22nd Annual CCR and DCEG Staff Scientists and Staff Clinicians Retreat

From Organoid to Bedside: Engineering the Future of Cancer Therapy

May 8th, 2026, NCI Shady Grove, Rockville, MD 20850

NATIONAL CANCER INSTITUTE

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Welcome to the 22nd Annual CCR and DCEG Staff Scientists and Staff Clinicians Retreat! On behalf of the Organizing Committee, we would like to thank you for joining us for a day dedicated to advancing cancer research and collaboration.

This year's theme, *"From organoid to bedside: Engineering the future of cancer therapy,"* explores how innovative model systems, cutting-edge technologies, and translational approaches are shaping the next generation of cancer treatments. We are honored to be joined today by renowned NIH scientists. To set the stage for a day of scientific exchange, the program will begin with opening remarks from Dr. James Gulley, NCI Clinical Director and Acting Co-Director of CCR. The keynote lecture on bioengineered cancer tissues will be delivered by Dr. Marc Ferrer, Director of the 3-D Tissue Bioprinting Laboratory, National Center for Advancing Translational Sciences (NCATS) and a leading expert in the field. To conclude the retreat, the program will close with remarks from Dr. Carol Thiele, Acting Co-Director of CCR.

We are especially excited to highlight the contributions of our staff scientists and clinicians, whose work bridges discovery and patient care. Featured today are 96 poster presentations and 10 short talks selected from the top-ranking abstracts. These presentations offer an opportunity not only to share findings but also to spark new ideas, collaborations, and directions. We encourage everyone to engage actively, ask questions, exchange perspectives, and take full advantage of the expertise gathered in this room.

In addition to the scientific program, we'll also hear updates from the different Staff Scientists Sub-Committees, recognize outstanding research achievements through awards, and celebrate the mentorship and leadership that sustain our community.

This retreat wouldn't be possible without the support from the Center for Cancer Training (CCT) and the Office of Training and Education (OTE). The Organizing Committee would like to especially thank Dr. Nastaran Zahir, Branch Director, CTB and Acting Director of CCT and Dr. Chanelle Case Borden, Branch Director, OTE, for their guidance and counsel, and Ms. Maria Moten, Program Specialist, CCT, Ms. Angela Jones, Lead Program Analyst, CCT, and the web team for their invaluable help.

We hope this retreat will inspire all of us to leverage innovative model systems and build meaningful collaborations that strengthen our ongoing efforts to combat cancer.

Best Regards,

Dr. Kellsye Fabian and Dr. Daniël Melters

Co-Chairs, 2026 CCR/DCEG Retreat Organizing Committee

2025-2026 Retreat Planning Committee Members

Abdul Waheed, Ph.D.
Acong Yang, Ph.D.
Bala Kuppusamy, Ph.D.
Brajendra Tripathi, Ph.D.
Brian Ko, Ph.D.
Chih-Shia Lee, Ph.D.
Daniël Melters, Ph.D.
Dong Kong, Ph.D.
Duane Hamilton, Ph.D.
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Jibran Ahmed, Ph.D.
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Sofia Gameiro, Ph.D.
Soyeong Sim, Ph.D.
Sukhbir Kaur, Ph.D.
Tiaojiang Xiao, Ph.D.
Usha Acharya, Ph.D.
Yanlin Yu, Ph.D.

22nd Annual CCR and DCEG Staff Scientists and Staff Clinicians Retreat

From organoid to bedside: Engineering the future of cancer therapy

Date:	**Friday May 8th, 2026**
Location:	**NCI Shady Grove Campus** **9609 Medical Center Drive** **Rockville, MD 20850**
Website:	**https://events.cancer.gov/cct/sssc-retreat**

8:30 a.m.	**Registration and Poster Set-up**
	Moderator: Dr. Kellsye Fabian
9:00 a.m.	**Welcome and Opening Remarks – Dr. James Gulley**
	Moderator: Dr. Daniël Melters
9:15 a.m.	**Keynote lecture - Dr. Marc Ferrer** – Bioengineered cancer tissue models with tumor microenvironment
10:15 a.m.	**BREAK**
	Oral Abstract Symposium I (10 min talk + 3 mi Q&A)
	Moderator: Dr. Balamurugan Kuppusamy
10:30 a.m.	**Dr. Andaleeb Sajid** – Paradigm shift in P-glycoprotein transport: evidence for a switch region in reversing the direction from an efflux to update of drugs
10:43 a.m.	**Dr. Brajendra Tripathi** – Mechanism-based combination therapies for mutant KRAS-driven pancreatic cancer
10:56 a.m.	**Dr. Jason Hoskins** – Characterization of a pancreatic cancer GWAS signal suggests PDX1 buffers stress and stabilizes homeostatic cell states in the exocrine pancreas
11:09 a.m.	**Dr. Feng Zhu** – IKKα serves as a molecular switch linking miRNA biogenesis and inflammation in skin tumorigenesis
11:22 a.m.	**Dr. Duane Hamilton** – Shed ERVMER34-1 drives formation of an immunosuppressive tumor microenvironment: a novel tumor-promoting role for an endogenous retroviral envelope protein

11:35 a.m. **Group picture**

11:45 a.m. **Poster Session I – 1hr (odd numbers)**

12:45 p.m. **LUNCH BREAK**

1:15 p.m. **Poster Session II – 1hr (even numbers)**

Oral Abstract Symposium II (10 min talk + 3 mi Q&A)

Moderator: Dr. Brajendra Tripathi

2:15 p.m. **Dr. Tzu-Ting Huang** – Targeting DHX9 helicase exposes a mitotic vulnerability that enhances paclitaxel response in ovarian and endometrial cancers

2:28 p.m. **Dr. Billel Gasmi** – From organoid to beside: establishment of patient-derived tumor organoids as *ex vivo* reagents to analyze personalized T-cell repsonses and predict clinical outcomes to adoptive cell therapy with TIL

2:41 p.m. **Dr. Zied Abdullaev** – Clinical assessment of the NCI Bethesda CNS Tumor DNA methylation classifier in a large consultation cohort

2:54 p.m. **Dr. Ranjodh Sandhu** – Decoding telomerase: harnessing telomerase RNA to decipher the enzyme's secrets

3:07 p.m. **Dr. Bahman Afsari** – Characterization of the microenvironment of Kaposi sarcoma using spatial transcriptomics identifies mechanisms of immune evasion

3:20 p.m. **BREAK**

Moderator: Dr. Nicolas Cuburu

3:40 p.m. **NIH Staff Scientist Organization Displaced Staff Scientist Committee**
Drs. Herman Edskes, Chin-Hsien (Emily) Tai, and Swati Choksi

4:10 p.m. **Information from SSSC Sub-committees**

The Dossier

Award Presentation

4:25 p.m. **CCR and DCEG Oral Presentation Travel Awards**

Outstanding Poster Awards

SSSC Outstanding Mentor Award

Moderator: Dr. Kellsye Fabian

4:40 p.m. **Closing remarks – Dr. Carol Thiele**

INVITED SPEAKERS

Biographies of Invited Speakers

Marc Ferrer Ph.D. Director, 3-D Tissue Bioprinting Laboratory, National Center for Advancing Translational Sciences (NCATS), NIH

Dr. Marc Ferrer is a leader in high-throughput screening innovation, specifically leveraging 3D bioengineered tissues for disease modeling and predictive assay platforms. He obtained his Bachelor of Science degree in Organic Chemistry from the University of Barcelona, Spain, in 1989. He went on to receive his Ph.D. in Biological Chemistry from the University of Minnesota in 1994. In his graduate work, he chemically synthesized small proteins to study protein folding using biophysical methods. He was a postdoctoral fellow at Harvard University from 1995-1999 where he used structure-based chemical approaches for the development of anti-HIV small molecules. He joined the Department of Automated Biotechnology at the Merck Research Laboratories in 1999, where he became the Director of Assay Development and High Throughput Screening. There he was involved in the miniaturization of biochemical and cell-based assays for high throughput screening of small molecule libraries for lead identification. He also led efforts in the implementation of genome-wide siRNA screening for target identification, developing new automation-friendly siRNA transfection protocols, improving data analysis tools for hit selection, and executing strategies for better on-target hit validation. In 2010, he joined the NCATS Chemical Genomic Center to work with academic investigators to implement high-throughput screening and medicinal chemistry programs for the discovery of small-molecule probes to study protein function. In 2018, Dr. Ferrer formally established the 3-D Tissue Bioprinting Laboratory within the Division of Preclinical Innovation at NCATS. Under his leadership, The NCATS 3DTBL has developed into a multidisciplinary group of bioengineers, assay development scientists, and drug discovery experts with the goal of creating and using 3D bioengineered tissues for disease modeling and as predictive assay platforms for drug discovery and development. Throughout his career, Dr. Ferrer has developed innovative assay paradigms for drug discovery, with emphasis on 3D cellular models such as tumor spheroids and 3D bioprinted tissues, iPSC-derived and primary cells for drug screening and has contributed to the development of matrix screening approaches for unbiased discovery of drug combinations. Dr. Ferrer has co-authored more than 150 peer-reviewed scientific publications and holds key roles as PI/co-PI in numerous successfully funded grants. He has established impactful collaborative translational programs with academic investigators and rare disease foundations.

James Gulley M.D. Ph.D. Clinical Director, Acting Co-Director, Center for Cancer Research, Co-Director, Center for Immuno-Oncology, National Cancer Institute, NIH

Dr. James Gulley is an internationally recognized expert in cancer immunotherapy with over three decades of experience translating laboratory discoveries into clinical applications. He earned his Ph.D. in 1994 in Microbiology and an M.D. in 1995 from Loma Linda University. As part of this eight-year MD/PhD Medical Scientist Training Program, he completed a dissertation on tumor immunology. He completed his residency in Internal Medicine at Emory University in 1998, followed by a Medical Oncology fellowship at the National Cancer Institute. Since joining the NCI's senior clinical staff in 2001, Dr. Gulley has ascended to several pivotal leadership roles. In 2013, he was appointed chief of the CCR Genitourinary Malignancies Branch and Director of the Medical Oncology Service. He was appointed NCI Clinical Director in 2023, and he currently also serves as the acting Co-Director of CCR. Dr. Gulley has been at the forefront of immuno-oncology for decades, contributing to the development of multiple immunotherapeutic approaches and advancing promising laboratory findings into clinical trials. These innovative studies involve the use of cancer vaccines and other immunostimulatory agents to modulate the immune response in cancer patients, and the addition of other strategies to enhance immune-mediated killing. He was the coordinating PI of an international trial of avelumab that led to regulatory approval. He was the PI of the first-in-human international study of a first in class bifunctional antibody, bintrafusp alfa, which targets PDL1 and TGF-beta. He also leads several rationally designed, cutting edge combination immunotherapy studies. He has been an investigator on over 200 clinical trials and has authored over 350 scientific papers. As the NCI Clinical Director, he oversees the day-to-day operations of more than 350 active clinical trials as well as all regulatory aspects of the clinical program, such as protocol approval and regulatory compliance. Dr. Gulley also serves on many national and NIH boards and committees. He has received multiple prestigious awards including the 2010 Presidential Early Career Award for Scientists and Engineers, the highest award bestowed by the US President on investigators early in their careers. He was awarded the 2018 Hubert H. Humphrey Award for Service to America for contributing to the health, safety, and well-being of the nation by helping to get FDA approval for avelumab for Merkel cell carcinoma and urothelial carcinoma and has received numerous NCI and NIH Director's Awards.

