. We also determined that a YAP-mediated mechanogenotype influenced the global dissemination in our in vivo and in vitro models and that reducing mechanical heterogeneity could reduce extravasation. Moreover, our high throughput analysis of mechanical phenotypes of patient samples revealed that this mechanics was in part regulated by the external hydrodynamic forces that the cancer cells experienced within capillary mimetics. Our findings indicate that disseminated cancer cells can continue to mutate within a continuum landscape of mechano-phenotypes, governed by YAP-mediated mechanosensing of hydrodynamic flow.

4. Longitudinal Immune Profiling and Biomarkers of Clinical Response in Castration-Resistant Prostate Cancer

Nicole J. Toney[1], Megan T. Lynch[1], Stephanie C. Pitts[1], Jason M. Redman[1], Patrick Soon-Shiong[2], James L. Gulley[1], Jeffrey Schlom[1], Renee N. Donahue[1]
1 Center for Immuno-Oncology, Center for Cancer Research, National Cancer Institute, NIH
2 ImmunityBio, Culver City, CA

5. *Interactions between Hsp90s and J-domain cochaperones are conserved across evolution*

Anushka C. Wickramaratne[1], Patrick Needham[1], Connor Jewell[2], Lisa Jenkins[2], and Sue Wickner[1]
1 Laboratory of Molecular Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD
2 Mass Spectrometry Section, National Cancer Institute

Hsp90 and Hsp70 are two ATP-dependent molecular chaperones that collaborate with one another in protein quality control by regulating protein folding, remodeling, unfolding, and activation together with co-chaperones. Previous work using E. coli and S. cerevisiae proteins demonstrated a direct interaction between Hsp90 and J-domain proteins (JDPs). We further explored the conserved nature of this interaction and, using a pull-down assay, BLI, and crosslinking followed by mass spectroscopy, demonstrated that H. sapiens Hsp90a interacts with DnaJB1. We also determined that H. sapiens, S. cerevisiae, and E. coli Hsp90-JDP complex formation involves conserved regions of interaction involving the JDP J-domain and in C-terminal domain I. Additionally, we demonstrated that the regions involved in ternary Hsp90-Hsp70-JDP protein complexes are conserved in E. coli and S. cerevisiae, involving the JDP J-domain or C-terminal domain I, the Hsp90 middle domain and the Hsp70 JDP binding region. Results also showed that Hsp90-JDP and Hsp90-JDP-Hsp70 interactions can occur between proteins from different species, confirming the highly versatile nature of J-domain proteins. Altogether, the interplay between Hsp90s and J-proteins is conserved across multiple species, suggesting a conserved evolutionary function. The versatile functionality of J-domain proteins underscores their ability to function with multiple clients and multiple chaperones such as Hsp90s and Hsp70s in protein remodeling.

Outstanding Postgraduate Fellow Finalists

6. *NAMPT inhibitors and pyrimidine agonist antimetabolites floxuridine and 5-FU in combination in rhabdomyosarcoma impair proliferation and survival*

Jamie Gudyka[1], Choh Yeung[1], Senna Munnikhuysen[1], Christina Robinson[2], Amy James[2], Xiaohu Zhang[3], David Holland[3], Michele Ceribelli[3], Simone Difilippantonio[2], Craig J Thomas[3], Christine M Heske[1]
1 Pediatric Oncology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA
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Pediatric rhabdomyosarcoma (RMS) is a soft tissue sarcoma of high unmet need that requires novel treatment approaches. Nicotinamide phosphoribosyltransferase (NAMPT) catalyzes the rate limiting step of the nicotinamide adenine dinucleotide (NAD+) salvage pathway. Previously, our lab showed that RMS is highly sensitive to NAMPT inhibition with treatment of in vivo RMS models with the clinical NAMPT inhibitor OT-82 resulting in tumor regression. However, we observed recurrence after cessation of treatment, suggesting single agent therapy is not durable. Thus, the aim of this project is to identify and evaluate drug combinations that synergize with NAMPT inhibitors in RMS, thus identifying new therapies for patients with RMS. To identify synergistic drug combinations, we used a matrix drug screen using 2 RMS cell lines (Rh30 (fusion-positive (FP)) and Rh36 (fusion-negative (FN)) testing 2 NAMPT inhibitors (daporinad and GNE-618) in combination with 62 other anticancer agents. We identified that floxuridine, a pyrimidine analog antimetabolite, was the most highly synergistic agent with both NAMPT inhibitors. Validation of the screen results using longitudinal proliferation assays confirmed synergy between floxuridine and an additional NAMPT inhibitor, OT-82, across additional FP and FN RMS cell lines. Doses of each drug that had minimal effect on proliferation as single agents resulted in durable cell death when combined. Notably, when this combination was tested in proliferating non-cancer cell lines, there was a minimal effect on proliferation, suggesting a therapeutic window exists. Rescue experiments using nicotinamide mononucleotide (NMN), the product of NAMPT, reversed the antiproliferative effect of the combination, indicating that the synergy is NAD+ dependent. Protein analysis revealed that floxuridine-related ternary complex formation of thymidylate synthase is blocked by NAD+ loss mediated by NAMPT inhibition. To evaluate this combination for potential translation into the clinic, we extended our testing to include clinical agents, including RPT1G (Remedy Plan Therapeutics), a NAMPT inhibitor currently under early phase evaluation, and 5-FU, a prodrug of floxuridine. Validation studies of these agents using longitudinal proliferation assays again demonstrated that single agent doses had minimal effect on proliferation but when combined, resulted in persistent cell death. Preliminary toxicity testing in Rh30 tumor-bearing NSG mice demonstrated tolerability of OT-82 plus 5-FU up to the highest doses tested (15 mg/kg and 25 mg/kg respectively) and reduced tumor volume. Efficacy experiments are ongoing and will be reported. These findings demonstrate that combining NAMPT inhibitors with floxuridine or 5-FU results in significant synergy and preliminarily suggest that this is a promising and feasible combination regimen for patients with RMS.

7. A T-cell Engager Antibody Targeting the Non-Shed Site of Mesothelin in Solid Tumors

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Mesothelin (MSLN) is a cell surface protein that is overexpressed in various cancers, including mesothelioma, pancreatic, and ovarian cancer. Its expression in normal tissue is limited to the mesothelial cells lining the pleura, peritoneum and pericardium; make it an attractive target for antibody-based therapeutics. Many efforts have been dedicated towards the development of these antibody-based approaches but proteases in the tumor

microenvironment promote the cleavage of MSLN from cancer cells. High concentrations of shed MSLN in the tumor microenvironment bind to the antibody inhibiting its activity and preventing the death of cancer cells.

To address the presence of shed MSLN, an antibody called 15B6 was designed. It binds the membrane-proximal, protease sensitive region of MSLN that is not shed in the tumor microenvironment. This study investigates the ability of 15B6-targeted bispecific antibodies to eliminate MSLN expressing cancers. We designed humanized and murine versions of a CD3x15B6 bispecific antibody, which binds the CD3 on T cells and the MSLN (15B6) epitope on cancer cells serving as a bridge to promote the activation of T cells to kill MSLN positive cancer cells. These 15B6-based antibodies were compared to SS1-based antibodies that target the distal N-terminal domain of MSLN that is shed by proteases. In-vitro, the SS1 and 15B6 antibodies hold similar cytotoxic activity, but when cocultured with MSLN 296-591, a recombinant protein mimicking shed MSLN, the SS1 antibodies activity is inhibited.

In-vivo, in a human mesothelioma model in immunodeficient mice, tumor shrinkage and growth inhibition were observed when treated with the 15B6-based antibody but not those treated with the SS1-based antibody. In an immunocompetent mouse model, the complete regression of colon and breast tumors was observed when treated with the 15B6-based antibody compared to the SS1 antibody and control samples. Transcriptional analysis revealed that 15B6-treated mice had higher levels of activation of both innate and adaptive immune cells, along with significant upregulation of cytokine and STAT5 signaling pathways. scRNA seq analysis revealed increased levels of neutrophil, monocyte, and macrophage infiltration within BsAb10 treat tumors, that held elevated levels of interferon-γ and tumor necrosis factor signaling, suggesting an enhanced pro-inflammatory tumor microenvironment that may be supporting the antitumor response within tumors. Through in-vitro and in-vivo studies, we have demonstrated that this 15B6-targeted antibody binds to the protease-sensitive region, is highly active against MSLN-expressing cancer cell lines in vitro, is not inhibited by shed MSLN, and promotes upregulation of immune cell populations and signaling pathways that support robust anti-tumor activity.

8. drGT: Attention-Guided Gene Assessment of Drug Response Utilizing a Drug-Cell-Gene Heterogeneous Network

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Accurate drug response prediction in cancer pharmacogenomics requires predictive performance and biological interpretability. Although deep learning approaches have demonstrated strong accuracy, their internal decision processes are often opaque, limiting their utility for mechanistic discovery. We hypothesized that integrating drug structures, cell line drug responses, and gene expression data within a unified heterogeneous graph using a graph neural network (GNN) would improve generalization and provide mechanism-oriented interpretability. We posited that attention coefficients could quantify gene-level contributions and recover drug-gene associations, including known DTIs and novel associations supported by external evidence. We developed drGT, a GNN model integrating drug fingerprints, gene expression, drug-cell line response, and DTI data into a unified drug-cell-gene graph. Drug-cell line edges encode IC50-derived sensitivity values for classification and regression tasks, while drug-gene and cell-gene edges encode biological prior knowledge. Graph Transformer layers perform over typed edges, generating node embeddings and attention coefficients. Drug and cell line embeddings are concatenated and passed through fully connected layers to predict binary sensitivity classification or continuous pIC50 values via regression.

The model was evaluated across four cancer pharmacogenomic datasets (GDSC1, GDSC2, CTRP, and NCI60) under three settings: 1) prediction of randomly removed values with five-fold cross-validation, 2) prediction of novel drugs or cell lines via leave-one-drug or leave-one-cell-line-out generalization, and 3) cross-dataset generalizability via zero-shot transfer. Interpretability was assessed by extracting top-ranked attention-derived drug-gene pairs and validating them against DrugBank DTIs, PubMed abstract co-occurrence, and predictions from the independent DTI model DeepPurpose. Pathway-level enrichment analysis was conducted using MSigDB Hallmark gene sets. Across

benchmarks, drGT achieved state-of-the-art regression performance while maintaining competitive classification accuracy. Under random masking, the model achieved AUROC values up to 0.945 and R^2 values up to 0.690, outperforming all baselines in regression. In leave-one-out evaluations, drGT achieved AUROC 0.844 for unseen drugs and R^2 0.692 for unseen cell lines, and it was the only model to obtain a positive R^2 for unseen drugs. In zero-shot transfer between GDSC1 and GDSC2, drGT achieved an AUROC of 0.786 and R^2 of 0.334, the highest overall performance among the compared methods.

Interpretability analyses demonstrated biological consistency. Among 4,880 attention-derived drug-gene pairs, 36.9% overlapped with known DTIs. Of the novel associations absent from curated DTI databases, 63.7% were supported by PubMed evidence or DeepPurpose predictions. Furthermore, the majority of drugs exhibited pathway enrichment patterns consistent with their mechanisms of action, indicating that attention coefficients capture biologically meaningful transcriptional programs. drGT provides a unified framework that bridges predictive modeling and mechanistic interpretation in pharmacogenomics. By modeling drugs, genes, and cell lines within a single heterogeneous graph, the approach enables cross-modal relational reasoning that surpasses many modern, as well as traditional, machine learning methods that fail to incorporate prior knowledge we have regarding drug targets and drug structures. Its strong generalization performance under unseen, combined with literature-supported interpretability, positions drGT as a powerful tool for hypothesis generation, mechanism exploration, and translational precision oncology research.

9. Anti-tumor Actions of Neutrophils on Bone Metastatic Prostate Cancer

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Drug Discovery: Disease Models, Pharmacology, & Therapeutics (Oral Abstracts)

10. Macrophage Reprogramming Establishes an Immune-Mediated Axis of Therapeutic Resistance in Metastatic Castration-Resistant Prostate Cancer (mCRPC)

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Metastatic castration-resistant prostate cancer (mCRPC) remains universally lethal due to rapid acquisition of therapeutic resistance and limited responsiveness to immunotherapy. Tumor-associated macrophages (TAMs) are abundant in advanced disease and have emerged as key mediators of immune suppression, tumor progression, and chemoresistance. However, the lack of physiologically relevant human systems has impeded the mechanistic interrogation of macrophage tumor interactions in mCRPC. We hypothesized that mCRPC tumors actively reprogram infiltrating macrophages into pro-tumorigenic effectors that sustain tumor growth and attenuate chemotherapy efficacy. Furthermore, we proposed that a 3D organoid–macrophage co-culture system could reveal immune-mediated mechanisms underlying tumor plasticity and therapeutic escape. To test these hypotheses, we developed a 3D co-culture platform using LuCaP167 and LuCaP 170.2 patient-derived xenograft organoids embedded in Matrigel, recapitulating key features in the prostate tumor immune microenvironment ex vivo. High-resolution confocal microscopy was employed to quantify macrophage infiltration, spatial organization, and engagement with organoids over time. Migration assays evaluated tumor-driven macrophage recruitment. Monocyte-derived macrophage polarization was assessed by flow cytometry for M2 associated markers CD206, CD163 ARG1, and TREM2. Finally, organoid viability and responsiveness to docetaxel and cabazitaxel were assessed in the presence of M1 or M2 polarized macrophages to establish a functional relationship between macrophage state and chemotherapeutic resistance. Confocal imaging revealed rapid, spatially organized macrophage infiltration and progressive matrix remodeling, concomitant with increased organoid growth. Migration assays confirmed active tumor-driven macrophage recruitment, consistent with bidirectional signaling that promotes tumor expansion. CRPC organoids induced robust M2-like polarization of macrophages, evidenced by time-dependent upregulation of CD206, CD163, ARG1, and TREM2, indicating classical pro-tumorigenic programming. Functionally, M2 but not M1 macrophages enhanced organoid viability and conferred resistance to docetaxel and cabazitaxel, establishing macrophage reprogramming as a functional driver of chemoresistance. These findings highlight immune-mediated mechanisms as crucial determinants of acquired resistance in mCRPC, resulting in a paradigm shift beyond tumor-centric therapies.
This study examines macrophage plasticity as an important contributor to therapeutic resistance in metastatic castration-resistant prostate cancer (mCRPC). The 3D organoid–macrophage co-culture platform provides a controlled system to investigate how TAMs influence tumor plasticity and to evaluate macrophage-targeted and combination immunotherapy strategies. By integrating mechanistic studies with functional validation, this work aims to provide a translational framework for understanding immune-mediated resistance and to inform the development of therapeutic strategies that complement tumor-directed treatments in advanced prostate cancer.

11. Exploiting MYC-Driven Oncogenic Stress for Lymphoma Therapy

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Diffuse Large B-cell Lymphoma (DLBCL) is the most frequently occurring form of Non-Hodgkin's Lymphoma. Gene expression profiling identified two types of DLBCL based on germinal center reaction cell-of-origin: germinal center B cell-like (GCB) or activated B cell-like (ABC) DLBCL. Subsequent classifications delineate further genetic subtypes of DLBCL, each with differentially perturbed cellular and biological pathways, and distinct clinical outcomes. Though GCB patients respond better than ABC to the standard immunochemotherapy regimen, one subtype of GCB patients paradoxically exhibit the lowest response rates. These represent a clinically and genetically distinct subset of DLBCL with both MYC and BCL2 translocations, known as 'double-hit' (DHIT)-GCB.

Recently, the ViPOR phase I/II clinical trial in relapsed/refractory DLBCL, designed to target survival pathways in ABC, demonstrated unexpectedly that DHIT-GCB had a 50% complete response (CR) rate. Venetoclax, a drug in ViPOR, inhibits BCL2, which is translocated and overexpressed DHIT-GCBs. In vitro drug sensitivity studies revealed that venetoclax is significantly more toxic in DHIT-GCB cell lines than in other GCB lines with BCL2 translocations but without MYC translocations. MYC is a ubiquitous transcription factor that promotes malignant cell growth but is toxic when introduced in normal cells. We hypothesized that inhibition of BCL2 renders DHIT-GCB malignant cells vulnerable to MYC-induced toxicity, explaining the selective sensitivity of DHIT-GCB lymphomas to venetoclax. To test this, we employed the degradation tag (dTAG) system to controllably degrade MYC protein. We generated DHIT-GCB cell lines ectopically expressing a MYC-dTAG fusion protein, with CRISPR-Cas9 inactivation of the endogenous MYC locus. In parallel, we use a knock-in strategy to express a MYC-GFP-dTAG fusion protein from the endogenous MYC locus. BCL2 inhibition in these lines induced rapid apoptosis, but concomitant degradation of MYC increased the proportion of live, non-apoptotic cells. I plan to use unbiased functional genomic screens in MYC-dTAG-expressing DHIT-GCB lines to study the mechanism behind MYC oncogenic stress that promotes sensitivity to BCL2 inhibition. These studies may suggest drug combinations strategies that would harness the synergism between MYC and BCL2 in DHIT-GCB, and hopefully improve therapeutic outcomes for these DLBCL patients.

12. Reducing renal radiation in SSTR2-targeted radiopharmaceuticals via glycosidase-cleavable linkers

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Radiopharmaceutical therapy (RPT) has revolutionized the management of somatostatin receptor (SSTR)-expressing neuroendocrine tumors (NETs). However, dose-limiting kidney toxicity remains a significant challenge, often requiring logistically burdensome amino acid infusions to mitigate nephrotoxicity. We hypothesized that incorporating enzyme-cleavable linkers, a strategy successfully utilized in antibody-drug conjugates (ADCs), could reprogram the biodistribution of RPT agents. Specifically, we proposed that linkers susceptible to naturally occurring glycosidases in the renal proximal tubules would facilitate the rapid excretion of radio-metabolites from the kidneys without compromising the delivery of the therapeutic payload to the tumor. This approach aims to reduce renal exposure while maintaining or improving tumor targeting. We designed and synthesized a library of 10 DOTA-linker-TATE constructs featuring diverse chemical moieties, including cleavable peptides, albumin-binding domains, and glycans. Initial screening was performed using ex vivo biodistribution of indium-111 (111In)-labeled variants in healthy mice to identify leads with reduced renal retention. Lead candidates, DOTA-MVK(epsilon)-TATE and DOTA-beta-Gal-TATE, were further evaluated in AR42J NET xenograft models via longitudinal PET/CT imaging with yttrium-86 (86Y). Finally, targeted alpha therapy (TAT) studies were conducted using actinium-225 (225Ac)-labeled versions to assess therapeutic efficacy, dosimetry, and histopathological safety. In parallel, in vivo cleavage efficiency and metabolic fate were analyzed to confirm linker processing and inform mechanistic interpretation. PET imaging revealed that 86Y-

DOTA-beta-Gal-TATE significantly enhanced tumor uptake (~90% increase) and achieved up to a 10-fold improvement in tumor-to-renal ratios compared to parental DOTA-TATE. Dosimetric calculations showed that the beta-Gal linker reduced the renal absorbed dose by 70% while delivering a 2.5-fold higher radiation dose to the tumor. In TAT studies, 225Ac-DOTA-beta-Gal-TATE markedly extended median survival to 86 days (single-cycle) and over 190 days (two-cycle), with complete tumor regressions observed. Histopathological evaluation confirmed superior renal safety for the beta-Gal construct, with 100% of samples showing grade 0 tubular damage, whereas the parental agent induced multifocal injury. These findings demonstrate that glycosidase-cleavable linkers can meaningfully reshape pharmacokinetics, improving therapeutic index without sacrificing antitumor efficacy. This work identifies a novel, translatable strategy to broaden the therapeutic window of radiotheranostics. By minimizing intrinsic renal toxicity and maximizing tumor delivery, this glycosidase-cleavable linker technology could be generalized to various peptide-based agents, antibody fragments, and other renally cleared small molecules. This approach offers a path toward safer and more effective cancer treatments that do not rely on additional supportive care, potentially transforming the design of next-generation radiopharmaceuticals. Such modular linker strategies may also accelerate the development of therapeutics requiring precise control of organ-specific exposure.

13. Activation of host anti-tumor immunity in CD47-targeted near-infrared photoimmunotherapy of solid tumors

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14. Immune-responsive 'ProTofaVET' peptide hydrogels: A spatiotemporal on-demand drug-delivery approach to improve vascular composite allotransplantation survival

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Vascularized composite allotransplantation (VCA) provides a restorative option for patients requiring the transplantation of multiple tissues as a single functional unit, such as a hand or abdominal wall transplant. Successful patient outcome after transplantation correlates with effective life-long application of immunosuppressive drugs such as Tofacitinib (Tofa), an effective JAK/STAT inhibitor proven to be effective in the inhibition of inflammatory cytokines which control the activation of T-cells in transplant recipients. However, debilitating side-effects such as nephrotoxicity, cardiovascular disease & cancer, associated with the systemic delivery of immunosuppressive therapy can offset their benefits. This presents an imminent need for developing less toxic therapeutic strategies that limit rejection and improve graft survival. We have designed and developed a syringe-injectable hydrogel platform - ProTofaVET, that stabilizes Tofacitinib and provides a localized and targeted drug-delivery profile, tuned to the intensity of the rejection response. The hydrogel, composed of self-assembling beta-hairpin peptides, encapsulates two Tofa-derived prodrugs – 1) microcrystalline deposits of Tofacitinib (cTofa) and 2) lipophilic Tofa sheathed within nanostructured lipid carriers (Tofa-NLC); each prodrug exhibiting independent release regimes. First, the cTofa deposits afford an extended-release profile via diffusion, enabling delivery to immune cells that infiltrate the transplanted tissue at the local injection site. Second, the Tofa-NLCs are programmed to release and traverse to the draining lymph nodes in response to matrix metalloproteinases upregulated during tissue rejection, which allows for targeted delivery of Tofa to the

physiological sites of the adaptive immune response. This on-demand drug release is achieved by engineering the peptide hydrogel to be susceptible to degradation via the incorporation of an enzyme-cleavable motif in the primary peptide sequence. The material properties of the composite hydrogel were characterized using CD and rheology. The kinetics of drug-release were studied using HPLC, MS and microscopy. We demonstrated that the ProTofaVET hydrogel platform, comprising the dual Tofa components, demonstrates suitable shear-thin recovery behavior. The localized release of cTofa can be tuned to range from a couple of days to several weeks by modulating its loading concentration. The lipophilization of Tofa proved to be effective in enhancing its retention within the lipid carriers and in vivo studies indicated that the released Tofa-NLCs accumulate in the lymph nodes proximal to the transplantation site. Further, their release from the gel is exclusively mediated by enzymatic action, complementing the timeline of graft rejection. Collectively, our therapeutic platform demonstrates a diverse set of release profiles ranging from fast, to intermediate and very slow, along with simultaneous regioselective and temporal control over the delivery of Tofa. The immune-responsive 'ProTofaVET' peptide gel platform presents a synergistic drug-delivery approach that targets both the innate and adaptive immune cells, with the potential to tailor our design for specific clinical applications and optimal therapeutic delivery timelines. The efficacy of this therapeutic platform is being currently evaluated toward aiding long-term transplant survival within pre-clinical VCA models, and the scope of our design is also being investigated for the site-selective delivery of other immunosuppressive drugs.

15. Development of novel GPC2-directed radiotheranostics and CAR T-cell therapy for neuroblastoma

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Genetics, Epigenetics, and Gene Regulation (Oral Abstracts)

16. E3 ubiquitin ligase UBR5 restricts FACT mediated CENP-A mislocalization for maintaining chromosomal stability

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17. Molecular Landscapes and Mechanistic Pathways in BAP1-Associated Mesotheliomas

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Patients with germline BRCA-associated protein 1 (BAP1) mutations are predisposed to a variety of cancers including malignant mesotheliomas (MMs). Whereas recent studies have demonstrated nonrandom DNA methylation alterations linked to tumor predilection in subjects with BAP1 Cancer Syndrome (BCS), the spectrum of genetic and epigenetic perturbations and their impact on the pathogenesis and clinical evolution of MMs in these subjects have not been systematically evaluated. The present study was performed to characterize secondary germline and somatic DNA mutations that might be associated with the pathogenesis and clinical evolution of MM arising in subjects with BCS. Eligible subjects >33 years old with germline BAP1 mutations with or without prior malignancies underwent dual energy CT imaging followed by bilateral thoracoscopies and diagnostic laparoscopies on a prospective IRB-approved protocol. Surgical biopsies with pathologically confirmed MM underwent comprehensive genomic profiling using the TruSight Oncology 500 (TSO500) NGS panel with subsequent pathway enrichment analysis using the Reactome Pathway Knowledgebase 2024. 65 subjects (28 males, 37 females; median age 49.4) with 37 unique variants spanning the coding region of BAP1 underwent surgical evaluation between March 2021 and November 2025. Known sites of disease were excluded from analysis. A spectrum of clinically occult, diffuse mesotheliomas was histologically confirmed in 55 of 65 (85%) subjects, including 132 of 170 (78%) compartments. Of 57 subjects with CLIA-certified multigene germline testing, 11 (19%) had at least one additional pathogenic germline mutation affecting 7 cancer-related genes associated with DNA repair, the most frequent of which was MITF (4 subjects). 9 of these 11 patients (82%) had confirmed mesothelioma. 25 additional germline variants of uncertain significance (VUS) were identified among the cohort, which were also enriched in DNA repair pathways. TSO500 analysis of 50 mesothelioma specimens (32 pleural; 18 peritoneal) from 47 patients identified 88 pathogenic or likely pathogenic somatic variants involving 15 genes in addition to their previously identified germline mutations. 74 of these were secondary BAP1 mutations identified across 41 (82%) samples, consistent with polyclonal field cancerization. The 14 remaining genes were linked to DNA damage signaling and cell fate regulation by MITF, though the specific targets were not significantly concordant with the secondary germline mutations. The 8 variants identified only in pleural mesotheliomas were associated with MET-driven cell growth and migration pathways, whereas 6 genes mutated only in peritoneal samples were enriched in TP53 tumor suppression and apoptosis pathways. 410 additional VUS involving 207 genes were identified, which were enriched in signal transduction and transcription pathways along the PI3K-AKT axis. No clear associations were evident between secondary germline or somatic mutations and tumor propensity possibly due to the limited sample size. The paucity of driver mutations suggests that epigenetic mechanisms are primarily responsible for initiation and early progression of germline BAP1-mutant MMs. These findings support further efforts

to identify and target epigenetic perturbations unique to MMs arising in subjects with BCS as a strategy to abort progression of these neoplasms to life-threatening disease states.

18. Cohesin loader NIPBL and STAG/eRNAs orchestrate the dynamic 3D chromatin architectures upon androgen receptor activation

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Transcription factor (TF) activation reconfigures the 3D genome, but the mechanisms linking chromatin dynamics to gene transcriptional regulation remain incompletely understood. Here, we show that the cohesin loader NIPBL and STAG proteins, together with enhancer RNAs (eRNAs), coordinate androgen receptor (AR)-mediated chromatin loop formation and gene activation in LNCaP prostate cancer cells. Using the highly sensitive transposase-mediated analysis of chromatin (Hi-TrAC) assay and AR Chromatin-immunoprecipitation sequencing (ChIP–seq), we identify androgen-dependent long-range chromatin interactions that dynamically change in both number and size. NIPBL and SMC1a are recruited to AR binding sites, and loss of NIPBL reduces AR occupancy and canonical loop formation. Surprisingly, reduction in NIPBL levels leads not only to a loss of loops but the creation of new loop structures, indicating a remarkable reorganization of chromatin interactions. In parallel, we find that increased eRNAs at AR binding sites contributes to enhancer-promoter chromatin loop formation and further activates AR responsive gene transcription in the absence of NIPBL. Co-immunoprecipitation reveals that XRN2, a 5′–3′ exoribonuclease, interacts with AR and NIPBL in a hormone-dependent manner. Knockdown of XRN2 leads to increased eRNAs at AR binding sites, suggesting a role for NIPBL-XRN2 in regulating eRNA turnover. Moreover, STAG1 and STAG2 are selectively recruited to these eRNA-enriched loci, implicating STAG-cohesin complexes in the formation of alternative chromatin loops in the absence of NIPBL. Our findings uncover a dual role for NIPBL in both cohesin loading and eRNA metabolism and highlight a previous unappreciated mechanism by which AR signaling integrates transcriptional activation with 3D genome reorganization.

19. mtKO: A dedicated guide RNA library for mitochondria redox biology research

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Mitochondria are multifunctional organelles essential for both physiological regulation and pathological progression. In malignant cancer cells, mitochondrial reprogramming establishes a metabolic foundation that supports tumor growth, which is essential for cancer cells to overcome intrinsic metabolic abnormalities and stress. To uncover key mitochondrial pathways involved in cancer development, we developed mitochondrial Knockout (mtKO) — a robust, flexible, and unbiased CRISPR/Cas9 guide RNA screening platform designed to systematically identify critical mitochondria-associated functions. The mtKO library target genes involved in diverse mitochondrial-guided processes, including biosynthesis, transmembrane transport, oxidative phosphorylation, and redox regulation. Through a mtKO dropout screen, we identified the mitochondrial antioxidant enzyme superoxide dismutase 2 (SOD2) as indispensable for the fitness and survival of cancer cells harboring oncogenic mutations in isocitrate dehydrogenase 1 (IDH1). Mechanistically, SOD2 mitigates mitochondrial reactive oxygen species (ROS) generated by dysfunctional Krebs cycle activity in IDH1-mutant glioma and chondrosarcoma cells. Functionally, SOD2 maintains redox homeostasis and preserves mitochondrial integrity thereby controlling disease manifestation both in vitro and in vivo. Overall, our study introduces a powerful functional genomics approach to interrogate mitochondrial biology

and uncovers a selective mitochondrial redox vulnerability in Krebs cycle-deficient cancers, highlighting mitochondrial redox imbalance as a potential therapeutic target.

20. DNA repair drives cisplatin-induced neuronal death

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21. The ubiquitin ligase HUWE1 controls B-catenin functions in WNT signaling and cell adhesion

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B-catenin plays two major roles in the cell, one as the key mediator of transcriptional responses to WNT signaling in the cytoplasm and nucleus, and the other as a structural component of adherens junctions, cell-cell adhesion complexes at the plasma membrane. WNT/B-catenin signaling is a fundamental signaling pathway, dysregulation of which can drive many types of cancer. Disassembly of adherens junctions, often caused by reduced B-catenin at the plasma membrane, is a hallmark of epithelial-mesenchymal transition (EMT), a process that promotes cancer metastasis. The goal of this study was to investigate the mechanisms that regulate the balance between these two spatially and functionally distinct pools of B-catenin. During WNT/B-catenin signaling, the main regulated step is B-catenin phosphorylation and degradation mediated by the destruction complex, composed of the scaffold proteins APC and AXIN1/2, and the kinases casein kinase 1-alpha and GSK3. Using CRISPR, we generated casein kinase 1-alpha knock-out haploid human (HAP1) cells, recapitulating the hyperactive WNT signaling state present in cancer. In this cell line, we observed by confocal microscopy that B-catenin accumulates in the cytoplasm and the nucleus, and promotes hyperactive WNT signaling. Importantly, we found that loss of the E3 ubiquitin ligase HUWE1 in casein kinase 1-alpha knock-out cells induced a marked change in the localization of B-catenin from the nucleus to the plasma membrane, which was accompanied by a substantial reduction in WNT/B-catenin signaling. Through proximity ligation assays, we found that localization of B-catenin to the plasma membrane caused by HUWE1 loss promotes B-catenin incorporation into adherens junctions. A new adherens junction-dependent cell adhesion assay that we developed revealed that the increase in B-catenin at adherens junctions also increases its functional contribution to cell-cell adhesion. Taken together, these results demonstrate that HUWE1 regulates the balance between B-catenin transcriptional activity in WNT signaling and its cell adhesion functions. Therefore, regulation of B-catenin functions through HUWE1 may open new therapeutic avenues in cancers caused by hyperactive WNT signaling, and/or in metastasis driven by EMT.

Cancer Biology: Initiation, Progression, & Metastasis (Oral Abstracts)

22. Mechanisms of tumor dormancy through immune niche formation, and its impact on immune therapeutic relapse

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Residual breast cancer cells can persist in a dormant state during prolonged clinical remissions, yet immune regulated mechanisms that actively maintain dormancy or permit metastatic reactivation remain poorly understood. Defining these mechanisms is critical to preventing late relapse following systemic therapy and immunotherapy. Here, we identify a myeloid driven immune-tumor circuit that enforces breast cancer dormancy and represents a therapeutically actionable vulnerability. Genetic abrogation of myeloid TGF-β receptor II generated an IFN-γ rich tumor microenvironment that induced durable quiescence of breast cancer cells. IFN-γ signaling activated a KLF4 dependent transcriptional program in malignant cells, leading to induction of SLURP1, which enforced dormancy by disrupting fibronectin–integrin signaling required for proliferative outgrowth. Dormant breast cancer lesions localized to spatially restricted immune niches enriched for NK cells, conventional dendritic cells, monocytes, and neutrophils. Despite this immune infiltration, dormant tumor cells evaded NK cell mediated elimination through engagement of the CD200-CD200R1 inhibitory axis. Therapeutically, disruption of CD200-mediated immune suppression reactivated immune surveillance and, when combined with chemotherapy and immune checkpoint blockade, resulted in robust eradication of dormant breast cancer cells. Collectively, these findings define the IFN-γ-KLF4-SLURP1 and CD200-CD200R1 axis as critical regulators of immune-enforced breast cancer dormancy and establish a late-breaking combinatorial strategy to eliminate dormant disease and prevent relapse following immunotherapy.

23. Remodeling the Tumor Extracellular Matrix Through Hyaluronic Acid Depletion to Enhance Cancer Therapy and Treat Fibrotic Disease

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Aberrant extracellular matrix (ECM) accumulation is a hallmark of solid tumors, driving progression, immune exclusion, and treatment resistance. Among ECM components, hyaluronic acid (HA), a glycosaminoglycan involved in tissue hydration and structural integrity, has emerged as a key regulator of tumor biology. Transcriptomic analyses across TCGA datasets revealed a consistent upregulation of hyaluronan synthases (HASes) in solid tumors (up to ~100-fold), which significantly correlated with poor overall survival. Immunohistochemical staining confirmed increased HA deposition. Hyaluronidases, the enzymes responsible for HA degradation, were downregulated and associated with significant adverse outcomes. Notably, early HA accumulation was observed in the pre-metastatic lungs of

osteosarcoma- and breast cancer-bearing mice, preceding the detection of metastases, suggesting that HA may initiate metastatic ECM remodeling. Similarly, in radiation-induced fibrosis, HA levels rose before classical fibrotic changes. These findings collectively identify disrupted HA homeostasis as a clinically significant and underexplored feature of the tumor and fibrotic stroma. To explore HA's functional role, we developed genetically engineered mesenchymal stromal cells (GEMesys) that express hyaluronidase to deplete HA. Using in vivo second harmonic generation imaging, multiplex immunofluorescence, and biochemical assays, we tested GEMesys in syngeneic orthotopic murine models of osteosarcoma (F42010), rhabdomyosarcoma (M-3-9M), pancreatic cancer (Panc02), and spontaneous pancreatic ductal adenocarcinoma (KPC). GEMesys homed to tumor sites and initiated ECM remodeling within 24 hours, resulting in substantial HA degradation and a three-fold reduction in collagen fiber density and alignment. Collagen reorganisation appeared secondary to HA depletion, underscoring HA's role in maintaining ECM structure. HA degradation also alleviated hypoxia, rapidly reducing Hypoxyprobe staining and HIF1α expression in these tumors. High HAS2/3 expression correlated with enrichment of glycolytic pathways and suppression of oxidative phosphorylation in TCGA datasets, and this was mirrored by downregulation of GLUT1 post-HA depletion in our models, implicating HA in modulating the Warburg effect. GEMesys further promoted vascular normalization (reduced $CD31^+$ and $CD105^+$ endothelial markers) and reprogrammed immune infiltration, enhancing $CD4^+CD44^+$ effector T cells while diminishing exhausted T cells and immunosuppressive myeloid populations. Therapeutically, GEMesys monotherapy reduced tumor burden, 1.4-fold in osteosarcoma, 2-fold in rhabdomyosarcoma, and 7.2-fold in Panc02. In the KPC model, GEMesys reduced HA/Col1 levels and extended overall survival, including one complete remission. Building on the immune reprogramming observed following HA depletion, we evaluated whether lymphodepleting chemotherapy could further enhance anti-tumor immunity. In the E0771 breast cancer model, combining GEMesys with cyclophosphamide/fludarabine resulted in durable responses, with 60% of treated mice achieving complete tumor regression and long-term survival. Critically, GEMesys also sensitized tumors to chemotherapy, achieving a four-fold reduction in growth and stable disease in combination regimens. In a radiation-induced fibrosis model, GEMesys treatment significantly extended survival, suggesting broader therapeutic potential beyond oncology. Together, these findings position HA as a master regulator of ECM architecture, tumor metabolism, immune suppression, and metastatic priming. Targeted HA depletion remodels the tumor microenvironment and sensitizes it to therapy, unveiling new treatment vulnerabilities. This work underscores the value of matrix-focused interventions and provides a compelling rationale for integrating anti-stromal approaches in the treatment of high-risk malignancies and fibrotic diseases.