Carol Thiele Ph.D. Acting Co-Director, Center for Cancer Research, Deputy Chief, Pediatric Oncology Branch, National Cancer Institute, NIH

Dr. Carol Thiele is a pioneering cancer biologist specializing in the development of novel

therapeutic strategies for pediatric tumors using state-of-the-art biologic and genomic analyses of tumors and normal counterparts. Dr. Thiele received her Ph.D. in Microbiology and Immunology from the University of California, Los Angeles. She completed her postdoctoral research at the NCI as a Cancer Research Institute and a Damon Runyon-Walter Winchell Fellow. Dr. Thiele's scientific interest is in the field of pediatric neuroectodermal tumors and neuronal development. 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. She pioneered studies using retinoids to “target” the MYCN oncogene and control tumor growth. These led to clinical studies which showed that retinoids improved outcomes for children with high-risk neuroblastoma. Her laboratory continues to innovate by developing pre-clinical models and genetically engineered mice to study mechanisms of neuroblastoma tumorigenesis and assess novel therapeutic interventions. Ongoing studies in her lab are also aimed at understanding epigenetic/chromatin-based mechanisms to re-program and differentiate neuroblastoma tumor cells. Beyond her research, Dr. Thiele has played a key role in advancing the scientific community. She has been involved in the organization of the Advances in Neuroblastoma Research Association (ANRA). She was one of the founding editors of Cell Death & Differentiation and has served on the editorial boards of Cell Death & Differentiation, Cancer Research and Molecular Cancer Therapeutics. She 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.

Biographies of SSSC Retreat Co-chairs

Kellsye Fabian Ph.D. Staff Scientist, Center for Immuno-Oncology, NCI

Dr. Fabian is an immunologist specializing in cancer vaccines and the development of innovative combination therapies for the treatment of cancer. Dr. Fabian completed her B. S. in 2007 and M.S. in 2011in Molecular Biology and Biotechnology from the University of Philippines. She received her Ph.D. in Immunology from the University of Pittsburgh in 2017. Her graduate work focused on targeting tumor blood vessel-associated antigens for cancer immunotherapy. She joined the Center for Immuno-Oncology (formerly the Laboratory of Tumor Immunology and Biology), CCR, NCI as a Postdoctoral Fellow in 2017 and was appointed as a Staff Scientist in 2021. Her focus is on the strategic combination of multiple immuno-oncology agents that can engage, expand, enable, and evolve the immune response to provide therapeutic benefits in several tumor types. Her current research is on novel recombinant vaccines and vaccine strategies to further enhance anti-tumor T-cell and NK cell responses through strategic combination with chemotherapy, targeted small molecules, immune checkpoint inhibitors, cell therapy, and other novel immunotherapeutic agents that modulate the immune response. In addition to her research endeavors, Dr. Fabian actively fosters the staff scientist community through service on the retreat organizing committee and provides mentorship to students and postdoctoral fellows.

Daniël P. Melters Ph.D. Staff Scientist, Laboratory of Receptor Biology and Gene Expression, NCI

Dr. Melters's research focuses on understanding how chromatin domains are formed, maintained,

and altered in cancer. Dr. Melters received his Bachelor's and Master's degree in Biomedical Sciences from the Leiden University in the Netherlands. For his M.S. degree, he studied important questions in immunology and neuroscience. After his M.S., Dr. Melters joined the endocrinology lab of Dr. Pearce's lab at UCSF. He then pursued his Ph.D. in the labs of Drs. Ian Korf and Simon Chan at UC Davis. In this collaboration, he used bioinformatic tools to identify candidate centromeric tandem repeats across the eukaryotic tree. During his Ph.D., he completed a biotechnology program internship at Genentech focusing on science policy. Dr. Melters joined the LRGBE/CSEM as a Postdoctoral Fellow and was later appointed as a staff scientist. The roles of histone variants, together with nucleosome-binding proteins, help orchestrate the functional aspects of chromatin domains, including chromatin accessibility and transcriptional output. Using cell biological, biochemical, and biophysical tools, Dr. Melters dissects how chromatin accessibility is modulated by histone variants and chromatin-binding factors in cancer. He is an expert in using AFM and high-speed AFM to study chromatin structure. These techniques allow for the direct, real-time visualization of nucleosomes and chromatin fibers providing insights into their dynamics at the single-molecule level. He has also developed automated, high-throughput AFM pipelines for analyzing large datasets of DNA–protein complexes. Dr. Melters is an engaged member of the staff scientist community, serving on the retreat organizing committee and mentoring the next generation of young scientists.

ABSTRACTS

Oral Presentations

6. Andaleeb Sajid, andaleeb.sajid@nih.gov **- Laboratory of Cell Biology, CCR**
Andaleeb Sajid, Rajan Guha, Nandhini Ranganathan, Stewart Durell, and Suresh V. Ambudkar1

"Paradigm shift in P-glycoprotein transport: Evidence for a switch region in reversing the direction from an efflux to uptake of drugs"

Background and Hypotheses
The multidrug transporter P-glycoprotein (P-gp) is a mediator of multidrug resistance (MDR) in cancer, exporting a wide range of amphipathic chemotherapeutic agents and thereby reducing intracellular drug retention. Structurally, P-gp consists of two homologous transmembrane domains, each containing six transmembrane helices (TMHs), and two cytosolic nucleotide-binding domains that drive substrate transport using the energy of ATP hydrolysis. Within the transmembrane region, TMH6 and TMH12 line the central drug-binding cavity and form a pathway for substrate interaction and translocation. Although P-gp is classically defined as an efflux pump, we previously observed that specific mutations in TMH6 and TMH12 impair efflux and can reverse transport direction for certain substrates, effectively converting P-gp into an uptake transporter. We hypothesized that TMH6 and TMH12 form a structural "switch" that governs transport directionality through regulated conformational transitions.

Study Design and Methods
To identify structural determinants of directional control, we engineered targeted mutations in TMH6 and TMH12. Alanine substitutions were introduced at three residues in TMH6 (V334, F336, F343) and four residues in the upper segment of TMH12 (V974, L975, V977, F978), generating 7All mutant P-gp. Functional outcomes were assessed using quantitative transport and ATPase assays. Additional mutagenesis within the central region of TMH12 evaluated the reversibility of the uptake phenotype. Molecular dynamics simulations were performed to examine conformational changes and coupling between ATP hydrolysis and substrate translocation.

Results and Conclusions
Combined alanine substitutions in TMH6 and TMH12 converted P-gp from a drug efflux pump into the uptake transporter. This shift was dependent on a specific group of residues in both helices, indicating that these residues function as a coordinated regulatory switch rather than as independent determinants. Further mutagenesis of residues in

TMH12demonstrated that the uptake phenotype is reversible, supporting the conclusion that transport direction is dynamically controlled rather than irreversibly altered by structural disruption. Biochemical and computational analyses revealed that the identified switch region primarily affects the substrate translocation step rather than initial binding. MD simulations showed significant changes to the lumen in the outer leaflet of the membrane in 7A-II, probably due to a drastic distortion of the helical conformation of TMH 12, effectively shortening it. The mutations modify conformational transitions that couple ATP hydrolysis to alternating access of the drug-binding cavity, thereby redirecting net substrate movement. Subtle rearrangements at the TMH6-TMH12 interface determine whether substrates are effluxed or imported. Similarly, by using a mutational approach, other bacterial and eukaryotic members of type IV ABC transporters have been converted to uptake pumps, indicating evolutionary preservation of this regulatory mechanism.

Collectively, these findings identify a previously unrecognized bidirectional switch within TMH6 and TMH12 that governs substrate transport by P-gp. Targeting this regulatory module provides a conceptual framework for selectively changing the direction of drug transport and offers a novel strategy to overcome drug resistance in P-gp-expressing cancers.

References:
1. Sajid A, et al. Proc Natl Acad Sci U S A.117:29609-17.2020
2. Sajid A, et al. J Mol Biol.437:168979.2025

15. Tzu-Ting Huang huangt2@nih.gov - Women's Malignancies Branch, CCR
Tzu-Ting Huang1, Jayakumar R. Nair1, Courtney Bowen1, Jennifer Castro2, Darryl Nousome3, Sunaina Nayak2, Serena J. Silver2 and Jung-Min Lee1

"Targeting DHX9 helicase exposes a mitotic vulnerability that enhances paclitaxel response in ovarian and endometrial cancers"

Background and Hypotheses
Recurrent high-grade serous ovarian carcinoma (HGSOC) and aggressive endometrial cancer (EC) exhibit limited durable responses to current therapies, creating an urgent need for new treatment strategies. DHX9, a helicase critical for R-loop resolution and chromosomal stability, is overexpressed in gynecologic cancers and associated with poor survival. However, its therapeutic potential and resistance mechanisms remain undefined, and existing biomarkers (BRCA mutation, microsatellite instability status) inadequately predict treatment response. We hypothesized that DHX9 inhibition exposes a mitosis-specific vulnerability distinct from DNA repair deficiency, and that combining DHX9

inhibition with microtubule-targeting agents can overcome intrinsic resistance mechanisms.

Study Design and Methods

We evaluated a selective DHX9 inhibitor (DHX9i, ATX968) across molecularly diverse HGSOC and EC cell lines and animal models encompassing platinum-resistance and treatment-refractory disease. Cellular responses were assessed through cell proliferation, DNA damage (Î^{3}H2AX), chromosomal instability, apoptosis, and mitotic integrity analyses. Multi-omics profiling (whole-exome and transcriptomic sequencing) identified genomic and pathway determinants of DHX9i sensitivity and resistance. Based on resistance-associated alterations in spindle assembly and microtubule regulatory pathways, we combined DHX9i with paclitaxel to enforce mitotic disruption.

Results and Conclusions

DHX9i induced DNA damage, chromosomal instability, and mitotic catastrophe independent of microsatellite instability status or prior platinum/PARP inhibitor resistance. Intrinsic resistance to DHX9i was unlikely driven by recurrent mutations but rather by coordinated copy-number alterations and transcriptional remodeling that preserved mitotic fidelity. Critically, the DHX9i-paclitaxel combination overcame intrinsic resistance, achieving enhanced cytotoxicity in vitro and durable tumor regression with significant survival benefit across multiple xenograft models without overt toxicity. This work establishes DHX9 as a therapeutically actionable target in gynecologic cancers and provides strong preclinical rationale for clinical translation of DHX9i-paclitaxel combinations in treatment-refractory disease.