24. Resolving the initiating changes in the immunomodulatory glycome during early tumorigenesis of head and neck squamous cell carcinoma in living animals

Sarah M. Hammoudeh, Thomas D. Madsen, Roberto Weigert

25. Knockout Of PPM1D Phosphatase Modulates Neutrophil Differentiation and Anti-Tumoral Activity Through Increased nAPCs Differentiation

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The wild-type p53-induced phosphatase (PP2Cdelta or PPM1D) is a member of the serine/threonine protein phosphatase 2C (PP2C) family known to de-phosphorylate DNA damage response regulators p53 and gH2AX. Due

to its capacity to inhibit p53, the PPM1D protein is overexpressed, or the Ppm1d gene is amplified, in several human cancers. Studies on human cells have shown that its overexpression compromises tumor suppressor functions, and studies of mice that lack PPM1D show that they are resistant to tumorigenesis. Previously, we used syngeneic tumor models to investigate the effects of ablating Ppm1d in the murine immune system on tumor progression. Myeloid-specific deletion of Ppm1d delayed the growth of both B10 melanoma tumors and LLC1 lung cancer tumors, confirming an important role of Ppm1d -deficient innate immune cells in anti-tumor immunity. Recently, we found that Ppm1d deletion in tumor infiltrating neutrophils (TANs) induces profound gene expression and phenotype changes, leading to an increase of their anti-tumor and immune functions and the emergence of a previously described specific antigen presenting population of neutrophils (nAPCs) characterized by increased phagocytotic capabilities. We identified CD48, H2-Dma, H2-Dmb and other members of the MHC-II complex as important markers of this subpopulation and used them to further characterize the phenotype. In vivo experiments showed that PPM1D deletion in neutrophils leads to increased nAPCs infiltration in tumors that is correlated with slower tumor growth and a reduction in lymphocyte exhaustion. Single cell RNAseq analysis of both human and mouse datasets showed that nAPCs are present in both human and murine tumors and interaction analysis revealed that nAPCs have the potential to interact with most immune cells present in the tumor micro-environment, notably with exhausted CD8 T-cells, exhausted Th1 cells and macrophages/monocytes. Interaction between nAPCs and exhausted T-cells activates pathways linked to immune activation and anti-tumor activity which explained the reduced tumor growth. Single-cell in-situ proteomic analysis showed that loss of PPM1D in neutrophils caused a change in TME spatial organization allowing closer interaction of T and B cells with cancer cells. We used induced pluripotent stem cells obtained from patients with a loss-of-function mutation to show that PPM1D also affected nAPCs differentiation in humans.
Overall, our findings demonstrate that PPM1D plays a role in neutrophil differentiation and more specifically in the differentiation of the anti-tumor nAPCs in the tumor microenvironment. They also reinforce the need to develop more efficient PPM1D inhibitors which could both reactivate p53 functions in tumor cells and increase the anti-tumor capabilities of the tumor micro-environment.

26. A whole genomic CRISPR-Cas9 screen identifies the amino acid transporter SLC43A1 (LAT3) as a major determinant of oxaliplatin sensitivity in colorectal cancer cells

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Colorectal cancer (CRC) is the second leading cause of cancer deaths in the United States, with a five-year survival rate of 65%. Oxaliplatin was the first platinum drug shown to improve CRC patient outcomes and is now a common adjuvant therapy for advanced disease, yet 90% of patients develop resistance. Oxaliplatin was developed as a third-generation derivative of cisplatin, but recent evidence points to divergent modes of action. Our objective is to utilize an unbiased genome-scale CRISPR approach to identify multifactorial mechanisms of oxaliplatin resistance in CRC.
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. Guide RNAs corresponding to the neutral amino acid transporter SLC43A1 (LAT3) were the most significantly enriched in knockout screens and depleted in activation screens, suggesting a potential role for LAT3 in modulating oxaliplatin resistance. In vitro CRISPR knockout and overexpression of LAT3 in SW620 and RKO cell lines confirm increased resistance or sensitivity to oxaliplatin, respectively. Further analysis demonstrates that increased LAT3 levels correlate with increased intracellular levels of oxaliplatin, increased levels of DNA-platinum adducts and DNA damage, demonstrating that enhanced LAT3-mediated uptake of oxaliplatin can exert its expected mechanism of action to induce cytotoxicity. Analysis of publicly available transcriptomic data corresponding to therapy response indicates that cell lines across tumor types and CRC cell lines that are responsive to OxPt have increased levels of LAT3 compared to those that are more resistant. CRC patient tumor cells display increased SLC43A1 RNA levels compared to normal tissue, while many other tumor types tend to downregulate LAT3 compared to normal tissue. This upregulation of the LAT3 transporter may explain the enhanced efficacy of oxaliplatin in CRC compared to other cancer types, suggesting a novel and specific role for LAT3 in modulating OxPt in CRC. These findings may lead to a better understanding of oxaliplatin's mode of action in CRC and can provide new insights

into the interplay between essential nutrient uptake and drug transport. We believe this global approach can clarify inconsistencies regarding the molecular basis of OxPt resistance and uncover potential targeted and personalized therapeutic strategies or biomarkers to improve patient outcomes for advanced CRC.

27. TLX3 represses IKZF2 to activate PI3K/AKT signaling: A novel transcriptional axis driving leukemia initiation

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Data Science, Bioinformatics, Epidemiology, and Genomics (Oral Abstracts)

28. Genome-wide association study of mantle cell lymphoma identifies novel loci suggesting a critical role for B-cell chromatin readers and DNA repair mechanisms

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29. Single-cell exon deletion profiling reveals splicing events that shape gene expression and cell state dynamics

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Alternative splicing is a pervasive regulatory mechanism that expands transcriptomic and proteomic diversity and plays essential roles in development, cell identity, and disease, particularly cancer. Despite the cataloguing of thousands of alternative splicing events, the functional significance of most remains unknown, largely due to the lack of scalable approaches for systematic interrogation. Functional analysis of individual exons typically requires years of bespoke experimentation, underscoring the need for high-throughput strategies to link splicing variation to cellular phenotypes. Here, we present scCHyMErA-Seq, a scalable CRISPR-based exon deletion screening platform integrated with 10x Genomics single-cell transcriptomics. A key technical advance is the engineering of the Cas12a guide scaffold to enable direct single-cell capture of Cas12a gRNAs, allowing simultaneous detection of Cas9 and Cas12a guides alongside polyadenylated transcripts while improving exon deletion efficiency. We applied scCHyMErA-Seq to systematically delete 224 alternative cassette exons across 161 genes previously implicated in cell fitness, profiling over 410,000 leukemic HAP1 cells. Using a dedicated analytical pipeline, we mapped transcriptional phenotypes at exon resolution, revealing distinct cellular states, exon-specific gene expression

programs, and widespread effects on cell-cycle regulation, which were independently validated using orthogonal assays. Mechanistic analysis of a strong hit, NRF1 alternative exon-7, demonstrates that exon inclusion modulates NRF1 transcriptional activity by altering its recruitment to target gene promoters. Notably, gene expression profiles generated by scCHyMErA-Seq closely recapitulate results obtained from traditional, labor-intensive orthogonal approaches. Overall, scCHyMErA-Seq provides a robust and scalable framework for systematically dissecting the functional consequences of alternative splicing at single-cell resolution, enabling prioritization of functionally relevant exons and opening new avenues for exon-targeted therapeutic discovery.

30. Seeing RNA as It Moves: A Direct Structural Perspective with HORNET

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RNA structural biology remains one of the last frontiers in understanding how molecular architecture encodes biological function. Unlike proteins, RNA molecules are inherently dynamic and structurally heterogeneous, yet our understanding of RNA function has largely relied on static models or structures inferred indirectly. This disconnect has limited our ability to fully explain RNA-mediated regulation, catalysis, and molecular recognition. To directly interrogate RNA structural dynamics, we pioneered the use of atomic force microscopy (AFM) to visualize individual RNA molecules in solution under near-physiological conditions. These experiments revealed that a single RNA sequence can populate multiple distinct, biologically relevant three-dimensional conformations. Rather than behaving as a single folded entity with flexible regions, RNA exists as an ensemble of dynamic structural states. To bridge the gap between 2D experimental visualization and 3D structural interpretation, we developed HORNET (Holistic RNA Structure Determination using AFM, Unsupervised Machine Learning, and Deep Neural Networks). HORNET integrates direct AFM imaging with data-driven computational modeling to reconstruct three-dimensional RNA topologies and estimate their structural accuracy. By combining experimental observation with AI, HORNET enables the analysis of highly heterogeneous and flexible RNA molecules that are inaccessible to traditional structure-determination approaches. This framework provides new insights into RNA dynamics, including correlated motions between structural elements, identification of invariant structural cores, and quantification of conformational heterogeneity. By directly observing RNA as it moves, HORNET reframes how we define RNA structure: shifting from static representations to dynamic structural landscapes. This work is described in Nature (Degenhardt et al., 2025) and Nature (Lee & Degenhardt et al., 2025).

31. Development of a CRISPRi Combinatorial Screening Platform for Enhanced Gene Repression

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CRISPR technologies are rapidly evolving, with novel gene editing strategies continuously emerging. The CHyMErA combinatorial screening platform utilizes Cas9 and Cas12a nucleases in combination with hybrid guide RNAs, enabling versatile targeting approaches. This allows among others for increased knockout efficiency of single genes or the simultaneous perturbation of gene pairs, facilitating the study of genetic interactions. In this project, we developed a CRISPR interference (CRISPRi) version of CHyMErA. CRISPRi employs catalytically inactive Cas nucleases bonded to transcriptional repressors to induce heterochromatin formation, thereby repressing gene expression without introducing DNA breaks. This DNA damage free approach minimizes cellular stress and off-target effects, improving phenotypic fidelity. We selected the ZIM3 KRAB domain as transcriptional repressor and tested 16 initial configurations, including N- vs. C-terminal binding, direct fusion vs. tag-based recruitment, and four Cas12a variants. Based on small-scale experiments we shortlisted four combinations for further evaluation in a high-

throughput optimization screen using 16,100 guides. The results demonstrated robust repression across two of the four cell lines. The optimal configuration involved direct fusion of ZIM3 to the N-terminus of Cas9 and the C-terminus of Cas12a, with LbHyper Cas12a identified as the most effective variant. Repression efficiency of individual Cas effectors was comparable to published CRISPRi systems with Cas9 outperforming Cas12a. However, dual-targeting by the CHyMErA platform significantly enhanced gene silencing. Our results highlight the potential of CRISPRi CHyMErA as a powerful tool for high-efficiency, multiplexed gene repression, expanding the applicability of CRISPRi in functional genomics such as the search for cancer gene dependencies.

32. Learning the language of cancer mutations using Artificial Intelligence

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Somatic mutation profiling is central to cancer diagnosis and treatment selection. However, current precision oncology workflows focus on individual actionable mutations, overlooking the broader mutational context that shapes tumor evolution and treatment response. Here, we introduce OncoBERT, a language model that learns contextual representations of somatic mutations from large-scale clinical sequencing data spanning >210,000 patients, 113 cancer types and 20 institutions. OncoBERT uncovers robust patient-specific mutational subtypes across diverse cohorts and targeted sequencing panels, revealing clinically meaningful mutation patterns that are associated with differential response to chemotherapy, targeted therapies, and immunotherapy. Importantly, integrating OncoBERT's contextual representations with clinically approved biomarkers of immunotherapy response, such as tumor mutational burden (TMB) and microsatellite instability (MSI), significantly improved prediction of clinical benefit. By further incorporating matched tumor transcriptomic profiles, we linked OncoBERT-defined mutational subtypes to distinct cancer hallmark programs and tumor microenvironment states. Together, OncoBERT provides a scalable framework for deciphering somatic mutation patterns, enabling improved patient stratification and advancing precision oncology.

33. Identifying clinically relevant cell state interactions in the tumor microenvironment using CSI-TME

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Interactions between distinct cell state in the tumor microenvironment (TME) play a critical role in cancer progression and therapeutic response. Systematic characterization of functional cell-state interactions (CSIs) remains challenging due to paucity of scRNA-seq cohorts with clinical information on one hand, and lack of cellular context in bulk RNA-seq cohorts on the other. To overcome these limitations, we developed CSI-TME, a data-driven computational pipeline that infers clinically relevant cell state interactions directly from bulk transcriptomic data. CSI-TME employs both supervised and unsupervised learning to deconvolute cell type-specific gene expression and identify distinct cell states in IDH-mutant gliomas. By modeling the joint activity of cell state pairs alongside clinical outcomes, our approach bypasses the need for prior ligand–receptor knowledge and leverages large-scale clinical datasets unavailable in most single-cell cohorts. Application of CSI-TME to an IDH-mutant glioma cohort from TCGA and an independent cohort from CGGA revealed a robust, reproducible cell state interaction network (CSIN) that is predominantly pro-tumor. A subset of interactions is mediated via direct cell-cell interaction mediated via ligand-receptor pairing and significantly co-colocalized in the sptial transcriptomic datasets. Notably, we identified a novel interaction between glioma stem cells and a unique T cell state that is intermediate between senescence and proliferation and is associated with poorer patient survival. Integration of the CSIN with somatic mutation data suggests that cell state interactions may exert an anti-tumor role in early-stage gliomas, shifting to a pro-tumor role as the disease progresses. Finally, we showed that CSI-TME can detect clinically relevant cell state interactions in multiple cancer types and provides a valuable tool for patient stratification in various therapeutic modalities.

Immunology & Virology (Oral Abstracts)

34. Therapy-induced host chromatin remodeling via AP-1/SWI-SNF drives BK Polyomavirus replication and Hemorrhagic Cystitis

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Hemorrhagic cystitis (HC) is a serious and sometimes fatal complication in patients undergoing chemotherapy or immune-conditioning regimens, particularly following hematopoietic stem cell transplantation. Reactivation of BK polyomavirus (BKPyV) is strongly associated with HC, yet the molecular mechanisms by which therapeutic exposures promote BKPyV amplification remain unclear. Current models emphasize immunosuppression and urothelial injury but do not explain why BKPyV replication increases. We hypothesized that subtoxic genotoxic and immune-conditioning therapies reprogram host transcriptional and chromatin states, generating a permissive cellular environment that enhances BKPyV replication. Human bladder epithelial cells (HBLAKs) were exposed to clinically relevant IC10 doses of chemotherapeutic agents (etoposide, cisplatin, 5-fluorouracil), immune-conditioning drugs (cyclophosphamide, fludarabine, busulfan), or antivirals (ganciclovir, acyclovir, cidofovir) prior to BKPyV infection. Viral replication was quantified by qPCR, immunofluorescence, confocal microscopy, and RNA sequencing. Host transcriptional responses were assessed by bulk RNA-seq, while chromatin accessibility changes were profiled using ATAC-seq with integrated motif enrichment analysis. Organotypic urothelial raft cultures were used to validate findings in a stratified epithelial context. Long-read nanopore sequencing and short-read DNA sequencing were performed on cell lines and longitudinal urine samples from HC patients to assess viral structural variation and mutational signatures. Functional chromatin dependence was tested using SWI/SNF complex inhibitors (AU-15330, FHD-286). CUT&RUN assays were performed to evaluate transcription factor and chromatin regulator occupancy. Subtoxic genotoxic therapies consistently increased BKPyV replication across monolayer and organotypic models, with cisplatin and etoposide producing the strongest effects without inducing host cell-cycle arrest. This response is not broadly stress-driven, as neither heat shock nor paclitaxel elevated BKPyV replication. Viral RNA-seq revealed marked induction of late viral genes (VP1, VP2), consistent with productive replication rather than transcriptional noise. Host RNA-seq identified common differentially expressed genes across treatments enriched for chromatin organization and stress-response pathways, including regulators such as ARID1A and P300. ATAC-seq demonstrated increased chromatin accessibility at promoter-proximal regions, with significant enrichment of AP-1 motifs. Intersection of RNA-seq and ATAC-seq datasets highlighted coordinated transcriptional and chromatin remodeling programs. Importantly, neither long-read nor short-read sequencing detected recurrent viral structural variants or mutational signatures in vitro or in patient urine samples, indicating that replication enhancement is host-mediated rather than mutation-driven. Pharmacologic inhibition of SWI/SNF activity significantly reduced BKPyV replication across all treatment contexts, supporting a chromatin-dependent mechanism. CUT&RUN indicates increased host regulator occupancy at the BKPyV non-coding control region under therapeutic stress. Patient urine samples demonstrated increased BKPyV load following immune conditioning, reinforcing clinical relevance despite limitations on host genomic analysis due to consent constraints. This study provides the first mechanistic evidence that therapy-induced host chromatin reprogramming, rather than viral genetic change, underlies BKPyV reactivation and hyper-replication associated with hemorrhagic cystitis. By identifying chromatin accessibility as a central gatekeeper of viral activation and demonstrating pharmacologic suppression of BKPyV replication, our findings reveal actionable host pathways with translational relevance. These results support enhanced virologic surveillance in oncology patients and suggest new therapeutic strategies to mitigate BKPyV-associated morbidity without compromising anticancer efficacy.

35. Pleiotropic Cytokine Signaling Orchestrates Immune Plasticity by Synchronizing Hematopoietic Proliferation

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36. Antibody-based γδTCR signaling overcomes antigen heterogeneity and drives metabolic reprogramming in hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) remains a leading cause of cancer-related mortality worldwide, and the effectiveness of chimeric antigen receptor (CAR)-T cell therapy in solid tumors is limited by antigen heterogeneity, low antigen density, and T-cell exhaustion. Glypican-3 (GPC3), an oncofetal proteoglycan highly expressed in HCC, represents an attractive target for cellular immunotherapy; however, heterogeneous expression and antigen escape can restrict therapeutic durability. To address these challenges, we developed a glypican-3–targeted antibody-based γδ T cell receptor platform (γδAbTCR-T) that integrates antibody-derived antigen recognition with endogenous γδTCR-CD3 signaling and a CD30 regulatory module. We engineered multiple GPC3-targeted γδAbTCR constructs incorporating distinct antibody epitopes and compared them with conventional GPC3 CAR-T cells. Engineered T cells were evaluated using in vitro cytotoxicity assays, cytokine profiling, and sequential tumor-killing assays against GPC3-positive HCC cell lines, including variants expressing clinically observed GPC3 missense mutations. Antitumor efficacy and persistence were assessed in multiple xenograft mouse models, including intraperitoneal, orthotopic liver, and large established tumor models. Mechanistic analyses included signaling studies, immunohistochemistry of tumor-infiltrating lymphocytes, and bulk RNA sequencing of tumor-infiltrating T cells. Among the engineered receptors, the dual-epitope hYP7-hYP7 γδAbTCR construct demonstrated superior cytotoxic activity and cytokine production compared with conventional GPC3 CAR-T cells. γδAbTCR-T cells maintained potent activity across clinically observed GPC3 variants and effectively controlled tumors with reduced antigen density. In multiple HCC xenograft models, a single infusion of γδAbTCR-T cells induced rapid and durable tumor regression, including in large established tumors. Mechanistically, γδAbTCR signaling triggered rapid proximal activation followed by controlled signal attenuation, avoiding sustained tonic signaling typical of CAR constructs. Transcriptional profiling of tumor-infiltrating lymphocytes revealed enhanced effector activation accompanied by metabolic downshifting and reduced exhaustion signatures. Together, these findings demonstrate that γδAbTCR architecture enables robust antitumor activity while maintaining controlled T-cell activation dynamics. This study establishes antibody-guided γδTCR signaling as a promising strategy to overcome antigen heterogeneity and T-cell dysfunction in solid tumors. By integrating high-affinity antibody recognition with physiologic TCR signaling, γδAbTCR-T cells achieve enhanced antigen sensitivity, improved persistence, and durable tumor control. These findings provide a mechanistic framework for next-generation cellular immunotherapies targeting hepatocellular carcinoma and other antigen-heterogeneous solid malignancies.

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

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Gammaherpesviruses are oncogenic pathogens that establish lifelong infections and causes cancer. There are no FDA-approved vaccines against Epstein-Barr virus or Kaposi sarcoma-associated herpesvirus (KSHV). Murine gammaherpesvirus 68 (MHV68) is a well-defined system to study gammaherpesvirus pathogenesis. We previously generated a replication-dead virus (RDV-50.stopDelM1-M4) that does not express the essential replication and transactivator protein (RTA) encoded by ORF50, leading to limited viral gene expression upon de novo infection, and also lacks non-coding tRNA-miRNA-encoded RNAs 6, 7, 8 and unique M1-M4 genes that promote latency and reactivation of MHV68 in vivo. Prime-boost intraperitoneal vaccination of WT-C57BL/6 mice with RDV-50.stopDelM1-M4 elicited robust neutralizing antibodies, virus-specific T-cell responses and provided near-complete protection against viral replication and reactivation. Although highly attenuated, RDV-50.stopDelM1-M4 still established low-levels of latency. Therefore, we generated a latency-dead virus (LDV) that does not express the latency-associated nuclear antigen (LANA) encoded by ORF73. However, the LDV retains an intact functional RTA, which could pose safety concerns, particularly in immunocompromised individuals. To address these limitations, we developed a third-generation vaccine (RDV-LDV) which does not express RTA and LANA, to eliminate the capacity for viral replication and latency establishment. The LDV and RDV-LDV were both generated on the DelM1-M4 backbone, using BAC recombineering, followed by virus production in the codon-swapped ORF50 producer cell line. We hypothesize that incorporation of an ORF73-stop mutation to RDV-50.stopDelM1-M4 would not impair vaccine-induced protective immunity against MHV68. WT-C57BL/6 mice were prime-boost intraperitoneally vaccinated with RDV-50.stopDelM1-M4 or LDV-73.stopDelM1-M4 or RDV-LDVDelM1-M4 -M4. Virus-specific antibody responses in sera and T-cell responses in lungs and spleen, respective sites of acute replication and latency, were analyzed on 28 days post-boost (dpb). Mice were then intranasally challenged with WT-MHV68 at 28 dpb. Acute replication (lungs) and reactivation from latency (spleen) was analyzed at 7 and 16 days post-challenge (dpc), respectively. Prime-boost intraperitoneal vaccination with LDV and RDV-LDV elicited MHV68 and viral glycoprotein gH/gL specific antibody responses, and stimulated effector T-cell responses in lungs and spleens that were comparable to RDV-50.StopDelM1-M4 vaccination. Vaccinated mice exhibited no splenomegaly and no reactivation at 28 dpb. Following intranasal WT-MHV68 challenge, vaccinated mice exhibited near-complete abolishment of virus replication and reactivation at 7 and 16 dpc, respectively. These findings indicate that RDV-LDV is a highly attenuated yet immunogenic platform that protects against MHV68 infection while eliminating viral replication and latency potential. 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. Future studies will evaluate RDV-LDV in combination with glycoprotein subunit immunization to further enhance protective immunity and define immune correlates of protection by testing whether passive transfer of IgG or T-cells from vaccinated mice protects naïve mice following WT-challenge. Together, these studies will provide a promising strategy for developing a safe and effective gammaherpesvirus vaccine.

38. Modulation of lymphocyte function by Zabadinostat facilitates PD-1 in checkpoint-refractory tumors

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While the advent of checkpoint therapy has revolutionized the cancer immunotherapy, resistance is common. T cell dysregulation plays a role in both primary and acquired checkpoint resistance. Epigenetic modifiers have been hypothesized to increase therapeutic efficacy by modulating immune cell function. The combination of nivolumab and the HDAC inhibitor Zabadinostat showed promising clinical efficacy against microsatellite-stable colorectal cancer; however, no mechanistic data has been reported. Healthy-donor PBMCs, human and murine tumor cell lines exposed to Zabadinostat in vitro were analyzed by flow cytometry and bulk RNA sequencing for changes in immune cell frequency, phenotype, and functionality. To assess anti-tumor activity, mice bearing CT26, EMT6, and EMT6 Jak1-/- tumors were treated with Zabadinostat and/or PD-1. Immune cell phenotyping of tumors, draining lymph nodes (dLN), and spleens from EMT6 Jak1-/- tumor-bearing mice was performed by flow cytometry and antigen-specific responses were determined by ELISpot. Zabadinostat increased activation of NK, CD8+ and CD4+ T cells, while reducing the frequency and suppressive function of Treg in healthy donor PBMCs. CD8+ T cells treated with Zabadinostat also displayed increased IFN and granzyme B expression in response to TCR stimulation. These effects were associated with increased expression of transcriptional activation marks H3K9ac and H3K27ac in multiple immune subsets, and CD8+ and CD4+ T cells displayed increased stemness upon Zabadinostat exposure. Minimal changes to histone acetylation marks, gene or protein expression of immune recognition markers in human and/or murine tumor cell lines were observed in vitro, with modest increases in PDL1, ULBP3, and MIC A/B expression observed in human tumor xenografts. Transcriptomic analysis of murine tumor cell lines revealed upregulation of hallmark IFN and TNF signaling pathways. In multiple murine tumor models, the combination of Zabadinostat and PD-1 elicited strong anti-tumor responses with >50% tumor resolution. Analysis of checkpoint-refractory EMT6 JAK1-/- tumors demonstrated combination therapy increased cytotoxic CD8+ T cells in the tumor, and neoepitope-specific CD8+ T cell responses in the periphery. Similar to findings with human PBMCs, CD8+ T cells in the dLN and spleen of combination therapy treated mice displayed increased multifunctional T cell responses following ex vivo TCR stimulation. Furthermore, CD8+ T cells in the dLN showed a strong shift towards resident memory phenotype with Zabadinostat treatment.
Together these data show that Zabadinostat activates immune cells critical for anti-tumor responses and offers mechanistic insights into the potential of Zabadinostat in combination with PD-1 for the treatment of checkpoint-refractory tumors.

39. MYCN and SPPL3 modulate cell-state plasticity as a tumor-intrinsic mechanism of CAR T-cell resistance in neuroblastoma

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Chimeric antigen receptor (CAR) T-cell therapy has shown clinical efficacy in hematologic malignancies but remains largely ineffective in solid tumors, in part due to poorly defined tumor-intrinsic resistance mechanisms. Neuroblastoma is a pediatric malignancy characterized by two transcriptionally distinct adrenergic (ADRN) and mesenchymal (MES) cell states that may interconvert during therapy in a context-dependent manner to achieve resistance. To systematically identify genetic determinants of CAR T-cell resistance, we performed genome-wide CRISPR–Cas9 loss-of-function screens in three genetically and cell state diverse neuroblastoma cell lines, ADRN (IMR5 and SH-SY5Y), and MES-like (SK-N-FI), exposed to CAR T cells targeting GD2, B7-H3, or GPC2, using multiple donors. Shared hits across cell lines, CAR constructs, and donors were validated in a small secondary focused CRISPR library, revealing STAT1, STAT3, MYCN, and SPPL3 as recurrent tumor-intrinsic regulators of resistance. To interrogate the tumor intrinsic mechanisms of resistance, we studied changes in antigen levels, proliferation rate,

chemotherapy sensitivity, and transcriptional programs in single-KO cell lines compared to WT. In addition, we performed a 7-day co-culture killing assay of tumor cells and CAR T-cells and analyzed tumor cell killing curves from Incucyte, cytokine production, and single-cell suspensions of tumor and T cells phenotypes using a 30-parameter panel. Rule-based regression modeling integrating tumor-intrinsic features, antigen density, cytokine production, and cell-state metrics identified two dominant predictors of CAR T-cell–mediated killing: tumor antigen abundance and CAR T-cell Fas ligand (FasL) expression at day 3 post-co-culture. By analyzing tumor antigen abundance, we found that loss of MYCN or SPPL3 is associated with diminished surface GD2 expression and impaired CAR-dependent cytotoxicity. Reduced GD2 expression was correlated with reduced ADRN transcriptional signatures and cell line-dependent enrichment of MES-associated signaling. Notably, antigen modulation alone did not fully explain resistance. Hierarchical rule analysis demonstrated that conditions with low antigen density, including GPC2-targeted conditions, required robust FasL expression by the T cells for effective tumor elimination, suggesting a shift toward cytokine-driven death receptor–mediated cytotoxicity. Consistent with this model, tumor cells exhibited increased Fas expression following exposure to IFNγ and TNFα, and enhanced susceptibility to FasL-mediated apoptosis. However, the magnitude of Fas induction and death sensitivity varied across genetic backgrounds, indicating that lineage state influences responsiveness to inflammatory signaling. These findings suggest that CAR T-cell resistance in neuroblastoma is governed by antigen-dependent mechanisms determined by maintenance of the ADRN transcriptional program, and by antigen-independent resistance to death receptor signaling driven by cytokine production. Further study of the signaling mechanisms governing cell plasticity might identify actionable targets to increase sensitivity to CAR T-cell therapy in neuroblastoma and other solid tumors.

Cellular Physiology: Metabolism, Microbiology, & Cellular/Molecular Biology (Oral Abstracts)

40. Using advanced cellular imaging to characterize progressive organelle membrane assembly regulated by Rab-GTPases from discrete vesicular pathways

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The primary cilium is essential for normal development and homeostasis, and defects in its assembly (ciliogenesis) are associated with ciliopathy and cancer. Primary ciliogenesis is initiated by signaling regulated trafficking and docking small vesicles to the distal appendages (DAs) of the mother centriole (MC). These distal appendage vesicles (DAV) subsequently fuse into a larger ciliary vesicle (CV) that covers the end of the MC and ensheathes the microtubule-based axoneme as it grows. Several membrane trafficking regulator Rab small GTPases, including Rab11, Rab8 and Rab34, have been linked to ciliogenesis, and a Rab cascade functions to convert Rab11 membranes to a Rab8 ciliary compartment. Together these observations suggest ciliogenesis involves a complex multistep membrane assembly mechanism at the MC. To investigate this poorly understood mechanism further we employed FIB-SEM 3D volume electron microscopy (vEM) and identified novel intermediate membrane structures at the MC between the DAV and CV steps, including C-shapes and membrane toroids. We next examined the organization of vesicle trafficking at the MC we examined ciliogenesis impairment in RPE-1 cells depleted of Rab8 (RNAi) and Rab34 (CRISPR-Cas9 knockout) by FIB-SEM. We discovered that Rab8 functions at the C-shape while Rab34 is linked to the downstream CV stage. Using SIM2 and expansion super-resolution microscopy we determined that Rab11 and Rab34 localize independently to the MC at pre-CV stages in Rab8 depleted cells, and the ciliary membrane marker Smoothened colocalizes with Rab11 but not Rab34. Moreover, multicolor fluorescence imaging shows that Rab34 (Golgi-associated) and Rab11 (endosomal recycling compartment) occupy distinct, non-overlapping domains around the centrioles. These findings reveal that early stages of ciliogenesis are regulated by coordinated membrane trafficking to the MC from the different membrane compartments to assemble the ciliary membrane in a progressive fashion. Our work also suggests a critical role for membrane shaping and fusion regulators in ciliogenesis. To investigate ciliary membrane fusion requirements, we performed a functional screen of the 38 human SNAREs and identified a novel ciliogenesis SNARE complex. Ongoing work is aimed at defining how Rab11, 8 and 34 coordinates with SNAREs, and the membrane shaping EHD1 protein, in remodeling membranes during the earliest steps of ciliogenesis. Together, these findings integrate 3D ultrastructure and super-resolution compartment mapping to establish a mechanistic framework for membrane trafficking regulation of ciliogenesis. Notably, these observations provide the first direct evidence that different Rabs organize stepwise progression of ciliogenesis membrane assembly from discrete vesicular compartments.

41. Mapping the CDK2 substrate network in stem cells reveals a role in regulating DNA repair via HMCES phosphorylation

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Cancer cells frequently sustain abnormally high cyclin-dependent kinase 2 (CDK2) activity that accelerates S-phase entry and maintains rapid proliferation. Similarly, early embryonic development is characterized by exceptionally rapid cell division cycles along with persistently high activity of CDK2. Accelerated cycling is expected to cause substantial replication stress, yet embryonic stem cells (ESCs) maintain remarkably high fidelity of DNA replication and genome integrity, suggesting CDK2 signaling may be mechanistically coupled to protective DNA repair and damage-tolerance programs. How highly proliferative ESCs protect their genomes and how cell cycle regulation is mechanistically coupled with DNA repair remain open questions. Here, we hypothesized that sustained CDK2 activity confers previously unrecognized functions beyond cell cycle progression. To define the CDK2 signaling network, we expressed an analog-sensitive CDK2 in mouse ESCs (mESCs), enabling direct labeling of CDK2 substrates for

identification by mass spectrometry. This approach confirmed several known CDK2 substrates and uncovered a broader substrate network enriched for proteins involved in DNA replication and repair. Together, our findings highlight an underappreciated role for CDK2 in safeguarding genome stability. Among the newly identified CDK2 targets, we focused on HMCES (5-hydroxymethylcytosine binding, embryonic stem cell–specific protein), a conserved sensor of apurinic/apyrimidinic (AP) sites. AP sites are among the most common endogenous DNA lesions, arising frequently during base excision repair and posing a significant threat to replication fork integrity. Notably, disruption of HMCES can sensitize tumor cells to DNA-damaging therapies. Here, we show that HMCES is selectively phosphorylated by CDK2, but not by other major cell cycle CDKs, establishing clear substrate specificity within the CDK family. Furthermore, we mapped three residues in the C-terminal tail of HMCES that are phosphorylated by CDK2. Next, we showed that CDK2-mediated phosphorylation of HMCES regulates its DNA binding activity in vitro. Consistently, phosphorylation-deficient HMCES mutants displayed impaired function in cells, marked by a global increase in genomic AP site burden. These defects were accompanied by reduced cell viability following DNA damage, indicating compromised genome maintenance. Together, our results demonstrate that CDK2-dependent regulation of HMCES is essential for efficient AP site repair and cell survival. In summary, our work uncovers a previously unrecognized CDK2-driven genome surveillance pathway in embryonic stem cells. These findings further raise the possibility that in addition to ESCs, cancer cells could also use CDK2-dependent genome maintenance mechanisms that sustain rapid proliferation. Overall, our data reveals potential vulnerabilities at the intersection of cell cycle control and DNA repair to develop novel cancer therapeutics.

42. Heparan Sulfate Chains Control GPC3-Mediated Assembly of Wnt Receptor Complexes at the Nanoscale

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Glypican-3 (GPC3), a heparan sulfate proteoglycan highly expressed in hepatocellular carcinoma, promotes tumor progression through Wnt3a/β-catenin signaling, yet how its nanoscale organization regulates signaling initiation remains unclear. Using Nano-Resolution MINFLUX imaging, single-molecule tracking, and functional assays, we define the spatial architecture and dynamic behavior of GPC3 at the hepatoma cell surface. GPC3 exists as monomers and nanoscale clusters and exhibits mixed diffusion with transitions between confined and Brownian states. Heparan sulfate (HS) chains stabilize nanoscale corralling domains that restrict GPC3 mobility, whereas HS deletion increases diffusive heterogeneity and disrupts confinement. Wnt3a stimulation induces higher-order GPC3 assemblies and enhances β-catenin activation, while loss of HS markedly impairs signaling. MINFLUX DNA-PAINT further shows that HS chains promote nanoscale coupling of Wnt3a with Frizzled-1, a prerequisite for pathway activation. Together, these findings link membrane proteoglycan architecture to dynamic confinement and signaling competence in cancer cells.

43. WDR44 ciliopathy variants disrupt interdomain interactions important for regulating primary cilium assembly initiation

Haizhen Liu, Christine Kettenhofen (Insinna), Christopher Westlake

44. Exploiting Sphingolipid–Cholesterol Crosstalk as a Metabolic Vulnerability in IDH1-Mutant Glioma

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Metabolic cross-regulation between sphingolipids and cholesterol remains poorly understood in cancer. IDH1-mutant (IDH1mut) gliomas exhibit distinct metabolic vulnerabilities that may be therapeutically exploitable. We previously identified sphingolipid metabolism as a selective weakness in IDH1mut gliomas. Here, we hypothesized that increasing sphingosine levels through combined treatment with C17 sphingosine and N,N-Dimethylsphingosine (NDMS) which is a sphingosine analog and sphingosine kinase 1 inhibitor, would synergistically promote cell death and disrupt cholesterol homeostasis in IDH1mut glioma cells. We further hypothesized that sphingosine-driven lipid remodeling would alter cholesterol synthesis and efflux independently of changes in total sphingosine-1-phosphate (S1P). IDH1mut glioma cell lines and patient-derived glioma cells were treated with C17 sphingosine and NDMS alone or in combination. Drug synergy was assessed using dose–response matrices and viability assays. Comprehensive lipidomic profiling was performed to quantify sphingolipids and cholesterol species, including free cholesterol and cholesteryl esters. Transcriptomic analysis (RNA-seq) was conducted to identify altered metabolic pathways, followed by protein-level validation of key cholesterol regulatory markers using immunoblotting. C17 sphingosine and NDMS acted synergistically to induce significant apoptosis in IDH1mut glioma cells, including patient-derived models. Lipidomic analysis demonstrated marked disruption of sphingolipid metabolism, with accumulation of sphingosine species without a corresponding increase in total sphingosine-1-phosphate. Concomitantly, we observed significant remodeling of cholesterol pools, characterized by increased ceramides and cholesteryl esters and a pronounced decrease in free (unesterified) cholesterol. RNA-seq analysis identified cholesterol homeostasis as one of the most significantly altered pathways. Transcripts encoding cholesterol efflux transporters (ABCA1 and ABCG1) were upregulated, whereas transcripts encoding key enzymes in cholesterol biosynthesis (HMGCS1 and HMGCR) were downregulated. Protein validation confirmed suppression of the cholesterol synthesis program and activation of efflux pathways. These coordinated changes indicate that sphingosine accumulation drives cholesterol redistribution toward esterified storage while simultaneously promoting efflux and suppressing synthesis, resulting in depletion of the accessible free cholesterol pool. This lipid remodeling is associated with cell death in IDH1mut glioma cells.
This study reveals a previously unrecognized mechanistic link between sphingosine accumulation and cholesterol homeostasis remodeling in IDH1mut gliomas. Importantly, synergistic targeting of sphingolipid metabolism using C17 sphingosine and NDMS induces cell death while reshaping sterol regulatory pathways independently of total S1P changes. These findings identify coordinated sphingolipid–cholesterol rewiring as a therapeutically exploitable metabolic vulnerability in IDH1mut glioma and provide a mechanistic framework for targeting lipid metabolism in brain tumors.