1Women's Malignancies Branch, CCR, NCI; 2Accent Therapeutics, Lexington, Massachusetts; 3CCR Collaborative Bioinformatics Resource, CCR, NCI.

24. Brajendra Tripathi tripathib@mail.nih.gov - Laboratory of Cellular Oncology, CCR
Brajendra K. Tripathi1, Sophia M. Shahin1, Elise Van Meter1, Marian E. Durkin1, Xiaolan Qian1, James H. Doroshow2, Dunrui Wang1, and Douglas R. Lowy1

"Mechanism-based combination therapies for mutant KRAS-driven pancreatic cancer"

Despite advances in cancer therapy, pancreatic cancer remains one the most lethal malignancies, with a five-year survival rate of only 13%. Pancreatic ductal adenocarcinoma (PDAC) accounts for more than 90% of all pancreatic cancer cases and is associated with a poor prognosis, largely due to late-stage diagnosis and limited responsiveness to currently available therapies. A major challenge in molecularly targeted therapy is the identification of effective drug combinations that selectively harness PDAC-specific vulnerabilities. Oncogenic mutations in the KRAS gene, present in over 90% of PDAC patients, play a central role in PDAC pathogenesis. Although KRAS inhibitors may be

clinically beneficial for the treatment of PDAC, drug resistance is likely to emerge with single-agent therapy. To overcome this anticipated limitation, the development of mechanism-based combination therapies that include KRAS inhibitors will be essential for effective treatment.

In this study, through integrated analyses of pancreatic cancer patient samples, cancer cell lines, and preclinical experimental model systems, we uncover a new, noncanonical, pro-oncogenic function of mutant RAS protein in pancreatic cancer. These findings suggest that drug combinations designed to cooperate with inhibition of this noncanonical, pro-oncogenic RAS function may provide an effective targeted treatment for pancreatic cancer. In preclinical pancreatic cancer models, we demonstrate that mutant KRAS facilitates the export of nuclear proteins to the cytoplasm and identify the DLC1 tumor suppressor protein as a critical downstream target of this KRAS-dependent nuclear export function. Perinuclear binding of KRAS-GTP to RanGAP1 promotes hydrolysis of RAN-GTP to RAN-GDP, resulting in the release of nuclear protein cargo, such as EZH2, into the cytoplasm. Cytoplasmic EZH2 directly methylates DLC1 protein at Lysine 678, making the protein susceptible to ubiquitin-dependent proteasomal degradation. Conversely, inhibition of KRAS activity blocks nuclear export of EZH2 to the cytoplasm, resulting in stabilization and restoration of DLC1 protein. Consistent with these findings, analysis of Clinical Proteomic Tumor Analysis Consortium (CPTAC) database indicates that DLC1 protein levels in PDAC tumors are markedly lower than would be expected based on the relatively high DLC1 mRNA expression in these tumors. Moreover, CPTAC data show that in adjacent normal pancreatic tissues, EZH2 protein levels are significantly lower, whereas DLC1 protein levels are significantly higher than in PDAC tumors. Notably, decreased DLC1 protein abundance in PDAC tumors is inversely correlated with increased levels of FBXW5, an E3 ubiquitin ligase responsible for the ubiquitin-dependent proteasomal degradation of DLC1 protein.

Our preclinical pancreatic cancer studies demonstrate that a three-drug combination consisting of a KRAS inhibitor together with AKT and SRC inhibitors exhibits significantly greater antitumor activity than the KRAS inhibitor alone. This cooperative antitumor effect arises because KRAS inhibition increases DLC1 protein levels, whereas AKT and SRC kinase inhibitors reactivate DLC1 by blocking phosphorylation events that attenuate its tumor-suppressive activity. Remarkably, DLC1 protein is an important biological target of the three-drug combination, as knockout of DLC1 abolishes most of the antitumor activity of the three-drug combination. Thus, this study introduces a new treatment strategy for pancreatic cancer that harnesses the noncanonical pro-oncogenic nuclear export function of the mutant KRAS protein.

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35. Billel Gasmi billel.gasmi@nih.gov - Cancer Genetics Branch, CCR
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"From Organoid to Bedside: Establishment of Patient-Derived Tumor Organoids as Ex Vivo Reagents to Analyze Personalized T-Cell Responses and Predict Clinical Outcomes to Adoptive Cell Therapy with TIL"

Background and Hypotheses
HER2+ breast cancer, defined by the overexpression or amplification of the HER2 gene, is an aggressive form of breast cancer. Standard of care includes combinations of monoclonal antibodies, antibody-drug conjugates (ADCs), chemotherapy and small molecule kinase inhibitors. Brain metastases are prevalent in the HER2 subgroup, often at initial relapse from first treatment in the metastatic setting. Both the lesions and their treatments confer serious physical and neurocognitive adverse effects. The limited efficacy of drugs for brain metastases is a major hurdle in therapeutic advances and has been attributed to the existence of blood-brain/tumor barrier (BBB/BTB). Trastuzumab Deruxtecan (T-DXd) is a third generation HER2 targeting ADC coupled with a topoisomerase-I inhibitor; has shown activity in the metastatic setting (DESTINY-trial) with intracranial activity (DEBBRAH-trial). In this study we evaluated the efficacy of T-DXd in preclinical mouse models of breast cancer brain metastasis and explored the mechanism of T-DXd crossing the BBB/BTB.

Study Design and Methods
We studied the efficacy of T-DXd in vivo using multiple models of breast cancer brain metastasis (SUM190-BR, HER2-high; JIMT1-BR, HER2+; and MDA-MB-231-BR, triple-negative) in both prevention and treatment settings. Mice were intracardiacly injected with brain-tropic breast cancer cells and dosed with 3 or 10 mg/kg T-DXd or 10 mg/kg control ADC, with endpoints of metastasis number and size. Following this we identified a potential receptor for T-DXd and established a possible mechanism of its crossing BTB using an in vitro BTB assay.

Results and Conclusions

In the JIMT1-BR model, T-DXd at both doses reduced metastasis number by 48-88% and size by 32-88%; a concordant loss of HER2 expression by lesions remaining at the experimental endpoint and low T-DXd distribution was observed. A distinct dose effect was observed in SUM190-BR with the 3 mg/kg dose inhibiting size and number by 24-39% and 10 mg/kg by 72-79%; HER2 expression was maintained together with heterogeneous T-DXd distribution. Surprisingly, T-DXd reduced metastatic colonies number by 33.76-52.15% in 231-BR triple negative prevention model while it failed to show any activity in treatment model. In all these models, homogeneously reduced tumor Ki-67 was observed, while increased cleaved caspase 3 primarily co-stained with T-DXd; little difference in DNA damage was observed. We used an in vitro model of the blood-brain and blood-tumor barriers (BBB, BTB) to ask how T-DXd crossed. Data demonstrated T-DXd endocytosis and transcytosis of brain endothelial cells, at least partially reliant on binding to the neonatal Fc receptor (FcRn) expressed not only in endothelial cells but also in Astrocytes, Pericytes and 231-BR cancer cells. T-DXd efficacy observed in 231-BR prevention model was further explored. Knockdown of FcRn or HER2 in 231-BR cells abrogated T-DXd efficacy observed in prevention settings, highlighting the role of FcRn and low level of HER2 in T-DXd efficacy observed in triple negative model.

Collectively, the data confirms T-DXd activity in HER2+ and triple negative brain metastases concordant with heterogeneous uptake, variable HER2 expression at endpoint, tumor cell cytotoxicity, and decreased proliferation that may be a bystander effect.

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"Clinical Assessment of the NCI Bethesda CNS Tumor DNA Methylation Classifier in a large consultation cohort"

Background and Hypotheses

Accurate classification of central nervous system (CNS) tumors is essential for prognosis,

treatment selection, and trial eligibility, and increasingly depends on integrating molecular features with histopathology. DNA methylation profiling provides an objective, genome-wide tumor signature that complements morphology and immunohistochemistry, particularly in diagnostically challenging or rare entities. The NCI Bethesda CNS Tumor DNA Methylation Classifier v3.1 (Bv3) is a machine-learning classifier optimized for formalin-fixed paraffin-embedded (FFPE) tissue that generates calibrated confidence scores and assigns tumors within a hierarchical schema (methylation families and methylation classes). We hypothesized that Bv3 would demonstrate (1) high agreement with an established methylation classifier (DKFZ Heidelberg Classifier v12.8; Hv12) at both high-level family and exact class levels, and (2) high clinical concordance with expert integrated diagnosis (IDx), with consistent performance across Illumina MethylationEPIC v1 and v2 platforms.

Study Design and Methods

We performed a retrospective method-comparison and clinical-concordance study of CNS tumor consultation cases evaluated at the NCI Laboratory of Pathology (January 2021-December 2025). Following DNA extraction and laboratory quality control, samples underwent genome-wide methylation profiling on Illumina Infinium MethylationEPIC v1 or v2 arrays. Cases meeting study QC requirements and Hv12 calibrated confidence criteria (match score >=0.9) were included. Bv3 and Hv12 outputs were compared at (a) a harmonized high-level tumor family category level using crosswalk mapping to address nomenclature/granularity differences and (b) exact methylation class name matching. Agreement was summarized with Wilson 95% confidence intervals and Cohen's kappa. Clinical performance was assessed against neuropathologist-rendered IDx incorporating histopathology, immunohistochemistry, ancillary molecular testing (when available), and clinico-radiologic correlation. Cases were assigned prespecified clinical impact levels, and discordant cases were reviewed in consensus conference.

Results and Conclusions

A total of 3,542 FFPE tumors were analyzed (EPIC v1: 2,110 [59.6%]; EPIC v2: 1,432 [40.4%]). Agreement between Bv3 and Hv12 was 81.0% at the tumor family level (2,868/3,542; 95% CI 79.7-82.3; κ =0.807) and 93.2% at the exact class level (3,300/3,542; 95% CI 92.3-94.0; κ =0.932), meeting or exceeding predefined acceptance criteria. Clinical concordance with IDx was 95.2% (Impact 1+2: 3,372/3,542; 95% CI 94.4-96.0), including 91.3% complete three-way concordance and 3.9% clinically concordant molecular refinement. Bv3 demonstrated potential diagnostic advantage in 2.3% of cases (Bv3 concordant with IDx while Hv12 was discordant), while 2.5% remained completely discordant across methods. Performance was comparable across EPIC v1 and v2

platforms. These findings support NCI Bethesda Classifier v3.1 utility for methylation-based classification for CNS tumors, with added value in select challenging cases and continued need for integrated interpretation and expert review in discordant scenarios.

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"Characterization of a pancreatic cancer GWAS signal suggests PDX1 buffers stress and stabilizes homeostatic cell states in the exocrine pancreas"

Background and Hypotheses

Pancreatic ductal adenocarcinoma (PDAC) remains one of the deadliest human cancers. The largest published Genome-Wide Association Study (GWAS) to date has identified 23 germline genetic signals influencing PDAC risk, but most lack sufficient characterization for biologically informative models. This study aimed to functionally characterize the PDAC GWAS risk signal in non-coding regions of the chr13q12.2 PLUT/PDX1 locus.