45. Uncovering a novel membrane trafficking mechanism important for ciliogenesis in multiciliated cells regulated by the small GTPase Rab36

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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.

Data Science, Bioinformatics, Epidemiology, and Genomics

1. Establishing a Combinatorial Genetic Screening Platform to Uncover Cancer-specific Dependencies in Renal Medullary Carcinoma

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2. Harnessing AI and Multi-Omics for Precision Cancer Immunotherapy

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Immunotherapy has transformed cancer treatment but is frequently limited by unpredictable efficacy and immune-related adverse events (irAEs) that reflect dysregulated immune activation. In this talk, I will present a computational framework that leverages artificial intelligence and multi-omics data to jointly model tumor immunity and autoimmunity, enabling more precise and safer immunotherapy strategies. I will introduce LORIS, a machine-learning model that integrates genomic and clinical features to accurately predict responses to immune checkpoint blockade, outperforming conventional biomarkers, particularly in low–tumor mutational burden settings. To overcome the limitations of tumor biopsies, I will present an immune-focused liquid biopsy approach that applies machine learning to blood-based data to non-invasively infer tumor microenvironment states and predict both treatment response and severe irAEs. Finally, I will highlight mechanistic insights into neutrophil-mediated T-cell suppression that drive immunotherapy resistance, as well as AI-guided discovery of logic-gated CAR targets that improve the efficacy and safety of cell therapies. Together, these studies illustrate how computational biology can bridge cancer immunity and autoimmunity to advance programmable, precision medicine.

3. From Sketch to SBGN: An AI-Assisted and Interactive Workflow for Generating Pathway Maps

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The Systems Biology Graphical Notation (SBGN) provides standardized visual languages for representing complex biological processes, enabling clearer communication, reproducibility, and sharing of models in systems biology. However, creating high-quality SBGN maps from scratch is often difficult, especially for new users, due to the steep learning curve of specialized editors and the challenge of converting informal ideas or sketches into structured diagrams. We propose that an integrated workflow combining automated sketch conversion, flexible map merging and splitting, and layout refinement can significantly simplify the creation of structured SBGN maps while maintaining flexibility for advanced users. The study introduces a workflow for SBGN map creation and refinement that supports both the Process Description (PD) and Activity Flow (AF) languages. The workflow consists of three main steps. First, hand-drawn SBGN sketches are automatically converted into SBGN-ML format using large language models with in-context learning, with an interactive interface allowing quick correction of recognition errors and automatic mapping of biological identifiers through an external library. Second, the system enables flexible merging and splitting of maps: digitized maps can be combined with existing ones by identifying nodes and edges with shared attributes or

reorganized into smaller components while preserving the original layout. Third, layout refinement techniques are applied, including a user-guided layout algorithm that uses sketch-based hints to adjust network or subgraph arrangements and a polishing step that improves readability through orthogonal or diagonal edge alignment and role-based node organization (input, output, modifier). The proposed workflow provides an end-to-end solution for converting informal sketches into structured, publication-ready SBGN maps. By integrating automated sketch recognition, flexible map composition, and guided layout refinement, the system enables efficient creation, correction, and organization of biological network diagrams while maintaining the structural integrity and readability required for SBGN standards. This approach lowers the barrier to entry for researchers who are new to SBGN while still offering advanced control for experienced users. By streamlining the transition from informal sketches to standardized diagrams, the workflow improves accessibility, supports iterative model development, and facilitates broader adoption of SBGN for representing complex biological systems.

4. Integrated Bulk and Single-Cell Transcriptomics Reveal Hepatocyte-Like Reprogramming in Metastatic Small Intestinal Neuroendocrine Tumors

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Liver metastasis is the primary determinant of poor prognosis in small intestinal neuroendocrine tumors (siNETs), yet the molecular mechanisms enabling metastatic adaptation remain poorly understood. We hypothesized that siNET cells undergo tumor-intrinsic transcriptional reprogramming during metastasis that allows them to acquire liver-adaptive functional traits. To investigate this, we integrated bulk and single-cell transcriptomic analyses to dissect the cellular and molecular programs associated with siNET liver metastasis. We first analyzed bulk RNA-seq data from 81 siNET patient samples, including 44 primary tumors and 37 liver metastases. Differential expression and pathway enrichment analyses revealed extensive transcriptional remodeling during metastasis. Metastatic tumors displayed significant upregulation of metabolic and xenobiotic-processing pathways alongside downregulation of intestine-specific transcriptional programs, suggesting a phenotypic shift toward hepatic functional states. To assess whether these transcriptional signatures could be attributed to contamination from normal liver tissue, we evaluated tumor purity using the ESTIMATE algorithm. Metastatic samples consistently showed high tumor purity, indicating that the observed hepatic transcriptional programs predominantly reflect tumor-intrinsic alterations rather than contributions from surrounding liver tissue. To validate these observations at single-cell resolution, we analyzed paired primary and liver metastatic tumors from five siNET patients using single-cell RNA sequencing. Integrative clustering revealed heterogeneous tumor cell populations, including neuroendocrine (NE) tumor states and hepatocyte-like tumor states enriched in metastatic samples. Trajectory and pseudotime analyses demonstrated a continuous progression from primary tumor enriched NE clusters toward metastasis-enriched hepatocyte-like clusters, indicating gradual transcriptional reprogramming during metastatic progression. These hepatocyte-like tumor states exhibited activation of liver-associated metabolic and xenobiotic-processing pathways, consistent with functional adaptation to the hepatic microenvironment. Together, these results demonstrate that siNET liver metastasis is characterized by tumor-intrinsic transcriptional plasticity, enabling tumor cells to transition toward hepatocyte-like functional states that support survival and metabolic integration within the liver niche. By integrating bulk and single-cell transcriptomic analyses, this study provides new insights into the mechanisms underlying metastatic adaptation in neuroendocrine tumors and highlights tumor cell-state plasticity as a potential driver of liver metastasis. These findings establish a conceptual framework for understanding organ-specific adaptation during metastasis and may help guide the development of therapeutic strategies targeting liver-adaptive tumor programs.

5. Using Transcriptomic, Immunopeptidomic, and Multi-Omic Approaches to Develop Novel Immunotherapeutics for Ewing Sarcoma

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Ewing sarcoma (EWS) is an aggressive pediatric cancer with 5-year survival of 70-80% in localized disease, falling to 10-30% in relapsed/refractory cases. The protein products of EWSR1-ETS fusion oncogenes are the primary drivers of EWS within an otherwise quiet genome lacking recurrent druggable mutations, underscoring the need for new therapies. Hypothesis: EWS tumors present intracellular peptides on class I MHC molecules. We hypothesize that the deregulation of gene expression promoted by the EWSR1-ETS fusion oncoproteins results in tumor-specific antigens exploitable for vaccine and TCR therapies. To identify EWS-specific genes and neogenes promoted by the transcriptional activity of the EWSR1-ETS fusion oncoproteins, including noncanonical transcripts and splice variants, we integrated short- and long-read RNA-seq with immunopeptidomics. Short-read RNA-seq was performed on 79 tumors, 43 cell lines, and 135 normal organs. Differential expression testing identified genes overexpressed in EWS vs. normal organs using Mann–Whitney U test with BH correction (FDR <0.1) and biologic filters (log2FC >0, median EWS log2(TPM+1) >10, mean <1 TPM in vital organs). Immunopeptidomics was conducted using HLA-A*02:01::FLAG or W6/32 antibodies under ±IFN-γ conditions, followed by LC-MS/MS and spectral matching to UniProt and long-read–derived predicted proteomes (neogenes). Long-read RNA-seq (PacBio Kinnex) is being performed on 59 EWS tumors and 6 cell lines to detect alternative splicing, fusion transcripts, and novel transcripts. ORFs are predicted using TransDecoder, and integration with EWSR1-FLI1 ChIP-seq defines direct fusion targets. Short-read analysis identified 90 differentially expressed genes (DEGs). Immunopeptidomics across 16 samples detected 3,042 proteins, of which 23 overlapped with the 90 DEGs (p=0.01, one-tailed hypergeometric test). To date, 8 tumors and 4 cell lines have undergone long-read RNA-seq, yielding on average 344,762 ± 44,873 (mean ± SD) unique high-confidence transcripts per sample. The top DEG was LIPI (TPM 56.9, FDR 2.08E-38); 5 LIPI-derived peptides were identified across 4/16 samples, and a prominent EWSR1-FLI1 ChIP-seq peak at its promoter indicated direct fusion control. Additionally, 15 NR0B1 peptides, a direct EWSR1-FLI1 target, were detected in 6/16 samples. We identified 90 EWS-specific DEGs, including 11 membrane-associated candidates. LIPI and NR0B1 emerge as highly EWS-specific targets with minimal normal tissue expression. Integrating transcriptomics with immunopeptidomics nominates multiple candidates for therapy development.

6. Identifying metabolic factors associated with the incidence of brain metastasis in breast cancer using All of Us Research Program data

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Breast cancer brain metastasis (BCBM) is a devastating, often fatal disease. Approximately 15-30% of patients with metastatic breast cancer develop brain metastasis (BM) and have a median overall survival of 8-17 months, compared to ~31 months in those without BM. Limited therapeutic efficacy due to the restricted passage through the blood-brain barrier and blood-tumor-barrier underscores the importance of prevention efforts for BCBM. However, limited understanding of metastatic mechanisms and lack of biomarkers that may predispose patients to BM make it challenging to design preventive clinical trials. Thus, identifying patients who are at high risk of future BM is an unmet clinical need. Emerging evidence suggests that systemic metabolic conditions, such as metabolic syndrome, can promote chronic inflammation and alter organ microenvironments in ways that may facilitate metastatic colonization. We therefore hypothesize that specific metabolic factors are associated with a higher incidence of brain metastasis in breast cancer and can be leveraged to identify patients at elevated risk for future BM. All of Us Research Program longitudinal electronic health records data for an observational retrospective cohort study was used to investigate the

association between metabolic factors and BCBM incidence. Univariate logistic regression analysis was used to identify variables associated with BM incidence which were included for a multivariate logistic regression model. A total of 11,166 breast cancer patients were identified. The BM incidence for this cohort was 2.1%. In univariate analysis, younger age (<50yo; p<0.001), black race (p=0.028), and lower HDL levels (<50 mg/dL; p=0.031) were significantly associated with higher incidence of BM. In multivariate analysis including age, race, HDL levels, and obese status, which is our variable of interest, younger age (Odds Ratio [OR]: 0.36, 95% confidence interval [95%CI]: 0.26 – 0.55, p=<0.001) and HDL<50mg/dL (OR:1.47, 95%CI:1.01 – 2.12, p=0.039) remained significant factors associated with higher incidence of BM. In conclusion, our study demonstrated that younger age and HDL<50mg/dL are associated with high incidence of BCBM. To identify preventive targets for BM, we are developing clinically relevant animal models of metabolic syndrome with disseminated breast cancer cells to investigate how metabolic syndrome reshapes the brain microenvironment and promotes BM formation. This work aims to identify mechanisms that can be targeted to prevent BM and ultimately improve and prolong overall survival in breast cancer patients at high risk of developing BM.

7. Investigating the role of somatic chromosome mosaicism events in liver cancer

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Somatic chromosome mosaicism events are acquired mutation events that occur in non-germline cells, which can lead to cell dysfunction. Many of these mutations are found in the X and Y chromosomes. Previous research has shown that higher rates of chromosome mosaicism events may lead to an increased risk of developing cancers such as lung, testicular, and blood cancers. Since 2010, liver cancer rates have increased in the United States. Globally, liver cancer remains a prominent public health issue in Asia and Northern Africa. Interestingly, liver cancer predominantly affects the male population with an incidence rate at least three times higher than in the female population. Previous research has been unable to explain why males develop liver cancer at a higher rate than females. We hypothesize that somatic chromosomal mosaicism may explain the increased rates of liver cancer in the male population. To investigate somatic chromosome mosaicism events in hepatocellular carcinoma patients, we are utilizing a cohort of 2,649 patient samples (695 – hepatocellular carcinoma, 509 – high-risk, and 1,445 population control). These samples were obtained from previously conducted population studies. We performed genetic analyses on these samples to identify the type and location of the somatic chromosome mosaicism events. This study aims to focus on the difference in somatic mosaicism events between the hepatocellular carcinoma and population control cases. First, we are investigating a difference in the number of mutation events in the hepatocellular carcinoma cases versus the population control. We will then investigate the frequency of mutation events between male and female samples, particularly within the hepatocellular carcinoma cases. Finally, we will determine if there is any association between somatic mosaicism events and age or viral infection. This study will allow us to identify whether somatic chromosome mosaicism events are elevated in hepatocellular carcinoma, potentially suggesting that mosaicism events may play a role in the development of liver cancer.

8. Astrocyte–myeloid cell interplay may contribute to an immunosuppressive myeloid landscape in glioblastoma

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Glioblastoma (GBM) is the most aggressive primary malignant brain tumor in adults and has a poor prognosis. GBM treatment using immunotherapy has not been successful. One possible reason for the poor response to immunotherapy in GBM is its immunosuppressive microenvironment. GBM is characterized by a high abundance of immunosuppressive myeloid cells, comprising up to 30 to 50 percent of the total tumor cells. The factors driving immunosuppressive myeloid cells are unclear. Astrocytes are glial cells that play a homeostatic role in the healthy human brain. However, in pathological conditions, astrocytes become reactive and can adopt a neurotoxic or neuroprotective phenotype. In brain metastasis, tumor-associated astrocytes (TAAs) modulate immune cells. However, their role in modulating immune cells within the GBM tumor microenvironment is not well characterized. In this study, we investigated potential roles of TAAs in modulating the immune cells in GBM. We applied a multi-omics analysis approach to investigate the role of TAAs in GBM. Correlative analyses were performed to establish associations between TAAs and myeloid cells. Potential interactions between TAAs and immune cells were investigated by cell-cell communication analyses. Spatial relationships between cell types were investigated using multiplex tissue imaging. We found that TAAs in GBM acquired a neurotoxic phenotype, showing high transcriptomic expression of established neurotoxic astrocyte markers FKBP5, C3, and C5. In correlation analysis, proinflammatory microglia with high expression of IL1A, TNF, and C1QA/B/C were correlated with TAAs. IL-1α, TNF, and C1q are known to induce neurotoxic reactive astrocytes; therefore, proinflammatory microglia may induce TAAs with a neurotoxic phenotype in GBM. These TAAs showed high expression of TGFB2, which induces a scavenger-suppressive myeloid cell program characterized by high expression of scavenger receptor genes. We found an anti-inflammatory macrophage population with the scavenger-suppressive program that showed a strong correlation with TAAs. TGF-B2 secreted by TAAs could be associated with the induction of anti-inflammatory macrophages with the scavenger-suppressive program. This was further supported by cell-cell communication analysis, revealing a significant probability of interaction between TAAs and myeloid cells via TGF-B2 signaling. Spatial analysis showed that the proinflammatory microglia and anti-inflammatory macrophages correlated with TAAs and were enriched in the cellular tumor region. In spatial proteomics analysis, tumor-associated neutrophils (TANs) also correlated with TAAs. Spatial analysis further showed that these myeloid cells were in proximity to TAAs, suggesting potential cell–cell interactions. This study suggests a paradoxical role for proinflammatory microglia, whereby IL-1α, TNF, and C1q secreted by these cells may drive neurotoxic TAAs to express TGF-B2. In turn, TGF-B2-expressing TAAs may promote the induction of anti-inflammatory macrophages exhibiting a scavenger-suppressive program. Collectively, these findings suggest a dynamic astrocyte–myeloid cell interaction that contributes to immune suppression in GBM and highlight TGF-B2 signaling as a potential therapeutic target.

Immunology & Virology (Posters)

9. Dosing-Dependent Effects of Post-Transplantation Cyclophosphamide (PTCy) on Human Early Immune Responses: Insights from Single-Cell Analysis

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Persisted despite PTCy; interestingly, given the HLA-haploidentical setting, alloreactive T cells commonly derived from memory cells in the donor. T-cell receptor diversity was more clonal at day +21 after Set 1 vs. Sets 2/3. Pathway analysis showed donor CD4+ and CD8+ T cells, natural killer cells, and monocytes had less DNA damage at days +5/+7 in Sets 2/3 vs. Set 1, an effect also seen within alloreactive T cells. At day +21, CD4+ T cells in Sets 2 and 3 had increased Th17 differentiation compared with Set 1, and Set 2 also had increased type I interferon signaling vs. Set 1. Donor CD8+ T cells and alloreactive CD8+ T cells within Sets 2/3 at days +5/+7/+21 had increases in pathways related both to GVHD but also responses to pathogens when compared to Set 1. Donor monocytes had increased interleukin-10 signaling at day +21 in Set 2 compared with Set 1. Cell chat analyses showed different patterns of cell-cell communications between Sets 2/3 and Set 1, particularly between donor and recipient T cells at day +5/+7 and between donor monocytes and T cells at day +21. Gene correlation network analysis across Sets and timepoints showed consistent associations at days +3, +5, +7, and +21 with non-relapse mortality. Conclusions: Reducing PTCy dosing results in less DNA damage, increased CD8+ T-cell pathways of GVHD and pathogen response, and modulations in cell-cell interactions. Even so, these effects did not increase clinical GVHD, even as they decreased infection. Gene modules were predictive of non-relapse mortality at day +3, suggesting that, irrespective of the PTCy dose, some patients already may be immunologically set up for failure very early post-HCT.

10. Age dependent collective trafficking of tissue resident T cells in zebrafish

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11. Vaginal tissue accumulation of the zinc finger protein inhibitor SAMT-247 Met-D drives vaccine-induced protective anti-inflammatory mucosal immunity against SIVmac251

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Topical administration of SAMT-247, a mercaptobenzamide thioester prodrug and zinc finger protein inhibitor, results in rapid metabolism into multiple metabolites (Met-A-D) and SAMT-247 inhibits HIV/SIV infectivity in vitro by targeting the Gag nucleocapsid. In vivo, delivery of SAMT-247 as either a vaginal gel or via an intravaginal ring (IVR) synergizes with the ΔV1DNA/ALVAC-SIV/ΔV1gp120/alum vaccine, markedly reducing the risk of vaginal SIVmac251 acquisition in vaccinated female macaques. This protection is associated with modulation of vaccine-induced protective innate and adaptive immune responses at mucosal sites. To define the mucosal distribution of SAMT-247 metabolites,

vaginal secretions and tissues were analyzed four hours after SAMT-247 gel administration in six macaques. High concentrations of Met-A, Met-B, and Met-C were detected in vaginal secretions, whereas Met-B and Met-C were not detectable in vaginal tissues using current analytical methods. In contrast, tissue samples contained low levels of Met-A and high levels of Met-D, suggesting differential metabolite penetration or retention within the mucosa. Ex vivo analyses of macaque mucosal tissues demonstrated that SAMT-247 modulates vaccine-induced innate immune signatures previously associated with protection. However, the immunological contributions of individual SAMT-247 metabolites to these responses remained undefined. To address this, we assessed ex vivo mucosal immune responses in ΔV1DNA/ALVAC-SIV/ΔV1gp120/alum-vaccinated macaques following in vitro exposure to distinct SAMT-247 metabolites. Met-A, which lacks virucidal activity, consistently dampened vaccine-associated protective immune signatures, including reductions in IL-17$^+$NKp44$^+$ ILCs and CD107$^+$NKG2A$^+$ NK cells linked to protection in vivo. Met-A exposure also reduced CD73$^+$ and IL-10$^+$ dendritic cells, increased TNF-α+ dendritic cells, and promoted a pro-inflammatory monocyte phenotype characterized by reduced CD73 and IL-10 expression. In contrast, the virucidal metabolites Met-D and Met-C preserved or enhanced SAMT-247 associated immune signatures. Met-D maintained or increased protective IL-17$^+$NKp44$^+$ ILCs and CD107$^+$NKG2A$^+$ NK cells and selectively expanded IL-10$^+$ dendritic cells and IL-10$^+$ monocyte subsets, consistent with an anti-inflammatory mucosal environment. Similarly, Met-C increased IL-10$^+$ dendritic cells and IL-10$^+$ monocytes. Collectively, these findings demonstrate metabolite-specific immunomodulatory effects of SAMT-247 and suggest that enrichment of Met-D in vaginal tissues contributes to the enhanced vaccine protection observed in vivo.

12. Epigenetic modulation synergizes with a novel TCR -targeted IL-2 to yield CD8+ T cell dependent tumor control in checkpoint-refractory tumors irrespective of MHC I status

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13. Modeling Human Immune Competent Patient-Derived Metastatic Prostate Cancer in Vivo

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As the most common non-cutaneous form of cancer in males, metastatic prostate cancer is expected to claim the lives of approximately 35,000 Americans in 2025. Notably, the rate of cancer death is highly dependent upon the site of metastasis, and thus the organ site directly affected by this hormone dependent cancer. Despite its prevalence, clinically relevant models recapitulating the histological, phenotypic, and molecular diversity of metastases are scarce and are often done in immune compromised systems. This prevents a detailed evaluation of potential immune effects which is especially relevant in the context of hormone deprivation treatments which may modify immunity. We have humanized NSG immunocompromised mice that express human Stem Cell Growth Factor, human M-CSF and human IL-3 that have been engrafted with human CD34+ cells (NSG-SGM3). Using both an androgen receptor (AR)-positive adenocarcinoma (PDM136) and a neuroendocrine (NE) prostate cancer (PDMLym1) model, we assessed metastatic properties by inoculating each PDX, via intracardiac injection. Immune engraftment and competence was determined via flow cytometry and response to ovalbumin challenge. Growth of tumors was monitored via bioluminescent imaging of a luciferase transgene expressed in each cell line. Metastatic development was noted in multiple organs. Androgen deprivation was performed before systemic tumor burden exceeded 108 radiance units. We demonstrate successful adoption of human immune cells in the murine model and the successful growth of tumors at distant metastatic sites. We compared these metastatic tumors to immunocompromised controls, noting differences in human macrophage populations within immunogenic metastatic bone niches before and after removal of testosterone. Given the influence of hormones on immune response in prostate tumors, this immunogenic response varies based on tumor location and hormone exposure. These data provide insight into how we approach combination therapies involving hormone modulation and immune therapies, such as checkpoint inhibition and cytokine traps.

14. The RNA dependent E3 ubiquitin ligase ZNFX1 acts as a Negative Regulator of Innate Immune Response in HLRCC Tumor Cells

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Cancer cells are notorious for escaping recognition by the immune cells, allowing them to survive and metastasize. In this project, we aimed to investigate the specific involvement of RNA modification related genes in shaping innate immune response, by modulating the interaction between tumor cells and Natural Killer (NK) cells, a type of innate lymphoid cells, that are localized to peripheral blood and are known to infiltrate solid tumors. Our model of study is Hereditary leiomyomatosis and renal cell carcinoma (HLRCC). Affected individuals with this autosomal dominant condition, characterized by a mutation in the fumarate hydratase (FH) gene, are at a high risk for developing highly aggressive kidney cancer. Loss of FH activity leads to the disruption of mitochondrial integrity, and the release of mitochondrial contents into the cytoplasm, subsequently activating the innate immune response. We performed a targeted pooled CRISPR-Cas9 screen to identify RNA regulatory pathways that protect target cells in a co-culture with NK-92, an immortalized NK cell line. Our top hit was NFX1-type zinc finger-containing protein 1 (ZNFX1), an interferon stimulated gene, previously described to regulate dsRNA sensing pathways. Human patients with bi-allelic pathogenic mutations in this gene are susceptible to infection and have early onset inflammatory disease leading to organ failure. We found that knocking out ZNFX1, sensitized HLRCC cells to NK cell mediated cytotoxicity and triggers of innate immunity such as dsRNA. To elucidate how ZNFX1 protects HLRCC cells from NK cell–mediated cytotoxicity, we performed single-cell RNA sequencing during NK–tumor cell co-culture. By comparing gene expression changes in both cancer cells and NK cells, we observed that ZNFX1 KOs exhibited upregulation of the interferon signaling pathway, leading to an enhanced innate immune response. These data suggest that ZNFX1 functions as a negative regulator of innate immune signaling in HLRCC. To delineate how ZNFX1 interacts with innate immunity pathways we targeted for silencing critical genes in this response. Our data shows that the hypersensitivity of ZNFX1 KO cells is dependent of IFN gamma receptor and dsRNA sensor PKR, suggesting that release of IFN gamma by the NK cells sensitizes the target cell for cytotoxicity in a PKR dependent manner when ZNFX1 is not expressed. To further dissect the molecular mechanism, we are conducting CLIP-seq and proteomic analyses to define ZNFX1 interaction partners and downstream regulatory networks. Preliminary CLIP-seq data indicate that, among other targets, mitochondrial transcripts are bound by ZNFX1. These transcripts most likely originate from mitochondrial dsRNAs that are released into the cytosol as a consequence of mitochondrial dysfunction. In parallel, proteomic analysis identified PKR as one of the binding partners of ZNFX1. We hypothesize that ZNFX1 plays a critical role in maintaining mitochondrial dsRNA homeostasis and regulating interferon signaling. Loss of ZNFX1 disrupts this balance, leading to aberrant activation of innate immune pathways and rendering HLRCC tumor cells hypersensitive to innate immune–related stress. Investigating the role of ZNFX1 in mechanisms of immune evasion, will help improve the efficacy of cancer immunotherapies, as well as in developing new therapeutic targets.

15. Rare Variants in DIS3L2 Implicate RNA surveillance in the Pathogenesis of Systemic Lupus Erythematosus

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Systemic lupus erythematosus (SLE) is a complex systemic autoimmune disease characterized by multi-organ inflammation and elevated Type I interferons. While SLE pathogenesis is incompletely understood, inappropriate nucleic acid sensing is known to be important in upregulating interferons. In analyzing genomic data, we have discovered that multiple children and adults with SLE carry rare exonic variants in DIS3L2, a gene that has not been previously associated with immune-mediated diseases. DIS3L2 encodes a ubiquitously expressed exoribonuclease that is important in cytosolic RNA surveillance; it degrades aberrant structured non-coding RNAs (ncRNAs) modified by non-templated 3′ oligouridine tails. We recently reported that depletion of DIS3L2 leads to upregulation of Type I Interferon-stimulated genes (ISGs) in a mouse macrophage cell line (RAW264.7). To determine whether these novel

rare variants in SLE patients functionally impact RNA surveillance and downstream interferon activation, we introduced patient-derived DIS3L2 variants into an expression vector using site-directed mutagenesis and are transfecting the plasmids into ISG reporter cell lines. This will help determine whether the rare variants found in patients contribute to interferon activation. Establishing a role for DIS3L2 in SLE will provide novel mechanistic insights into disease pathogenesis and could inform the development of novel therapeutics aimed at mitigating dysfunction in RNA surveillance in interferon-based immune diseases.

16. Single-cell profiling identifies unique peripheral blood immune signatures following extracellular matrix scaffold-assisted cancer vaccine delivery with long-term immune memory

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Implantable biomaterials have advanced cancer care by enabling targeted and sustained drug delivery and promoting tissue regeneration and recovery after surgical tumor resection. Extracellular matrix (ECM) scaffolds derived from decellularized mammalian tissues are clinically used to repair damaged tissues following tumor resection. These scaffolds initiate a Type 2 immune response driven by the cytokine IL-4, promoting scaffold remodeling and tissue regeneration. Previously, we demonstrated that integrating ECM scaffolds with Type 1 immune adjuvant CDA recruited substantial antigen-presenting cells at the implant site, without suppressing the IL-4-driven Type 2 immune signature of SIS-ECM. Therapeutically, the SIS-CDA vaccine showed a complete response in 60-75% of mice in the E.G7-OVA lymphoma tumor model, via CD8+ T cells. In contrast, the bolus vaccine (without ECM scaffold) showed no anticancer efficacy. We hypothesized that the SIS-CDA vaccine induces an early innate immune response that potentiates an effective CD8+ T cell-mediated response at later timepoints. Porcine small intestine submucosa (SIS) was decellularized, lyophilized, and cryomilled into particles. Sterile SIS-ECM was hydrated with CDA and ovalbumin (OVA) to form the SIS-CDA-OVA vaccine. E.G7-OVA tumors were subcutaneously implanted in C57BL/6 mice. Once tumors reached around 75mm3, mice were randomized to 1) Untreated Control, 2) Saline-CDA-OVA (without SIS), and 3) SIS-CDA-OVA vaccine. Peripheral blood was collected on Days 3 and 13 post vaccination to assess immune markers associated with early and late vaccine responses. Single-cell RNA sequencing was performed using 10X Chromium Next GEM Single-cell 5' platform for Gene expression (GEX), and TCR and BCR repertoire analysis. Data from three groups at two time points were normalized, subjected to dimensionality reduction, and clustering in R using the Seurat package, followed by DEG analysis and BCR/TCR clonality analysis. Single-cell RNA sequencing identified 37 immune cell clusters spanning myeloid and lymphoid populations. A strong Type 2 immune profile was observed exclusively in SIS vaccinated groups, consistent with our previous RT-PCR findings from draining lymph nodes. At Day 3, differential gene expression analysis showed that SIS-CDA elicited a robust early innate response marked by upregulation of the NF-kB pathway (Nfkbia, Nfkbid), enhanced antigen presentation (H2-Q4), and early TCR signaling (Nr4a1, Ier2). This early response led to a strong immune metabolic reprogramming response on day 13, evident by upregulation of mitochondrial gene (mt-Nd1/2/3/4/5), indicating increased metabolic activity of immune cells with a shift towards oxidative phosphorylation (mt-Atp6, mt-Cytb, mt-Co2/3) during the adaptive immune response. TCR repertoire analysis on the CD8+ T cell subset revealed that SIS-CDA induced strong antigen-specific CD8+ T cell clonal expansion with focused TCR clonotypes on Day 13, consistent with the previously observed CD8+ T cell-mediated effector response.In conclusion, the SIS-CDA vaccine induces an early coordinated immune response characterized by NF-kB-driven innate response that transitioned later into robust antigen-specific CD8+ T cell clonal expansion with durable memory formation. ECM scaffolds function as an effective immunomodulatory platform that enhances cancer immunotherapy while preserving tissue-regenerative Type 2 immune characteristics, supporting their clinical potential for local immunotherapy.

17. IL-13 levels are elevated in floxuridine-treated patient plasma and ex vivo cystic duct perfusates

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Hepatic artery infusion pump floxuridine is an increasingly employed management strategy for patients with liver-limited colorectal liver metastases. However, floxuridine results in biliary injury, which limits the amount of drug administered and thereby its efficacy in order to avoid biliary sclerosis. To mitigate the injury, dexamethasone (a potent immunosuppressant) is administered concomitantly with floxuridine, although the immune pathways activated by the injury are unstudied. Serum samples were collected from 9 patients undergoing hepatic artery infusion pump therapy at baseline and following two weeks of floxuridine/dexamethasone treatment (no systemic therapy). Autologous plasma perfusate was collected following 96 hours of ex vivo perfusion of cystic duct (n=1) treated with floxuridine. All samples were evaluated with the Olink Target 96 Immuno-Oncology panel. Following 96 hours of floxuridine treatment, a significant increase (≥100% change) in IL-13 levels was observed in perfusate of ex vivo cystic duct compared to control perfusates. IL-10 demonstrated similar elevation (≥100% change). Correlation with patient serum demonstrated significant elevation of IL-13 levels following two weeks of floxuridine/dexamethasone treatment and significantly decreased levels of IL-6. Further investigation demonstrated no correlation of IL-13 levels with liver function test levels (alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, direct and total bilirubin) during this time frame.Our data suggests that IL-13, a well-established promoter of hepatic fibrosis, may play a role in biliary injury following long-term (multiple cycles) floxuridine treatment. Further investigation of IL-13 throughout the treatment course may aid in the development of mitigating therapies.

18. Non-Viral Site-Directed hYP218 CAR T Cells for Mesothelin-Positive Solid Tumors

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19. ICOS supports the IL-2-independent survival and effector function of small intestine intraepithelial Foxp3+ Treg cells

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Foxp3+ T regulatory cells (Tregs) correspond to a subset of CD4 T cells that play a critical role in suppressing the autoimmune effector functions of the immune system. Tregs are generated in the thymus upon strong TCR signaling in the presence of IL-2, and then exported to the periphery, where they inhibit the activation of effector cells through direct cell-cell contact or by producing immunosuppressive molecules, such as IL-10. Unlike conventional T cells, which depend on the cytokine IL-7, Tregs require IL-2 for their maturation and survival. Notably, Tregs in the small intestinal (SI) epithelium express very low levels of the IL-2 receptor alpha-chain (CD25), indicating that IEL Treg cells would survive and undergo homeostasis without IL-2 signaling. Consequently, SI epithelial Tregs must rely on alternative pathways for their maintenance. Based on high-throughput LEGENDScreen surface protein analysis of IEL Treg cells, we identified ICOS as a promising candidate to promote Treg cell survival in the absence of IL-2 signaling. Using genetically engineered mouse models, here, we demonstrate that IL-2 ablation leads to a significant increase in ICOS expression in Treg cells. Finally, using ICOS-deficient and ICOS/IL-2 double-deficient mice, we demonstrate that ICOS deletion impairs Treg cell survival and maintenance in the SI. These findings not only reveal the role for IL-2 signaling in regulating ICOS expression but further suggest that ICOS plays a major role in regulating Treg survival and function in the SI epithelium.

20. Drug-regulatable, inducible, and membrane-bound interleukin 12 for controlled expression in adoptive cancer cell therapies

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Interleukin-12 (IL-12) is a proinflammatory cytokine that can potentiate adoptive cell therapies (ACT) for cancer. However, its systemic toxicities have limited clinical use. Here, we present drug-regulatable, inducible, and membrane-bound IL-12 (DRIM-IL-12) designed for ACT. The lenalidomide-responsive degron and the NFAT-inducible system achieved tight regulation of DRIM-IL-12 expression. Membrane-anchoring of IL-12 minimized systemic release and was essential for lenalidomide-induced degradation. Functionally, DRIM-IL-12 improved ACT efficacy across multiple settings, including TCRs targeting p53 or RAS neoantigens, melanoma-infiltrating lymphocytes, and the CD19-chimeric antigen receptor against CD19-low leukemia cells. In mice, ACT with DRIM-IL-12 and mutant p53 or KRAS-targeting TCRs caused significant tumor regression and improved survival. Interestingly, our data revealed that uncontrolled DRIM-IL-12 expression upregulated TIGIT and other exhaustion-related genes. Treatment with low-dose lenalidomide, which achieved 20-40% of maximal DRIM-IL-12 expression, reversed T-cell exhaustion, enhancing DRIM-IL-12's efficacy. These findings support further clinical evaluation of DRIM-IL-12 for safety and efficacy.

21. Methyl quercetin attenuates radiation-induced cellular senescence and macrophage polarization through pSMADs/TGF-β signaling.

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Quercetin, a plant-based flavonoid, has been investigated for its therapeutic potential in fibrotic diseases. In our previous study, continuous oral administration of quercetin for 150 days attenuated radiation-induced dermal fibrosis in C3H/HeN mice exposed to 35 Gy irradiation. Notably, following oral administration, quercetin underwent extensive metabolism and was predominantly detected as methylated metabolites in plasma, liver, and urine. Building on these findings, the present study examined whether methylated quercetin (MQ) exerts biological effects that mitigate radiation-induced skin fibrosis. In particular, we focused on radiation-induced cellular senescence and macrophage polarization, using both in vivo skin tissue and in vitro NIH/3T3 fibroblasts. Skin tissue was harvested at 150 days post-irradiation from C3H/HeN mice exposed to 0 Gy or 35 Gy and fed either quercetin-formulated chow or control chow. NIH/3T3 fibroblasts were exposed to 0 Gy or 17.5 Gy irradiation in the presence of 3-O-methylquercetin (MQ) or vehicle.In skin tissue irradiated with 35 Gy, mice fed quercetin chow exhibited reduced epidermal thickness, reduced vimentin expression, and decreased numbers of cells positive for the senescence markers p21 and p16, compared with mice fed control chow. NIH/3T3 fibroblasts exposed to 17.5 Gy showed increased cellular senescence 5 days after irradiation, as assessed by SA-β-gal (X-gal) staining and p21 expression. Treatment with MQ attenuated radiation-induced cellular senescence, and reduced p21 expression. The expression of TGF-β1 and Pai-1, two senescence-associated secretory phenotype (SASP) factors, was significantly increased in the conditioned media from irradiated NIH/3T3 fibroblasts, but decreased with MQ pretreatment. Additionally, pretreatment of NIH/3T3 fibroblasts with MQ prior to TGF- β treatment reduced cell proliferation and vimentin expression through canonical pSMADs/TGF-β signaling. In 35 Gy-irradiated skin, the numbers of CD206- and F4/80- positive macrophages were elevated in the dermis of mice fed control chow, whereas they were reduced in mice fed quercetin chow. Similarly, CM collected from 17.5 Gy- irradiated fibroblasts induced M2 polarization in the Raw264.7 macrophage cell line, whereas CM from MQ pretreated, 17.5 Gy-irradiated fibroblasts exhibited attenuated M2 polarization, as determined by qPCR. Collectively, these findings suggest that MQ act as a senomorphic agent by suppressing SASP expression, thereby attenuating radiation-induced cellular senescence and macrophage polarization and ultimately protecting against radiation-induced skin fibrosis.

22. Bit by Bit: Uncovering the regulators of monocyte-tumor trogocytosis.

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23. CAR T Cells Targeting the gamma-delta TCR Constant Region Eradicate gamma-delta T Cell Malignancies

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Chimeric antigen receptor (CAR) T cell therapy is an immunotherapy in which patient T cells are genetically modified to express synthetic receptors that redirect cytotoxicity toward cancer cells. This strategy has transformed the treatment of B-cell malignancies by enabling potent tumor elimination. However, no tumor-specific targeted therapies exist for gamma-delta T cell malignancies, which are rare but aggressive cancers associated with high relapse rates and poor survival. As these malignancies arise from gamma-delta T cells, the gamma-delta T cell receptor (TCR) represents a lineage-restricted antigen uniquely expressed on gamma-delta T cells and absent from other cells, providing a rational and potentially safe therapeutic target. We hypothesized that CAR T cells directed against the gamma-delta TCR constant region could selectively eradicate gamma-delta T cell malignancies. We engineered a novel CAR incorporating a binding domain derived from an antibody against the constant region of the gamma-delta TCR. We systematically optimized the antigen-binding, hinge, transmembrane, and intracellular signaling domains of the CAR to produce the optimized construct, MGDL-28Z. MGDL-28Z CAR T cells specifically recognized gamma-delta TCR–expressing leukemia cell lines (Loucy, BE-13, and MOLT-13), producing IFN-γ and IL-2 and mediating potent cytotoxicity in vitro while sparing gamma-delta TCR-negative cells. In two disseminated xenograft models of MOLT-13 and BE-13, MGDL-28Z induced dose-dependent tumor regression, significantly reduced tumor burden, and prolonged survival compared to a control CAR. These findings establish targeting of the gamma-delta TCR constant region as a novel immunotherapeutic strategy and provide strong preclinical rationale for clinical translation of MGDL-28Z CAR T cells.

24. FAM111A-knockdown restores host range in SV40 large T mutants

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25. Modulation of Vaginal Microbiota to Prevent HIV Infection in Females

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In 2022, women accounted for approximately 19% new HIV diagnoses in the United States. Currently, there are no licensed vaccines or topical microbicides proven to effectively reduce HIV acquisition risk, and the efficacy of pre-exposure prophylaxis (PrEP) remains less well established in women compared to men. A vaccine regimen that partially reduced HIV acquisition was evaluated in humans in the RV144 trial and we have improved this platform in a non-human primate model removing SIV V1 env region from the DNA and the gp120 components resulting in a V1-

DNA/ALVAC/V1gp120/alum. We also have demonstrated that topical administration of S-acrylic-2-mercaptobenzamide thioester-247 (SAMT-247) gel synergized with vaccination, resulting in a vaccine efficacy of 92.7%, with 80% of rhesus macaques remaining uninfected following repeated SIV challenges. SAMT-247 microbicide is a small-molecule targeting HIV-1 nucleocapsid, with potent antiviral activity and immunomodulatory properties. Building upon these findings, we hypothesize that combining vaccination with sustained delivery of SAMT-247 via an intravaginal ring (IVR) will provide direct antiviral activity and favorably modulate the vaginal microenvironment, including the local bacterial flora, enhancing protection against HIV acquisition. In this project, we aim at to evaluate: (1) how the V1-DNA/ALVAC/V1gp120/alum anti-SIV vaccine regimen affects the vaginal microbiota in the rhesus macaques model of HIV infection, (2) how the V1-DNA/ALVAC/V1gp120/alum anti-SIV regimen in combination with SAMT-247 microbicide delivered by IVRs influences the vaginal microbiota in macaques, and (3) how the continuous delivery of SAMT-247 microbicide by intravaginal rings alone impacts the vaginal microbiota of naive macaques compared to empty IVR. The study aims to identify the types of microbiomes present in the vaginal mucosa of non-human primates, how these relate to vaccine efficacy and how they affect the levels of mucosal cells and cytokines during inflammatory responses. Preliminary results have suggested that SAMT-247-IVRs can influence the mucosal microbiome environment while providing drug-based protection. Overall, analyses have shown that SAMT-247 IVRs administered in vaccinated animals significantly changed the overall microbiome taxa. Specifically, 64 least known taxons (LKTs) increased and 12 decreased 2 weeks post-insertion when compared to before insertion. Further analyses will be done to investigate single microbes that may be affected by vaccination, as well as the products produced by the whole microbiota. In addition, correlative studies between the microbiome and the immune responses will be conducted to assess the microbiome's effect on the host immune system. Developing IVRs that deliver microbicides offers an effective approach for women to prevent HIV. Unlike gels or oral PrEP, IVRs release antiretroviral drugs or microbicides steadily and directly into the vagina. This method enhances adherence and effectively protects the primary site of HIV transmission. This strategy is vital for women in regions where HIV rates remain high and social and structural barriers limit prevention options. By providing a discreet, long-lasting, and user-controlled protection, the microbicide-releasing vaginal ring addresses the gaps left by current PrEP methods and directly promotes women's reproductive independence.

26. Propagation and Characterization of Low-Passage Primary Effusion Lymphoma Patient-Derived Xenograft Cell Lines

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Primary effusion lymphoma (PEL) is a rare, aggressive B-cell malignancy universally infected with Kaposi sarcoma herpesvirus (KSHV) and often coinfected with Epstein–Barr virus (EBV). PEL occurs predominantly in people living with HIV and other immunocompromised individuals. Despite therapeutic advances, chemotherapy-resistant disease remains associated with poor prognosis. Targeted monoclonal antibody therapies, including anti-CD38 and cereblon modulatory agents, are under investigation; however, most preclinical studies rely on long-established cell lines that may not reflect the heterogeneity of patient tumors. We hypothesized that low-passage patient-derived xenograft (PDX) models of PEL could preserve key molecular and phenotypic features of primary disease and provide a scalable platform for therapeutic testing and single-cell characterization. Primary PEL samples were obtained from five HIV-positive patient volunteers diagnosed with KSHV+EBV+ PEL, including individuals with concurrent multicentric Castleman disease (n=2) or Kaposi sarcoma (n=1). Five primary and one low-passage samples were implanted intraperitoneally (IP; n=6 mice) or intravenously (IV; n=3 mice) into NOD-scid-IL2Rγnull (NSG) mice. Tumors from first

passage (P1) were re-implanted IP for secondary passage (P2). Neoplasms were assessed by histopathology, cytology, qPCR for viral coinfection, and multiparameter flow cytometry. Selected tumors were expanded ex vivo and purified by FACS. Single-cell RNA sequencing (scRNA-seq) was performed to compare transcriptional profiles from primary patient samples through serial xenograft passages and against established PEL cell lines (BCBL1 and BC1). IV implantation in P1 produced disseminated neoplasia primarily involving spleen, with additional liver, lung, lymph node involvement, and malignant ascites. IP implantation in P1 and P2 resulted in ascites and mesenteric tumors. Cytology demonstrated atypical plasmacytoid lymphoid cells consistent with PEL. Tumors exhibited sheets of large lymphoid cells with high mitotic index. KSHV and EBV coinfection was confirmed by qPCR. Flow cytometry demonstrated preservation of plasmacytic immunophenotype (CD45+CD19−CD20−CD38hi) with variable HLA-DR and CCR7 expression. Four neoplasms derived from two patient effusions were successfully expanded ex vivo and purified. The PDX pipeline enabled propagation of PEL from limited clinical material within 54–155 days. Low-passage PEL-PDX maintained key molecular and pathological characteristics of primary tumors. scRNA-seq analysis is being used to define population-level and transcriptomic changes occurring during serial passage and to compare PDX-derived cells with established lines. These findings demonstrate that low-passage PEL-PDX models preserve essential disease features and are suitable for downstream genomic and functional analyses. This work establishes a scalable platform for expanding primary PEL that captures patient-specific molecular heterogeneity beyond established cell lines. Low-passage PEL-PDX models have the potential for personalized drug susceptibility testing and mechanistic evaluation of immunologic and targeted therapies in vivo. These models provide a critical translational bridge for studying chemotherapy-resistant PEL and for identifying novel therapeutic vulnerabilities in this high-risk population.

27. Enrichment for Intact Proviruses Identifies Clinically Relevant Archived HIV-1 Resistance Mutations

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28. Tumor-targeting IL-12 immunocytokine therapy increases peripheral memory T cells with self-renewing properties

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Stem-like memory T cells (TSCM) are a rare subset of multipotent, self-renewing cells important for antitumor control. Accumulation in the tumor or tumor-draining lymph node of TSCM or T cells expressing TCF1, critical in self-renewal, are associated with improved response of patients to immune checkpoint blockade. These tissues, however, are often not available for study. Here, we evaluated the effects of a tumor-targeting IL-12 immunocytokine PDS01ADC (previously termed NHS-IL12), with promising activity in preclinical and clinical studies, on peripheral T cell stemness in murine hosts and cancer patients. Prevalence of TSCM and expression of the murine T cell stemness marker SCA1 were evaluated by flow cytometry in spleens of mice treated with PDS01ADC. Similarly, TSCM and TCF1+ T cells were assessed in peripheral blood of 28 patients with advanced solid tumors treated with PDS01ADC (NCT01417546). PDS01ADC treatment in mice increased peripheral CD8+ and CD4+ TSCM and effector memory T cells (TEM) expressing SCA1. In patients, treatment with PDS01ADC increased CD8+ and CD4+ TSCM and TEM in the periphery expressing TCF1, with increased TCF1+ T cells associated with disease control. Most peripheral TSCM were negative for PD-1 and TIGIT throughout treatment, indicating their continued quiescence and self-renewal. Additionally, expanded TCF1- CD8+ TEM expressing PD-1 and/or TIGIT showed increased intensity of granzyme B, indicating that treatment did not induce CD8+ T effector cell dysfunction. This is the first report of a cytokine-based therapy increasing peripheral T cell stemness in mice and humans. Increases in peripheral T cell stemness induced

by PDS01ADC correlated with disease stabilization and may serve as an indicator of which patients are more likely to benefit from therapy. These data support future studies combining PDS01ADC with other agents to synergize with this increase in T cell stemness.

29. Decoding the Hallmark Features of Modular CAR Domains and Their Impact on CAR T Cell Tumoricidal Activity

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Engineering T cells to express a Chimeric Antigen Receptor (CAR) directs potent tumor cytotoxicity, and this cellular immunotherapy has yielded robust success in hematological malignancies. However, in solid tumor models, including neuroblastoma, regression after CAR administration is only transient. Barriers to CAR T cell success in solid malignancies include the development of T cell exhaustion, limited T cell persistence, and tumor antigen expression levels below the activation threshold. CARs are modular receptors composed of linked domains that determine the threshold of antigen recognition and the magnitude of activation signal transduced. As such, CAR design can be optimized to overcome these efficacy barriers, but there remains limited insight as to how each modular domain and its variants influence T cell phenotype and function, and there are no current guidelines instructing the best design or phenotype required for CAR T cell efficacy. We generated a library of 12 constructs that combinatorially link 3 distinct hinge regions (CD8, CD28, or IgG), 2 transmembrane domains (CD8 or CD28), and 2 co-stimulatory regions (41BB or CD28), all frequently used in FDA-approved CARs. We used a scFv targeting the oncofetal antigen GPC2, an antigen with moderate to low expression in neuroblastoma with minimal expression in healthy tissues. Expressing these unique CARs in human T cells, we will evaluate differences in T cell cytokine production, killing capacity, phenotype, and persistence using preclinical models of neuroblastoma. These assays will generate a high-dimensional dataset, and we will employ machine learning to identify an in vitro biosignature of CAR T cells that predicts in vivo CAR T cell killing, as well as a favorable phenotype for persistence and effector qualities. Preliminary results identified a broad range of CAR T cell cytotoxicity against neuroblastoma in vitro, with the transmembrane domain driving the greatest variation in killing, as well as significant interaction effects between domains. Further, large multicolor flow cytometric analysis identifies distinct activation, stem, and effector phenotypes across CAR T cell constructs after co-culture with neuroblastoma. Overall, we aim to create a model establishing the role of each domain and how they interact to propagate an activation signal, recruit a unique signalosome, and dictate the phenotype and function of CAR T cells in the hopes of guiding future CAR T cell design and testing.

30. Targeted Lung Microbiome Modulation with Aerosolized Antibiotics Enhances Immune Checkpoint Blockade in Advanced NSCLC

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31. A recombinant murine gammaherpesvirus that expresses KSHV vIL-6 modulates STAT3 in mouse cells

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Kaposi sarcoma herpesvirus (KSHV) causes Kaposi sarcoma, primary effusion lymphoma, KSHV associated multicentric Castleman disease (MCD), and KSHV inflammatory cytokine syndrome. Viral interleukin-6 (vIL-6), encoded by KSHV gene K2, is a key pathogenic cytokine that amplifies the JAK/STAT pathway to promote cell survival, proliferation, and angiogenesis. Mice transgenic for H2 K promoter-driven vIL 6 expression developed MCD-like disease which was abrogated on an IL 6 knock-out background and suggests that vIL 6 cooperates with host IL 6 in vivo. However, the role of IL-6 and vIL 6 in the context of latency establishment and reactivation of a

gammaherpesvirus in B cells in vivo remains unknown. Murine gammaherpesvirus 68 (MHV68) is colinear with KSHV, infects B cells, and leads to lymphoproliferation in immunosuppressed mice, but does not encode a K2 homolog. We hypothesize that expression of vIL-6 in MHV68 will increase virus-driven B cell proliferation and survival of infected B cells in mice.To define the contribution of IL-6 in MHV68 viral pathogenesis, we infected WT and IL-6 KO mice with 5000 plaque forming units (PFU) of MHV68 expressing a YFP reporter by intranasal route of infection. To assess the KSHV vIL-6 signaling in murine cells, we examined STAT3 activation in vitro. To query the in vivo role of KSHV vIL-6, we constructed a recombinant MHV68 that expresses KSHV vIL 6 and a P2A linked mNeonGreen reporter gene, under the control of the native KSHV K2 promoter (MHV68-vIL6-mNG) which was used to infect WT mice with 5000 PFU by intranasal route of infection. We found that host IL-6 promotes splenomegaly, the germinal center (GC) response, the establishment and reactivation in the spleens of MHV68-infected mice. Robust STAT3 activation, evidenced by phospho-Y705 and ptgs2 target gene induction, was observed in murine 3T12 fibroblasts upon direct transfection or when treated with secreted KSHV vIL-6 in the conditioned media of 293T transfected cells. KSHV vIL-6 also activated STAT3 in A20 murine B lymphoma cells. We validated KSHV vIL 6 and mNeonGreen expression from the recombinant MHV68-vIL6-mNG virus upon infection of murine fibroblasts which demonstrates that the native KSHV K2 promoter is functional in murine cells in vitro. Infection of WT mice with MHV68-vIL6-mNG virus has a lytic replication defect as was observed by lower viral titers in lungs at days 7 and 9 post-infection but still led to an enhanced GC response in the lymph nodes on day 16 post-infection, compared with MHV68-H2B-YFP infected mice. We found that the host IL-6 and vIL-6 have different and tissue specific roles. Infection of WT and IL-6 KO mice with MHV68-vIL6-mNG and MHV68-vIL6-mNG STOP viruses will help us define how KSHV vIL-6 shapes both germinal center responses and the B cell expansion and survival of infected cells, alone as well as in combination with the host IL-6. This infection based model will expand our understanding of the in vivo contribution of the KSHV vIL 6 to lymphoproliferative disorders in people.

32. Neuropilin 1 (NRP1) is a novel biomarker for a group of KSHV infected primary effusion lymphomas and plays vital roles in virus replication

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Many people living with HIV are coinfected with Kaposi sarcoma associated herpesvirus (KSHV), a biphasic virus with latent and lytic replication cycles. KSHV causes primary effusion lymphoma (PEL), an aggressive malignancy characterized by poor prognosis and atypical B cell marker expression. To identify surface proteins as prognostic markers, we conducted DNA sequencing on a patient derived PEL cell line, BCBL1, and identified NRP1. Studies show that NRP1 can be used by viruses such as SARS CoV2 as an entry receptor into host cells. Therefore, we hypothesized that NRP1 may play a role in KSHV infection of B cells. Using loss of function assays, we demonstrated that NRP1 is essential for KSHV lytic reactivation, virion production, and infectivity in BCBL1 and JSC1 PEL cell lines. NRP1 depletion reduced lytic gene transcription, virion production and infectivity in PEL cell lines during reactivation. We next investigated the role of NRP1 in de novo KSHV infection of BJAB cells, an EBV negative Burkitt lymphoma cell line. Unlike PEL cells, BJAB cells are not naturally infected with KSHV; therefore, they can serve as a comparison to determine whether the effects of NRP1 in PEL cells extend to other B cell lymphomas. To assess whether NRP1 affects lytic transcription and replication in BJAB cells, KSHV infected, NRP1 overexpressing BJAB cells were incubated with 2.5 mM valproic acid, a histone deacetylase inhibitor that induces lytic reactivation. Three days post induction, total RNA was extracted from cell pellets and underwent qPCR to measure changes in lytic gene expression. Additionally, KSHV genomic DNA was extracted from culture supernatant and quantified by qPCR as an indicator of virion content. Subsequently, HEK293 epithelial cells were incubated with the supernatant for 6 hours, followed by RNA extraction and qPCR quantification of lytic gene expression at 3 days post infection. Harvested total RNA was reverse transcribed into cDNA and probed for the following KSHV transcripts: RTA (early lytic), LANA (latency), K8.1 (late lytic), and ORF6 (late lytic). These genes initiate lytic translation, maintain viral latency, evade immune response, and drive replication, respectively. Harvested DNA was probed for RTA and ORF6 to confirm presence of virions in the supernatant. De novo infected, NRP1 overexpressing BJAB cells displayed greater increases in viral gene expression after lytic reactivation compared to control groups. However, infectivity of virions did not differ significantly between NRP1 overexpressing and control cell lines. Thus, the data suggest that NRP1 aids in accelerating virion production and viral transcription, but infectivity effects are cell specific among B cell lymphomas. Because PEL cells lack typical B cell markers, NRP1 may serve as a novel biomarker for the JSC1 and BCBL1 cell lines. As NRP1 expression correlates with lytic replication and virion production, it may also help characterize lytic

KSHV infected B cells in terms of disease progression. Future transcriptomic experiments comparing lytic transcripts between BJAB NRP1 overexpressing cells and control cells could help identify other differentially expressed lytic genes that affect infectivity.

33. Persistent Immune Activation in Persons with HIV-2 During Antiretroviral Therapy

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HIV-2 is less pathogenic than HIV-1, with lower levels of plasma HIV-2 RNA levels and slower rates of CD4 lymphopenia. Cellular immune activation (CD8+DR+, CD8+CD38+DR+) is common in untreated persons with HIV-2 (PWH2) and has been reported to be associated with levels of viremia or cell-associated HIV-2 DNA. Effects of anti-retroviral therapy (ART) on immune activation in PWH2 have not been extensively studied. To investigate the effects of antiretroviral therapy on immune activation, we quantified levels of lymphocyte cell subsets in participants with HIV-2 prior to and following ART initiation and in otherwise healthy controls. We hypothesize that 1) pretherapy levels of activated CD8 cell subsets will be elevated in viremic, but not aviremic PWH2 compared with matched otherwise healthy controls and 2) levels of activated CD8 cell subsets will normalize after introduction of ART in all PWH2 and will not be significantly different than matched otherwise healthy controls. PWH-2 (N=6) enrolled in NIH protocols and otherwise healthy controls without HIV (N=64) in the NIH volunteer program provided plasma and peripheral blood mononuclear cell (PBMC) samples. Otherwise healthy controls were matched with PWH-2 by age (+/- 5 years), sex, and race. PBMCs underwent immunophenotyping using fluorescence-activated cell analysis for CD4, CD8, CD8+DR+, and CD8+CD38+DR+. Longitudinal samples from PWH were obtained prior to (n=13) and following ART initiation (n=83). In PWH2, proviral DNA was assayed in 2.4-6 million PBMC in 4/6 PWH2. Parametric statistics were used to analyze data. PWH2 and matched controls were studied. As expected, pretherapy levels of CD8+38+DR+ and CD8+DR+ were significantly higher in PWH2; mean CD8+38+DR+ PWH2 (35.31%) vs. controls (11.24%; $p<0.0001$); CD8+DR+ (62.15% vs. 22.62% $p<0.0001$). After >3 years on ART, levels of CD8 subsets were still significantly elevated (both $p<0.0001$) indicating immune activation persisted. Proviral DNA levels were not detected in 2/3 individuals undergoing ART; levels in one PWH2 were low (7 c/million PBMC). Interestingly, in one individual, HIV-2 RNA and proviral DNA could not be detected pretherapy, despite significant CD8 subset elevations. Elevated immune activation persists despite long-term ART, and low or undetectable levels of HIV-2 DNA. In PWH2, viral sources of immune activation are likely based in tissues, not blood. Though investigated in people with HIV-1, levels of immune activation in PWH2 undergoing ART have not been well-characterized. Further investigation is needed to elucidate the role of immune activation in morbidity and mortality of PWH2 undergoing ART.

34. Regulation of Oncoprotein Survivin Expression by Cereblon-Binding Immunomodulators in Primary Effusion Lymphoma

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Primary effusion lymphoma (PEL) is a Kaposi sarcoma-associated herpesvirus (KSHV)-driven lymphoma with limited therapeutic options and relatively poor outcomes. About 80% of PELs are co-infected with EBV. We have previously found that cereblon-binding immunomodulators (CBIs), particularly the next-generation agent golcadomide (Golc), have strong activity in PEL. Survivin (BIRC5), a critical inhibitor of apoptosis protein (IAP) known to be essential for PEL cell survival, was found to be one of the most downregulated proteins by CBIs in PEL cell lines. Here, we assessed the mechanism by which CBIs downregulate survivin and investigated transcriptional and post-transcriptional processes that might contribute to its downregulation. PEL cell lines JSC-1 (EBV+/KSHV+) and BCBL-1(EBV-/KSHV+) were treated with golcadomide (Golc) and survivin level was assessed at the protein level through western blot and at the mRNA level by qRT-PCR. Cycloheximide (CHX), a translation inhibitor, was used to determine survivin half-life and test whether Golc exposure alters survivin protein. The proteasome inhibitor MG132 was used to evaluate whether proteasome-dependent degradation contributes to CBI-mediated survivin decrease. Parallel studies

were performed on EBV-positive and negative Burkitt lymphoma cell (BL) lines. Golc treatment reduced survivin protein levels in both JSC-1 and BCBL-1 PEL cell lines. JSC-1 cells also showed a significant and substantial decrease in survivin mRNA while BCBL-1 cells showed only a small non-significant decrease, suggesting that Golc may also affect survivin protein stability post-transcription. While published literature in non-PEL cancer models reported survivin to be a short-lived protein with a half-life of less than 1 hour, CHX chase studies showed that survivin has prolonged stability in PEL cells with half-life exceeding 18 hours. By contrast, MCL-1, another inhibitor of apoptosis and known to be short-lived, was degraded within 1 hour of CHX exposure in PEL cells. In KSHV-negative BL and osteosarcoma lines, survivin half-life was observed to be around 1-2 hours, suggesting that enhanced survivin persistence may be an unusual feature of KSHV-associated PEL. While Golc treatment reduced survivin levels in PEL cells, the survivin still showed prolonged stability similar to control cells, suggesting that the unique stability of survivin in PEL is not directly affected by Golc. Inhibiting proteasomal degradation using MG132 showed a minor increase in survivin protein levels in both control and Golc-treated cells. Together, these findings support a model in which survivin protein is unusually stable in PEL, and CBIs like Golc may downregulate survivin production primarily through effects on transcription as well as other steps in protein production. The unique prolonged stability of survivin in PEL cells is worthy of further study. This study advances mechanistic understanding of how next-generation CBIs target a key survival pathway in PEL and identifies survivin stability as a potentially unique and therapeutically relevant feature of KSHV-associated PEL. By showing that CBIs can overcome abnormal survivin persistence, these findings strengthen the rationale for clinical evaluation of newer CBIs in PEL.

35. The CEBPD Transcription Factor Regulates Granulocyte Lineage Commitment and Mature Neutrophil Functions

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Genetics, Epigenetics, and Gene Regulation (Posters)

36. Investigating Transcriptional Regulation of Cisplatin Sensitivity in Small Cell Lung Cancer Using a Functional Overexpression Screen

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Small cell lung cancer (SCLC) is a highly aggressive neuroendocrine malignancy characterized by initial sensitivity to platinum-etoposide chemotherapy, yet patients commonly experience early relapse and the development of drug resistance. In the absence of recurrent actionable genomic alterations, accumulating evidence points to epigenetic dysregulation as a central driver of progression and therapy resistance. SCLC exhibits remarkable molecular heterogeneity and phenotypic plasticity, with recent classification systems defining distinct molecular subtypes based on differential expression of lineage-defining transcription factors. Dynamic changes in subtype identity during treatment have emerged as a potential mechanism of acquired resistance and differential responses to platinum-based chemotherapy. Defining the transcriptional programs that govern these patterns of chemoresistance therefore represents a critical unmet need in SCLC. To identify transcriptional regulators that confer cisplatin resistance, we performed a pooled, barcoded open reading frame (ORF) over expression screen in an SCLC cell line model under prolonged cisplatin treatment. This unbiased functional genomics approach revealed selective enrichment of members of the Forkhead box (FOX) family of transcription factors. FOX proteins are an evolutionarily conserved family defined by a characteristic winged helix DNA binding domain that regulate diverse cellular processes, including stress responses, cell cycle control, DNA damage repair, and cell fate determination. Across cancer types, FOX factor dysregulation has been linked to drug resistance through regulation of DNA repair pathways, apoptotic signaling, and pro-survival pathways. We have confirmed that FOX transcription factors emerging from the ORF overexpression screen are mediators of cisplatin resistance. We are systematically characterizing resistance phenotypes across major SCLC molecular subtypes using a comprehensive panel of cell line models. Integrating transcriptomic analysis with functional assays, we will delineate the FOX-driven transcriptional programs and assess whether resistance is mediated through shared or subtype-specific mechanisms, including validation inpatient-derived datasets. The impact of these findings could inform SCLC management by identifying transcriptional biomarkers predictive of cisplatin response and early drug resistance. Mechanistic insights into FOX-mediated resistance programs may also support rational combination strategies that pair platinum chemotherapy with targeted inhibition of key resistance pathways.

37. AMPK γ2 Is a Direct Thermosensor: Linking Metabolic Signaling to Heat Stress.

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Cells must coordinate energy balance with proteome stability to survive stress, yet how metabolic and thermal stress pathways are mechanistically integrated remains incompletely understood. AMP-activated protein kinase (AMPK) is a central metabolic regulator that integrates stress signals to maintain cellular energy homeostasis. While its γ subunits are established nucleotide sensors, whether AMPK can directly sense temperature to couple heat stress to metabolic reprogramming has remained unclear. Here, we identify the γ2 regulatory subunit from intrinsic disorder region at the N terminal act as a thermosensitive module that converts physiologically relevant. Whenever cellular temperature increases N terminal IDR region from γ2 subunits undergo LLPS (Liquid-Liquid Phase Separation) formation through LCST (Lower Critical Solution Temperature) type of phase behavior which leads to AMPK deactivation and HSF1 activation. Using In Vitro by recombinant γ2-containing AMPK complexes, as well as γ2-containing IDR, W/O IDR and Full Lenth region, we found elevation in the temperature change the secondary structure or confirmation from IDR region of γ2 but not with W/O IDR region that clearly leads to droplet formation of IDR region. At the cellular level we found AMPK complex is major suppressor of HSF1 during non-stress condition, by mathematically 95% of HSF1 is repressed by AMPK validated by immunodepleting technique, after knocking out AMPK complex (α1α2,β1β2,γ1γ2)

HSF1 spontaneously activated , we also showed that modest heat elevations decreases adenylate-dependent activation and signaling output selectively through γ2, without equivalent responses from γ1- or γ3-containing complexes. In cells, γ2-dependent heat sensing coordinates dephosphorylation of canonical AMPK targets, reshapes proteostasis networks through HSF1 activation by direct interaction or releasing from their repressive state, and promotes survival under repeated heat stress, whereas disruption of γ2 blunts these adaptive responses. These findings reveal AMPK γ2 as a dedicated molecular heat sensor that links thermal stress to metabolic and proteostatic control, with implications for fever responses, cancer cell stress tolerance, and tissue vulnerability to hyperthermia-like brain and heart.

38. Stress controls heterochromatin inheritance via histone h3 ubiquitylation

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Heterochromatin, marked by histone H3 lysine 9 methylation, can be epigenetically inherited through cell division, maintaining gene repression that preserves cell identity and enables adaptation to environmental challenges. Studies in S. pombe have shown that heterochromatin propagation depends on the read-write mechanism, wherein a sufficient density of H3K9me3-modified nucleosomes, stabilized by histone deacetylases (HDACs), concentrates Clr4SUV39H on chromatin to promote further deposition of H3K9 methylation. Whether additional mechanisms control heterochromatin propagation via Clr4SUV39H, a subunit of the E3 ubiquitin ligase complex ClrC, was unknown. Here we uncover a ubiquitin-dependent heterochromatin heritability regulatory hub (HRH) that broadly governs heterochromatin propagation, even without HDAC activity. The HRH is tuned by the limiting factor Raf1DDB2, a substrate receptor for the ClrC ubiquitin ligase. In addition to linking Clr4SUV39H to other ClrC components on chromatin, Raf1DDB2 acts in a dosage-dependent manner to promote ubiquitination of histone H3 at lysine 14 (H3K14ub), which is critical for heterochromatin self-propagation. Importantly, HRH is intricately linked to environmentally responsive pathways, including nonsense-mediated decay (NMD) and TOR signaling, enabling cells to adapt to changing conditions. By modulating heterochromatin propagation, cells leverage the HRH to gain resistance to antifungal agents and adapt to high temperature. Thus, heterochromatin self-propagation is actively regulated via H3K14ub in response to external stimuli, with broad implications for understanding mechanisms governing rapid changes in the epigenetic landscape in physiology and disease.

39. Patient-derived Atlas of Structural Variants Underlying Castration-Resistant Prostate Cancer

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Progression to lethal, castration-resistant prostate cancer (CRPC) is shaped by molecular heterogeneity, including structural variants (SVs) that perturb multigene regulatory landscapes and contribute to therapy resistance. Despite substantial genomic profiling of prostate tumors, most datasets rely on short-read sequencing, in part because commonly available archival specimens are often suboptimal for long-read approaches. Consequently, SV architecture and function remain incompletely resolved. In other cancers, patient-derived xenograft (PDX) models have enabled long-read whole-genome sequencing (WGS) and regulatory genomic profiling at scale; we seek to adapt this strategy for prostate cancer. We will compile an atlas of SVs and their functional impact using long-read sequencing across the patient-derived xenograft/organoid (PDX/PDO) LuCaP series (developed by the NCI Genitourinary Malignancy Branch), which recapitulates key features of CRPC. We hypothesize that the distinct

phenotypes and therapy outcomes of CRPC patients modeled by the LuCaP series will be functionally explained by SVs regulating multiple genetic programs. We will profile matched intact and castrated PDX tumor samples for each LuCaP model using long-read WGS to resolve SVs. Currently, a sequencing pilot on intact-castration pairs from models LuCaP136 and LuCaP167 is underway, targeting ~50x-100x depth for comprehensive SV identification. Host/graft disambiguation will generate tumor-enriched BAMs. Multiple tumor-only SV callers and filters will derive a consensus callset in the absence of matched normal controls. In parallel, short-read WGS will support SNV/CNV-based clonality inferences and phylogenetic modeling to estimate cancer cell fractions (CCFs) and compare clonal dynamics across the intact-to-castrated transition. To interpret regulatory impact, we will integrate epigenomic profiling (5mC/5hmC methylation) and transcription factor-associated regulatory analyses to understand SV-linked activation/inactivation not evident from DNA sequences alone. Our pilot long-read WGS data currently reaches ~10x–15× mean depth—sufficient to validate our SV discovery workflow but insufficient for low–allele frequency and subclonal events. As coverage increases, we expect to resolve recurrent CRPC-relevant SVs, including AR copy number gains and tandem duplications (particularly AR enhancer duplications and noncoding AR locus rearrangements), AR intragenic deletions, inversions, and complex rearrangements, as well as SVs affecting additional CRPC loci (e.g., CDK12, BRCA2, TP53, RB1, PTEN, LSAMP, MYC, FOXA1, and TMPRSS2-ERG). Thus far, this project establishes feasibility for processing PDX long-read data and positions the project for downstream SV cataloging of tumor-only samples once target coverage is achieved. Conclusions: These pilot studies demonstrate a working long-read WGS pipeline to assemble a patient-derived atlas of SVs found in CRPC progression. Achieving the planned ~50x–100× depth and triangulation across SV callers and filters will enable robust identification of SVs and nomination of castration-selected events using within-pair allele-frequency shifts to distinguish clonal versus subclonal alterations. We anticipate CRPC tumors to display acquired AR-centric SVs that increase AR dosage or reconfigure AR regulatory architecture by enabling constitutively active signaling or potentially promoting AR-bypass signaling and/or lineage plasticity. More broadly, we expect CRPC phenotypes will be driven by non-AR SVs regulating distinct genetic programs supporting cell-intrinsic adaptation to the tumor microenvironment and facilitating clonal selection under treatment pressures.

40. PARP3 suppresses the Alternative Lengthening of Telomeres pathway by promoting G4 quadruplex resolution

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42. Systematic Single-Cell Exon Deletions Map Alternative Splicing-Driven Gene Regulatory Programs

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CRISPR-Cas technology has transformed functional genomics, yet our understanding of how individual exons shape cellular phenotypes remains limited. We previously developed CHyMErA, a combinatorial Cas9 and Cas12a platform that enables massively parallel exon deletion screens in human cells, identifying over 2,000 exons that regulate cellular fitness. Notably, these fitness-altering exons are disproportionately enriched in genes involved in gene expression regulation, underscoring the need for scalable approaches to systematically investigate how alternative exons shape transcriptional phenotypes. Here, we present single-cell CHyMErA-sequencing (scCHyMErA-Seq), a novel platform that combines ultra-efficient exon deletion using CHyMErA with the simultaneous capture of Cas9 and Cas12a guide RNAs at single-cell resolution. scCHyMErA-Seq directly links individual exon deletions, identified via

gRNA sequencing, to transcriptional profiles across thousands of single-cells, enabling massively parallel analysis of exon-specific gene expression phenotypes. As proof-of-concept, we applied scCHyMErA-Seq to delete 224 frame-preserving alternative cassette exons across 161 genes, prioritizing exons previously implicated in cellular fitness and enriched in gene regulatory pathways. Dimensionality reduction revealed that specific exon deletions generate distinct cell clusters, indicating reproducible exon-specific transcriptional phenotypes. Moreover, exons from genes within the same protein complexes or pathways frequently occupy nearby transcriptional states, suggesting coordinated yet distinct effects on gene expression. Strikingly, 45% of the profiled exons (101 out of 224) influence the expression of at least 200 genes. Among these, we identified NRF1 alternative exon-7. Focused analyses using engineered cell lines expressing either full-length or Δexon-7 NRF1 isoforms revealed that exon-7 modulates NRF1 transcriptional activity by regulating its recruitment to the promoters of target genes. Furthermore, scCHyMErA-Seq uncovered exons from distinct genes that coordinately regulate specific pathways, as exemplified by TAF5 exon-8 and LSM11 exon-3, whose deletion resulted in shared transcriptional signatures associated with histone pre-mRNA processing. Collectively, our work shows that the gene expression phenotypes captured by scCHyMErA-Seq recapitulate findings from traditional, labor-intensive approaches while offering substantially greater scalability and efficiency. Overall, scCHyMErA-Seq provides a robust platform for uncovering the functional impact of alternative splicing on gene regulation. Its modular design further supports combinatorial perturbation strategies, including genetic interaction mapping and targeted interrogation of specific regulatory or structural genomic elements to define their impact on transcriptional programs.

43. RACK1-Mediated Proteolysis of CENP-A Preserves Chromosomal Stability by Preventing Mislocalization of CENP-A

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44. Mechanical Insights into Centromeric Evolution

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Faithful chromosome segregation, which is indispensable for the survival of all organisms, is evolutionarily conserved across most eukaryotes. Despite the function being preserved, both centromeric DNA and kinetochore proteins are rapidly evolving. These contrasting aspects of centromere biology are known as the “centromere paradox”. This motivates us to understand the mechanistic basis of centromeric evolution. Unlike canonical histone proteins which are nearly invariant through evolution, centromeres are epigenetically defined by the fast-evolving histone H3 variant CENP-A. Recently we reported that CENP-A nucleosomes are two-fold more elastic than canonical H3 nucleosomes, and get stiffer when bound to the essential kinetochore protein CENP-C. In this work, we investigate whether human CENP-A elasticity is a conserved feature across eukaryotes. To study nucleosome elasticity in different eukaryotic models, we selected CENP-A from fungi kingdom (Saccharomyces cerevisiae), plant kingdom (Arabidopsis thaliana), and compared with animal kingdom (Homo sapiens). Our results indicate that human CENP-A nucleosomes are the most elastic followed by canonical H3, budding yeast Cse4 and Arabidopsis CenH3 nucleosomes. Although the size of human centromere tandem repeat (171 bp) is in the same range as that of Arabidopsis with 178 bp in length, there is no resemblance in elasticity between human CENP-A and Arabidopsis CenH3. Whereas budding yeast point centromere is 125 bp in size with AT rich CDEII DNA elements. Therefore, considering the genetic diversity of centromeres, CENP-A elasticity may be centromere specific and not a conserved feature among eukaryotes. Particularly for human CENP-A elasticity, CATD domain plays a vital role as H3 nucleosome containing CENP-A

CATD domain is found to be more elastic compared to canonical H3 nucleosome. Then we determined nucleosome dynamics by mobility measurement via High-speed AFM. Rigid Arabidopsis CENH3 nucleosome found to be less mobile and elastic H3CATD nucleosome is more mobile. So, a correlation was observed between the nucleosome elasticity with mobility.