Study Design and Methods

Statistical and epigenomic fine-mapping, luciferase reporter assays, and electrophoretic mobility shift assays (EMSAs) were conducted to identify likely functional variant(s) within the GWAS signal, and GTEx v8 whole pancreas eQTL results and colocalization analyses were used to identify likely target gene(s). CRISPR/Cas9 single-base editing validated the expression regulatory role of the putative functional variant. Single-nuclei RNA-seq analyses of pancreatic acinar and ductal cells from neonatal, adult, and chronic pancreatitis sample, and single-cell RNA-seq analyses of pancreatic tumor samples were

performed to clarify the functional role of the putative target gene in acinar, ductal, and tumor cells across varied contexts.

Results and Conclusions
Fine-mapping, luciferase reporter assays, and EMSAs implicated rs9581943, a PDX1 promoter SNP, as a functional variant underlying this GWAS signal. Expression QTL (eQTL) results from GTEx indicated significant association between rs9581943 genotype and PDX1 expression in whole pancreas tissue, and single-base CRISPR/Cas9 editing of the variant in PDAC-derived cell lines confirmed its functional status by recapitulating the eQTL, where the risk allele associated with lower PDX1 expression. PDX1 is a transcription factor with well-established roles in early pancreas development and Î²-cell homeostasis, but its role in exocrine pancreatic cells (acinar and ductal) is less clear. Single-nucleus RNA-seq analyses of pancreatic acinar and ductal cells from neonatal, adult, and chronic pancreatitis donors suggested PDX1 activity alleviates high secretory load and ER-stress in acinar and biases ductal cells toward more homeostatic phenotypes. Similarly, single-cell RNA-seq analyses of pancreatic tumors revealed PDX1 activity correlates with reduced biosynthetic and inflammatory stress and increased epithelial differentiation. Our study therefore implicates rs9581943 as a causal variant for the chr13q12.2 PDAC GWAS signal wherein the risk allele reduces PDX1 expression, thereby eroding PDX1’s capacity to buffer stress and stabilize epithelial cell fate in the exocrine compartment.

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“IKKa serves as a molecular switch linking miRNA biogenesis and inflammation in skin tumorigenesis”

Ikka mutations or reduced expression have been linked to human squamous cell carcinoma (SCC), autoimmunity and increased susceptibility to infections. In mice, absence of IKKa leads to development of spontaneous SCC in the skin, lungs, and esophagus through aberrant epigenetic alterations and inflammation. However, the interplay between epigenetic and inflammatory changes remains largely unknown. Here, we show that IKKa deletion in the mouse epidermis results in impaired miRNA processing, with markedly reduced expression of ribonuclease Dicer, an enzyme required for miRNA maturation, due to Dicer gene silencing through DNA methylation. Consequently, the maturation of miR-203-3p, the most abundant miRNA in keratinocytes, is blocked in Ikka-null keratinocytes. We further identified Myd88 as a miR-203-3p target by Western blot and

luciferase report assays. As expected, we observed increased activity of caspase 1 cleavage, an indicator of inflammasome activation, in Ikka-null keratinocytes. Interestingly, Myd88 deletion or 5-aza-2'-deoxycytidine treatment partially rescued the phenotypes of Ikka$^{f/f}$;K5Cre mice. By analyzing a TCGA dataset of human head and neck SCCs, we found that Dicer expression is positively correlated with IKKa expression. Moreover, patients with reduced IKKa expression and/or reduced expression in both IKKa and Dicer had poor prognosis. Thus, our study revealed a novel IKKa/Dicer/miR-203-3p/Myd88 signaling loop, in which IKKa serves as a molecular switch linking microRNA biogenesis and inflammation in skin tumorigenesis.

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"Decoding Telomerase: Harnessing Telomerase RNA to Decipher the Enzymes Secrets"

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"Shed ERVMER34-1 drives formation of an immunosuppressive tumor microenvironment: A novel tumor-promoting role for an endogenous retroviral envelope protein"

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"Characterization of the Microenvironment of Kaposi Sarcoma Using Spatial Transcriptomics Identifies Mechanisms of Immune Evasion"

We applied spatial transcriptomics to Kaposi sarcoma (KS) skin lesions to define how Kaposi sarcoma-associated herpesvirus (KSHV) infection reshapes the cellular microenvironment and enables immune evasion. By integrating spatial gene expression, viral transcript detection, cell-type deconvolution, and a predictive gene signature, we provide a detailed map of infected versus uninfected niches within KS tumors.

BIOCHEMISTRY, BIOPHYSICS AND STRUCTURAL BIOLOGY

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"Structures of dynamic interactors at native proteasomes by PhIX-MS and cryoelectron microscopy"

Proteasome function depends on a network of transient interactions that remain structurally and functionally unresolved. We developed PhIX-MS (Photo-induced In situ Crosslinking-Mass Spectrometry), a structural proteomics workflow that stabilizes transient interactions in cells by UV-activated crosslinking to capture topological information. Applying PhIX-MS with cryo-electron microscopy (cryo-EM), we mapped redox sensor TXNL1 at the proteasome regulatory particle (RP), placing its PITH domain above deubiquitinase RPN11 and resolving its dynamic thioredoxin domain near RPN2/PSMD1 and RPN13/ADRM1, ideally located to reduce substrates prior to proteolysis. We also resolved chaperone PSMD5 bound to RP without the proteolytic core particle (CP) where its C-terminus inserts into the ATPase pore blocking CP binding. PhIX-MS and AlphaFold modeling tether ubiquitin ligase UBE3C/Hul5 along the RP placing its catalytic site above the RPN11 active site, enabling their coupled activities. Our integrative approach enables the localization of native, low-affinity protein interactions and is broadly applicable to dynamic macromolecular assemblies.

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"Proteasome activity maintains cell type-specific gene expression"

In eukaryotes, regulated proteolysis depends on the ubiquitin-proteasome system, which is critical to myriad cellular functions, including protein quality control, cell cycle regulation, and DNA repair. Here, we provide evidence that proteasome activity is also essential for maintaining cell identity. This discovery was made by evaluating the impact of losing the ubiquitin-binding activity of proteasome substrate receptor hRpn10/PSMD4. We precisely edited the colon cancer HCT116 cell line to delete the hRpn10 ubiquitin interaction motifs (UIMs) and hRpn10 E3 ligase E6AP/UBE3A-binding RAZUL domain to leave only an intact hRpn10 von Willebrand factor type A (VWA) domain (hRpn10VWA). We found the most dysregulated proteins in hRpn10VWA cells are transcriptionally altered, with striking overrepresentation of proteins canonically restricted in expression to specific tissues. Similar aberrant transcriptional responses were also observed in an edited human kidney HEK293T cell line expressing reduced hRpn10 full-length protein and comparable levels of the intact hRpn10VWA domain. This dysregulation is partly driven by accumulation of the proteasome substrate and deubiquitinase OTUD5, a known chromatin and transcriptional regulator. Altogether, we identified previously unrecognized proteasome-dependent mechanisms to safeguard cell-type specific gene expression programs and expand proteasome biology to include governance of cell identity.

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"Distinct associations of pioneer factor Ascl1-E12a with nucleosomes drive changes in cell fate"

Understanding how pioneer transcription factors target nucleosomal DNA and initiate chromatin accessibility reveals the earliest events in cell fate control. We integrated structural, biochemical, and genomics approaches to assess how a pioneer factor Ascl1-E12a heterodimer perturbs nucleosomes in vitro and in vivo to induce a neural cell fate. Two Ascl1-E12a heterodimers shift and unwrap 15 bp of nucleosomal DNA in a stepwise manner, while eliciting solvent exchanges within the octamer. Nucleosome binding, but not free DNA binding, by Ascl1-E12a is enhanced by two types of associations with the nucleosome that differentially affect the kinetics of DNA unwrapping and shifting. Nucleosome association mutants of Ascl1 perturb chromatin opening on linker histone-compacted nucleosome arrays, independent of nucleosome remodelers, and closed chromatin targeting in vivo, with consequent deficiencies in cellular reprogramming. Our findings establish that distinct associations with nucleosomes are essential for pioneer factor Ascl1 to overcome chromatin barriers to reprogram cell fate.

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"Identification of molecular determinants of gene-specific bursting patterns by high-throughput imaging screens"

Stochastic transcriptional bursting is a universal property of active genes. While different genes exhibit distinct bursting patterns, the molecular mechanisms that govern gene-

specific stochastic bursting are largely unknown. We have developed a high-throughput-imaging-based screening strategy to identify cellular factors that determine the bursting patterns of native genes in human cells. We identify protein acetylation as a prominent effector of burst frequency and burst size acting via decreasing off-times and gene-specific changes in the on-time. These effects are not correlated with promoter acetylation. Instead, we demonstrate acetylation of the Integrator complex as a key determinant of gene bursting that alters Integrator interactions with transcription elongation and RNA processing factors but without affecting pausing. Our results suggest a prominent role for non-histone acetylation of a transcription cofactors as a mechanism for modulation of bursting via a far-downstream checkpoint.

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"Yra2 restricts CENP-A mislocalization to preserve chromosomal stability"

Background and Hypothesis
The centromere-specific histone H3 variant Cse4 (CENP-A in humans) is essential for accurate chromosome segregation, and mislocalization of overexpressed Cse4 contributes to chromosomal instability (CIN) and aneuploidy. Elevated CENP-A levels are observed in multiple human cancers and are associated with poor prognosis, underscoring the importance of mechanisms that restrict its localization to centromeric chromatin.

Study Design and Methods
The study was performed using budding yeast as a model system. Wild type and mutants were assayed for chromosome segregation using genetics, cell biology, biochemical, and molecular biology approaches. Experiments were performed with three biological replicates and data were analyzed using the analysis of variance and Student's T-test.

Results and Conclusions
Using genome-wide genetic screens in Saccharomyces cerevisiae, we identified gene deletions that confer growth defects upon Cse4 overexpression and uncovered a previously uncharacterized role for Yeast RNA Annealing protein Yra2 in regulating Cse4 homeostasis. We show that Yra2 promotes ubiquitin-mediated proteolysis of Cse4, thereby preventing its accumulation and mislocalization to non-centromeric chromatin. Previous

biochemical approaches identified Yra2 in a complex with mRNA export proteins Yra1 and Mex67. We determined that loss of YRA2 does not impair poly(A)+ RNA export and yra1-2 and mex67-ts5 strains do not exhibit growth defects upon Cse4 overexpression suggesting a distinctive role for Yra2 in regulating cellular levels of Cse4. Strain deleted for YRA2 show defects in ubiquitin-mediated proteolysis of Cse4, increased protein stability with an enrichment of Cse4 in chromatin, mislocalization to non-centromeric regions and defects in chromosome segregation. Suppression of growth defects and CIN phenotype of yra2Δ strain by reduced histone H4 dosage or increased histone H3 levels shows that balanced histone stoichiometry is critical for localization of Cse4. In summary, our findings identify Yra2 as a key regulator of Cse4 proteolysis and localization, providing insights into mechanisms that safeguard chromosomal stability with an opportunity to explore the role for this pathway in preventing mislocalization of CENP-A in human cancers.