45. Dynamic MYCN Amplification Topology Links ADRN–MES Plasticity in Neuroblastoma

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Neuroblastoma (NB) tumorigenesis is driven by chromosomal instability and recurrent copy-number alterations, most notably MYCN amplification (MNA). Oncogene amplification occurs either as extrachromosomal DNA (ecDNA) or as chromosomally integrated homogeneously staining regions (HSRs), two topologies associated with distinct levels of oncogene expression and intratumoral heterogeneity. NB tumors comprise two interchangeable cell states: an adrenergic (ADRN) state with high proliferative capacity and a mesenchymal-like (MES) state associated with stress tolerance, chemoresistance, and relapse. An inverse correlation between MNA and the MES state has been reported, suggesting a functional link between oncogene dosage, amplification topology, and cell identity. However, how these features interact to shape NB heterogeneity and plasticity remains unclear. We established an ex vivo model of NB plasticity using patient-derived xenografts (PDXs) from MYCN-amplified tumors. PDX-derived spheroids were propagated in neural stem cell medium and subsequently transferred to two-dimensional Matrigel-coated cultures in serum-containing medium, generating a transient parental population with mixed ADRN/MES identity. Matched ADRN and MES sublines were isolated by adherence-based selection. MYCN expression and cell identity were assessed by immunofluorescence, Western blotting, and single-cell RNA sequencing (10x Genomics). Chromosomal instability, structural variation, and MYCN amplification topology were characterized using Oxford Nanopore long-read sequencing, spectral karyotyping, and multiplex FISH across matched tumor, spheroids, parental cultures, and ADRN/MES sublines. Parental tumors and spheroids were predominantly composed of high MYCN-expressing ADRN cells harboring mainly ecDNA-based MNA, with a minor fraction of HSR-positive cells. Upon isolation, ADRN sublines were uniformly ecDNA-positive and maintained high MYCN expression. ADRN cells also acquired additional state-specific alterations, including trisomy 1 with a t(1;2) translocation involving MYCN, indicating divergent karyotypic evolution between cell states. In contrast, MES sublines exclusively displayed chromosomal MYCN configurations, including HSRs on different chromosomes or complete loss of amplification, accompanied by reduced or absent MYCN expression. MES non-amplified cells progressively accumulated during culture. Long-read sequencing revealed that the MYCN amplicon is conserved across cell states but exhibits dynamic structural remodeling, shifting from highly complex ecDNA architectures in ADRN cells to simplified configurations in MES, consistent with loss of ecDNA species and chromosomal integration. Our study identifies MYCN amplification topology as a dynamic and state-dependent feature of NB heterogeneity. Transition to the MES state involves silencing, chromosomal sequestration, and loss of MYCN amplification, whereas the ADRN state selects for ecDNA-driven MYCN amplification. Whether these changes arise from genetic drift, unequal segregation of ecDNA, or active mechanisms of ecDNA reintegration remains under investigation. We propose that reversible transitions between chromosomal and extrachromosomal MYCN configurations may enable a plastic cycle in which MYCN-driven proliferation is transiently attenuated in stress-tolerant MES states and reactivated upon re-entry into adrenergic transcriptional programs, providing a potential framework for tumor persistence and relapse in high-risk neuroblastoma.

46. A novel enzyme responsible for the acp3U20 modification in P. aeruginosa

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tRNAs, necessary adaptor molecules in protein synthesis, are highly modified. These chemical modifications have been shown to regulate folding, translational efficiency, and mRNA decoding. We previously generated a map of tRNA modification sites in Pseudomonas aeruginosa, a pathogenic bacterium that is a common comorbidity for cancer patients. In this map, we identified sites with functions that remain unexplored. The presence of a modification at position 20B is conserved in P. aeruginosa, other pathogens, and human, suggesting the presence of 3-(3-amino-3-carboxypropyl)uridine (acp3U). This modification is dynamically regulated in some bacterial species with a possible role in stabilizing the tRNA D-loop. We previously identified the enzyme, PA_17650, that deposits acp3U at position 46 and 47; sequence analysis of the P. aeruginosa genome revealed a homolog enzyme, PA_46020. Mutational signature analysis confirmed this enzyme deposits a modification at position 20 of tRNAGlu(UUC) in P. aeruginosa. To confirm the identity of the modification, we are using primer extension analysis and mass spectrometry to confirm the role of this enzyme. To determine the functional role of acp3U20 in P. aeruginosa, we designed a system to test the effect of this modification on translational efficiency using a GFP reporter under different stressors. Altogether, we aim to identify the origins and functional roles of tRNA modifications such as acp3U in pathogenic strains. Ultimately, identifying the molecular function of these tRNA modifications may offer insight into prokaryotic translation and the function of the tRNA D-loop.

47. A Regulatory Variant at rs3747579 Controls DNAJA3 Through Chromatin Looping and Associates With MASH Related Liver Cancer

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Metabolic dysfunction associated steatohepatitis, previously called NASH, is an increasingly important cause of hepatocellular carcinoma, yet inherited regulatory mechanisms linking metabolic liver injury to malignant progression remain poorly defined. We hypothesized that common noncoding variants modulate mitochondrial stress resilience in steatohepatitis by altering long range gene regulation, thereby influencing risk and clinical behavior of steatohepatitis related liver cancer. We focused on a candidate locus regulating DNAJA3, a mitochondrial Hsp40 co chaperone involved in proteostasis and stress adaptation. We integrated human genetics with liver expression quantitative trait locus analysis to prioritize candidate regulatory variants and target genes. We evaluated associations with steatohepatitis related hepatocellular carcinoma and examined outcome relationships using tumor expression data. To define mechanism, we assessed chromatin topology and enhancer promoter interactions at the locus, analyzed transcription factor occupancy using ChIP seq, and tested function using RBFOX2 knockdown and allele specific luciferase reporter assays. We synthesized results into a genetics to mechanism model linking variant dependent regulation to mitochondrial gene expression. We identified rs3747579 as a common noncoding variant significantly associated with steatohepatitis related hepatocellular carcinoma. Liver eQTL analysis prioritized DNAJA3 as the target gene and showed that the rs3747579 TT genotype is associated with reduced DNAJA3 expression. Lower DNAJA3 levels correlated with unfavorable clinical outcomes in hepatocellular carcinoma, consistent with a clinically relevant DNAJA3 low axis. Mechanistic analyses indicated allele specific enhancer looping at the rs3747579 locus consistent with long range regulation of the DNAJA3 promoter. ChIP seq suggested that the rs3747579 CC allele resides within an RBFOX2 binding motif, implicating RBFOX2 in local chromatin regulation in addition to canonical RNA splicing functions. Functionally, RBFOX2 knockdown reduced DNAJA3 mRNA, and reporter assays showed higher transcriptional activity for the CC allele compared with TT. Collectively, these results support a model in which rs3747579 modulates RBFOX2 linked enhancer activity and chromatin looping to suppress mitochondrial DNAJA3, associating with increased risk and poor prognosis of steatohepatitis related hepatocellular carcinoma. This work

provides a mechanistic example of how a common regulatory variant can couple three-dimensional genome organization to mitochondrial quality control in metabolic liver cancer. The rs3747579 DNAJA3 axis supports patient stratification by genotype and tumor expression state and motivates therapeutic strategies that exploit mitochondrial stress handling pathways in steatohepatitis related hepatocellular carcinoma.

48. Deciphering the role of NSD3 in HPV-negative HNSCC

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49. Transfer RNA acetylation regulates in vivo mammalian stress signaling

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Transfer RNA (tRNA) modifications are fundamental to translational fidelity and cellular homeostasis, yet the position-specific physiological roles of individual modifications remain incompletely understood. Here, we present our investigations into the impact of N4-acetylcytidine (ac4C), a highly conserved tRNA modification catalyzed by the acetyltransferase Nat10. By targeting Thumpd1, a nonessential adaptor protein required for Nat10-catalyzed tRNA acetylation, we demonstrate that loss of tRNA acetylation leads to reduced tRNALeu levels, increased ribosome stalling, and activation of eIF2α phosphorylation. Thumpd1 knockout mice exhibit growth defects and sterility, and concurrent knockout of Thumpd1 and the stress-sensing kinase Gcn2 causes penetrant postnatal lethality, revealing a critical genetic interaction. These findings demonstrate that a modification at a single position within type II cytosolic tRNAs can regulate ribosome-mediated stress signaling in mammalian organisms. We also detail preliminary efforts to model the impact of acute, spatiotemporal inhibition of tRNA acetylation in Thumpd1-dependent cancer cell lines, with the goal of dissecting the immediate translational consequences of ac4C loss free from long-term compensatory adaptation. Together, this work advances our understanding of epitranscriptomic control of translation and highlights new avenues for therapeutic intervention.

50. Centromere Dysfunction as a Driver of Age-Associated Chromosomal Instability

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Associated genome instability remains poorly defined. Chromatin structure deteriorates over time, but the chromosomal drivers of this decay are unclear. Here, we investigate aging in the context of the centromere, the epigenetically defined locus required for faithful chromosome segregation. Centromere identity is specified by the histone H3 variant CENP-A, which is replenished independently of DNA replication. We demonstrate that aging is accompanied by progressive loss of CENP-A and CENP-C from centromeres, coupled with their redistribution to chromatin-accessible regions, revealing age-associated CENP-A mislocalization. Using chemically aged human cells, primary cells from individuals of varying ages, and human tissues, we provide a mechanistic dissection of centromere erosion during aging. We find that essential DNA-binding inner kinetochore proteins are depleted through a p53-dependent pathway, leading to impaired centromere integrity and mitotic defects. Additionally, centromeric noncoding RNA levels decline in aged cells and tissues, compromising proper CENP-A deposition. We identify the molecular basis of this RNA depletion and demonstrate a strategy to functionally restore centromere activity in aged cells.

Together, these findings establish a mechanistic link between chromatin decay, centromere dysfunction, and genomic instability during aging, offering insight into why chromosomes become increasingly fragile with advancing age.

52. Investigating why DNMT3A Methylates CA DNA in Neurons

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DNA cytosine methylation is an epigenetic mark regulating gene expression. While CG methylation occurs in all cell types, neurons have high CA methylation (mCA), a less-characterized modification linked to human disease. mCA defines the neuronal epigenome, accumulating during postnatal brain development alongside synaptic refinement. DNMT3A knockout in mouse brain eliminates mCA but not mCG, indicating DNMT3A is the primary mCA methyltransferase. DNMT3A mutations cause the neurodevelopmental disorder Tatton-Brown-Rahman syndrome and cancers such as T-cell acute lymphoblastic leukemia (T-ALL). While DNMT3A is expressed across cell types, mCA appears unique to neurons. It is unknown why DNMT3A methylates CA DNA in neurons. We hypothesize that neuron-specific protein partners, high DNMT3A expression, and the non-dividing nature of neurons contribute to this specificity. To test whether neuron-specific DNMT3A protein partners contribute to mCA, we performed DNMT3A immunoprecipitation (IP) followed by mass spectrometry from mouse brain and lung tissue. We chose lung because DNMT3A levels are comparable to brain, but mCA is undetectable. We identified brain-enriched DNMT3A interactors, including BCL11B, a zinc-finger transcription factor essential for immune and nervous system development. BCL11B mutations are associated with intellectual disability and cancers, including T-ALL. We validated by IP western blot that DNMT3A interacts with BCL11B in brain but not lung tissue, likely due to higher BCL11B expression in brain. Notably, the consensus binding motif for BCL11B contains a CAC sequence, corresponding to the neuronal mCA sequence context. To assess whether BCL11B contributes to CA methylation activity of DNMT3A, we used shRNAs to knock down (KD) BCL11B in cultured mouse neurons. In preliminary results, we observed a decrease in mCA levels following BCL11B KD, assessed by DNA dot blot. We also generated preliminary low-depth Enzymatic Methyl-seq (EM-seq) data to assess genome-wide mCA levels in BCL11B KD neurons and confirmed reduction in mCA levels. In ongoing work, we will repeat BCL11B KD experiments with additional shRNAs and generate high-depth EM-seq data to map and compare mCA levels following BCL11B and DNMT3A KD in neurons. These data will be integrated with DNMT3A ChIP-seq to determine if BCL11B KD reduces DNMT3A binding at CA sequences where mCA decreases. To determine whether high DNMT3A expression in neurons contributes to mCA, we overexpressed DNMT3A via transfection in non-neuronal cells using HEK293T cells. DNMT3A overexpression in HEK293T was shown by western blot, and dot blot showed increased mCA levels upon DNMT3A overexpression compared to empty vector and catalytic mutant control. To test in a more biologically relevant cell type, we repeated the experiment in Mouse Embryonic Fibroblasts (MEFs). Similarly, we found overexpression of DNMT3A was sufficient to induce mCA. Our results suggest neuron-specific DNMT3A-interacting proteins and high DNMT3A expression drive CA methylation in neurons. In future work, we will co-overexpress DNMT3A and BCL11B in non-neuronal cells to test if this yields more mCA than DNMT3A overexpression alone. We will investigate whether blocking cell division in non-neuronal cells increases mCA. These findings provide insight into why DNMT3A methylates CA DNA in neurons and why DNMT3A mutations cause neurodevelopmental disorders in neurons but cancer in cells.

53. Cell type-specific effects of common germline variation on molecular phenotypes and pancreatic cancer risk

Gauri Prasad, Thuc Nhi Truong Vo, Jun Zhong, Jason W. Hoskins, Laufey T. Amundadottir, H. Efsun Arda

54. Characterizing enhancer dysfunction in human pancreatic ductal adenocarcinoma using a massively parallel reporter assay

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Precise regulation of pancreatic cell identity is integral for organ function, and its disruption contributes to diseases including diabetes and pancreatic cancer. Yet, the cis-regulatory mechanisms governing cell identity transitions remain poorly understood. Enhancers, through interactions with transcription factors (TFs) and gene promoters, play a central role in regulating cell type-specific gene expression. Additionally, genome-wide association studies have revealed that over 90% of disease-associated genetic variants are found in non-coding regions, underscoring the importance of cis-regulatory enhancer elements as main drivers in disease susceptibility. Our lab has identified over 100,000 putative cell type-specific enhancers across different human pancreas cell types. We have found that these regions harbor DNA binding sites for lineage-specific TFs, suggesting they may function as enhancers that maintain cell identity. However, experimentally assessing the enhancer activity of these thousands of putative genomic elements across relevant cellular contexts, and understanding the cis-regulatory mechanisms that drive cell identity present significant challenges. Massively parallel reporter assays (MPRAs) are a powerful tool to assess the intrinsic transcription-enhancing activity of thousands of candidate cis-regulatory sequences simultaneously. Using this tool, we have evaluated candidate enhancer activities in primary pancreas cells obtained from healthy donors, as well as in a human pancreatic ductal adenocarcinoma (PDAC) model, and identified differentially active sequences driven by key lineage-specific TFs. Further, by overexpressing these TFs in PDAC cells, we measured how TF modulation impacts enhancer activity, and identified a subset of enhancers that could be reverted to a normal cell-associated activity level upon TF overexpression. With this systematic approach, we are advancing our understanding of the regulatory mechanisms underlying pancreas cell identity through the creation of a comprehensive and biologically relevant map of pancreatic enhancer activity in human cells, and identifying regulatory pathways whose modulation may inform pancreas cancer risk prediction and treatment.

55. Discovery of atypical enhancer-promoter topologies in distinct pancreatic cell types

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The pancreas comprises multiple specialized cell types – including endocrine α, β, and δ cells, and exocrine acinar and ductal cells – that coordinate critical functions such as hormone secretion and digestive enzyme production. Understanding how these distinct pancreatic cell types maintain their specialized gene expression programs remains a central challenge in deciphering pancreas function and disease. While enhancer-promoter interactions are key to regulating cell type-specific transcription, the organizational principles that govern these regulatory interactions are not fully understood. In our prior work, we used 3D chromatin conformation capture and network modeling to map enhancer-promoter "tree" structures across purified pancreas cell types. These tree models typically feature a promoter as the root node connecting to multiple enhancers – a pattern consistent with canonical models of gene regulation. However, in approximately 20% of enhancer trees, we observe atypical tree structures in which an enhancer, rather than the promoter, occupies the central position in the interaction network. This deviation from expected topology suggests either mis-annotation of regulatory elements or alternative modes of gene regulation. We found that these atypical enhancer trees are not uniformly distributed and show high specificity across cell types, suggesting previously unrecognized features of pancreatic transcriptional control. To investigate the biological relevance of these atypical trees, we are integrating Precision Run-On Sequencing (PRO-seq) and its variant, PRO-cap, to refine transcription start sites and enhancer annotations to uncover potential alternative promoters or regulatory elements. We are further combining this with disease-associated variant mapping and network perturbation modeling to explore how these non-canonical regulatory structures contribute to cell type-specific gene expression and may be altered in diseases, including diabetes and pancreatic cancer.

56. A Small Retained Intron controls MALAT1 Nuclear Speckle Localization to Regulate Gene expression and Cell Migration

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57. Profiling the Epigenome of Micronuclei Across Cancer and Senescence

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Micronuclei (MN) are cytosolic bodies of membrane-bound chromatin that promote cancer progression through activation of the cGAS-STING pathway. While the contribution of MN to cancer progression is well characterized, the properties of MN found in senescent cells remain poorly understood. Using a combination of immunofluorescent microscopy and immunoblotting, we profiled MN in senescent cells and found that cGAS–STING activation and its associated epigenetic signatures are conserved across cancer and senescent contexts. Moreover, our work analyzing cancer cell MN reveals that this cGAS-STING signaling can be abrogated through inhibition of histone modifying enzymes. This finding establishes the potential for drugs that can limit MN derived pathological signaling across both cancer and senescence.

58. Control of epithelial cell fate by YAP1/TAZ-TEAD transcriptional networks *Briana Branch[1,2],*

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59. Ndfip1 supports viability of BRCA2-deficient cells by enhancing DNA repair by non-homologous end joining.

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Drug Discovery: Disease Models, Pharmacology, & Therapeutics (Posters)

60. Study of RNA-Binding Interactions of FDA-Approved Molecules For Drug Repositioning

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RNA targeting represents an original and promising approach to the discovery of new therapeutic tools against numerous diseases. Indeed, the majority of intracellular RNAs are noncoding RNAs that play key regulatory functions in many physiological processes such as translation, splicing, and RNA decay. Like proteins, many RNAs can fold into diverse secondary and three-dimensional structures with hydrophobic pockets in many structures throughout the protein databank (PDB). Those structures can be recognized by proteins or small molecules, resulting in functional changes caused by altering the thermodynamic stability or conformation of the RNA. This is the case of RNA G-quadruplexes (rG4s), bearing guanine-rich tracks that fold to form the high-order structure, mostly located in the untranslated regions (5' or 3'-UTR) of mRNAs. Thus, targeting rG4s represent a powerful opportunity to control the expression of pathogenic proteins that are challenging to target as a polypeptide structure. Thanks to the experience in small molecule microarray (SMM) technology, our lab developed a novel SPRi-SMM screening method to efficiently and rapidly identify rG4s small molecule ligands belonging to an FDA-approved drug library. Prior to this work, a bioinformatic approach was employed to inform RNA-binding chemical space and identify a rG4 structure in the 5’ UTR of our RNA overall in the human transcriptome, meaning that there was the potential for translational regulation. Our pipeline combining bioinformatics with biochemically competitive HTS efforts demonstrates a practical strategy to identify druggable structured regions within complex mRNAs as well as ligands with defined binding modes. Moreover, this study represents a good way to find out whether FDA-approved drugs and their analogs have off-target interactions with an mRNA of interest.

61. Clinical outcomes of muscle-invasive and metastatic, bladder and urethral pure adenocarcinoma (BUAdenoCa): an international study from the Global Society of Rare Genitourinary Tumors (GSRGT)

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BUAdenoCa are rare and their management options are limited. The GSRGT assembled an international cohort of pts with BUAdenoCa to evaluate the natural history and clinical outcomes of this rare cancer in the perioperative and metastatic settings. We retrospectively collected data on pts with locally advanced or metastatic BUAdenoCa receiving systemic therapy between 2000-2026 at 25 medical centers in the US, Europe, South America, and Asia. Median overall survival (mOS), disease-free survival (mDFS), and progression free survival (mPFS) were estimated by the Kaplan-Meier method for perioperative and metastatic pts. Observed objective response rate (ORR) was determined for metastatic pts receiving systemic therapy by the clinical investigator per RECIST v1.1 criteria when feasible. Among 328 pts with BUAdenoCa, including urachal (47%), urethral (11%), and non-urachal-nonurethral bladder AdenoCa/other (42%). Median age was 61 years; 61% were male; 68% were White, 17% were Black, 12% were Asian; 87% were non-Hispanic. At time of study, most patients were metastatic (65%), followed by muscle-invasive/locally advanced (16%), and non-muscle-invasive/NED (19%). 80% of stage IV pts had visceral metastases, including lung (44%), bone (36%), and liver (17%), while 10% had LN-only disease. Other metastatic sites included peritoneal/mesentery, gynecologic organs, soft tissue, muscle, CNS, and other abdominal or retroperitoneal locations. Among pts with molecular data, 98% were microsatellite stable. Common alterations included TP53 (18%), KRAS (9%), and SMAD4 (5%). Of 220 pts undergoing definitive surgery, mDFS was 1.5, 1.0, and 1.8 years (yrs) and mOS was 6.3, 4.2, and 3.2 yrs for urachal, urethral, and other BUAdenoCa, respectively. In pts receiving surgery and perioperatively treatment with 5-FU-based therapy vs. other systemic therapy, the mDFS was 2.7 vs. 2 yrs and mOS was NR vs. 2.7 yrs. In pts receiving systemic therapy in the metastatic first-line (1L) setting (n=138), mPFS was 0.68, 0.37, and 0.54 yrs and mOS was 2.3, 2.9, and 1.6 yrs for urachal, urethra, and other BUAdenoCa, respectively. Median PFS was 0.64 and 0.57 yrs and mOS was 2.3 and 1.5 yrs with 5-FU-based vs non-5-FU therapy, respectively, across types. Among evaluable pts (n=131), ORR was 29.3% (24/82; median DoR 203 days) with 5-FU-based therapy and 22.5% (11/49; median DoR 260 days) with non-5-FU therapy. To date, this represents the largest retrospective analysis of BUAdenoCa. These data suggest outcomes differ by type of BUAdenoCa and an association between 5-FU based therapy improved outcomes in both the perioperative and metastatic settings.

62. Targeting nuclear export and translation initiation uncovers synergistic antitumor activity in lethal prostate cancer

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Metastatic castration-resistant prostate cancer (mCRPC) remains a lethal disease with significant therapeutic challenge, as patients frequently develop resistance to standard of care treatments. A central mechanism of resistance involves the emergence of the AR splice variant AR-V7, which promotes tumor growth and is strongly associated with poor clinical outcomes. We hypothesized that rationally designed drug combinations targeting vulnerabilities revealed by unbiased high-throughput screening could synergistically overcome AR-driven resistance, improve therapeutic efficacy at low doses, and minimize toxicity.We performed an unbiased high-throughput matrix drug screen to identify highly synergistic combinations. Using a robotic automation platform, we evaluated 2,480 mechanistically annotated compounds targeting over 800 biological pathways across 8 prostate cancer cell lines. Single-agent cytotoxicity assays identified key vulnerabilities, including the nuclear export receptor XPO1. Based on these results, 42 compounds were selected for pairwise testing in two AR-V7-expressing mCRPC models, LNCaP-95 and VCaP-CR, using 10×10 dose-response matrices to evaluate all 861 possible dual-drug combinations. ExcessHSA synergy modeling was applied to identify highly synergistic interactions. Validation studies were conducted across 7 prostate cancer cell lines and 3 patient-derived organoid models. Mechanistic effects were assessed by live-cell imaging, global proteomic profiling, gene set enrichment analysis, subcellular fractionation, immunoblotting, and immunofluorescence. In vivo tolerability, toxicity, and efficacy were evaluated in LNCaP-95 CDX and LuCaP167-CR PDX models. ExcessHSA analysis revealed pronounced synergy between XPO1 and EIF4A1 inhibitors. Validation across multiple cell lines and organoid models confirmed robust synergy at low, clinically achievable concentrations. These findings guided the development of a combination strategy simultaneously targeting XPO1-mediated nuclear export (Eltanexor) and EIF4A1-regulated translation initiation (Zotatifin). Mechanistic analyses demonstrated that a single administration of the combination markedly suppressed proliferation and triggered rapid apoptosis. Global proteomic profiling showed coordinated downregulation of key oncogenic regulators, including AR, CCND1, SLC2A1, and CDK4, along with suppression of AR-V7-specific transcriptional programs. Gene set enrichment analyses revealed concordant reductions in cell cycle and metabolic pathways, including G2M checkpoint, E2F, glycolysis, and MYC targets. Subcellular fractionation, immunoblotting, and immunofluorescence confirmed substantial reductions in AR-FL, AR-V7, and MYC protein levels, with nuclear accumulation of p53, implicating it as a critical initiator of combination-induced apoptosis. In vivo, the combination significantly reduced tumor volume in LNCaP-95 and LuCaP167-CR models at doses more than tenfold below established tolerability limits, without weight loss. These studies identify XPO1-EIF4A1 co-inhibition as a potent and mechanistically defined therapeutic strategy that synergistically disrupts oncogenic signaling and overcomes AR-V7-driven resistance. The combination demonstrates efficacy at low, clinically achievable doses with minimal toxicity and provides a strong rationale for clinical translation, representing a promising avenue for improving outcomes in mCRPC patients.

63. Novel T cell activation via bifunctional antibody combined with immune checkpoint blockade overcomes resistance in checkpoint-refractory tumor models

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While immune checkpoint inhibition (ICI) via antibodies against the PD-1/PD-L1 axis have transformed cancer therapy, low response rates and acquired resistance remain persistent clinical challenges. Interestingly, multiple clinical reports have revealed expanded exhaustion signatures among T cells and exhausted T cell proportions in tumors post checkpoint blockade therapy. Therefore, new therapies and combinations are required to better serve numerous patient populations. In this study, we combined ICI with a novel T cell activation method via a bifunctional fusion molecule, anti-TCRβ-IL-2, comprised of a monovalent Fab, that binds and activates the T-cell receptor (TCR) through distinct variable beta (Vβ) chain residues, and an IL-2 molecule for co-stimulation. We hypothesize that we can overcome ICI-resistance using a potent T cell activator by reviving T cell function and combatting exhaustion.ICI-refractory, syngeneic mouse models for lung and colon cancer were used to assess anti-tumor responses to combination of the TCRβ-IL-2 agent and PD-1 blockade. Different sequences of drug administration were investigated since ICI had been shown in the literature to induce T cell exhaustion. Single cell RNA-sequencing, flow cytometric, and histological analyses of tumor infiltrating lymphocytes (TIL) from treated tumor-bearing mice were performed to reveal potential mechanisms of action for the benefit of the combination therapy over monotherapies. Finally, tumor associated antigen- and neoantigen-specific T cell responses were detected via flow cytometry-based and ELISpot assays to assess overall immune response efficacy and diversification. The different sequences of drug delivery yielded significant anti-tumor efficacy discrepancies in both lung and colon cancer models leading to enhanced tumor cures and long-term protection when PD-1 blockade is delivered after or with the TCRβ-IL-2 agent as opposed to before the agent. Transcriptomic analysis of TIL interestingly revealed PD-1 blockade monotherapy to greatly expand the terminally exhausted T cell population. However, tumors treated with the TCRβ-IL-2 agent followed by anti-PD-1 contained reduced proportions of CD8+ T cells with markers of terminal exhaustion, CD8+ and CD4+ T cells with increased proliferation and cytotoxic functional markers, and lower proportions of regulatory CD4+ T cells. Furthermore, only the combination therapy cohort generated significantly more neoantigen-specific T cells, while the anti-TCRβ-IL-2 monotherapy and combination therapy groups generated similar numbers of tumor-associated antigen-specific T cells. Altogether, these data reveal the combination therapy to reduce T cell exhaustion and immune suppression, enhance T cell effector function, and diversify the immune response overall resulting in enhanced anti-tumor efficacy when the drugs are delivered in the optimal sequence. Our work assessing administration sequence and profiling TIL phenotypes and states is significantly informative for any T cell-targeting therapy being combined with ICI, which is a primary focus in the field of immunotherapy as many researchers and clinicians are working toward overcoming ICI-resistance. Finally, a clinical trial is planned based on these studies to evaluate the combination of a human anti-TCRβ-IL-2 agent (invikafusp alfa) and anti-PD-1 in ICI-refractory non-small cell lung and castration-resistant metastatic prostate cancers.

64. Enabling Structure-Guided Design in the Development of Peptide Hydrogels

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Self-assembling peptide hydrogels with tunable biochemical and biophysical properties hold immense therapeutic potential in the treatment of complex tumors with irregular morphologies and microenvironments. The rational design and development of such hydrogels, however, requires a high-resolution understanding of the fundamental atomic and molecular interactions that underlie their emergent properties and functions at the mesoscopic scale. The Schneider group has developed and studied a family of fibril-forming amphiphilic β-hairpin peptides that form hydrogels with tunable viscoelastic properties, immunogenicity, molecular recognition, and rates of cargo delivery. However, uncovering the structural basis of these desirous properties is difficult and often limited to indirect and low-resolution spectroscopic techniques due to the disordered nature of the peptide fibrils in the bulk hydrogel network. The absence of high-resolution structural data limits opportunities for the structure-guided design of next generation peptide hydrogels. Herein, we have developed a molecular design strategy to modulate the phase state of β-hairpin peptides prone to form fibrillar gels into ones that assemble into crystal lattices, making high-resolution structural analysis possible. Truncating, cyclizing, and Cα-methylating a hydrogel forming peptide, affords macrocyclic β-hairpin peptides that assemble in the crystal state to form fibril-like assembles stabilized by extended arrays of hydrogen bonded β-sheets and hydrophobic packing. This approach has allowed us to investigate stereocomplexation, complementary electrostatic interactions, and covalent stabilization as atomically resolved design elements in the development of next-generation peptide hydrogels.

65. LC-MS Lipidomics Reveals Unique Dysregulation in Temozolomide-MF-438 Combination Treatment of Isocitrate Dehydrogenase 1-Mutant Oligodendroglioma

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Stearoyl-CoA desaturase (SCD1) overexpression has been implicated in isocitrate dehydrogenase 1-mutant (IDH1mut) glioma. MF-438, a SCD1 inhibitor, has demonstrated therapeutic efficacy in both cellular and murine models of human oligodendroglioma. Temozolomide (TMZ), which induces DNA alkylation, has long been the standard of care for oligodendroglioma, especially in tandem with radiotherapy. Combination treatments of drugs with different mechanisms of action have been standard in oncology for some time. In this study, MF-438 and TMZ in combination were hypothesized to induce cell death in IDH1mut oligodendroglioma models. Cell samples were collected after treatment and proliferation and lysed via sonication probe. Lipids were extracted from cell lysates per modified Bligh-Dyer methodology. All work was performed on an Agilent 1290 Infinity II UHPLC coupled to an Agilent 6545 Q-TOF. Mobile phases A and B were 10 mM ammonium formate, pH 3.5, in 3:2 acetonitrile:water and 45:4:1 isopropanol:acetonitrile:water, respectively. Column was Agilent Poroshell 120 C18, 1.9 μm, 2.1 x 100 mm. Gradient was 25 % to 95 % B over 13 minutes. MSConvert, mzMine 3.9.0, and Metaboanalyst 6.0 were used for file conversion, data processing, and statistical analysis, respectively. Cellular lipid dysregulation was assayed via LC-MS. Survivorship benefit from TMZ + MF-438 treatment in mice bearing human xenografts of IDH1mut oligodendroglioma was also contemporaneously determined.Preliminary results indicated MF-438 inhibited SCD1 activity. Phospholipids, lysophospholipids, ceramides, and triglycerides that contained palmitic (16:0), palmitoleic (16:1), stearic (18:0), and oleic (18:1) acids were upregulated in the treatment group, whereas phospholipids with polyunsaturated fatty acids were reduced. TMZ treatment resulted in subtler dysregulation: expression of one phospholipid with polyunsaturated fatty acids was decreased, and several saturated phospholipids, diglycerides, and triglycerides were increased. Thresholds for inclusion were $p < 0.05$ for all groups and fold change (FC) > 2.0 and FC > 1.5 for MF-438 and TMZ, respectively. Combination TMZ + MF-438 treatment resulted in unique upregulation of four triglycerides: TG 14:0/16:0/18:1 (FC = 3.6, p = 0.0028), TG 16:0/16:0/18:0 (FC = 13, p = 0.0073), TG O-16:0/16:0/18:1 (FC = 3.7, p = 0.024), and TG 14:0/16:0/16:1 (FC = 3.6, p = 0.050) were all differently changed compared to therapy with either TMZ or MF-438 alone. Results indicated that MF-438 acts cooperatively with TMZ to

increase expression of triglycerides with saturated and monounsaturated medium-chain fatty acids in cells. Survivorship benefit was demonstrated for combination therapy relative to either TMZ or MF-438 alone in mice bearing xenografts of human IDH1mut oligodendroglioma. Decreased proliferation via Ki67 was also observed in the combination treatment group, as well as reduced intensity of H&E stain. Therefore, the observed changes in triglyceride expression correlate with increased therapeutic efficacy of combined TMZ + MF-438 treatment. Uncovering metabolic changes associated with improved therapeutic outcomes will help to determine drug development targets. Increasing dysregulation of similar pathways could enhance the combinatorial effect observed in this study. In addition, mechanistic research on the elucidated pathways could deepen understanding of oligodendroglioma tumorigenesis and further enhance treatment efficacy.

66. Isolation and structure elucidation of Dm-CVNH, a new cyanovirin-N homolog with activity against SARS-CoV-2 and HIV-1 from the marine aqueous library of the National Cancer Institute.

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An anti-HIV screening of natural product extracts resulted in the discovery of a new antiviral protein through bioassay-guided fractionation of an aqueous extract of the ascidian Didemnum molle. The protein was sequenced through a combination of tandem mass spectroscopy and N-terminal Edman degradation of peptide fragments after a series of endoproteinase digestions. The primary amino acid sequence and disulfide bonding pattern of the 102-amino acid protein were closely related to the antiviral protein cyanovirin-N (CV-N). This new CV-N homolog was named Dm-CVNH. Alphafold2 prediction resulted in a tertiary structure, highly similar to CV-N, comprised of two symmetrically related domains that contained five β-strands and two α-helical turns each. Dm-CVNH showed specificity for high mannose and oligomannose structures, bound to HIV-1 gp-120 and potently inactivated HIV in neutralization assays (EC50 of 0.95 nM). Dm-CVNH inhibited infection in a SARS-CoV-2 live virus assays (EC50 = 11-18 nM) and was shown to bind to the S1 domain of SARS-CoV-2 Spike glycoprotein. Dm-CVNH behaved in a manner similar to CV-N, binding with a 2:1 stoichiometry to Spike (both to WH-1 and Omicron variants) and preferring the Omicron variant (Kd 42 nM) to original WH-1 (Kd = 89 nM) Spike. This sensitivity to emergent strains was mirrored in viral neutralization assays where Dm-CVNH potently inhibited the infection of Omicron strains XBB.1.16 and JN.1 (IC50 = 11-18 nM).

67. Benzyl α-Ammonium Carbamates: A Polar Linker Strategy to Improve Bioconjugate Properties and Enable ADC Discovery

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68. Fluorescence Polarization High-Throughput Screening to Discover Small-Molecule Disruptors of the Rpn13–Rpn2 Proteasomal Interface

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69. Non-viral PD-1 locus-targeted anti-GPC3 CAR-T cells regress solid tumors in a mouse model

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Although CAR-T therapy has achieved significant success in hematologic malignancies, its broader application is limited by reliance on lentiviral transduction, which carries risks of random genomic integration and variable transgene expression. These limitations underscore the need for precise and safer genome engineering strategies. In addition, T cell exhaustion, largely driven by inhibitory receptors such as programmed cell death protein 1 (PD-1), remains a major barrier to durable efficacy, particularly in solid tumors. Here, we used CRISPR/Cas9 technology to generate non-viral anti-GPC3 CAR-T cells via precise integration at the PD-1 locus, thereby simultaneously eliminating PD-1 expression. Primary human T cells were electroporated with CRISPR/Cas9 ribonucleoprotein complexes targeting the PD-1 locus, together with a nanoplasmid encoding anti-GPC3 CAR constructs (hYP7 and HN3) to generate PD1-integrated hYP7 or HN3 CAR-T cells (PD1-hYP7 or PD1-HN3). Lentivirus-transduced hYP7 or HN3 CAR-T cells (LV-hYP7 or LV-HN3) were prepared as a control. We used T cells from seven healthy donors to verify the protocol established in our lab, and the average CAR knock-in efficiency was 32%, while PD-1 knockout efficiency reached 98%. In ex vivo killing assays, both PD1-hYP7 and LV-hYP7 demonstrated comparable cytotoxicity against GPC3-expressing liver tumor cell lines. PD1-HN3 CAR-T cells exhibited greater cytotoxic activity than LV-HN3 CAR-T cells, with a higher proportion of $CD8^+$ T cells and increased expression of activation markers, including CD69 and CD137. When comparing PD1-hYP7 and PD1-HN3, both showed similar killing activity across liver tumor cell lines. In a liver tumor mouse model, both the PD1-HN3 and LV-HN3 groups exhibited complete tumor regression, with comparable survival rates. Blood PD1-HN3 CAR-T cells exhibited a higher frequency of stem cell-like memory T cells than LV-HN3 cells. Within the $CD8^+$ CAR-T population, PD1-HN3 cells showed lower CTLA-4 expression than LV-HN3 cells. PD1-HN3 CAR-T cells exhibited enhanced activation profiles, improved memory phenotypes, reduced expression of exhaustion markers, and tumor control comparable to that of lentiviral CAR-T cells in solid tumor models. This precision genome-editing strategy provides a potentially safer and more effective platform for next-generation CAR-T therapies targeting solid malignancies.