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"Ubiquitin-independent degradation of CENP-A protects centromere integrity in budding yeast"

CENP-A, an evolutionally conserved centromeric histone H3 variant, (Cse4 in Saccharomyces cerevisiae, CENP-A in humans), is an essential component of the kinetochore (centromeric DNA and associated proteins) that regulates faithful chromosome segregation. Stringent regulation of CENP-A expression restricts its localization to centromere as mislocalization of overexpressed CENP-A promotes aneuploidy in model organisms and human cells. One of the key mechanisms to regulate cellular levels of Cse4 is ubiquitin-mediated proteolysis of Cse4 by E3 ubiquitin ligases such as Psh1 that prevents Cse4 mislocalization to non-centromeric regions. Previous study showed that Cse4 16KR (all 16 lysines are mutated to arginine), which is more stable than Cse4, is still degraded (Collins et al., Curr Biol, 2004) suggesting ubiquitin-independent proteolysis of Cse4. However, studies to date have not explored the alternative pathway for Cse4 degradation. In this study, we demonstrate that Sse1, a member of heat shock protein Hsp110, regulates ubiquitin-independent degradation of Cse4 to protect centromere integrity.

Our results demonstrate that overexpressed Cse4 16KR is stable at 25°C, but rapidly degraded at 37°C, suggesting that high temperatures promote ubiquitin-independent degradation. Mass spectrometry of Cse4 16KR (Ranjitkar et al. 2010) identified several heat shock proteins such as Hsp26, Sse1, Hsc82, and Hsp82 as primary interacting partners. Protein stability assays further revealed that overexpressed Cse4 16KR is stabilized at 25, 30, and 37°C in a strain carrying a deletion of SSE1. To further investigate this pathway, we utilized strains overexpressing the Cse4 Y193A mutant which retains all 16 lysine residues and is rapidly degraded. We determined that Cse4 Y193A is also stabilized upon deletion of SSE1. To examine the physiological relevance of Sse1-mediated Cse4 proteolysis, we analyzed phenotypes of strains expressing Cse4 Y193A from its endogenous promoter. We found that these strains exhibit sensitivity to the anti-microtubule drug benomyl, temperature sensitivity in the context of deletion of SSE1 or CTF19 (kinetochore protein), and defects in centromere integrity. Subcellular fractionation showed an interaction of Sse1 with Cse4 within the soluble pool. Taken together, our results provide evidence for a novel quality-control mechanism in which Sse1-mediated, ubiquitin-independent proteolysis of misfolded Cse4 (such as Cse4 Y193A) is essential for centromere integrity. Given the evolutionary conservation of Cse4/CENP-A, it will be of significant interest to determine if ubiquitin-independent proteolysis of CENP-A contributes to protecting the integrity of centromeric chromatin in human cells.

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"Molecular dynamics simulations give insight into the synergistic structural perturbations by which major and minor drug-resistant mutations give rise to HIV's drug resistance against protease inhibitors."

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“pH-Dependent Regulation of LRRK1/2 Kinase Activity and LRRK2 Oligomerization: Implications for Parkinsons Disease”

Leucine-rich repeat kinases 1 and 2 (LRRK1/2) are large multidomain signaling proteins implicated in membrane trafficking and cytoskeletal organization. Hyperactivation of LRRK2 is a major driver of familial and sporadic Parkinsons disease (PD) and has also been linked to cancer, yet the upstream signals that regulate its kinase activity remain incompletely understood.

Here, we identify solution pH as a robust regulator of LRRK1/2 enzymatic activity and assembly state in vitro. Using purified proteins in kinase assays, we observe enhanced catalytic activity under mildly acidic conditions (pH 6-7) and reduced activity at alkaline pH (8-10). Biophysical analyses of LRRK2 further reveal a pH-dependent shift in oligomeric equilibrium, with acidic pH favoring monomeric LRRK2 and alkaline conditions promoting dimerization. Together, these findings reveal a previously unrecognized layer of regulation coupling proton concentration to both kinase activity and oligomeric equilibrium. Because intracellular pH can fluctuate during cellular perturbations linked to neurodegeneration, these findings motivate a testable working model in which pH dynamics may modulate LRRK2 signaling capacity in disease-relevant contexts. Notably, oxidative stress and endolysosomal dysfunction in neurodegeneration models can perturb cellular pH homeostasis, raising the possibility that pH-dependent regulation provides a mechanistic link between cellular stress and LRRK2 activation. Ongoing and future work will assess whether physiological pH shifts in cells are sufficient to tune LRRK2 activity and downstream substrate phosphorylation.

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"Functional Implications of the Conformational Landscape of a Multidrug Transporter Revealed by Structures of Zebrafish Abcb4"

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"CRAF Flexibility as a Determinant of its Structure and Function"

The RAF kinases (ARAF, BRAF, and CRAF/RAF1) are central mediators of RAS/MAPK signaling, regulating cellular proliferation, survival, and differentiation. Each isoform plays a key role in propagating upstream signals from RAS to ERK, but with distinct requirements for both activation and stability. BRAF exists in a cytosolic autoinhibited complex that prevents aberrant activation in the absence of RAS activation. This complex is stabilized by intramolecular interactions between the cysteine-rich domain (CRD) and the kinase domain, as well as by a 14-3-3 dimer that sterically blocks the dimerization interface. While CRAF and ARAF also adopt inactive conformations, whether they form a similarly structured autoinhibited complex remains unclear. These structural differences parallel intrinsic differences in kinase activity and stability. BRAF exhibits relatively high basal kinase activity and requires tight regulation to prevent inappropriate signaling. In contrast, CRAF and ARAF display lower intrinsic kinase activity and reduced structural stability, suggesting distinct regulatory requirements. The molecular chaperone Hsp90 plays a critical role in stabilizing structurally labile proteins. CRAF and ARAF are strong Hsp90 clients and depend on Hsp90 and its co-chaperone Cdc37 for stability, whereas BRAF is a comparatively weak client that transiently associates with the complex. This differential chaperone dependence likely reflects sequence divergence in regions governing structural stability, including the CRD. Under physiological conditions, these isoform-specific properties enable fine-tuned control of MAPK signaling. However, disease-associated

mutations can override normal regulatory constraints. BRAF is the most frequently mutated RAF isoform in cancer, whereas CRAF rarely serves as a primary oncogenic driver. Notably, CRAF-but not BRAF or ARAF-is required for maintenance of certain KRAS-driven tumors, including lung adenocarcinoma, highlighting unique functional dependencies. Both BRAF and CRAF also harbor recurrent mutations in Rasopathies, often within regulatory domains, underscoring the importance of structural control in modulating kinase activity. To define how structural stability influences RAF regulation, we used a proximity-based NanoBRET assay to monitor RAF interactions with Hsp90 and KRAS in live cells. Disease-associated mutations in both CRAF and BRAF increased Hsp90 dependence, consistent with reduced intrinsic stability, while exerting variable effects on KRAS binding, indicating that RAS association and Hsp90 engagement are mechanistically distinct. Using a stable NanoBRET system, we further show that RAF inhibitors disrupt CRAF-Hsp90 complexes by promoting RAF dimerization, particularly in the presence of activated RAS. Together, these findings delineate separable mechanisms regulating RAF stability and RAS engagement and highlight isoform-specific features that may inform more precise therapeutic strategies targeting dysregulated RAS/MAPK signaling.

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“High-Throughput Drug Discovery Architecture: Integrating SLICE for Rapid Library Generation with AI-Driven GPU-Accelerated Screening”

Background and Hypotheses
While virtual libraries of synthetically accessible compounds have expanded into the billions, the capacity to extract valuable drug leads is severely bottlenecked by limited computational resources and the lack of agile library generation tools. We hypothesized that an integrated computational framework combining a "no-code" library generator with high-performance, AI-enhanced GPU screening engines would allow for the rapid exploration of massive chemical spaces. Our goal was to demonstrate that this workflow could identify potent hits for undruggable" or challenging cancer targets, where conventional virtual ligand screening (VLS) often fails.

Study Design and Methods
We developed a two-pronged computational suite designed for the modern drug discovery pipeline: SLICE (SMARTS and Logic In ChEmistry): A computer program featuring a user-friendly graphical interface (SLICE Designer) that allows chemists to define reaction chemistries and constraints without manual coding. The SLICE Engine then generates diverse virtual libraries from specified building blocks.

RIDGE and RIDE Engines: To screen these libraries, we developed the Rapid Docking GPU Engine (RIDGE) and the Rapid Isostere Discovery Engine (RIDE).
To maximize predictive accuracy, RIDGE employs a hybrid scoring architecture. It integrates a traditional physics-based scoring function with advanced AI-based scoring, specifically utilizing both Radial and Topological Convolutional Neural Network (CNN) scores. This dual approach allows the engine to capture both fundamental thermodynamic interactions and complex structural patterns within the binding pocket.

Results and Conclusions
The SLICE Engine demonstrated high-speed library generation, producing between 0.6 and 2.5 million compounds per hour. In screening benchmarks across 102 proteins from the DUD-E (a Database of Useful Docking Decoys-Enhanced) dataset, RIDGE performance met or exceeded existing methods. When applied to challenging targets, this integrated workflow successfully identified five novel inhibitors for PD-L1 and three for K-Ras G12D, with affinities ranging from single-digit to sub-micromolar levels.
Results indicate that the integration of Radial and Topological CNN scores within GPU-accelerated methods provided superior predictors of binding compared to conventional VLS. These novel tools expand the screenable chemical space and provide the agility required for efficient lead generation. Future optimization, including libraries with higher molecular weight cutoffs, may further improve the targeting of non-druggable proteins

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“Molecular Basis for the Auto-inhibition of Heterochromatin Protein 1”

(ITC) to define the structural and thermodynamic basis of this regulation in human HP1Î± and the S. pombe homolog, Swi6. Our data demonstrate that the N-terminal extension

CLINICAL/ TRANSLATIONAL

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"FGFR4-Targeted Exatecan Antibody-Drug Conjugate Achieves Durable Tumor Control in Preclinical Rhabdomyosarcoma and FGFR4-Positive Breast Cancer"

Background
Antibody-drug conjugates (ADCs) offer practical advantages over chimeric antigen receptor (CAR) T-cell therapy including scalable manufacturing, outpatient friendly administration, lower cost, shorter pharmacologic exposure, and a more predictable safety profile. Fibroblast growth factor receptor 4 (FGFR4) is selectively expressed in rhabdomyosarcoma (RMS) and subsets of other cancers, with minimal normal-tissue expression, making it a promising immunotherapy target. We generated FGFR4-targeted ADCs using a high affinity monoclonal antibody 3A11, the same binder in an FGFR4 CAR currently under clinical evaluation at the NCI (NCT06865664).

Methods
Two chimeric 3A11 antibody-based ADCs were constructed with either monomethyl auristatin E (MMAE) or an exatecan derivative payload using 2 distinct linkers. Internalization and FGFR4-dependent cytotoxicity were assessed in vitro. Antitumor activity was evaluated in subcutaneous RMS xenografts, including aggressive fusion-negative (FN-) RMS559 and fusion-positive (FP-) RH4 cell line derived xenografts (CDXs) and a FP patient-derived xenograft (PDX). Efficacy was also tested in an FGFR4's expressing MDA-MB-453 breast cancer model, with a retreatment paradigm at relapse. To enable translational development, a humanized version of 3A11 (hu3A112) was engineered and characterized for affinity and species cross-reactivity. Cytotoxicity of ADCs conjugated with hu3A112 was tested across FGFR4-positive lines (RMS559, RH4, MDA-MB-453) versus RH4 FGFR4-knockout and non-target anti-RSV ADC controls.

Results
Antibody hu3A112 was rapidly internalized in FGFR4-positive RMS cells. It showed improved affinity over its chimeric counterpart and demonstrated cross-reactivity to human and cynomolgus FGFR4. hu3A112ADCs showed selective killing of FGFR4-expressing cells, with minimal cytotoxicity in FGFR4-knockout RH4 and in anti-RSV ADC

controls. In vivo, each ADC significantly inhibited tumor growth and extended survival in RMS xenografts. The exatecan-based ADC demonstrated superior efficacy, with durable tumor control across FN RMS559, FP RH4, MDA-MB-453 CDXs and in an FP-RMS PDX and was well tolerated in mice. Furthermore, retreatment with the exatecan ADC eradicated relapsed tumors.

Conclusions
FGFR4-targeted ADCs, especially an exatecan-based ADC, drive durable antitumor activity across multiple FGFR4-positive preclinical models, including RMS CDX/PDX and a breast cancer model, and enable effective retreatment at relapse. The humanized hu3A112 antibody, with enhanced affinity and cynomolgus cross-reactivity, supports translational toxicology and further clinical investigation of FGFR4-directed ADCs.

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“Smad4 loss alters TGF-beta dependent alternative mRNA splicing in human colon cancer cells”

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“Epigenetic plus cytokine therapy activates TCF-1+CD8+ T cells in stem-immunity clusters to overcome anti-PD-1 resistance”

Purpose

Checkpoint blockade (CPB) as limited efficacy in patients with colorectal carcinoma (CRC), warranting improved therapeutic strategies capable of remodeling the tumor microenvironment. Stem-like TCF1+CD8+ T cells, cytotoxic CD8+ T cells, and lymphoid aggregates have each been associated with CPB responsiveness, either individually or as coordinated stem-immune clusters.

Experimental design

We evaluated the combination of anti-PD-1, the class I histone deacetylase inhibitor entinostat, and the IL-15 superagonist N-803 in CPB-resistant murine CRC and breast cancer models with varying antigen presentation deficiencies. Proteomic, transcriptomic, spatial, and functional analyses were completed to identify treatment-specific immune remodeling. Translational relevance was assessed using publicly available CPB-treated cancer genomic datasets and functional assays with patient-derived peripheral blood mononuclear cells (PBMCs).

Results

Triple therapy increased stem-like TCF1+CD8+ T cells in tumor-draining lymph nodes and tumors, concomitant with increased intratumoral cytotoxic GZMB+CD8+ T cells. Therapeutic efficacy was associated with remodeling of the tumor microenvironment, uniquely marked by enrichment of stem-immunity clusters comprising TCF1+ and cytotoxic CD8+ T cells, type 1 conventional dendritic cells, and B cells, along with concerted cytokine and chemokine production. A transcriptional signature derived from triple therapy induced stem-immunity clusters predicted favorable clinical responses across multiple CPB-experienced cancer cohorts. These responses were functionally corroborated by increased markers of cytotoxicity in patient-derived PBMCs in vitro.

Conclusions

Treatment with Entinostat, N-803 and anti-PD-1 boosts anti-tumor multicellular stem-immunity clusters. Collectively, these findings suggest that TCF1+ stem-immunity clusters associated with anti-PD-1 responsiveness and lacking in non-responders, including CRC, may be restored by entinostat and N-803 in anti-PD-1-refractory patients.

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"Peripheral memory T cells with self-renewing properties increase with tumor-targeting IL-12 immunocytokine therapy"

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"E-cadherin Fuels Metastatic Breast Cancer by Reprogramming Mitochondrial Metabolism: a targetable vulnerability"

Background and hypotheses
Breast cancer (BC)-specific mortality is due to metastases, which remain understudied compared to primary tumors. While epithelial-mesenchymal transition and glycolytic metabolism are well-characterized drivers of cancer progression, accumulating evidence also points to a role for oxidative mitochondrial metabolism (Oxidative phosphorylation; OXPHOS) in BC. In addition, most metastases express epithelial E-cadherin, a cell adhesion molecule long considered a tumor suppressor. However, our recent study demonstrated that E-cadherin expression was associated with increased circulating tumor cells and lung metastasis in preclinical triple-negative breast cancer (TNBC) models, but the mechanisms remained unclear. Furthermore, we found that a 3D ex vivo culture paradigm with mild mechano-stimulation increased OXPHOS only in E-Cadherin+, but not in E-Cadherin- cells. These findings raised the hypothesis that E-Cadherin is required for OXPHOS. In the present study, we determined how E-cadherin supports BC malignancy using mechanistic studies and their potential relevance to in vivo physiology and as tools to discover new therapeutic vulnerabilities.

Study Design and Methods
a. Approaches to study molecular and metabolic features of metastatic breast cancer cell lines stably depleted for E-Cadherin: RNA and protein expression analyses, In vivo experimental metastasis assays, Seahorse-based metabolic assays, multiplex immunofluorescence, and cell viability, along with clinically relevant inhibitors/drugs.

b. Cell lines and mouse models: SUM149, MDA-MB-468 (epithelial TNBC subtype), and IBC-3 (Epithelial, HER2+ subtype), SUM159 and MB-231-LM2 (mesenchymal TNBCs), NSG mice with cell-line derived xenografts (CDX), and BC patient specimens.

Results and Conclusions
Loss of E-cadherin reduced cell viability, mitochondrial respiration, and lipid content while increasing reactive oxygen species. Through pharmacologic and genetic manipulations in several epithelial BC cell lines, we identified the mitochondrial anaplerotic enzyme pyruvate carboxylase (PC) as a downstream effector of E-cadherin, induced by AKT activation of the YAP/TEAD transcription factor complex. Clinically relevant AKT and TEAD inhibitors reduced PC expression and oxidative respiration. In contrast, restoring PC expression in E-cadherin silenced cells rescued survival, cluster formation, and mitochondrial oxygen consumption and protected cells from oxidative stress. Additionally, E-Cadherin overexpression in mesenchymal breast cancer cell such as MDA-MB-231-LM2 and SUM159, induced PC expression and PC-dependent mitochondrial respiration. Analysis of experimental lung metastases and TNBC tissues confirmed in vivo co-expression of E-cadherin and PC. Importantly, PC inhibition as monotherapy attenuated or reduced established experimental lung metastasis burden in mice. Collectively, these data demonstrate that E-cadherin mediated cell-cell adhesions directly support mitochondrial metabolism through AKT-YAP/TEAD-PC signaling and suggest a therapeutic vulnerability in metastatic epithelial triple-negative breast cancer

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"Modeling Rare Molecular Subtypes of Gastrointestinal Stromal Tumors (GIST)"

Background and Hypothesis
Gastrointestinal stromal tumors (GIST) are rare mesenchymal malignancies that arise predominantly in the stomach and small intestine, with lower incidence at other gastrointestinal sites. GIST originate from c-KIT/DOG1-positive interstitial cells of Cajal (ICC) and are most commonly driven by activating gain-of-function mutations in c-KIT or PDGFRA. While these tumors often respond to tyrosine-kinase inhibitors, a subset known as Wild-Type GIST (WT-GIST) lacks these mutations and instead harbors alterations in RAS pathway genes (e.g., NF1, KRAS, BRAF), succinate dehydrogenase (SDH) subunit genes, or rare gene fusions. WT-GIST do not respond to standard therapies, and currently there are no established treatments or robust preclinical models for these tumors. Our objective is to develop novel mouse models that faithfully recapitulate rare molecular subtypes of

human WT-GIST, enabling mechanistic studies and preclinical evaluation of targeted therapies.

Study Design and Methods
We generated conditional mouse models mimicking two of the most frequent WT-GIST alterations: an activating BRAFV600E mutation and loss of SDHB. These genetic alterations were selectively induced in c-Kit-positive ICC at defined time points using a tamoxifen-inducible c-Kit-CreERT2 system.

Results and Conclusions
Mice harboring the BRAFV600E mutation developed GIST-like tumors and moderate to severe ICC hyperplasia along the gastrointestinal tract, whereas mice with isolated SdhB loss did not develop neoplastic lesions. Interestingly, mice with combined BRAFV600E/SdhB loss exhibited increased tumor penetrance and developed GIST-like lesions with molecular features characteristic of human SDH-deficient GIST, including elevated HIF2-alpha and Fgf-4 expression and reduced 5-hydroxymethylcytosine levels. Importantly, these tumors responded to treatment with the BRAF inhibitor dabrafenib and showed that SdhB deficiency exacerbates tumorigenesis of BRAF-driven GIST. These studies establish the first fully penetrant mouse models of BRAF-driven GIST initiated in c-Kit-positive ICC. The models recapitulate key histopathological and molecular features of human WT-GIST and provide powerful platforms for studying tumor initiation, progression, and therapeutic resistance. Importantly, it allows for preclinical testing of targeted therapies in WT-GIST, offering new insights into treatment strategies

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"ClpP Agonist-Induced Cellular Senescence Promotes Vulnerability to Apoptosis and Ferroptosis in Triple Negative Breast Cancers"

Background and Hypothesis

Mitochondrial metabolism is widely reprogrammed in cancers to meet its energy demand. Caseinolytic mitochondrial matrix protease (ClpP) plays a crucial role in mitochondrial protein quality control by degrading unfolded or misfolded proteins. ClpP agonists are new class of anti-tumor drugs. Mechanistically, ClpP agonists induce hyperactivation of ClpP, resulting mitochondrial dysfunction and leading to impairment of cell viability. While ClpP agonists moderately impair tumor growth of TNBC, it also induces cellular senescence characterized by increased senescence associated-beta-galactosidase activity, induction of senescence-associated secretory phenotype genes, DNA damage, elevated reactive oxygen species, and increased expression of the anti-apoptotic protein BCLxL. We hypothesized that targeting the senescence induced by ClpP agonists will improve its therapeutic efficacy.