70. Optimizing a fluorescent polarization assay targeting the ATG5-ATG16L1 protein-protein interaction to enable high-throughput screening of the NCI natural product prefractionated library

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71. ABC-Transporters Limit Proteolysis Targeting Chimeras (PROTACs) Efficacy in Cancer Cells

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Proteolysis-targeting chimeras (PROTACs) are an emerging class of small molecules enabling selective targeted protein degradation. These heterobifunctional molecules consist of two ligands joined by a linker: one binds a protein of interest (POI), and the other recruits an E3 ubiquitin ligase, inducing ubiquitination-mediated proteolysis of the POI. Currently, there are thousands of PROTACs developed and over 120 ongoing clinical trials; yet the intracellular uptake and transport dynamics governing the efficacy of PROTACs remain poorly understood. ATP-binding cassette (ABC) transporters such as ABCB1 and ABCG2 are known to limit cellular uptake of structurally unrelated drugs leading to resistance. These transporters are located in the gut and blood-brain barrier where they have shown to have a role in limiting oral bioavailability and brain penetration of many therapeutics. In this study, we seek to evaluate and structurally assess the transport dynamics of a variety of PROTACs with the goal of characterizing transporter-mediated PROTAC resistance. HT1080 fibrosarcoma cells were transfected with empty vector or vectors containing the ABCB1 or ABCG2 genes. Clones were selected and transporter expression was validated by flow cytometry. ABC transporter mediated resistance to several commercially available PROTACs targeting multiple kinases, BRD (bromodomain), CDK (cyclin-dependent kinase), and HDAC (histone deacetylase) proteins, as well as their corresponding target ligands, were assessed in transfected cells following a 72-hour drug treatment and GI50 concentrations (50% growth inhibitory concentration) were determined.

Overexpression of ABCB1 confers significant change in cell viability as compared to control cells for a majority of the PROTACs tested. Of the PROTACs assessed, 11 demonstrated greater than a 5-fold increase in ABCB1-overexpressing cells, indicating likely transporter mediated efflux. In contrast, inhibitors in isolation exhibited no significant GI50 shift, indicating transport effects independent of the target ligand. PROTACs containing the same POI and E3 ligase components but a 2-fold longer linker length showed over 46-fold increase in cell viability among ABCB1-overexpressing cells while incorporation of oxygen atoms in the linker further amplified resistance, potentially due to increased flexibility due to hydrogen bonding at the ternary binding interface. ABCG2-overexpressing cells showed changed in PROTAC sensitivity in two of the evaluated PROTACs as compared to the control cells. Ongoing studies include cell cycle analysis to assess cell growth inhibition, as well as Western blot analysis and further cell viability assessment using ABCB1 and ABCG2 inhibitors to confirm recovered protein degradation. Collectively, we demonstrate that ABCB1 and ABCG2-overexpressing cells confer increased cell viability in many PROTACs, independent of target protein ligand, and consistent with ABC transporter-mediated efflux influenced by structural features such as linker length. These findings suggest that ABC transporters may confer resistance to PROTACs and that co-administration with transporter inhibitors could enhance PROTAC efficacy. Moving forward, we aim to systematically evaluate effluxed PROTACs to identify concrete structural distinctions such as linker length and composition that drive transporter-specific resistance. This will inform rational design strategies and proactively address efflux-mediated resistance as these agents progress towards clinical application.

72. Beyond the Core: Uncovering Dynamic Regulators of the Proteasome

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The proteasome is the central machinery responsible for degrading ubiquitinated proteins, thereby maintaining cellular protein homeostasis. Although its structure is well characterized, its function relies on dynamic and transient interactions that remain poorly resolved. In particular, numerous accessory proteins associate with the proteasome regulatory particle (RP), yet their binding sites, activities, and biological relevance are still largely unclear. To address

this, we integrated cryo-electron microscopy with PhIX-MS (Photo-induced In-situ Crosslinking Mass Spectrometry), a structural proteomics workflow that stabilizes transient interactions in living cells using UV-activated crosslinking. This approach allowed us to capture and structurally position proteasome-interacting partners with high confidence. Using this strategy, we resolved the redox sensor TXNL1 at the RP and positioned its PITH domain above the deubiquitinase RPN11, while its thioredoxin-like domain localizes dynamically near RPN2 and RPN13. This strategic placement suggests that TXNL1 may reduce substrates prior to proteolysis, linking redox control to proteasomal degradation. We also observed the assembly chaperone PSMD5, which binds in a way that sterically blocks interaction between the RP and the core particle. Its C-terminus inserts into the ATPase channel, inducing a flatter ATPase ring conformation. These findings provide structural insight into how proteasome assembly and regulation may be controlled. We further identified multivalent interactions between the poorly characterized protein ECPAS/ECM29 and hRpn10, with ECPAS binding to the UIM1 region of hRpn10. Loss of ECPAS impairs clearance of ubiquitinated proteins, which we propose results from disruption of a peptide exit site for proteolyzed substrates. While promoting substrate exit, ECPAS also protects the proteasome from autoproteolysis. These critical activities are foundational to proper proteasome function. Together, our findings define previously unresolved structural and functional roles of accessory proteins at the proteasome, revealing new regulatory layers that shape substrate processing and proteostasis. These insights expand our understanding of how protein degradation is regulated within the cellular environment.

73. Mesothelin-Targeted Nanobody-Drug Conjugates to Treat Pancreatic Cancer

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Development of targeted therapies that can penetrate the dense stroma of the pancreatic ductal adenocarcinoma (PDAC) tumor microenvironment (TME) is essential to improve outcomes for pancreatic cancer patients. We have discovered a camel VHH nanobody (A101) using phage display, which effectively binds to mesothelin (MSLN), a cancer antigen that is expressed in PDAC. Using the A101 VHH nanobody with an MMAE payload, we generated nanobody-drug conjugates (NDCs) that can be advantageous compared to existing biologic therapies by enhancing tumor penetration through the PDAC TME and improving pharmacokinetic distribution, while minimizing systemic toxicity. The structure of the A101 nanobody was computationally predicted and molecular dynamic simulations were run to understand its interaction with MSLN. Site-specific modifications were engineered and small linker-MMAE molecules were synthesized, then bioconjugated to the modified A101 nanobody using maleimide chemistry. The chemical linkers used in these constructs were valine-citrulline, glycine-proline, and AB (a novel small molecule). The NDCs were tested for binding to MSLN via bio-layer interferometry assays. Internalization and cell viability were also assessed using mouse and human PDAC cell lines. Finally, in vivo activity was assayed in MSLN+ and MSLN knockout (MLSN-KO) subcutaneous mouse PDAC tumor models to determine efficacy and specificity of each therapeutic. We found that modified A101 nanobody retains binding to human (KD = 18 nM) and mouse (KD = 5.6 nM) MSLN. A101 NDCs displayed a statistically significant difference in internalization into MSLN+ as compared to isogenic MSLN-KO cells. Additionally, the NDCs efficiently killed PDAC cell lines in vitro (IC50 = 20-200 nanomolar). In mice, the A101-AB-MMAE NDC (at 20 mg/kg, 3x per week) displayed the most promising inhibition of tumor growth in MSLN+ tumors and had the highest specificity compared to other A101-NDCs tested, showing limited efficacy in analogous MSLN-KO tumors. We have synthesized A101-based NDCs with high-affinity MSLN binding and promising anti-tumor activity in PDAC cell lines. Our preliminary animal studies suggest that the A101-AB-MMAE conjugate, which has a novel exclusively intracellular cleavable linker, has superior efficacy and specificity compared to other A101-based NDCs tested with known linkers. Future studies will explore A101-AB-MMAE activity in orthotopic humanized MSLN mouse models of PDAC to assess safety, biodistribution, and anti-tumor efficacy.

74. Vaginal tissue accumulation of the zinc finger protein inhibitor SAMT-247 Met-D drives vaccine-induced protective anti-inflammatory mucosal immunity against SIVmac251

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SAMT-247, a mercaptobenzamide thioester, exerts virucidal activity by targeting HIV NCp7 zinc finger protein required for viral RNA encapsidation. In vivo delivery of SAMT-247 via gel or intravaginal ring (IVR) synergizes with the ΔV1DNA/ALVAC-SIV/ΔV1gp120/alum vaccine regimen, significantly reducing vaginal SIVmac251 acquisition (VE: 92.7% and 82.8%, respectively; $p<0.0001$). SAMT-247 enhances mucosal immunity and reduces inflammation by promoting zinc release from NCp7. Intravaginal administration of SAMT-247 generates four metabolites (Met-A, -B, -C, -D). While parent SAMT-247 and metabolites B, C, and D exhibit virucidal activity in vitro, the role of metabolites in immunomodulation remains unknown. We investigated the distribution of SAMT-247 metabolites in the vaginas of six rhesus macaques by collecting vaginal swabs and biopsies four hours after topical application of SAMT-247. To dissect, in vitro, the biological effects of the SAMT-247 metabolites, we collected and differentially stimulated ex vivo mucosal biopsies from 11 vaccinated macaques. High levels of Met-D, Met-B, and Met-C were detected in cervicovaginal fluids, whereas vaginal tissue showed higher Met-D levels than Me-A, with Met-B and Met-C undetectable. Met-D preserved or increased the number of protective IL-17$^+$NKp44$^+$ ILCs and CD107$^+$NKG2A$^+$ NK cells, and selectively expanded IL-10$^+$ dendritic cells and IL-10$^+$ macrophages, thereby creating an anti-inflammatory mucosal environment critical for reduced SIVmac251 acquisition. Strikingly, Met-A, a non-virucidal metabolite, reduced IL-17$^+$NKp44$^+$ ILCs and cytocidal CD107$^+$NKG2A$^+$ NK cells, likely by decreasing surface CD73 expression on tolerogenic dendritic cells and reducing IL-10 production. SAMT-247 and its metabolites shape mucosal immunity through multidimensional effects on mucosal NK/ILC, dendritic cell, and monocyte responses. Met-D demonstrates both virucidal and anti-inflammatory activity in vaginal tissues and contributes to the enhanced vaccine protection observed in vivo. Met-A promotes inflammation and diminishes protective responses. These results not only identify SAMT-247 metabolite-specific immune pathways relevant to HIV/SIV prevention in women but also highlight the broader concept that microbicide metabolism can differentially affect mucosal immune responses. Chemical modification of SAMT-247 may be performed to prevent Met-A formation, potentially yielding a more potent microbicide.

75. Engineering chimeric antigen receptor (CAR) T cells targeting a tumor associated isoform of glypican-1 for cancer immunotherapy

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76. Structure-based design of next generation small molecule degraders against proteasome subunit Rpn13

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The ubiquitin-proteasome system maintains cellular proteostasis by targeting misfolded or excess proteins for degradation. Ubiquitinated proteins are recognized by receptors in the 19S regulatory particle (RP)1 and degraded within the 20S core particle (CP)2. Dysregulation of this pathway is implicated in numerous cancers, and FDA-approved CP-targeting proteasome inhibitors show strong efficacy in hematological malignancies, motivating alternative proteasome-targeting strategies.3 Proteolysis-targeting chimeras (PROTACs) promote selective degradation by linking targets to ubiquitination machinery4 or directly to proteasome subunits such as Rpn1 or Rpn13.5-6 Rpn13 is a therapeutic target, as PROTACs6-8 and small-molecule degraders9 that induce its depletion trigger apoptosis in multiple cancer types8-11. Rpn13 functions on and off the proteasome, binding the C-terminus of Rpn2 and ubiquitin chains on substrates12-15. Off the proteasome, Rpn13 interacts with disordered protein regions14 and regulates additional processes through epigenetic regulators such as PADI4 and HDAC8.16-17 These roles in proteolysis and gene expression support continued development of Rpn13-targeting compounds. We previously identified small molecules, including XL44, that selectively target a truncated form of Rpn13 (Rpn13Pru) that is variably expressed across cell lines and abundant in myeloma cells9. This fragment contains the pleckstrin-like receptor for ubiquitin domain and mediates interactions with ubiquitin15-17, proteasomes19, and epigenetic regulators16-17. XL44 covalently modifies C88 of Rpn13 and engages surrounding hydrophobic residues9. Structure-guided optimization produced XL69 and XL80, which bind Rpn13 with higher affinity and improved cellular potency: XL44 acts at a micromolar IC50, whereas XL69 and XL80 act at submicromolar concentrations. We evaluated the efficacy and mechanisms of Rpn13-targeting compounds (XL69 and XL80) in depleting Rpn13Pru and inhibiting viability in myeloma cell lines. RPMI-8226 cells were treated with varying concentrations of XL69 or XL80, and viability was measured using MTT assays. Rpn13Pru abundance was assessed by immunoblotting, and depletion kinetics were quantified over time. We also solved crystal structures of Rpn13Pru bound to XL69 or XL80. XL69 and XL80 caused a concentration-dependent reduction in viability in RPMI-8226 cells, with IC50 values in the nanomolar range. Immunoblotting revealed pronounced depletion of Rpn13Pru, indicating direct targeting and depletion of this post-translationally processed form of Rpn13. XL69 and XL80 showed sustained activity, with an approximately 15-hour half-life for Rpn13Pru depletion. Crystal structures reveal a new binding mode, validated by NMR, in which interaction surfaces are fully stabilized, correlating with enhanced potency. These findings demonstrate potent activity of XL69 and XL80 in restricting cell viability and targeting/depleting Rpn13Pru in myeloma cells. The compounds show potential as alternatives and/or synergistic agents for current proteasome inhibitors by disrupting Rpn13 and related pathways. Further optimization may enable novel therapeutic strategies targeting Rpn13-mediated pathways in cancer and other diseases.

77. TR-107, a Novel Mitochondrial ClpP Activator, Exhibits Potent Antitumor Activity in Adrenocortical Carcinoma Models

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78. Selective Cytotoxicity of Base Excision Repair inhibitor, TRC102, in DNA Damage Response Hyperactivated Glioblastoma

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Glioblastoma is the most aggressive and lethal type of brain tumor. Tumor heterogeneity and DNA repair pathways contribute strongly to poor outcomes, highlighting the need for more personalized approaches. TRC102 (methoxyamine hydrochloride) is a small-molecule inhibitor of base excision repair that acts by covalently binding to abasic sites and preventing downstream repair enzymes from repairing the lesion. Clinical studies utilizing TRC102 in glioblastoma have identified hyperactivated DNA damage response (DDR) gene signature among exceptional responders, suggesting DDR-hyperactivity as a potential clinical biomarker. Here, we evaluated whether glioblastoma cells reliant on DDR signaling are particularly vulnerable to TRC102 treatment.We performed gene set enrichment analysis using RNA-seq data across 24 glioblastoma cell lines within the NCI, Neuro Oncology Branch database and identified three patient-derived cell lines (L0, L1, GSC627) with hyperactivated DDR signature. Treatment efficacy was evaluated using cell viability, colony formation, DNA damage, and immunofluorescence assays.TRC102 treatment alone induced significant DNA damage and cell death in DDR-hyperactivated glioblastoma cell lines, whereas DDR-hypoactivated cells showed minimal sensitivity. In L0 and L1 cell lines, TRC102 treatment significantly inhibited colony formation and cell proliferation over 72-hour treatment. Mechanistic studies through western blotting revealed that TRC102 led to significant suppression of CHK2 activity, a key kinase involved in the DDR that controls cell cycle progression. Abrogation of cell cycle checkpoint blockades through CHK2 depletion may allow glioblastoma cells to initiate mitosis without fully resolving DNA damage, leading to mitotic catastrophe and cell death. Interestingly, TRC102 also suppressed HIF protein expression in hypoxic conditions, leading to eventual cell death. The antitumor effect of TRC102 appears to be mediated through targeting of the newly identified ATM-CHK2-HIF axis, allowing for selective cytotoxicity in DDR-hyperactivated glioblastoma. These results suggest a specific vulnerability to TRC102 in DDR-hyperactivated glioblastoma that are typically resistant to conventional chemotherapy and radiation therapy, supporting biomarker-driven patient selection in future clinical studies.

79. Autophagy Modulation for Anticancer Drug Development: Discovery of Small-Molecule Inhibitors of the ATG5-ATG16L1 Protein-Protein Interaction

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80. Enabling High-Density Antibody Labeling with Cysteine-Reactive Heptamethine Cyanines

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Heptamethine indocyanines have been widely used in both clinical and preclinical settings due to its near-infrared (NIR) absorbance and emission properties. However, the lack of effective methods for synthesizing these molecules has limited their broader application. Previous efforts have found that chemically diverse indocyanines with varied conjugation chemistry can be synthesized in high yields using pyridinium benzoxazole (PyBox) salts as precursors. This approach allowed us to develop an optimized lysine-labeling probe, FNIR-766-NHS, with reduced dye-dye interactions and enhanced tumor uptake and selectivity compared to other commonly used probes. However, lysine-labeling strategies are heterogenous, and the development of homogenous imaging remains an important goal. To address this limitation, we are seeking to create thiol-reactive NIR cyanines that target disulfides in the hinge of the antibody. This study aims to achieve homogenous conjugation with minimal cyanine self-quenching. Using Pybox chemistry, we synthesized three thiol-reactive FNIR-766 derivatives variated in their reactive moiety: bromo-acetamide, maleimide, and self-hydrolyzed maleimide. When conjugated to antibodies, these molecules exhibit reduced self-aggregation, even at high labeling densities. Moreover, the resulting conjugates generate strong fluorescent signals both in vitro and in vivo, outperforming conventional persulfonated heptamethine cyanines. Their site-specificity, homogeneity, and reduced self-aggregation make these fluorophores promising candidates for preclinical and clinical applications.

81. Elucidating the molecular determinants for disrupting ATG14L-Beclin1 Interactions as a Strategy for Selective Autophagy Inhibition

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82. Targeting CDK4/6 with Palbociclib Reduces Tumor Burden in Murine Osteosarcoma Models

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Osteosarcoma (OSA) is the most common form of primary bone cancer found in both humans and canines. Dogs closely recapitulate the clinical and molecular features of OSA in humans, positioning them as a promising model for investigating novel therapeutic approaches. Palbociclib is an FDA-approved breast cancer drug that inhibits CDK4/6, preventing the G1 to S phase transition of the cell cycle. Genes involved in cell cycle control are frequently altered in human and canine OSA and may represent druggable targets. In this study, we assessed the use of palbociclib in vitro and in vivo as a therapeutic strategy for treating canine and human OSA. In vitro growth inhibition of palbociclib was assessed across a panel of canine (MCKOS/SM1/GFP, OSCA 29, and Payton) and human (HOS-MNNG/GFP, MG63.3/GFP) OSA cell lines using IncuCyte Live-Cell Analysis, followed by CellTiter 96® AQueous One Solution cell proliferation assay. Western blots were conducted to explore the downstream effects of palbociclib treatment. Additionally, somatic copy number variations were determined using GATK CNV caller in tumor-normal mode (v4.6.0.0). Following an orthotopic injection of 1 x 10^6 human OSA cells, murine models were treated with palbociclib (100 mg/kg) via oral gavage for 4 weeks. Tumor volume was measured weekly and calculated using the equation (D x d2)/6 × 3.12 (where D = the maximum diameter and d = the minimum diameter) to determine palbociclib's impact on primary tumor growth. Palbociclib treatment resulted in variable IC50 values across canine and human OSA cell lines, ranging from 3-11 µM. Murine xenografts treated with palbociclib exhibited a decrease in primary OSA tumor burden compared to untreated mice. The final tumor volume of palbociclib-treated mice was approximately 56% and 13% of the volume of untreated mice for MNNG/GFP and MG63.3/GFP, respectively. Western blotting revealed hypophosphorylation of retinoblastoma, as well as a decrease in cMYC expression following treatment. Contrastingly, we observed an increase in CCNE1 and CDC25A expression after treatment, suggesting a potential compensatory escape mechanism for cells to re-enter S phase. Palbociclib is a novel treatment for canine and human OSA. These findings support further investigation of the anti-OSA activity of palbociclib in both pediatric and veterinary medicine. Approximately 1,000 children and over 10,000 pet dogs are diagnosed with OSA annually. The highly metastatic, aggressive, and heterogeneous nature of OSA makes it an extremely challenging disease to treat. Despite several advancements in OSA therapeutics, treatment outcomes have remained largely unchanged over the past 40 years. This study offers a robust preclinical assessment of a commercially available CDK4/6 inhibitor in both canine and human OSA, supporting its clinical role in veterinary and pediatric settings as an addition to the current standard of care backbone. The canine patient serves as a strong, comparative model for understanding this aggressive cancer in humans, and our data support a clinical trial concept in dogs to better understand this disease.

83. P-glycoprotein overexpression confers acquired resistance to mirvetuximab soravtansine in folate receptor-alpha-positive ovarian cancer

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84. Synthetic optimization of a small-molecule ATG14L-Beclin1 protein-protein interaction inhibitor for selective autophagy inhibition

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Autophagy is a catabolic process that degrades and recycles cellular components to maintain homeostasis. Cancer cells use autophagy for survival in response to stresses, including hypoxia, nutrient deprivation, and treatment with chemotherapeutics. Clinical trials have used chloroquine or hydroxychloroquine, late-stage inhibitors of autophagy that disrupt lysosome function, but their lack of selectivity leads to off-target effects. To improve selectivity, inhibitors of the lipid kinase VPS34 have been developed; however, VPS34 is involved in two multi-protein complexes: the autophagy initiation complex (Complex I) and the vesicle trafficking complex (Complex II), thus, direct inhibition leads to inhibition of both pathways. ATG14L is only found in the autophagy initiation complex, where it interacts with Beclin1 through a coiled-coil domain and is critical for autophagy activation. We hypothesized that inhibition of this protein-protein interaction (PPI) would selectively inhibit autophagy without inhibiting vesicle trafficking. We developed cellular nanoBRET assays to monitor the interaction of the ATG14L-Beclin1 and UVRAG-Beclin1 PPIs to identify compounds that selectively inhibit autophagy by only inhibiting the VPS34 Complex I PPI. A synthetic strategy was developed to synthesize the initial hit, Compound 19, and analogues for structure-activity relationship studies. Through these studies, we were able to identify key structural features of Compound 19 that are required for activity and selectivity Unfortunately, Compound 19 and selected analogues had half-lives of less than five minutes as determined by microsomal stability experiments. We performed metabolite identification studies with Compound 19 and determined that the major metabolites include oxidation of the aryl methyl groups and oxidation of the cyclobutyl substituent in the oxadiazole. Current efforts are focused on the synthesis of additional analogues to incorporate aryl methyl bioisoteres and a variety of substituted heterocyles to replace the oxadiazole. Successful completion of this work will provide a selective, small-molecule autophagy inhibitor with improved metabolic stability that will be used to evaluate in vivo efficacy in cancer models.

85. Overexpression of TmtA1A and TmtA1B confers resistance to small molecule prenyltransferase inhibitors

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Protein prenylation is an irreversible covalent posttranslational modification found in eukaryotic cells. Protein prenylation inhibitors (PTIs) were developed after discovering that oncogenic Ras proteins rely on farnesylation for membrane localization and oncogenic signaling. Initial development of inhibitors focused on thiol-containing peptide mimetics that target farnesyltransferase. Previously, our lab found that the thiolmethyltransferases TMT1A and TMT1B confer resistance to thiol-based HDACis (histone deacetylase inhibitors) by methylating and inactivating the zinc-binding thiol. TMT1A and TMT1B can methylate thiol groups, a modification that can inactivate thiol-containing PTIs by preventing their interaction with target proteins. Similarly to thiol-containing HDACis, we hypothesized that PTIs, such as geranylgeranyltransferase transferase inhibitors (GGTIs) and farnesyl transferase inhibitors (FTIs), that also contain zinc-binding thiols could be inactivated by overexpression of TMT1A or TMT1B. In this study we utilized HT1080 cells, known to be sensitive to PTIs, to observe the effects of TMTAs on PTIs. We transfected the cells to overexpress TMTA1A and TMTA1B and performed western blots to confirm protein expression. To understand how the HT1080 cells expressing TMTA1A or TMTA1B interact with thiol-containing PTIs, we conducted cytotoxicity assays on several FTIs and GGTIs that either contain or lack thiol groups. With FACS analysis, we then analyzed changes in cell cycle in the presence of the thiol containing drugs in the HT1080 cells with and without TMTA overexpression. Finally, we utilized mass spectrometry analysis to visualize changes to the drug structure after exposure to cells expressing TMT1A or TMT1B. From the cytotoxicity assays, we observed that cells overexpressing TMTAs were more resistant to PTIs containing a thiol group. Cycle analysis revelaed that while the empty vector

HT1080 cells were primarily arrested in G0/G1 with depletion of the S and G2/M phases, such changes were not observed in the HT 1080 cells overexpressing TMT1A or TMT1B. Using mass spectrometry to analyze changes to the PTI drug structure, we observed that exposure to cells that overexpressed TMT1A or TMT1B, PTIs containing thiol groups exhibited methylation at the thiol moiety. These results suggest that TMTA is partially responsible for resistance to PTIs, especially those containing thiol groups, through methylation. Taken in combination with our lab's previous study demonstrating the role of TMTA in methylation of HDACis, we demonstrate that thiol containing drugs may be broadly metabolized by the body in a way that renders them inactive. Despite emerging evidence that TMTs methylate drugs at their thiol moiety and render then inactive, drug companies continue to develop drugs that contain these thiol groups. PTIs are emerging as potential therapeutic agents targeting the prenylation process, which is crucial for the function of various proteins involved in cancer progression. However, the development of resistance to PTIs poses a significant challenge to their clinical efficacy. The involvement of TMTs in resistance mechanisms highlights the importance of considering these enzymes as potential targets for overcoming resistance to PTIs and improving therapeutic outcomes.

86. Multiplexed Anti-Glycan Antibody Discovery Using DNA-Barcoded Neoglycoproteins Coupled with Single Cell Sequencing.

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Anti-glycan monoclonal antibodies play a critical role in human health and fundamental research. However, the availability of high-quality anti-glycan mAbs remain limited, underscoring the need for improved technologies to enable their discovery. Beyond the general challenges associated with antibody development, anti-glycan antibodies are particularly hindered by inherently low binding affinity and suboptimal selectivity. To help overcome this barrier, we developed a multiplexed strategy for rapidly identifying glycan-binding B cells. The approach uses a set of DNA barcoded, multivalent neoglycoconjugates as bait to target glycan-binding B cell via their B cell receptor. Positive cells are then sorted by FACS, and the pool is then subjected to single cell sequencing to determine the sequences of the heavy chain and light chain as well as profile binding of the barcoded glycoconjugates. Using this strategy, we obtained a variety of different anti-glycan antibodies in a single experiment. To verify the specificity of these antibodies, we tested these antibodies using glycan microarray technology. Glycan microarray experiments lead to discovery of multiple good antibodies against various targets. We carried out additional experiments on couple of antibodies (anti-rhamnose and anti-chitin) that had better specificity and affinity based on the glycan microarray to verify their binding to natural targets. We carried out ELISA experiments and showed the anti-rhamnose antibody binds to rhamnolipids isolated from Pseudomonas aeruginosa and anti-chitin binds to chitin from shrimp. Confocal microscopy showed that the anti-chitin antibody binds very well to the cell walls of yeast (S. cerevisiae) and fungi (Aspergillus fumigatus). The positive results demonstrate the potential of the approach for rapidly isolating glycan-binding B cells and their associated antibodies.

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87. The CST complex suppresses the toxicity of transcription-replication conflicts in ARID1A deficient cancer cells

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Mutations in genes encoding SWI/SNF chromatin remodeler subunits are mutated in more than 20% of human cancers and ARID1A is the most frequently mutated SWI/SNF component. Loss of function driver mutations in ARID1A occur in many cancer types and its loss compromises multiple DNA repair pathways, including Mismatch Repair and 2 major pathways of DNA double-strand break repair, Homologous Recombination and Non-Homologous End Joining. ARID1A deficiency is associated with high tumor mutational burden and is being investigated as a biomarker for immunotherapy, and its loss sensitizes to radiotherapy and inhibitors of the DNA damage response, including ATR and PARP inhibitors. To identify genetic vulnerabilities of ARID1A deficient cancers, we carried out CRISPR/Cas9 screens in multiple isogenic cell line pairs. Among the genes common to both cell lines, we identified CTC1 and STN1, both members of the CST (CTC1-STN1-TEN1) complex. The CST complex plays critical roles in DNA repair pathway choice by inhibiting DNA resection and is required for telomere and genome maintenance through the recruitment of Polymerase alpha-primase to carry out fill in DNA synthesis. We validated the CST complex as a vulnerability of multiple ARID1A deficient cell lines and identified increased DNA damage resulting from elevated R-loop and G4 quadraplex stability as the likely driver of toxicity. Consistent with this, we found that ARID1A deficient cancer cells were sensitized to G4 stabilizers, and this was further exacerbated by depletion of the CST complex. Treatment with inhibitors of the MRE11 nuclease partially mitigated the sensitivity of ARID1A cancers to CST loss, implicating excessive DNA resection in the absence of CST as a driver of toxic genomic instability. Our results suggest that G4 stabilizers may benefit the treatment of ARID1A deficient tumors and identify the CST complex as a critical genetic vulnerability that could be targeted in future work.

88. Deriving and Characterizing CRISPR/Cas9 Knock-In Murine Oral Squamous Cell Carcinoma Cell Lines in C57BL/6 mice for Improved Understanding of Immune Checkpoint Blockade Inhibitors in Head and Neck Cancer

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Head and Neck Cancer (HNC) is the 7th most common cancer globally. While standard therapies are multimodal, including surgery followed by chemo-/radio- therapy, personalized treatments tailored to an individual's immune context are still developing. Immune checkpoint blockade inhibitors (ICB) offer improved results in subsets of HNC patients. However, many patients do not benefit from ICB therapy, and how particular oncogenes contribute to immune resistance remains uncertain. To enable rapid testing of gene-specific tools within an immune-competent tumor microenvironment, CRISPR/Cas9 gene editing provides a powerful approach for dissecting key tumor–immune interactions in vitro. However, Cas9-expressing murine oral squamous cell carcinoma (mCas9-OSCC) models that can grow in immune-competent mice were unavailable. To address this gap, two novel mCas9-OSCC cell lines have been derived and characterized, offering new models to investigate oncogenic drivers, their contributions to immune evasion, and their resistance to immune checkpoint blockade therapies in immune competent mouse models.

89. CRISPR-Cas9 Screens in Mice to Identify Tumor Suppressors of Aggressive Human Lymphoma

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Diffuse Large B Cell Lymphoma (DLBCL), the most common form of aggressive Non-Hodgkin's Lymphoma, has been subdivided into genetic subtypes that respond differentially to therapy. The MCD subtype, characterized by MYD88L265P and CD79B mutations, is a subtype of DLBCL with some of the worst clinical outcomes. Patients have the lowest response rates to the standard of care – R-CHOP immunochemotherapy. To study MCD biology, we have developed a mouse model harboring four MCD-associated genetic alterations. With age, these mice develop DLBCL that arises in the spleen following an accumulation of spontaneous germinal center B Cells (GC Bs) suggesting they play a role in the transformation to malignancy. As MCD mice develop DLBCL with age, this suggests additional genetic events are necessary to accelerate tumor development. The MCD genetic subclass of DLBCL is enriched for mutations in many genes that could potentially function as tumor suppressors. The inclusion of a Cas9 allele in the MCD mouse model enables loss-of-function assays to study putative tumor suppressors in the premalignant cell-of-origin. Screening these candidate tumor suppressors in this system revealed that loss of either Setd1b, a histone methyltransferase, or Tbl1xr1, a transcriptional corepressor, caused the largest increase in premalignant splenic GC Bs, suggesting they play a key role in driving MCD pathogenesis. Furthermore, the combined loss of these genes caused an even larger increase in this premalignant subpopulation, indicating a functional relationship between these two genes. Single cell RNA sequencing data showed that loss of Setd1b and Tbl1xr1 alters the phenotype of splenic GC Bs, resulting in a neomorphic cell state with features of both GC Bs and memory B cells. The combinatorial loss of these genes also induced gene expression signatures characteristic of the oncogenic signaling pathways that human MCD tumors depend upon. These data point towards a cooperative functional relationship between these genes, a discovery that is further supported by analysis of human MCD tumors, in which SETD1B and TBL1XR1 are genetically inactivated in the same tumors significantly more often than expected by chance. As we continue to investigate the role of these now validated tumor suppressors in the development of MCD-DLBCL, we hope to shed valuable light on their contribution to lymphomagenesis, which may reveal new therapeutic vulnerabilities in this aggressive cancer.

90. Extracellular vesicle determinants of metastatic potential

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91. Comprehensive characterization of the peritumoral tumor microenvironment in metastatic osteosarcoma

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The non-tumor tissue adjacent to metastases can appear morphologically unremarkable under a microscope; however, it is exposed to a milieu of secretory factors and proteins derived from tumor cells, stromal cells, and immune cells within the surrounding tumor microenvironment. Studies investigating the peritumoral tissue (PTT) or so-called Normal tissue Adjacent to Tumor tissue (NAT) have identified distinct differences between the genomic and transcriptomic profiles of healthy and tumor-adjacent non-tumor tissues. These alterations are hypothesized to have

significant implications in local tumor progression, metastasis, and patient outcome. Most NAT/PTT studies focus on the primary tumor microenvironment (TME). In this study we investigated the metastatic TME with comparisons between met-recipient and met-free tissues, both derived from a canine osteosarcoma clinical trial. We hypothesize secretory factors derived from the metastatic tumor tissue and may play a role in TME remodeling that is favorable to tumor progression. Samples were collected during necropsy of pet dogs with a confirmed diagnosis of appendicular osteosarcoma that participated in a multisite prospective clinical trial. Non-tumor tissues derived from osteosarcoma patients with and without metastases are referred to as met-recipient and met-free tissues, respectively. Using FFPE tissue blocks, non-tumor tissue proximal to osteosarcoma metastases was obtained via hand macro-dissection by a veterinary pathologist (JAB). All other samples were collected via scrolls. RNA isolation from tissues was performed using (Cat. #80204) and QA/QC was assessed by Agilent Tape station (DV200≥50%). All samples were loaded onto nanoString Canine Immuno-oncology panels, which contain 800 of genes that span oncology and 47 immunologic pathways. Data was analyzed with the nCounter System (Bunker Spatial Biology); downstream analysis was conducted using the nanostringr (v.0.4.2) platform. Genes and pathways found to be enriched in met-recipient tissues were either tissue-specific or shared across tissues (lung, liver, kidney). Examination of site-specific changes identifies key components (such as SP-C, DMBT1, GFAP, CXCL10, CCL2) of the metastatic osteosarcoma TME. Our findings also suggest that activation of CCL2-CCR2 chemotactic signaling axis is present across geographically distinct sites of metastasis. Furthermore, comparison of our findings in non-tumor tissue adjacent to human cancers suggests that CCL2-CCR2 signaling pathway is also observed in multiple human cancers.These findings improve our understanding of the landscape of the peritumoral TME of metastatic osteosarcoma and further underscore the translational relevance of the canine patient as a model of human disease. These finding also provide insight into shared therapeutic targets with potential benefit for both species. One example is the ongoing clinical evaluation of losartan in dogs and humans with metastatic osteosarcoma, where losartan inhibits CCL2 signaling, illustrating a promising cross-species therapeutic strategy.

92. TLR4 Loss Synergizes with Keratinocyte IKKα Haploinsufficiency to Promote Epidermal Microbiota and Drive IL-4R–Mediated Squamous Carcinogenesis

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Epithelial cells continuously sense commensal microbes to preserve barrier integrity and immune homeostasis. Disruption of this sensing may contribute to carcinogenesis. Although Toll-like receptors (TLRs) are implicated in cutaneous tumorigenesis, the epithelial-specific role of TLR4 in microbiota-dependent squamous cell carcinoma (SCC) initiation remains poorly understood. Analyses of human SCC datasets reveal recurrent alterations in TLR4, suggesting that impaired microbial sensing may influence early tumor development. We hypothesized that germline TLR4 deficiency cooperates with keratinocyte-specific IκB kinase alpha (IKKα) haploinsufficiency to promote epidermal microbiota expansion and activate IL-4R/STAT6-dependent epithelial reprogramming that drives SCC initiation. K5-Cre–mediated IKKα-flox mice, with or without germline Tlr4 deletion, were followed longitudinally to assess tumor development and microbiota dynamics using 16S rRNA sequencing. Temporal profiling determined whether microbial alterations preceded tumor formation. Single-cell RNA sequencing characterized epithelial transcriptional changes, and ATAC-seq assessed chromatin accessibility at the Il4r locus. To dissect epithelial-intrinsic mechanisms, we established a three-dimensional murine skin organoid system derived from primary keratinocytes and exposed organoids to live tumor-associated microbiota. DNA damage (γH2AX), IL-1β induction, and STAT6 phosphorylation were quantified. Dependence on IL-4R signaling and the microbiota was tested using epithelial Il4r deletion and antibiotic-mediated microbial depletion in vivo. Keratinocyte-specific IKKα haploinsufficiency alone resulted in infrequent tumor formation; however, combined TLR4 deficiency significantly increased SCC incidence and accelerated tumor onset ($p<0.05$). Importantly, pre-tumor epidermis from TLR4-deficient mice demonstrated a marked increase in microbial burden, indicating that microbiota expansion precedes carcinogenesis. Microbial profiling revealed enrichment of Gram-positive taxa, including Staphylococcus and Corynebacterium, which correlated with reduced macrophage phagocytic efficiency, suggesting impaired microbial clearance. In the organoid model, direct exposure to tumor-associated microbiota induced epithelial-intrinsic DNA damage, elevated IL-1β expression, and robust STAT6 activation in the absence of immune cells. Single-cell transcriptomic analysis revealed epithelial reprogramming toward a type 2 inflammatory and pro-tumorigenic state. Consistent with these findings, ATAC-seq demonstrated increased chromatin accessibility at the Il4r locus, indicating epigenetic priming of IL-4R signaling.