Study Design and Methods
TNBC cell lines (MDA-MB231, BT549) and Patient Derived Cells (PDCs) obtained from NCI PDMR (Patient-Derived Models Repository) were used as in vitro model systems. National Center for Advancing Translational Sciences (NCATS) drug combination screen was conducted to identify class of drugs that show a synergy with ClpP agonists. Identified candidates and ClpP agonists were combined in vitro and the synergistic effect on cell viability (CellTiterGlo 2.0) and cell lethality (propidium iodide uptake) was analyzed with SynergyFinder 2.0. siRNA was used to evaluate the role of specific genes on the drug effect. Flow cytometry was used to assess clearance of senesced cells. Protein levels were analyzed with Western blotting. Intracellular iron levels were analyzed with fluorescent probes (FerroOrange, Lyso-FerroRed) using plate reader and confocal microscopy.

Results and Conclusions
NCATS drug combination screen identified Navitoclax (an inhibitor of BCL2, BCLxL and BCLw) as a synergistic drug with ClpP agonist in TNBC. Targeting specific BCL2 family members by siRNA or specific pharmacological inhibitors revealed that BCLxL is the target of Navitoclax. When combined, ClpP agonist and Navitoclax showed a synergistic effect in multiple TNBC. Additionally, Navitoclax acted as a senolytic and caused apoptosis when senescence was induced by a ClpP agonist. Further, we observed that ClpP agonists induce the anti-ferroptosis protein glutathione peroxidase 4 (GPX4), suggesting that ClpP agonists induce iron accumulation in cells. Indeed, we observed that ClpP agonists induce iron accumulation at the lysosome. RSL3, an inhibitor of GPX4, induced ferroptosis in cells pre-treated with ClpP agonist, especially in the senescent cells. RSL3-induced senolysis of cancer cells is not previously reported. When Navitoclax and RSL3 were combined, significant senolysis was observed in cells pre-treated with the ClpP agonist. In conclusion, we found that ClpP agonist-induced senescence in TNBC cells increases the sensitivity to apoptosis and ferroptosis. Therapeutic efficacy of the combination is currently under investigation using PDCs and in vivo xenograft models.

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"Scalable and Efficient Deep Learning based Pipeline for Whole-Slide Mitotic Detection and Analysis with Prognostic Signal in Early-Stage TCGA-BRCA"

Background
Mitotic activity is a cornerstone of tumor grading and prognosis, yet manual mitotic assessment on gigapixel whole-slide images (WSIs) is labor-intensive and variable due to subjectivity in hotspot selection and interpretation. Scalable, standardized AI-based mitosis quantification can serve as a reproducible digital pathology biomarker that supports both clinical workflows and large-cohort translational research. Beyond mitosis counts, mitotic morphology – particularly atypical mitoses – may capture deeper biological information and offers a path to integrate histopathologic phenotypes with molecular and genotype-level correlates.

Methods
We developed a three-stage deep learning pipeline for WSI-scale mitotic analysis. Stages 1-2 provide high-sensitivity detection: (1) a YOLOv11 detector to generate mitosis candidates with high recall and (2) an ultra-light EfficientNet classifier to suppress false positives with minimal compute overhead. Stage 3 uses an EfficientViT classifier to distinguish atypical mitotic figures (AMFs) from normal mitotic figures (NMFs), addressing a newly formalized benchmark task (MIDOG 2025 Track 2). Detection was trained/validated on MIDOG++ (11,937 annotated mitoses; 503 tumor cases; 7 tumor types). AMF/NMF classification used the MIDOG 2025 Track 2 dataset (13,938 mitotic image patches across 7 tumor types after deduplication). Translational relevance was evaluated in TCGA-BRCA (around 1,000 patients with pathology slides and clinical follow-up), focusing disease-specific survival (DSS) analyses on early-stage (I-II) cases (n=755).

Results
On MIDOG++ test, Stage 1 achieved high recall (0.864-0.917 across YOLOv11 variants), supporting sensitive screening for rare mitotic events. Adding Stage 2 improved overall detection performance (F1 increasing from ~0.54-0.57 to ~0.73-0.75 across variants), consistent with effective false-positive suppression at low compute cost. For Stage 3, on the MIDOG 2025 Track 2 final test set (3,800 images from 12 cancer types), AMF/NMF classification achieved ROC-AUC=0.9616 and balanced accuracy=0.9089. In TCGA-BRCA early-stage (I-II) cases (n=755), higher mitosis density was associated with worse DSS (3-year p=0.00089; 4-year p=0.000475). The pipeline operated at cohort scale with few-

minute-per-slide throughput (58.5% <2 minutes; 96.0% <4 minutes) and feasibility on a mid-range 8GB VRAM GPU.

Conclusions
This scalable WSI pipeline combines high-recall mitosis detection (Stages 1-2) with mitotic morphology analysis (Stage 3), validated on large public benchmarks and linked to patient outcomes in early-stage TCGA-BRCA. It enables standardized, high-throughput digital pathology biomarkers for translational research and clinically relevant workflows and provides a foundation for future genotype-phenotype analyses that connect mitotic patterns to underlying tumor biology

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"Improved natural killer cell targeting of prostate carcinoma with ATR inhibitor tuvusertib in combination with IL-15 receptor superagonist N-803"

Introduction Given its role in DNA damage repair, ataxia telangiectasia and Rad3-related (ATR) represents a viable target for cancer therapy with ATR inhibitors (ATRi) demonstrating anticancer activity and synergy with other therapies. We hypothesize that the ATRi tuvusertib could induce immunogenic modulation, thereby sensitizing prostate cancer (PCa) cells to natural killer (NK) cell-mediated lysis.

Methods DU145 human PCa cells were treated with tuvusertib (provided by Merck KGaA, Darmstadt, Germany; CrossRef Funder ID: 10.13039/100009945) for 48 hours. Resulting immunogenic changes were evaluated via flow cytometry. PBMC-derived NK cells were exposed to interleukin-15 (IL-15) receptor superagonist N-803 (nogapendekin alfa inbakicept; provided by ImmunityBio, Culver City, CA, USA) overnight. Tuvusertib-treated DU145 were co-cultured with NK cells (untreated or N-803-treated), PD-L1 targeting high-affinity NK (t-haNK) cells (provided by ImmunityBio), or KillerTRAIL. Anti-PD-L1 avelumab or anti-TRAIL antibody was included in select assays. Cell lysis was quantified using impedance-based real-time cell analysis. The efficacy of tuvusertib + N-803 combination therapy was assessed in DU145 tumor-bearing NU/NU mice.

Results Increased expression of TRAIL-R2, ULBP-1, and PD-L1 was observed in tuvusertib-exposed DU145. Tuvusertib rendered PCa cells more susceptible to NK-mediated lysis and PD-L1-targeting by avelumab-mediated antibody-dependent cellular cytotoxicity (ADCC) and PD-L1 t-haNK cells. N-803 pretreatment of NK cells further enhanced killing of tuvusertib-treated DU145, while NK-mediated lysis was partially blocked by TRAIL signaling blockade. In the DU145 xenograft model, concurrent tuvusertib and N-803 treatment improved tumor growth inhibition and animal survival.

Conclusion Immune-mediated targeting of prostate carcinoma is improved by tuvusertib ATRi which can be combined with avelumab and/or N-803

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“Biomarker-Driven Therapy for Glioblastoma: Exploiting SLFN11 Expression with the Long-Acting SN-38 Prodrug PLX038A”

Background
Glut of chemotherapy failures in central nervous system (CNS) tumors stems from the blood-tumor-brain barrier (BTBB), which restricts most therapeutics. While the enhanced permeability and retention (EPR) effect is well-documented in solid tumors, its efficacy in glioblastoma (GBM) remains controversial due to molecular and structural heterogeneity.

Methods
We evaluated PLX038A, a 15 nm long-acting prodrug consisting of a 40 kDa PEG scaffold tethered to SN-38 (a TOP1 inhibitor). Sensitivity was assessed across GBM models with varying expressions of SLFN11, a known DNA-damage response biomarker. PET imaging was utilized to track the systemic vs. intratumoral accumulation of the nanocarrier.
Results: 1. Biomarker Sensitivity: SLFN11-high GBM cells demonstrated exquisite sensitivity to SN-38, whereas SLFN11-low tumors remained resistant.
2. Efficacy: Systemic administration of PLX038A significantly suppressed intracranial tumor growth and extended survival in mice. 3. Mechanism: PET imaging confirmed that the intact 40 kDa PEG nanocarrier - not just the released drug - effectively penetrates

3 the BTBB. The carrier is retained within the tumor microenvironment, where it slowly releases its SN-38 payload. Systemic conversion of the prodrug was shown to be insufficient for the observed therapeutic effect.
Conclusions: PLX038A successfully bypasses the BTBB to deliver high-dose SN-38 directly to the tumor site. By pairing this nanomedicine with SLFN11 stratification, we propose a precision-medicine framework to treat molecularly defined subsets of GBM, offering a promising alternative to current ineffective systemic therapies

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“Multi-omics analysis delineates metabolic dysfunction in Myelodysplastic Syndromes”

Introduction
Myelodysplastic syndromes (MDS) are stem cell malignancies with bone marrow dysplasia and ineffective hematopoiesis. Despite growing evidence for disease driving activity of somatic gene mutations, including those found in the splicing factor U2AF1, the molecular mechanisms of dysregulated hematopoiesis have yet to be fully elucidated. Here, we utilized a multi-omics approach to identify mitochondrial dysfunction as a likely driver of disease pathogenesis.

Methods
Bone marrow mononuclear cells (BMNC) were isolated from MDS patients with wild type (WT) U2AF1or the U2AF1 S34F mutation (mutMDS) and age-matched healthy donors (n=3 each). Primary CD34+ BMNC and isogenic U2AF1 S34F and WT induced pluripotent stem cells (iPSC) were evaluated by mass spectrophotometry, untargeted metabolomics, multiplexed cytokine analysis (Olink assay), and/or electron microscopy.

Results

Gene set enrichment analysis (GSEA) of normalized peptide intensity by mass spectrophotometry identified oxidative phosphorylation (OXPHOS) and related metabolic pathways as those most significantly altered in MDS vs age-matched donors and more profoundly so in mutMDS cases. Mitochondrial proteins, particularly those found in the inner membrane and mitochondrial matrix, are downregulated in MDS with greater decreases in found in mutMDS cases. These findings were reproduced by mass spectrophotometry of isogenic U2AF1 S34F and WT iPSC. Further, electron microscopy identified noticeable deformation of mitochondrial cristae in primary CD34+ MDS BM cells vs donors. Cristae are larger, less organized, and contain noticeable voids indicative of mitochondrial stress. Multiplex cytokine analysis (n=21 MDS/AML/CHIP/CCUS, 6 non-malignant) demonstrated that, in addition to EPO and IL1b, known to be upregulated in MDS, mitokines including FGF21, FGF23, and PGF were significantly upregulated in BM plasma of pre/myeloid malignancy cases compared to those without. BM plasma untargeted metabolomics showed that MDS cases had decreased levels of vitamin D and cholesterol metabolites. Notably, the proteins catalyzing these processes are predominantly housed in the mitochondrial inner membrane.