Critically, microbial-induced STAT6 activation and DNA damage were significantly attenuated in Il4r-deficient organoids. In vivo, epithelial IL-4R ablation markedly reduced tumor formation, and antibiotic-mediated microbiota depletion similarly suppressed tumor development, demonstrating both pathway and microbial dependence. These findings establish that epithelial TLR4 deficiency promotes a pro-tumorigenic microbial shift that precedes tumor formation and drives IL-4R/STAT6-dependent epithelial reprogramming. Cooperative TLR4 loss and keratinocyte IKKα haploinsufficiency create a permissive epithelial state that amplifies microbiota-driven SCC initiation. Targeting epithelial IL-4R signaling may therefore represent a therapeutic strategy to intercept early carcinogenesis. Moreover, our organoid platform provides a tractable system to mechanistically dissect epithelial–microbial interactions and evaluate preventive interventions in microbiota-driven skin cancer.

93. Tacrolimus-Resistant, TCR-Deficient, GPC3-Specific CAR-T Cells for Safe and Effective Treatment of Hepatocellular Carcinoma after Liver Transplantation

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Liver transplantation is a curative treatment for early-stage hepatocellular carcinoma (HCC), while immunotherapy remains the first-line therapy for advanced disease. However, managing recurrent HCC after liver transplantation poses a significant therapeutic challenge, as immune checkpoint inhibitors are contraindicated due to the high risk of allograft rejection. Tumor-specific chimeric antigen receptor (CAR) T cells targeting glypican-3 (GPC3) offer a promising alternative strategy. Here, we describe a CAR T cell platform designed for post-transplant application, featuring CRISPR/Cas9-mediated knockout of the FK506-binding protein 12 (FKBP12) locus to confer tacrolimus-resistance and maintain CAR T cell function under continuous immunosuppression. Concurrent disruption of the TRAC locus eliminates T cell receptor (TCR) expression, reducing the risk of allograft rejection mediated by the endogenous TCR of these tacrolimus resistant CAR T cells. Resting human T cells were electroporated with a ribonucleoprotein (RNP) complex composed of a 1:1 mixture of single-guide RNAs targeting FKBP12 and TRAC, pre-incubated with recombinant high-fidelity Cas9 protein. Following electroporation, cells were expanded in culture with CD3/CD28 beads and supplemented with IL-2, IL-7, and IL-15 and transduced with a second-generation anti-GPC3-CAR. Engineered T cells were validated and analyzed by genomic sequencing, flow cytometry, cytotoxicity assays using HCC cell lines, and antigen-specific stimulation assays. Knockout efficiencies for TRAC and FKBP12 following electroporation ranged from 70-90%, as determined by flow cytometry and genomic sequencing, respectively. TCR-negative CAR T cells were enriched to high purity (> 99%) using conventional Magnetic-Activated Cell Sorting for CD3-negative cells. Upon GPC3-specific antigen stimulation, tacrolimus significantly inhibited interferon-gamma production in wildtype (WT) CAR T cells, but not in FKBP12 knockout (KO) cells (169.6 pg/ml vs. 9903 pg/ml, $p = 0.0035$). The cytotoxic activity of WT CAR T cells co-cultured with Hep3B (1.49% vs. 54.03%, $p < 0.0001$) and HepG2 (23.35% vs. 92.30%, $p < 0.0001$) tumor cells was markedly reduced in the presence of tacrolimus, whereas KO CAR T cells maintained a robust killing capacity under the same conditions.This dual-edited CAR T cell platform offers a safe and effective approach for the development of cellular immunotherapy for recurrent HCC in the post-transplant setting while continuing tacrolimus-based immunosuppression.

94. RRM1 modulates cellular tolerance to ATR-CHK1 inhibition through regulation of replication stress independent of global dNTP abundance

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Replication stress is a defining feature of many cancers, particularly high-grade serous ovarian cancer (HGSOC), which is characterized by near-universal TP53 mutations. These alterations impair replication fork progression and generate stretches of single-stranded DNA (ssDNA) that activate the ATR–CHK1 checkpoint pathway. The ATR–CHK1 axis stabilizes stalled replication forks, suppresses excessive origin firing, coordinates DNA repair, and prevents premature mitotic entry. Pharmacologic inhibition of CHK1 disrupts these protective mechanisms, resulting in unscheduled CDK activation, increased origin firing, impaired fork stabilization, and depletion of replication resources. This cascade culminates in widespread fork collapse, accumulation of double-strand breaks, and extensive γH2AX formation. To identify genetic determinants of resistance to CHK1 inhibition, we performed a genome-wide CRISPR screen in the HGSOC cell line OVCAR8 treated with the CHK1 inhibitor Prexasertib, currently in clinical trials for advanced ovarian cancer. The catalytic ribonucleotide reductase subunit RRM1 emerged as a top resistance hit. Ribonucleotide reductase (RNR), composed of the catalytic subunit RRM1 and regulatory subunits such as RRM2, is essential for maintaining deoxyribonucleotide (dNTP) availability for DNA synthesis. We hypothesized that RRM1 modulates cellular tolerance to checkpoint inhibition by influencing replication stress adaptation.A genome-wide CRISPR activation screen was performed in OVCAR8 cells treated with Prexasertib to identify modulators of drug resistance. RRM1 was validated using gain- and loss-of-function approaches, including overexpression of wild-type RRM1 and two catalytic mutants (C218A and C429A). DNA damage signaling was evaluated by immunoblotting for γH2AX and related markers. Intracellular dNTP pools were quantified by chromatography following drug treatment. RRM1 overexpression conferred resistance to both CHK1 and ATR inhibition, whereas RRM1 depletion enhanced sensitivity and increased γH2AX accumulation. Surprisingly, RRM1-mediated resistance occurred without substantial changes in global dNTP pools, even after 18 hours of drug exposure. Prexasertib treatment reduced RRM2 protein levels, may indicating impaired ribonucleotide reductase activity under checkpoint inhibition. Notably, combined overexpression of RRM1 and RRM2 sensitized cells to CHK1 inhibition, may suggesting that balanced RNR subunit activity—rather than maximal nucleotide production—is required for survival under replication stress. The catalytic mutants RRM1-C218A and RRM1-C429A failed to confer resistance, demonstrating that RRM1 enzymatic activity is necessary for its protective effect. In non-transformed HEK293 cells, RRM1 overexpression similarly conferred resistance to CHK1 inhibition, indicating that its role in modulating checkpoint dependency extends beyond replication stress specific contexts. RRM1 modulates cellular tolerance to CHK1 inhibition independently of global dNTP abundance. Beyond its established function in nucleotide biosynthesis, RRM1 appears to define the replication stress threshold at which ATR–CHK1 inhibition becomes catastrophic. These findings clarify a mechanism of resistance to CHK1 inhibitors and may inform strategies to improve therapeutic responses to replication checkpoint–targeted therapies.

95. CRISPR-guided multiomics unveils direct mTOR phosphorylation of CDCP1 at Serine 154

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Deciphering the unique roles of mTOR signaling has been a significant challenge. Here, we utilize comparative multiomics to analyze CRISPR/Cas9 gene-edited RPTOR-/-, RICTOR-/-, and MEAK7-/- cells. MEAK7-/- cells downregulated canonical mTOR signaling broadly. CUB-domain containing protein 1 (CDCP1) was downregulated in both RPTOR-/- and MEAK7 -/- cells, which is associated with poor prognosis in cancer patients. mTOR directly binds and phosphorylates CDCP1 at Ser154. CDCP1 was demonstrated to bind to mTOR, SRC and LATS1. Serine->Alanine mutation of Ser154 CDCP1 results in sequestration of LATS1 and YAP, whereas Aspartic Acid mutation releases LATS1 and YAP. S154D CDCP1 results in enhanced cell proliferation, demonstrating a direct link for mTOR inhibition of Hippo.

96. MYBBP1A is part of a mechanically modulated system that alters breast cancer metastatic potential by regulating focal adhesion

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MYB binding protein 1a (MYBBP1A) was first identified for its ability to bind and repress the proto-oncogene c-MYB. Most research focuses on its role in regulating ribosomal RNA (rRNA) transcription; However, its impact on metastasis remains unclear. Analysis of TCGA breast cancer patient data reveals higher MYBBP1A levels are associated with shorter survival times in breast cancer patients, while a Mybbp1a knockdown (KD) gene signature, generated by machine learning, indicates significantly improved patient survival outcomes. Using mouse spontaneous metastasis model, we confirmed that MYBBP1A loss suppresses breast cancer cell metastasis. Further analysis revealed that Mybbp1a KD cells exhibit robust abnormalities in cell morphology, which is associated with reduced cell invasion and disrupted F-actin organization. Cell morphological changes are partially rescued in type I collagen-coated high stiffness extracellular matrix (ECM) culture, indicating MYBBP1A could be part of a mechanically modulated system that alters breast cancer metastatic potential. This is supported by the observation that MYBBP1A levels exhibit a gradually increase in response to the increases of ECM stiffness, which is coupled with increased cell invasion capacity. Mechanical stimuli from the tumor microenvironment play an important role in mediating breast cancer

metastasis. The pathways that cancer cells use to sense and leverage mechanical cues are largely driven by the assembly and disassembly of focal adhesions, which regulate the dynamics of F-actin stress fibers and couple F-actin with the extracellular matrix (ECM) to transduce mechano-signaling to nucleus and regulate cell motility. Focal adhesion can be visualized by immunofluorescence (IF) staining of phospho-FAK (focal adhesion kinase) plaques at the points of F-actin filaments and be measured by FAK level. Our data shows focal adhesion is reduced in Mybbp1a KD cells, evidenced by weakened level and number of phospho-FAK clustering at the points of F-actin. In parallel, overexpression of FAK rescues breast cancer cell phenotypes and partially restores the reduced cell metastasis capacity observed in Mybbp1a KD. In exploring the function of MYBBP1A in regulating mechanosignal transduction, we found that SUN2, an important component of the Linker of Nucleoskeleton to the Cytoskeleton (LINC) complex, was stripped from nuclear envelop and relocated to heterochromatin dense areas, upon Mybbp1a KD. That breaks the connection between nucleus and cytoskeleton and could explain the reduced focal adhesion assembly following Mybbp1a KD. Future work will focus on the role of MYBBP1A as part of a mechanically modulated complex that is important in promoting breast cancer metastasis.

97. Unmasking Previously Unknown DLBCL-specific, Essential Genes to Determine New Therapeutic Targets in Lymphoma

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98. Longitudinal Immune Profiling and Biomarkers of Clinical Response in Castration-Resistant Prostate Cancer

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99. Myeloid Selenoh Loss Disrupts Metastatic Niche Formation via M1 Macrophage Shift

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100. Viscoelastic conditioning promotes metastatic competence via coordinated actin–tubulin networks and epigenetic regulation

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101. COX2 Spatially Reorganizes B7H4 and CD44v6 Promoting Immunosuppression and Therapy Resistance in ER- Breast Cancer

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Aggressive estrogen receptor-negative breast tumors have limited treatment options, warranting the identification of novel therapeutic targets. COX2 is a promising tumor biomarker indicative of disease progression. Spatial profiling of accumulated $COX2^{+}$ cell clusters at the tumor-stroma interface reveals high-density, contiguous tumor masses that create localized prostaglandin E2 gradients, promoting tumor immunosuppression in deceased patient tumors. Colocalization of $COX2^{+}$ tumor clusters with B7H4 and CD44v6 defines distinct, immunosuppressive, and therapy-resistant niches predictive of poor survival. COX2 acts as both a biochemical inducer and a structural organizer of resistant niches, recruiting $B7H4^{+}$ and $CD44v6^{+}$ cells into tight immunosuppressive neighborhoods under hypoxic conditions. Moreover, radiation therapy increases cox2, b7h4, and chemoresistant markers, which were partially altered by COX2 inhibition, indicating potential enrichment of cancer stem cells. These findings highlight COX2 spatial clustering as a key factor in the formation of immune deserts and drug-resistant niches that could be targeted by COX inhibitors.

102. A tumor-targeting IL-12 immunocytokine therapy increases peripheral natural killer (NK) cells with phenotypes positively associated with tumor cell lysis in an NCI first-in-human clinical trial

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103. Modeling and Profiling Metastatic Ewing Sarcoma Using Patient-Derived Xenografts to Reveal Novel Therapeutic Vulnerabilities

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Although improvements in local therapy have increased the 5-year survival rate for localized Ewing sarcoma (EWS) from less than 20% to 70%, little progress has been made in the treatment of metastatic disease, which has a 5-year survival rate of less than 30%. A major challenge in the field is a lack of preclinical models that can recapitulate spontaneous metastatic disease and can be used to better understand the disease and identify new vulnerabilities. In this study, we present and characterize a murine model of spontaneous distant EWS metastasis derived from human patient-derived xenografts (PDX) in two different mouse backgrounds that mimics the clinical progression of the human disease. A panel of seven molecularly diverse patient-derived xenograft (PDX) models (SJ18, SJ17, S049, NCH1, NCH4, PDMR-098, PDMR-077) were injected orthotopically into the gastrocnemius muscle in the left hind leg of athymic nude and NOD SCID gamma (NSG) mice. Once primary tumors reached 1500 mm3, hind limb amputation survival surgery was performed, and we observed animals for the development of distant spontaneous metastases. EWS PDXs formed spontaneous macrometastases in multiple sites including lymph nodes, lung, liver, and kidney in both NSG and nude mice. Each PDX model exhibited a distinct pattern of macrometastasis formation, based on site, metastasis frequency, and mouse strain. Immunohistochemical analysis for CD99 positivity revealed the presence of micrometastases in some locations where no macrometases were evident. The highest frequency of distant metastases (macro- plus micro-) was seen in PDMR-098 and SJ18 models in NSG mice (75% and 73.3% respectively); SJ18 most frequently metastasized to lung, whereas PDMR-098 most frequently metastasized to liver and mesenteric lymph nodes. Comparing results in NSG versus nude mice, we observed variations in metastasis frequency and site preference, with NSG mice demonstrating a higher rate of metastasis overall. Here we describe a preclinical model of spontaneous distant EWS metastasis that recapitulates the characteristics of human disease. The site and frequency of metastases vary based on the specific PDX model as well as the mouse background, highlighting that modeling metastasis formation is a multifactorial process driven by complex interactions between the tumor and host. Metabolomic profiling conducted to compare primary and macrometastatic tumors between models and metastatic sites revealed differences in multiple metabolic pathways. Future studies will focus on identifying potentially targetable metabolic vulnerabilities that could be used in the development of treatments for metastatic EWS.

104. CAMAT1 lncRNA interacts with the co-integrator complex to promote MAPK signaling and cell migration

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Long non-coding RNAs (lncRNAs) are the largest group of genes transcribed from the human genome. Although some lncRNAs have been functional characterized, the cellular and molecular functions of the vast majority of lncRNAs remain to be elucidated. Here, we report the initial functional characterization of a poorly characterized human lncRNA LINC02582 that we termed CAMAT1 (Colorectal Adenocarcinoma Migration Associated Transcript 1). CAMAT1 is upregulated in colorectal cancer (CRC) and high CAMAT1 expression is associated with poor prognosis suggesting oncogenic functions. At the cellular level, CAMAT1 is very abundant in well-differentiated CRC cells and is predominantly localized to the nucleus. Transcriptome analysis from CAMAT1-depleted cells (using CRISPRi and CRISPR knockout) revealed that CAMAT1 activates the expression of multiple members of the S100 family of genes including S100A4 that is known to regulate actin cytoskeleton, MAPK signaling and cell migration. Consistent with this, CAMAT1-depleted cells exhibited defects in actin cytoskeleton organization, decreased ERK1/2 phosphorylation and reduced cell migration. Mechanistically, CAMAT1 RNA pulldowns followed by mass spectrometry uncovered specific binding of this lncRNA with multiple proteins of the activating signal co-integrator complex 1 (ASCC1), including the RNA-binding protein ASCC1. Indeed, RNA bind-n-seq analysis identified that ASCC1 recognizes ACCTCC/ACCTCT motifs for its interaction with CAMAT1. Importantly, silencing ASSC1 in CAMAT1 wild-type cells upregulated the expression of S100A4 indicating that ASCC1 plays a transcriptional inhibitory role on S100A4. Interestingly, depletion of ASCC1 in CAMAT1 knockout cells did not significantly rescue the expression of S100A4

and other CAMAT1-target genes suggesting that ASCC1 requires CAMAT1 to exert its transcriptional inhibitory function on CAMAT1 target genes. Collectively, our results suggest that CAMAT1 exerts its oncogenic function in CRC cells by enhancing the expression of specific genes involved in MAPK signaling and promoting cell migration.

105. Rpn13Pru-targeting degraders inhibit APC/C pathway inducing mitotic arrest in breast cancer.

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Protein degraders are structurally-modified small molecules that promote selective degradation of a target protein. XL44 is a small molecule previously identified as a potent hRpn13Pru degrader that enhances hRpn13Pru degradation via ubiquitin-proteasome system (UPS), subsequently triggering hRpn13Pru-dependent apoptosis in myeloma cells. Notably, XL44 has also been associated with causing ubiquitin-independent cell cycle defects thereby expanding its functional scope. Our current study evaluates two other XL44 derivatives: XL69 and XL80, with similar cell cycle arrest mechanisms. Here, we unravel the anticancer activity of XL69 and XL80 elucidating their underlying molecular mechanism in breast cancer cell line. MCF-7 cells were treated with an increasing concentration (1-100 μM) of XL69 and XL80 for 24h, and the IC50 values were determined using MTT assay. For cell cycle analysis, cells were treated with 10 μM of each compound for 24h and analyzed via flow cytometry. The spindle assembly checkpoint (SAC), and anaphase-promoting complex/cyclosome (APC/C) activity were evaluated using western blotting and immunofluorescence microscopy. Tubulin organization and centrosome movement were quantified using ImageJ software. The MTT assay for XL69 and XL80 showed increased cytotoxicity at concentrations above 25 μM, however cells exhibited rapid detachment at lower concentrations (1 μM). The detached, floating cells remained viable for the next 48-72 h post-treatment before cell death and exhibited marked variation in nuclear morphology and cytoskeleton organization. XL69 and XL80 treated MCF-7 cells accumulated in G2/M (65.766% and 54.166%, respectively, vs Veh 25.201%; $p < 0.001$). In addition, elevated levels of phospho-histone 3 were detected 24 h post-treatment (31.566%, 32.500% vs Veh 10.773%, p-value <0.01) indicating robust mitotic arrest. Immunofluorescent detection of Aurora B with DAPI nuclear staining confirmed prometaphase-metaphase arrest. Tandem mass tag (TMT) protein profiling and western blot showed upregulation of Bub1 and BubR1, confirming SAC activation. Activation of the SAC led to formation of the mitotic checkpoint complex (MCC), subsequently inhibiting the APC/C, as evidenced by the accumulation of cyclin B1 and securin. Furthermore, the inhibition of APC/C also prevented degradation of Aurora B kinase, resulting in impaired tubulin assembly. Immunofluorescent images showed disordered microtubule network with XL69 and XL80 treatment, similar to nocodazole, a microtubule polymerization inhibitor. Furthermore, a significant reduction ($p<0.0001$) in centriole movement during the prometaphase stage was observed in the treated group compared to both Veh and nocodazole. These findings suggest that XL69 and XL80 induce mitotic arrest by targeting the APC/C pathway, disrupting microtubule dynamics and centriole movement. Additionally, preliminary in vivo screening of XL69 showed a significant decrease ($p=0.004$) in tumor volume in MCF-7 xenograft models. These studies demonstrate that the small molecules XL69 and XL80 effectively induce mitotic arrest, impair microtubule dynamics, disrupt cellular attachment, leading to cell death in MCF-7 cells. The treatment led to sustained cell cycle arrest, SAC activation, APC/C pathway inhibition, and impaired microtubule organization and centriole movement. As such, the study highlights XL69 and XL80 as promising anti-cancer agents that target mitotic progression.

106. Investigating the Role of Alveolar Pneumocytes in the Lung Microenvironment of Osteosarcoma Metastases

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Pulmonary metastasis is the leading cause of mortality in human and canine osteosarcoma (OS) patients. However, the contribution of lung-resident epithelial cells—particularly surfactant protein C (SPC) expressing type II alveolar pneumocytes (TIIPs)—to metastatic progression remains undefined. This study investigates the transcriptional as well as spatial changes in pneumocytes in OS lung metastases and explores their interactions with tumor cells. Firstly, spatial transcriptomics was used to determine alveolar TIIPs' abundance and location in the tumor microenvironment of human OS. Further, immunohistochemistry staining for SPC+ cells was carried out in both canine and murine (orthotopic injection of K7M2 murine OS cell line) models of OS lung metastases. HALO quantitative image analysis was used to quantify SPC+ cells, and a paired t-test was performed for statistical analysis. To assess direct OS cell–pneumocyte crosstalk, human primary TIIPs were co-cultured with MG63.2 human OS cell line followed by RNA sequencing and cytokine analysis to identify gene expression changes and cytokine levels respectively. Furthermore, ELISA was used to detect and quantify functional protein levels. Spatial transcriptomics of human patient tissues showed an accumulation of TIIPs in the region immediately adjacent to the tumor. Quantification of SPC+ cells in canine and murine tissue also demonstrated a significant increase of TIIPs within the tumor:non-tumor interface compared to non-tumor lung tissue. RNA sequencing, cytokine activity and ELISA demonstrated significant enrichment of the p53 pathway and elevated secretion of pro-fibrotic cytokines and TGFB1 in TIIPs co-cultured with OS cells versus TIIPs in monoculture. Together these findings suggests that there is overlap between the TIIP response in OS and idiopathic pulmonary fibrosis, a chronic lung disease driven by TIIPs. This investigation highlights a previously under-recognized epithelial component of the OS metastatic microenvironment and suggests that pneumocytes play a role in the lung metastatic niche of OS. Future work aims to identify new approaches for targeting host–tumor interactions to limit metastatic progression of OS in both human and canine patients.

107. Spatial Transcriptomics Reveals Zonation-Dependent Metabolic Reprogramming in Premalignant Hepatocarcinogenesis

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Von Hippel-Lindau is a hereditary disease caused by a mutation in the VHL tumor suppressor gene characterized by the development of many different tumors including bilateral and multifocal renal cell carcinomas. The prognosis of patients with RCC has improved significantly with a longer than 10-year survival in some studies. Germline mutations in the BAP1 gene, also a tumor suppressor gene, predisposes to the development of high-grade tumors with poor prognosis including renal cell carcinoma. This study evaluates the clinicopathologic and genomic features of RCC with co-occurring BAP1 and VHL alterations, including germline mutations, with emphasis in prognosis, and modalities of treatment. Twenty-three patients with RCC and documented BAP1/VHL alterations were retrospectively analyzed, comprising 24 tumor samples (20 primary renal tumors and 4 metastatic biopsies). Clinical information and morphologic evaluation of all the tumors was done, as well as next-generation sequencing using the TSO-500 platform, to identify pathogenic variants, variants of uncertain significance, and additional genomic alterations with focus in identifying potentially targetable molecular pathways. Patients ranged from 25 to 72 years of age (male: female = 15:8). Bilateral disease was present in eight patients, and tumor size ranged from 0.9 to 18 cm. Metastatic disease occurred in twelve patients, most commonly affecting soft tissue, liver, and lung, and resulted in disease-related death in three patients. Primary tumors were typically ISUP grade 2–3, however grade 3 and 4 was observed

in some primary tumor and in all metastatic lesions, supporting the possibility that an association between combined BAP1/VHL mutations may confer aggressive clinical behavior. All tumors demonstrated BAP1 alterations (Variant Allele Frequency (5.7%–67%), and 21 of 24 harbored VHL mutations detected by NGS (Variant Allele Frequency (4.3%–64%). Two patients with clinical features of Von Hippel–Lindau syndrome lacked detectable pathogenic variants by NGS: one showed an intragenic exon 2 deletion consistent with germline VHL alteration, and another had a history of cerebellar hemangioblastoma suggestive of germline disease. These findings highlight the importance of complementary genomic studies in patients with VHL for proper diagnosis and treatment. Morphologically, 14 tumors exhibited classic clear cell RCC, four demonstrated papillary or cystic features, one showed sarcomatoid differentiation, and one displayed mixed tubular, eosinophilic, and clear cell architecture. Across the cohort, 356 genomic alterations were identified. Beyond VHL and BAP1, the most recurrently altered genes were LRP1B (29%), RECQL1 (25%), and FAT1 (25%), suggesting involvement of DNA damage response, chromatin regulatory, and immune-modulatory pathways that may be associated with differential sensitivity to immune checkpoint blockade, hypoxia-inducible factor–directed therapy, or emerging targeted agents. RCC with VHL and proven concurrent BAP1 alterations, appear to be characterized by morphologic more aggressive tumors and the possibility of metastatic progression. Comprehensive genomic profiling, including detection of intragenic deletions and co-mutational landscapes, is critical for accurate diagnosis, prognostication, and treatment selection. These findings support molecularly stratified management of RCC and provide a rationale for integrating hypoxia-pathway inhibitors, DNA damage response targeted therapies, and immunotherapy approaches in patients with BAP1/VHL-altered renal cell carcinoma.

108. Renal cell carcinoma with both, VHL and BAP 1 tumor suppressor gene mutations, an association for the development of more aggressive tumors?

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Von Hippel-Lindau is a hereditary disease caused by a mutation in the VHL tumor suppressor gene characterized by the development of many different tumors including bilateral and multifocal renal cell carcinomas. The prognosis of patients with RCC has improved significantly with a longer than 10-year survival in some studies. Germline mutations in the BAP1 gene, also a tumor suppressor gene, predisposes to the development of high-grade tumors with poor prognosis including renal cell carcinoma. This study evaluates the clinicopathologic and genomic features of RCC with co-occurring BAP1 and VHL alterations, including germline mutations, with emphasis in prognosis, and modalities of treatment. Twenty-three patients with RCC and documented BAP1/VHL alterations were retrospectively analyzed, comprising 24 tumor samples (20 primary renal tumors and 4 metastatic biopsies). Clinical information and morphologic evaluation of all the tumors was done, as well as next-generation sequencing using the TSO-500 platform, to identify pathogenic variants, variants of uncertain significance, and additional genomic alterations with focus in identifying potentially targetable molecular pathways. Patients ranged from 25 to 72 years of age (male: female = 15:8). Bilateral disease was present in eight patients, and tumor size ranged from 0.9 to 18 cm. Metastatic disease occurred in twelve patients, most commonly affecting soft tissue, liver, and lung, and resulted in disease-related death in three patients. Primary tumors were typically ISUP grade 2–3, however grade 3 and 4 was observed in some primary tumor and in all metastatic lesions, supporting the possibility that an association between combined BAP1/VHL mutations may confer aggressive clinical behavior. All tumors demonstrated BAP1 alterations (Variant Allele Frequency (5.7%–67%), and 21 of 24 harbored VHL mutations detected by NGS (Variant Allele Frequency (4.3%–64%). Two patients with clinical features of Von Hippel–Lindau syndrome lacked detectable pathogenic variants by NGS: one showed an intragenic exon 2 deletion consistent with germline VHL alteration, and another had a history of cerebellar hemangioblastoma suggestive of germline disease. These findings highlight the importance of complementary genomic studies in patients with VHL for proper diagnosis and treatment. Morphologically, 14 tumors exhibited classic clear cell RCC, four demonstrated papillary or cystic features, one showed sarcomatoid differentiation, and one displayed mixed tubular, eosinophilic, and clear cell architecture. Across the cohort, 356 genomic alterations were identified. Beyond VHL and BAP1, the most recurrently altered genes were LRP1B (29%), RECQL1 (25%), and FAT1 (25%), suggesting involvement of DNA damage response, chromatin regulatory, and immune-modulatory pathways that may be associated with differential sensitivity to immune checkpoint blockade,

hypoxia-inducible factor–directed therapy, or emerging targeted agents. RCC with VHL and proven concurrent BAP1 alterations, appear to be characterized by morphologic more aggressive tumors and the possibility of metastatic progression. Comprehensive genomic profiling, including detection of intragenic deletions and co-mutational landscapes, is critical for accurate diagnosis, prognostication, and treatment selection. These findings support molecularly stratified management of RCC and provide a rationale for integrating hypoxia-pathway inhibitors, DNA damage response targeted therapies, and immunotherapy approaches in patients with BAP1/VHL-altered renal cell carcinoma.

109. p53 Activates the cGAS-STING-IFNβ Pathway to Regulate M2 Macrophage Polarization in Anaplastic Thyroid Cancer

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110. Investigating the Myeloma Microenvironment

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Multiple myeloma is a hematologic neoplasm of plasma cells that originates within the bone marrow. Despite recent therapeutic advancements, it remains an incurable disease characterized by increasing drug resistance and eventual refractory disease. Growing evidence implicates the bone marrow microenvironment as a pro-tumor niche that promotes disease progression and contributes to therapeutic resistance. A key component of this microenvironment is bone marrow stromal cells (BMSCs), which are pluripotent cells capable of differentiating and maturing into various mesenchymal cell types including fibroblasts, osteoblasts, and adipocytes. These cells provide critical growth factors and survival signals that are essential for appropriate hematopoietic cell maturation. Co-culture experiments using myeloma cell lines and the immortalized healthy bone marrow stromal cell line HS-5 have demonstrated enhanced myeloma cell survival following in vitro exposure to chemotherapeutic agents, suggesting that this model may recapitulate interactions that support disease progression and drug resistance. To explore indirect interactions supporting myeloma cell survival, the myeloma cell line L363 was cultured in the presence and absence of conditioned media from HS-5 cells. Supernatants were collected and analyzed for protein composition using data-independent acquisition mass spectrometry (DIA-MS). In this pilot study, 2283 proteins were detected, of which 267 exhibited at least a 2-fold increase in abundance in co-culture conditions compared to both stromal and myeloma monocultures. Among these, 160 proteins were uniquely identified in the co-culture condition. These findings suggest that stromal-derived soluble factors induce the expression or secretion of these proteins in or from the myeloma cells. Functional analyses revealed significant enrichment in pathways involving mRNA metabolism and processing. Future experiments with biological replicates are planned to validate and extend these findings with the eventual goal of identifying ways to interrupt the stromal-mediated mechanisms supporting myeloma cell survival even in the presence of chemotherapeutic agents.

111. Deciphering neutrophil heterogeneity to enhance therapeutic efficacy of TRAIL (Tumor necrosis factor–related apoptosis-inducing ligand) in triple negative breast cancers

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TRAIL induces apoptosis in many preclinical cancer models, including breast cancer, and has been extensively studied as a potential therapeutic target. However, the limited clinical efficacy of TRAIL agonists suggests that additional modulatory mechanisms may restrict their activity in patients. Our prior work showed that, in triple-negative breast cancer (TNBC), TRAIL induces cytokines including CXCL1, CXCL2, CXCL3, CXCL8, CXCL11, and IL6 through

an NFKB2-dependent pathway. These factors promote recruitment of immunosuppressive neutrophils, suggesting that neutrophil-mediated remodeling of the tumor immune microenvironment may influence TRAIL responsiveness in TNBC. We hypothesized that TRAIL and TRAIL-induced factors from TNBC remodel neutrophil heterogeneity, creating potential to be used as a targeted strategy improving the efficacy of TRAIL therapy. Neutrophils isolated from a healthy donor were cultured under four conditions: serum-free media (SFM), SFM supplemented with TRAIL (SFM-T), conditioned media from TNBC cells (CM), or conditioned media from TRAIL-treated TNBC cells (T-CM). Single-cell RNA sequencing (scRNA-seq) was performed across all conditions, generating data from 43,420 neutrophils. Findings were independently validated in a second scRNA-seq experiment using pooled neutrophils from 5 healthy donors (35,500 cells). Transcriptional heterogeneity was analyzed by clustering and t-SNE, and candidate populations are being further validated by gene expression analysis and surface marker staining. scRNA-seq identified 10 transcriptionally distinct neutrophil clusters across the four conditions. Similar transcriptional states were observed in the validation dataset, including programs related to inflammation, antigen presentation, and ribosome biogenesis, demonstrating substantial neutrophil heterogeneity. Two clusters were consistently expanded following T-CM treatment in both experiments. One cluster was enriched for antigen-presentation and immunosuppressive genes, including CCL4, CD274, IL1A, and IL1B. A second cluster was enriched for ribosome biogenesis-related genes, including DDX21, UTP18, LAGE3, and NPM1. Both clusters showed low expression of IFN-γ/IFN-α response genes, further supporting an immunosuppressive phenotype induced by T-CM. In contrast, SFM-T treatment generated a distinct neutrophil population expressing genes associated with canonical neutrophil effector functions, including NETosis and degranulation. These findings suggest that TRAIL may have indirect effects on the tumor microenvironment by inducing tumor-derived factors that polarize neutrophils toward immunosuppressive states. By identifying neutrophil subsets associated with conditioned media from TRAIL-treated TNBC cells, this work highlights the innate immune system as a potential therapeutic target in TNBC. Reprogramming these neutrophil populations toward an anti-tumor phenotype may represent a strategy to enhance the therapeutic efficacy of TRAIL and improve clinical outcomes.

112. Bone Metastatic Prostate Cancer Progression Augments the Bone Marrow Immune Compartment and Metabolism of the Tumor-Bone Microenvironment

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Prostate cancer (PCa) affects around 1 in 8 men in the US every year and while localized disease has a 97% 5-year survival rate, metastasis to distant sites drops survival to ~37%. Disseminated PCa cells most frequently home to bone. Current therapeutics are mostly palliative rather than preventive of growth of bone metastatic PCa (BM-PCa), emphasizing that the mechanisms of BM-PCa remain poorly understood. The bone contains a milieu of immune cells (dominated by neutrophils) and bone stromal cells, including bone remodeling osteoblasts and osteoclasts, all of which interact with BM-PCa. Previous findings from the Cook lab show that neutrophils can directly target BM-PCa cells in vitro and in vivo but lose this ability as the tumor continues to progress in the bone environment via unknown mechanisms. We thus set out to understand the effects of PCa cells on the bone-stromal microenvironment throughout BM-PCa progression. To do this, we first utilized the intratibial bone metastasis model with injection of mouse RM1 PCa into immune competent C57BL/6 mice. Tumor-containing bone marrow was collected every other day for 8 days and single cell RNA sequencing (scRNA-seq) was performed. We found that neutrophils and B cells were altered in both the tumor limb and saline control limb and also changed in maturity and activation states peaking on day 5, then returning to baseline by day 7. We previously showed that BM-PCa alter neutrophil redox metabolism which contributes to tumor burden. Next, we investigated whether the immune changes translated into altered metabolic processes in the bone microenvironment. To do this, RM1 bone marrow flushes were collected after 3 days of tumor growth and liquid chromatography/mass spectrometry (LC/MS) performed, with a focus on the tricarboxylic acid (TCA) cycle metabolism. We observed an overall suppression of the TCA cycle in bone tumors, supported by an increase in lactate production suggesting a shift in metabolism compared to non-tumor bone. Finally, based on the propensity of RM1 cells to metastasize to liver, lung, and bone and suggested roles for tissue matrix on tumor-immune interactions, we sought to determine the impact of tissue matrix of these sites on RM1 tumor growth. To do this, we developed RM1 spheroids with incorporated decellularized extracellular matrix (dECM), which are referred to as Matrispheres,

from human liver, lung, and bone tissues. RM1 cells were seeded liver, lung, and bone dECM (or no matrix as a control) and cultured in low attachment plates for 4 days prior to imaging and RNA collection. Bulk RNA-sequencing revealed an upregulation of an ER stress/hypoxia gene signature of RM1 grown in bone dECM, which was separated from an overlapping proinflammatory and IFN signature induced by both lung and liver dECM. Collectively, our findings demonstrate that the presence of PCa in the bone affects both the metabolism and maturation of immune cells which can be leveraged for novel therapeutic interventions. Future experiments will interrogate how evolution of the immune population and metabolome contributes to BM-PCa growth in bone.

113. Allelic Variation in Mtor Modulates Downstream Signaling in Response to DNA Damage

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The mechanistic target of rapamycin (mTOR) is a serine/threonine kinase that functions within multiprotein complexes to coordinate cell-cycle progression, integrating growth and stress signals to regulate checkpoint engagement. Kinase activity is required for cells to progress through G1/S and G2/M transitions, and it modulates how cells respond to DNA damage by influencing whether checkpoints arrest the cycle for repair or permit continued proliferation. Altered regulation of these processes can lead to excessive plasma cell proliferation, contributing to plasma cell neoplasms such as plasmacytoma and multiple myeloma, where defects in B cell differentiation promote abnormal survival. BALB/cAnPt (BALB) and DBA/2NPt (DBA) mice have been used to study plasmacytoma susceptibility. BALB and NZB mice are uniquely susceptible to developing tumors following pristane injection, while DBA and most other strains of mice do not. Subsequent genome-wide association studies attributed the increased tumor susceptibility of BALB mice to allelic variation in Mtor. BALB and NZB mice carry a rare variant (C1977T) that encodes cysteine at amino acid 628 (R628C), whereas most other strains, including DBA, carry arginine at this position. We hypothesize that the R628C allelic variant of Mtor alters the assembly of mTOR complexes and the phosphorylation of downstream substrates in response to DNA damage. To test this hypothesis, cell lines expressing either allele were generated by stably transfecting HEK293T cells with N-terminal FLAG-tagged Mtor coding sequences corresponding to the R628 (DBA) or 628C (BALB) variants, whose expression is responsive to doxycycline induction. DNA damage in the form of double-strand breaks was induced by exposure to gamma-irradiation (4 Gy). Protein lysates were collected for immunoprecipitation and Western blot analyses to quantify phosphorylation of mTOR substrates and interactions with regulatory partners. RNA sequencing (RNA-Seq) was used to identify downstream transcriptional responses influenced by the allelic variation. Flow cytometry (FACScan) was performed to assess cell-cycle phase distribution and indirectly measure the extent of checkpoint engagement following DNA damage. RNA sequencing followed by Gene Set Enrichment Analysis revealed differences in DNA damage response pathways associated with the R628C variant, which were corroborated by immunoprecipitation and Western blot. Cells expressing the 628C variant exhibited reduced G2/M arrest and more rapid cell-cycle recovery following gamma-irradiation compared to R628-expressing cells. These results suggest that the 628C variant allows continued proliferation despite DNA damage, potentially increasing the risk of mutation accumulation. mTOR functions within multiprotein complexes, including mTORC1 and mTORC2, which are assembled with binding partners such as RAPTOR, RICTOR, and DEPTOR. The R628C substitution alters these interactions and could therefore modulate complex activity and downstream signaling, providing a mechanistic explanation for the altered cell-cycle progression observed in R628C-expressing cells. By defining how this substitution modulates mTOR complex composition and downstream signaling in response to DNA damage, this work will further elucidate our understanding of the implications of allelic variation in Mtor for plasmacytoma susceptibility.

114. Developing a Mechanistic Understanding of Cancer Stem Cell Resistance to Chemotherapy

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Cancer stem cells (CSCs) are a small subset of tumor cells uniquely capable of self-renewal and the ability to generate differentiated progeny (nonCSCs). Importantly, they are also intrinsically resistant to many therapies. These distinct properties make them key drivers of metastasis and therapy failure. While CSC-associated chemoresistance has been widely proposed, directly tracking CSC state dynamics during treatment has remained challenging. To address this challenge, we developed a lentiviral-based destabilized fluorescent reporter system that reports on stemness as a dynamic phenotypic state, allowing us to visualize CSC fate decisions in real time. We used this reporter in a model of metastatic triple negative breast cancer (TNBC) to study the effects of paclitaxel treatment. Paclitaxel is an approved chemotherapy drug used to treat breast cancer; it works by destabilizing microtubules during the cell cycle, leading to cell death. We confirmed that paclitaxel treatment enriches for CSCs in vitro and in vivo, and we hypothesized that direct observation of CSC and nonCSC behavior in response to paclitaxel would give insights into the mechanism of paclitaxel resistance.: Incucyte live cell imaging was used to track single cells over 2+ days in a TNBC model: MDA-MB231 LM2. The fluorescent reporter system was used to identify CSCs. Movies from the incucyte were generated to allow manual cell tracking in the forward (fate mapping) and reverse (origin mapping) directions. The fate decisions from these mappings were separated into “early” and “late” events, taking place about 1 day and 2 days after paclitaxel treatment, respectively. With the same time points recorded under normal conditions, the fate decisions of CSCs and nonCSCs were compared between normal conditions and conditions after paclitaxel treatment. Cell fate mapping showed that nearly all CSCs and nonCSCs stopped dividing in response to paclitaxel, but that the rate of cell death was significantly higher in nonCSCs. Origin mapping of CSCs revealed that 13% of the CSCs present at the end of the mapping period originated from nonCSCs under control conditions. This proportion increased to 42% following paclitaxel treatment. This observation suggests that paclitaxel treatment may 3-fold increase the frequency of de-differentiation of nonCSCs, and that this induced phenotypic plasticity may contribute to the observed enrichment of CSCs following chemotherapy. Combined origin and fate mapping of CSCs also revealed a large population of CSCs that transiently differentiated following paclitaxel treatment, but then recovered their stem cell phenotype. The functional significance of this transient de-differentiation is unclear. A future direction for this research would be to identify the molecular mechanism underlying the increase in nonCSC plasticity under paclitaxel treatment, as blocking this should reduce the CSC population and enhance therapeutic efficacy. Standard of care chemotherapy fails to fully eliminate CSCs, making patients vulnerable to the development of metastasis. Understanding CSC resistance mechanisms can help reveal therapeutic vulnerabilities of CSCs, which can lead to the development of new therapies to limit metastasis.

115. Pericyte-derived lipid transfer promotes tumor cell quiescence

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Triple-negative breast cancer (TNBC) is a highly lethal breast cancer subtype, for which metastasis accounts for most disease-related mortality. Metastatic relapse can occur when disseminated tumor cells (DTCs) persist undetected in a dormant state before proliferating into deadly metastasis. The perivascular microenvironment (milieu surrounding blood vessels) can alter DTC fate. Pericytes, key support cells in the perivascular niche, critically influence metastasis. We seek to identify how lung pericytes influence quiescence of disseminated breast cancer tumor cells and, therefore, affect propensity for metastatic relapse. We previously reported that pericytes promote DTC quiescence through transient cell-cell contact during extravasation. Along with this phenotypic change, there is evidence of lipid transfer from pericytes to tumor cells, including transfer of lipid dye during co-culture and increased lysophospholipid content

after co-culture. We hypothesize that pericytes promote tumor cell quiescence through a lysophospholipid-mediated mechanism by transferring lipids to tumor cells during transient interaction. Our studies utilize murine primary lung pericytes and the 4T1 metastatic TNBC tumor cell line. Lipid content within tumor cells after co-culture with pericytes or monoculture (control) will be evaluated with liquid chromatography – mass spectrometry (LC/MS) and Raman spectroscopy. LC/MS allows us to identify and quantify the lysophospholipids in tumor cells following co-culture. Raman spectroscopy allows us to visualize the location of lipids within a tumor cell. Novel applications of lipid tracing techniques with LC/MS and Raman spectroscopy allow us to identify pericyte-derived lysophosphatidylcholines (LPC) within tumor cells: First, we provide primary murine pericytes with propargylcholine (a traceable modified component of LPC) or deuterated LPC precursor, dioleoyl phosphatidylcholine (DOPC). Then, we perform a co-culture and detect these traceable molecules in tumor cells with LC/MS, Raman spectroscopy, and/or fluorescence microscopy. We will use LC/MS to quantify the metabolites of pericyte-derived lysophospholipids, thus providing insight into how they are metabolically processed by the tumor cell. We utilize click chemistry and subsequent fluorescent imaging to locate propargylcholine-containing (pericyte-derived) lipids and metabolites in tumor cells. Coherent Raman spectroscopy may help elucidate the molecular character of tumor cell regions that are rich in pericyte-derived lipids. We will also implement RNA sequencing, western blots and drug screens of co-cultured tumor cells implicate LPC-mediated mechanisms driving quiescence such as changes in cell cycle regulation and lipid metabolism. LC/MS replicates the finding that lysophospholipid content, including LPC(18:1), is elevated in co-cultured tumor cells relative to monocultured cells. We observe deuterated LPC(18:1) in tumor cells co-cultured with pericytes containing deuterated DOPC, supporting that elevated tumor cell LPC(18:1) derives from pericytes. We, so far, visualized transfer of propargylcholine from pericytes to tumor cells with fluorescence microscopy. These preliminary data demonstrate that pericyte-derived lipids are transferred to tumor cells, in a capacity that correlates with quiescence. They also demonstrate feasibility of lipid tracing methods. We aim to elucidate mechanisms by which pericytes induce tumor cell quiescence via changes in tumor cell metabolism and cell cycle regulation. These findings may reveal vulnerabilities in dormant disseminated tumor cells and potentially inform detection, diagnosis and treatment of latent metastatic TNBC before relapse.

116. The role of SRGN in metastasis of triple-negative breast cancer

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117. Preclinical Evaluation of a CD276-targeted Antibody-Drug Conjugate in Renal Medullary Carcinoma

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Renal medullary carcinoma (RMC) is a rare, highly aggressive kidney cancer originating within the renal medulla, almost exclusively in patients with sickle cell trait and is hallmarked by biallelic loss of SMARCB1, a tumor suppressor critical for chromatin remodeling. Despite its lethality, effective therapeutic options and patient outcomes remain poor. Here we identify CD276 (B7-H3), a transmembrane immune checkpoint protein of the B7 family, as a novel therapeutic target in RMC, demonstrating its overexpression in RMC patient-derived cell lines. Consistent with this, CD276 is highly expressed across multiple solid tumors including renal cell carcinoma and ovarian cancer, while exhibiting minimal expression in normal tissues. This tumor-selective expression pattern provides the rationale for evaluating m276-SL-PBD, a CD276-targeted antibody-drug conjugate (ADC), that delivers a potent DNA-crosslinking pyrrolobenzodiazepine (PBD) payload to CD276-expressing cells, as a novel therapeutic strategy in RMC. We hypothesize that m276-SL-PBD will selectively target CD276-expressing cells and inhibit tumor growth in RMC. This study aims to characterize CD276 protein and mRNA expression in RMC, histologically localize and quantify its expression in patient-derived tumor specimens, and evaluate the efficacy of m276-SL-PBD in vitro and in vivo. To accomplish this, cell-surface CD276 expression was analyzed on four RMC (UOK 360, UOK 353, RMC 219, RMC 2C1) and normal kidney cell lines using flow cytometry through indirect staining with a FITC-conjugated anti-CD276 antibody. In addition, total CD276 protein expression was characterized across cell lines via Western blot and using ImageJ to quantify band intensities while normalizing to beta-actin. In vitro drug response assays with m276-SL-PBD

were performed using serial dilutions, and cell growth was monitored via live-cell confluence imaging with percent confluence calculated to quantify cell growth inhibition of RMC cell lines relative to untreated and normal kidney controls. Flow cytometry demonstrated an increase in CD276 surface expression across all RMC cell lines, with mean B530-A fluorescence 2.1-, 2.0-, 1.7-, and 1.9-fold higher than normal kidney cells for UOK 360, UOK 353, RMC 2C1, and RMC 219, respectively. These findings were corroborated by ImageJ Western blot analysis, revealing robust total CD276 protein expression compared to normal kidney cells across all RMC cell lines (UOK 360: 20-fold; UOK 353: 52-fold; RMC 2C1: 38-fold; RMC 219: 77-fold). In vitro drug response assays showed that m276-SL-PBD strongly inhibited RMC cell growth across all four cell lines with the mean inhibition ranging from 62% to 76% across 0.125–8 µg/mL, while normal kidney cells responded minimally with a mean 17% inhibition across the same concentration range. Future studies include immunohistochemical analysis of CD276 expression and localization in patient tumor specimens, quantification of CD276 mRNA expression, and assessment of m276-SL-PBD efficacy in cell line-derived and patient-derived xenograft mice models. Altogether, these findings demonstrate the efficacy of m276-SL-PBD in RMC cell lines and provide early preclinical support for a CD276-targeted therapeutic strategy for renal medullary carcinoma, warranting further in vivo evaluation.

118. MRI Features Predict Adverse Pathology and Long-term Outcomes After Radical Prostatectomy: A Prospective Cohort Analysis

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Multiparametric MRI (mpMRI) is now routinely used in prostate cancer diagnosis and staging, enabling more accurate tumor localization and risk assessment. The availability of MRI-derived prostate volumes has also made PSA density a standard clinical variable, commonly applied in biopsy decisions and active surveillance protocols. Despite its widespread use, few studies have evaluated PSA density alongside MRI features to predict both adverse pathology and long-term oncologic outcomes. This study examined the association of preoperative MRI features and PSA density with adverse pathology at radical prostatectomy and with biochemical recurrence–free and metastasis-free survival in a contemporary cohort. We analyzed a prospectively maintained cohort of 499 patients who underwent RP at the National Cancer Institute from 2007 to 2023 (NCT02594202). Patients who received neoadjuvant therapy, lacked complete MRI data, or underwent salvage RP were excluded. Multivariable logistic regression assessed the association of MRI features and PSA density with adverse pathology, defined as Gleason Grade Group (GG) ≥ 4, extraprostatic extension (EPE), seminal vesicle invasion (SVI), or lymph node involvement. Cox proportional hazards regression evaluated biochemical recurrence–free survival (BRFS) and metastasis-free survival (MFS). The median follow-up was 47 months. On multivariable analysis, PSA density was associated with GG ≥ 4 (OR = 16.539, $p < 0.001$) and pathology-confirmed EPE (OR = 14.345, $p < 0.001$). MRI evidence of SVI was associated with pathology-confirmed SVI (definite: OR = 13.970, $p < 0.001$; possible: OR = 7.067, $p = 0.013$). MRI evidence of ECE was associated with pathology-confirmed EPE (definite: OR = 15.305, $p < 0.001$) and lymph node involvement (possible: OR = 3.342, $p = 0.022$). Higher PI-RADS scores correlated with SVI (PI-RADS 4: OR = 5.029, $p = 0.008$; PI-RADS 5: OR = 3.606, $p = 0.035$). On Cox regression, PI-RADS 5 (HR = 1.961; 95% CI, 1.111–3.463; $p = 0.020$), definite MRI ECE (HR = 3.631; 95% CI, 1.736–7.598; $p = 0.001$), and PSA density (HR = 3.057; 95% CI, 1.577–5.924; $p = 0.001$) were associated with shorter BCRFS. For MFS, MRI SVI (HR = 5.419; 95% CI, 1.556–18.872; $p = 0.007$) and definite MRI ECE (HR = 4.151; 95% CI, 1.003–17.183; $p = 0.049$) were significant predictors of metastasis. Preoperative PSA density and adverse MRI features, including PI-RADS score, MRI-detected ECE, and MRI-detected SVI, are independently associated with adverse pathology and worse survival following RP. These imaging and biomarker parameters may improve preoperative risk assessment and guide treatment decision-making in localized prostate cancer.

119. Examining the Role of Pericyte-Secreted Extracellular Matrix on Breast Cancer Metastatic Progression

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Metastasis is the primary cause of mortality in breast cancer, yet its underlying mechanisms remain unknown. Increasing evidence supports that tumor cell dissemination occurs early during tumor progression and that the fate of disseminated tumor cells (DTC) is shaped by the microenvironment of distant organs—the pre-metastatic niche (PMN). This microenvironment contains specific environmental signals, including other cells, biochemical factors, and mechanical cues, that drive metastatic colonization. While immune cells and fibroblasts are established contributors to PMN formation, the functional role of pericytes, vascular support cells involved in endothelial stability, remains understudied. Our prior research in melanoma models indicate that pericytes aid tumor survival and colonization by transmitting soluble factors and metabolites to DTCs during extravasation [1]. We hypothesize that pericyte-secreted extracellular matrix (ECM) components promote pro-metastatic behaviors in breast cancer cells. This hypothesis will be investigated through the following aims: (1) Investigate the role of pericyte-secreted ECM components on tumor cell migration and/or proliferation time-lapse microscopy and image-based analysis, and (2) develop an engineered 3D microfluidic “niche-in-a-dish” device to investigate the spatiotemporal role of pericyte ECM remodeling in the earlier stages of metastatic colonization. A deeper understanding of these interactions could clarify the mechanisms underlying metastatic switching and identify therapeutic targets. This project addresses the critical gap in understanding how pericyte-secreted ECM influences pro-metastatic behaviors in breast cancer cells. Leveraging a recently developed protocol to isolate and culture phenotypically stable pericytes under physiologically relevant oxygen tensions1, this study will investigate how pericyte-derived ECM alters tumor cell morphology, migration, and proliferation. Time-lapse microscopy will be used to track the migration trajectories and morphology of fluorescently labeled tumor cells. Quantitative analysis of cell shape and motility via ImageJ and a MATLAB-based 2D migration code enabled identification of phenotypic shifts in response to pericyte-promoted environments. Preliminary results suggest MDA-MB-231 cells migrate farther and exhibit increased directional exploration (Langevin motion) on tumor-conditioned media (TCM)-educated pericyte ECM compared to naïve pericyte ECM. Consistent with the established relationship between migration and morphology, this enhanced migration correlates with morphological changes, including increased area and reduced circularity. Future work will identify specific pericyte ECM components through mass spectrometry and siRNA treatments, and then use both 2D migration assays and a custom 3D microfluidic "niche-in-a-dish" to track tumor cell behavior in response to activated pericyte ECM. Metastasis is the leading cause of cancer-related deaths, making it crucial to elucidate the role of pericytes in DTC survival and PMN formation. This study aims to provide fundamental information necessary to develop a 3D “niche-in-a-dish” model that mimics aspects of the pericyte/vascular interface, models tumor cell adhesion and extravasation, and allows assessment of tumor cell survival and colonization in the perivascular space. This device will also be utilized to identify pericyte activation dependent ECM remodeling, including structural and organizational changes over the later stages of the metastatic cascade. The anticipated outcomes will advance our mechanistic understanding of pericytes in breast cancer metastasis and could inform targeted therapies to disrupt early metastatic colonization and limit disease progression across multiple cancer types.

120. Interferon-β1 suppresses oncogenic signaling and impairs cancer stem cell activity in anaplastic thyroid cancer

Manju Acharya and Sheue yann Cheng

Anaplastic thyroid cancer (ATC) is a highly aggressive malignancy characterized by rapid progression, early metastasis, and poor clinical outcomes. Despite advances in targeted therapies, effective treatments remain limited. Cancer stem cells (CSCs) are key drivers of ATC progression, therapeutic resistance, and recurrence due to their abilities in self-renewal, differentiation, and tumor initiating capacities. Targeting CSCs may therefore improve therapeutic outcomes. Interferon-β1 (IFNβ1), a type I interferon, has demonstrated anti-proliferative and immunomodulatory effects in several cancers; however, its role in ATC and CSC regulation remains unclear. This study investigates the effects of IFNβ1 on apoptosis, oncogenic signaling, immune responses, and CSC activity using in vitro and in vivo ATC models. Human ATC (hATC) cell lines (THJ-11T harboring KRASG12V mutation, THJ-16T

expressing PIK3CA/TP53/RB mutations, and 8505C expressing BRAFV600E/TP53 mutations) were treated with recombinant human IFNβ1 (rhIFNβ1). In addition, the effects of endogenous IFNβ1 expression were analyzed using hATC cells stably expressing doxycycline-inducible human IFNβ1. Cellular proliferation was measured by cell counting and Ki67 immunohistochemical (IHC) staining. The apoptosis was evaluated by fluorescence-activated cell sorting (FACS) and by detecting apoptotic markers, including PARP and cleaved caspase-3, via western blotting. To investigate the underlying mechanisms, key signaling pathways (JAK1-STAT1, ERK, p38 MAPK, and TRAIL) were analyzed by western blotting and RT-qPCR. IFNβ1-mediated immune responses were evaluated by measuring the cytokines and chemokines expression along with major histocompatibility complex class I (MHC-I) levels. CSC self-renewal activity was evaluated using tumor sphere formation assays and analysis of CSC-associated markers in vitro. The in vivo anti-tumor and CSC effects of IFNβ1 were further evaluated using three limiting dilutions (5×10^6, 5×10^5, and 5×10^4 cells) of IFNβ1 expressed THJ-16T xenograft models to determine its impact on tumor growth and tumor-initiating potential. Both exogenous and endogenous IFNβ1 expression significantly inhibited proliferation of hATC cell lines and induced apoptosis. These effects were evidenced by reduced Ki67, increased apoptotic cell populations and elevated levels of PARP and cleaved caspase-3. Mechanistic studies revealed upregulation of signaling pathways involved in cell survival and apoptosis, including JAK1, STAT1, ERK, p38 MAPK, and TRAIL. IFNβ1 expression also elicited immune-related responses in hATC cells, characterized by upregulation of pro-inflammatory cytokines and chemokines, including CXCL9, IL-6, and IL-8, along with enhanced HLA-A, HLA-B and HLA-C expression, suggesting increased immune recognition potential. Importantly, IFNβ1 significantly suppressed CSC self-renewal activity, as demonstrated by reduced tumor sphere formation and downregulation of CSC-associated markers such as SOX2, OCT4, CD44 and ABCG2 in vitro. Consistent with these findings, IFNβ1 reduced tumor growth, tumor-initiating capacity, and CSC marker expression in xenograft models. This study identifies IFNβ1 as a key regulator of CSC-driven tumorigenesis in ATC, demonstrating its ability to inhibit tumor growth, disrupt oncogenic signaling, enhance immune responses, and suppress CSC self-renewal. These findings support future clinical evaluation of IFNβ1-based therapies and targeting interferon pathways in aggressive cancers.

121. SLK Inhibition Leads to DNA Damage in XPO7-overexpressing Cholangiocarcinoma

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Cholangiocarcinoma (CCA) is a rare, aggressive cancer that originates from the biliary epithelium. Patients often present with metastatic disease, and 5-year survival rates remain less than 10% despite recent advances in targeted molecular therapies. To expand therapeutic options to more patients with this disease, we investigated functional crosstalk, which revealed Exportin-7 (XPO7) as a highly expressed protein. Immunohistochemical (IHC) analysis of 170 patients with CCA demonstrated overexpression of XPO7 in 30% of the samples, which correlated with abbreviated survival. To understand the role of XPO7 in CCA, we carried out co-immunoprecipitation, proteomic analysis and protein modeling. We demonstrated that XPO7 interacts with the Ste-20 like kinase (SLK) and forms a molecular complex, with interruption of either protein resulting in decreased tumor xenograft formation. To identify potential pharmacologic inhibitors, a kinome screen was run for agents with activity against SLK. We identified tivozanib, a potent VEGFR2 tyrosine kinase inhibitor, as having substantial activity against the kinase activity of SLK, and used x-ray crystallography to confirm ATP-binding pocket with “DGF-out” conformation. Importantly, tivozanib regressed tumors in established CCA patient-derived xenografts overexpressing XPO7, which we confirmed in human tumors using our ex vivo tumor slice culture system. In vitro experiments, tivozanib treatment in cholangiocarcinoma cell lines showed G2/M cell cycle arrest. Both shRNA-mediated knockdown of SLK and tivozanib treatment demonstrated a reduction in phospho-PLK1 activation (T210), with subsequent DNA damage response characterized by upregulation of phospho-DNAPKc (S2056), phospho-H2AX (S139), and p27 with decreased signaling through the mTORC1 pathway characterized by phospho-mTOR (S2448) and phospho-RPS6 (S235/236). These results were

confirmed in human tumors (n=11) using mass spectrometry, IHC, and Western blotting. Lastly, tivozanib monotherapy demonstrated in vivo efficacy by RECIST criteria in patients with XPO7 over-expressing CCA. On treatment biopsies in a responding CCA patient to tivozanib, we confirmed marked upregulation of phospho-H2AX (S139) with decreased expression of Ki67, phospho-mTOR (S2448) and phospho-RPS6 (S235/236), confirming DNA damage and reduction in proliferation. Overall, our findings show SLK as a promising therapeutic target in patients with XPO7-overexpressing CCA.

122. Characterization of Cas9-Integrated Murine Cell Lines in Immunocompetent Mice Model for Oral Squamous Cell Carcinoma

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Oral squamous cell carcinoma (OSCC) is among the most common cancers worldwide, with approximately 380,000 cases and 180,000 deaths annually. Despite therapeutic advances, the 5-year survival rate remains at 50% due to late-stage diagnosis and high rates of recurrence and metastasis. Immune checkpoint blockade, introduced for OSCC in 2016, has improved survival outcomes and patient quality of life compared to conventional multimodal therapy. However, the overall response rate remains low (18%) and is limited to a subset of patients, highlighting the need to identify novel immunotherapeutic targets. To address this, we generated a Cas9-expressing OSCC cell line derived from immunocompetent mice that recapitulates key mutational features of human OSCC (90.4% similarity). This model enables in vivo CRISPR/Cas9-mediated oncogene knockout screening to identify and analyze cancer driver genes that modulate tumor–immune interactions and therapeutic response within the tumor microenvironment. It provides a platform to discover mechanisms of immunotherapy resistance and develop effective combination treatments for OSCC.

128. Discovery of Genomic Events in Oral Squamous Cell Carcinoma using Whole Exome Sequencing

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Oral squamous cell carcinoma (OSCC) is a major subtype of head and neck squamous cell carcinoma that develops through stepwise progression from oral epithelial dysplasia. This process is thought to involve accumulation of genetic and chromosomal alterations, yet the specific mutations that drive progression from dysplasia to invasive OSCC remain incompletely understood. We hypothesized that paired dysplasia and carcinoma samples would contain both shared early genomic alterations and additional mutations enriched in invasive disease, allowing identification of candidate progression-associated events. This study included 36 FFPE biopsy samples overall, from which eight paired oral dysplasia and OSCC samples from eight patients were selected for high-confidence analysis. Whole-exome sequencing was performed, and pairwise alignment was conducted using DRAGEN. Somatic variant calling was carried out using Mutect2 from GATK and DeepSomatic with FFPE-aware parameters. Mutation calls were then annotated against the COSMIC Cancer Gene Census. The analysis focused on comparing mutational burden between matched dysplasia and carcinoma samples, identifying shared mutations, and characterizing recurrently altered genes and pathways associated with clonal progression. The mutational landscape demonstrated substantial heterogeneity across paired samples. In most cases, carcinoma samples had a higher mutational burden than their matched dysplastic lesions, supporting continued genomic evolution during malignant progression. At the same time, exact shared mutations were identified between dysplasia and OSCC in several pairs, indicating clonal continuity and suggesting that some driver events arise early and persist through progression. Shared founding-like mutations were

observed in biologically relevant pathways, including RAS/RTK, PI3K/mTOR, NOTCH/WNT, NRF2/stress response, and DNA repair. Limited overlap in some pairs may reflect clonal evolution, field cancerization, or sampling differences. Overall, these findings support a model in which OSCC progression involves both persistence of early alterations and acquisition of additional mutations during invasion. These findings are relevant because reliable molecular markers of progression from oral dysplasia to OSCC remain limited. By identifying shared and progression-associated alterations in matched precursor and invasive lesions, this study highlights candidate early genomic markers that may improve risk stratification and biological understanding of oral cancer development. Despite the small cohort and use of FFPE specimens, the results provide a foundation for future whole-genome studies to better define copy number changes, clonal architecture, and genomic events associated with malignant transformation.

Cellular Physiology: Metabolism, Microbiology, & Cellular/Molecular Biology (Posters)

123. Replication stress induces fork stalling in expressed genes with convergent forks

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Faithful DNA replication is vital to cell division and genome integrity. However, this process is constantly challenged by various kinds of extracellular and intracellular stresses. Replication stress will cause wide-spread fork stalling during replication. Currently, it remains unclear which genomic regions experience replication fork stalling. In this study, we hypothesize that we can monitor fork movement by capturing free 3'-ends that accumulate at stalled replication forks. We used low-dose Aphidicolin (a DNA polymerase inhibitor) to mimic replication stress in cells, and TrAEL-seq was used to capture free 3'-ends from leading strand DNA synthesis. To better understand how replication fork movement was affected by stress, we did a time-series experiment to monitor the distribution of free 3'-ends after Aphidicolin treatment. Other genome-wide assays, such as ChIP-seq, ATMP-seq, and TT-seq, were used to study changes in factors like RPA binding, DNA supercoiling, and transcription. Fork stalling induced by replication stress occurs specifically in expressed genes where two convergent replication forks merge within the gene body, including well-known common fragile site genes such as WWOX, FHIT, and PARD3B. Most genes with stalled forks recover and complete replication, but a small fraction enter mitosis with incomplete replication. Strong positive DNA supercoiling accumulates in these genes after prolonged replication stress, particularly in longer genes, providing a mechanistic explanation for why complete replication of long genes is especially challenging under stress. Finally, we show that persistent fork stalling ultimately blocks transcription in these genes. Our findings reveal a previously unrecognized mechanism of genome instability under replication stress. By identifying that fork stalling occurs specifically in expressed genes where convergent forks merge, and demonstrating that positive DNA supercoiling accumulates in these regions, we provide a mechanistic explanation for why certain genomic loci, including common fragile sites, are particularly vulnerable to breakage and structural variations. This is especially relevant for cancer biology, as cancer cells experience chronically elevated replication stress and show enriched structural variations at these precise locations. Understanding this supercoiling-mediated vulnerability opens new avenues for therapeutic intervention: drugs targeting the resolution of replication stress or DNA supercoiling in these genes could selectively exploit cancer cells' dependence on completing replication under stress. Our work thus provides both a mechanistic framework for understanding fragile site instability and a potential therapeutic strategy for cancer treatment.

124. N-acetyl Aspartate Induces a Pro-Inflammatory Phenotype in Peritoneal Resident Macrophages, Driving Metabolic and Transcriptional Changes

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Peritoneal cavity (PC) tissue resident macrophages (TRM) are key players in immune defense and tissue homeostasis. The metabolite N-acetyl aspartate (NAA) is elevated in the PC. NAA is synthesized from acetate and

aspartate by Nat8l and metabolized back to aspartate by Aspa. TRM depend on their tissue niche, PC TRM uniquely express Aspa, and NAA levels are associated with worse outcomes in ovarian cancer patients. This suggests NAA is key in the biology of the PC and leads to the hypothesis that NAA is metabolized as a fuel by PC TRM.The PC niche was assessed by flow cytometry, metabolomics, 13C carbon tracing and single cell RNA sequencing in wild type and Aspa-/- or Nat8l-/- mice. In addition, multiple type 2 inflammatory models and orthotopic tumor models carried out in vivo and macrophage responses to NAA were assessed in vitro.
We reveal the expression of Nat8l by PC TRM, indicating the NAA metabolic loop can run within the PC. Moreover, PC NAA levels are dynamic in type 2 mouse models, however, there is low incorporation of metabolized NAA-derived aspartate into the TCA cycle. In vitro, NAA alters the intracellular metabolome and increases metabolic rates. These metabolic changes are also seen in vivo, where Aspa-/- mice display increased PC NAA levels and glycolytic intermediates following stimulation. The role of NAA is not only metabolic, as macrophages exposed to NAA have an increased pro-inflammatory gene signature. Lastly, in contrast with previous literature, genetic perturbation of the NAA metabolic loop within the PC leads to reduced growth in a murine intraperitoneal tumor challenge model. This work shows a role for the NAA metabolic loop within the PC, anchored by the TRM. NAA within the PC serves as a metabolic and pro-inflammatory modulator rather than a fuel, leading to increased cellular energetics, and an altered metabolome. Therefore, the primary role of Nat8l and Aspa in PC TRM may be to modulate NAA levels and control the inflammatory potential of the peritoneal cavity. Previous literature on the NAA metabolic loop has been limited, especially within the PC. This work defines the role of NAA within the PC as a metabolic and pro-inflammatory modulator rather than directly as a metabolite and reveals a new mechanism of cellular control. The production and consumption of metabolites not for energy or macromolecule requirements but as a method of controlling the inflammatory and metabolic milieu is a novel and exciting development, which may be broadly applicable to multiple other metabolites, tissue sites and disease states.

125. Peroxiredoxin 1 safeguards the nucleolar genome from oxidative damage

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Peroxiredoxin 1 (PRDX1) is a highly conserved, thiol-dependent peroxidase that rapidly scavenges reactive oxygen species to modulate redox signaling. PRDX1-null mice exhibited genomic instability, shortened lifespan, and accelerated tumorigenesis, including development of lymphomas, sarcomas, and carcinomas. Despite extensive characterization of these phenotypes, the molecular mechanism by which PRDX1 loss causes genomic instability remains poorly understood. Here we show that PRDX1 deficiency alters nucleolar morphology, impairs RNA Polymerase I (POL-I)-dependent transcription of pre-ribosomal RNAs and triggers nucleolar genomic instability. This oxidative stress-induced nucleolar dysfunction promotes the stability of secondary DNA structures, such as RNA-DNA hybrids and G-quadruplex DNA, contributing to nucleolar genomic instability. We demonstrate that PRDX1 loss reduces nascent rRNA levels and impairs rRNA processing, further affecting ribosome biogenesis. Mechanistically, we established that PRDX1 loss triggers activation of the nucleolar DNA damage response including activation of DNA repair kinase ATM and the nucleolar factor TCOF1 within the nucleolus, and recruitment of the MRE11-RAD50-NBS1 (MRN) complex subunit NBS1 to ribosomal DNA (rDNA) loci. NBS1 accumulation correlates with the repression of rDNA transcription by POL-I, potentially delaying rRNA synthesis, and safeguarding the nucleolar genome from further oxidative damage. Collectively, these findings uncover a previously unrecognized, but critical role, for PRDX1 in maintaining nucleolar integrity and ribosomal biogenesis through redox-dependent regulation of rDNA transcription and processing machinery.

126. R-spondin 1 potentiates WNT signaling in colon cancer cells without its known co-receptors, ZNRF3, RNF43 and LGR4/5/6.

Praveen Sonkusre[1], Rebecca Kim[1], Jeremy Ritchey[1] and Andres Lebensohn[1]
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127. Defining the roles of docking interactions in cyclin D-CDK4/6-mediated RB phosphorylation.

Devin Bradburn[1] and Mardo Koivomagi[1]
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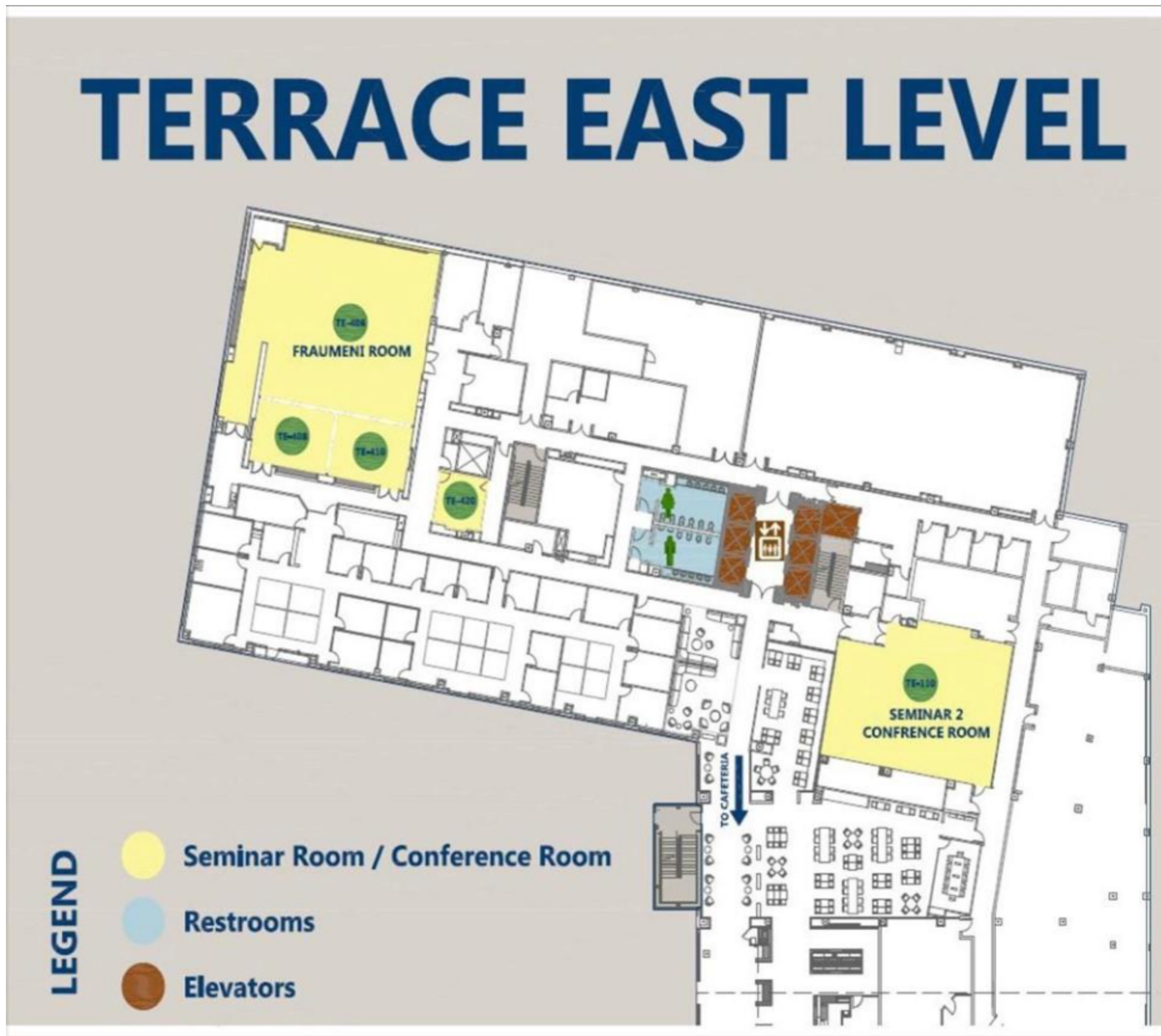
Terrace East (TE) Level Map



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

NCI Shady Grove Order Ahead:
https://www.toasttab.com/local/order/corporate-chefs-nci

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