Conclusions

Collectively, these data suggest deficient loading of mitochondrial proteins in the inner mitochondrial membrane resulting in morphologic changes, mitochondrial dysfunction, and extracellular stress signal secretion likely contributing to the observed defective hematopoiesis in these patients. Notably, our previous data showed U2AF1 involvement in mitochondrial protein translation and trafficking (Garcia et al. Molecular Cell, in press). U2AF1 S34F substantially altered mitochondrial protein composition supporting the phenotypes found in primary progenitor cells here and further defining the disease driving activity of U2AF1 S34F in MDS.

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"Targeting of stem-like adenoma cells with a vaccine for the tumor-associated antigen brachyury reduces colorectal adenoma development in the APCmin/DSS mouse model"

Colorectal cancer (CRC) poses a substantial public health risk as the third most diagnosed cancer globally. Both genetic and environmental factors such as family history, diet, and inflammation contribute to the development and progression of CRC which occurs in a multistage developmental process. Many research groups are currently investigating the biological processes and antigenicity of early precancerous lesions with the goal of intercepting or preventing cancer formation through potential medical intervention. Most CRC cases arise through sporadic somatic mutations with more than 80% of those tumors having mutations in the adenomatous polyposis coli (APC) gene. In this study, we utilize the APCMin/+ mouse model which harbors a heterozygous nonsense germline mutation in the APC gene leading to the development of numerous adenomas in the small intestine. These mice are subsequently exposed to the colitis-inducing agent, dextran sodium sulfate (DSS), which increases the formation and multiplicity of adenomas and, eventually, carcinomas in the colon. In this study, colon lesions were extensively characterized through multiplex immunofluorescence, RNA in situ hybridization, and single cell RNAseq analyses. We identified the upregulation of the embryonic transcription factor brachyury in a significant portion of cells with stem-like characteristics in early-stage adenomas and in late-stage carcinomas. Expression of brachyury in APC-DSS lesions co-localized with multiple markers of intestinal stem cells, including LGR5 and ASCL2, while brachyury positive colonic stem-like cells also overexpressed pathways related to overall stemness and features of epithelial-to-mesenchymal transition. To target these stem-like cells in the APC-DSS mouse model, APC-DSS mice were vaccinated with an adenovirus-based brachyury vaccine that encodes the full-length mouse brachyury protein. We demonstrated via ELISPOT assays that administration of weekly doses of Ad-brachyury vaccine significantly increased the number of splenic brachyury-specific T cells, and flow cytometry analyses demonstrated increased number of cytotoxic CD8+ T cells in spleens and colons compared to unvaccinated mice. This resulted in vaccinated APC-DSS mice showing decreased number of colon lesions, with a corresponding increase in median survival time over that of non-vaccinated, control mice. Further testing of the treatment schedule revealed that vaccination of mice against brachyury was able to reduce colon lesion burden in both the interception, prior to lesion induction with DSS, and treatment, after DSS lesion formation, settings. Brachyury is a tumor-associated antigen currently being investigated in the clinic as a target for cancer vaccine approaches; therefore, there are clinical grade agents available for use in CRC. Extensive immunohistochemistry analysis of human tissue microarray slides and patient tissues revealed that brachyury is expressed in early-stage colon adenomas, primary carcinomas, and metastatic carcinomas. Further mouse studies are currently planned to identify candidate immune-oncology agents to combine with this vaccination approach and further enhance anti-lesion efficacy in our mouse model to be translated in the clinic

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"Single-cell analysis of a TCR Vβ6/10 targeted bifunctional fusion protein (invikafusp alfa) in the START-001 clinical trial"

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"HDAC and CDK Inhibitor Combinations Suppress Neutrophil Activation in Myeloma"

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

Background and Hypotheses
HER2+ breast cancer, defined by the overexpression or amplification of the HER2 gene, is

an aggressive form of breast cancer. Standard of care includes combinations of monoclonal antibodies, antibody-drug conjugates (ADCs), chemotherapy and small molecule kinase inhibitors. Brain metastases are prevalent in the HER2 subgroup, often at initial relapse from first treatment in the metastatic setting. Both the lesions and their treatments confer serious physical and neurocognitive adverse effects. The limited efficacy of drugs for brain metastases is a major hurdle in therapeutic advances and has been attributed to the existence of blood-brain/tumor barrier (BBB/BTB). Trastuzumab Deruxtecan (T-DXd) is a third generation HER2 targeting ADC coupled with a topoisomerase-I inhibitor; has shown activity in the metastatic setting (DESTINY-trial) with intracranial activity (DEBBRAH-trial). In this study we evaluated the efficacy of T-DXd in preclinical mouse models of breast cancer brain metastasis and explored the mechanism of T-DXd crossing the BBB/BTB.

Study Design and Methods

We studied the efficacy of T-DXd in vivo using multiple models of breast cancer brain metastasis (SUM190-BR, HER2-high; JIMT1-BR, HER2+; and MDA-MB-231-BR, triple-negative) in both prevention and treatment settings. Mice were intracardiacly injected with brain-tropic breast cancer cells and dosed with 3 or 10 mg/kg T-DXd or 10 mg/kg control ADC, with endpoints of metastasis number and size. Following this we identified a potential receptor for T-DXd and established a possible mechanism of its crossing BTB using an in vitro BTB assay.

Results and Conclusions

In the JIMT1-BR model, T-DXd at both doses reduced metastasis number by 48-88% and size by 32-88%; a concordant loss of HER2 expression by lesions remaining at the experimental endpoint and low T-DXd distribution was observed. A distinct dose effect was observed in SUM190-BR with the 3 mg/kg dose inhibiting size and number by 24-39% and 10 mg/kg by 72-79%; HER2 expression was maintained together with heterogeneous T-DXd distribution. Surprisingly, T-DXd reduced metastatic colonies number by 33.76-52.15% in 231-BR triple negative prevention model while it failed to show any activity in treatment model. In all these models, homogeneously reduced tumor Ki-67 was observed, while increased cleaved caspase 3 primarily co-stained with T-DXd; little difference in DNA damage was observed. We used an in vitro model of the blood-brain and blood-tumor barriers (BBB, BTB) to ask how T-DXd crossed. Data demonstrated T-DXd endocytosis and transcytosis of brain endothelial cells, at least partially reliant on binding to the neonatal Fc receptor (FcRn) expressed not only in endothelial cells but also in Astrocytes, Pericytes and 231-BR cancer cells. T-DXd efficacy observed in 231-BR prevention model was further explored. Knockdown of FcRn or HER2 in 231-BR cells abrogated T-DXd efficacy observed

in prevention settings, highlighting the role of FcRn and low level of HER2 in T-DXd efficacy observed in triple negative model.

Collectively, the data confirms T-DXd activity in HER2+ and triple negative brain metastases concordant with heterogeneous uptake, variable HER2 expression at endpoint, tumor cell cytotoxicity, and decreased proliferation that may be a bystander effect.

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“Using Organoids and Tumor Infiltrating Lymphocytes to Elucidate the “Dark Matter” of Human Tumor Antigens”

The Surgery Branch, NCI is conducting clinical trials using tumor-infiltrating lymphocytes (TILs) to treat common epithelial malignancies, including colorectal, pancreatic, and breast cancers. In an effort to identify predictors of therapeutic benefit, we demonstrated that patient-derived tumor organoids (PDTOs) serve as a robust platform for assessing TIL potency. TIL infusion products from 30 patients (colorectal, n=21; pancreatic, n=4; breast, n=2; other, n=3) were evaluated for reactivity against autologous PDTOs. Objective clinical responses were observed in 53% (8/15) of patients whose TILs recognized PDTOs, compared with only one response among the 15 patients without such recognition. We next examined the nature of the PDTO antigens recognized by TILs that could not be identified through screening of neoantigens defined by exomic sequencing. Using a transcriptional signature of tumor-reactive TILs derived from single-cell RNA sequencing of fresh TIL, we identified sets of organoid-reactive clonotypes that showed no reactivity to the patients’ known neoantigens-referred to as “orphan TCRs.” Through organoid-derived cDNA library expression screening, we successfully identified five distinct target antigens and their corresponding epitopes recognized by ten orphan TCR clonotypes from patients with non-small cell lung cancer (NSCLC) and colorectal cancer (CRC). Importantly, all identified antigens originated from non-coding genomic regions, including lncRNAs, lincRNAs, 5[2]-UTRs, and gene/non-coding sequence fusions. These findings provide

definitive evidence that natural human anti-tumor T-cell responses target genomic "dark matter," thereby expanding the tumor antigenic landscape to include previously overlooked regions of the genome. Incorporating this knowledge into TIL screening and selection strategies may enhance TIL diversity and help overcome tumor immune escape.

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"Spatial transcriptomic profiling of radiation-induced cutaneous fibrosis reveals senescence phenotypes and indicates senolytic therapy response"

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"Spatial Analysis of Kaposi Sarcoma Identifies a Dramatic Shift in the Transcriptional Landscape between Conventional and Anaplastic Tumor Variants"

Background/Significance
Kaposi sarcoma (KS) is an angioproliferative neoplasm driven by endothelial cells infected with Kaposi sarcoma herpesvirus (KSHV). Typical KS is characterized histologically by KSHV $LANA^{+}$ spindle shaped cells and extravasation of immune cells in skin and mucosal tissues. We established patient derived xenograft (PDX) models of KS by subcutaneous implantation into immunodeficient mice that retained the histological features of their respective tumors. Recently, three patients presented with a highly aggressive, fatal variant termed anaplastic Kaposi sarcoma (aKS). The KSHV $LANA^{+}$ tumor cells of aKS exhibited marked nuclear atypia and angiosarcomatous morphology. These features were also observed in aKS PDX tumors, which display rapid growth following renal capsule implantation. To expand beyond known clinical and pathological features, we defined molecular features distinguishing KS and aKS using spatial transcriptomic profiling with the NanoString GeoMx DSP platform.

Methods

Digital spatial profiling was performed on biopsies from eight KS tumors with four matched KS PDXs, and one aKS tumor with a matched aKS PDX. Regions of interest (ROIs) were selected based on $CD31^+$ endothelial cells and KSHV LANA expression. LANA segmented tumor ROIs and vessel ROIs were analyzed for differential gene expression and pathway enrichment. These comparisons enabled identification of host gene expression programs associated with tumor ROIs in both KS and aKS and assessment of their conservation in PDXs.

Results

In KS tumors, differentially expressed genes (DEGs) between LANA ROIs and vessel ROIs defined a lymphatic endothelial program, reduced antigen processing capacity, and inflammatory signaling, consistent with previous KS profiles. LANA ROIs of aKS retained lymphatic endothelial cell markers, yet indicated downregulation of hypoxia associated signatures, inflammatory pathways, extracellular matrix organization, angiogenic signaling, and epithelial-mesenchymal transition. aKS DEGs also indicated upregulation of metabolic genes supporting survival under stress conditions. Taken together, spatial transcriptomic profiling indicated loss of terminal differentiation and gain of plasticity in aKS. Our collaborators identified mutations in host tumor suppressors and regulatory genes associated with cell cycle control, DNA repair, chromatin remodeling, apoptosis, and genomic stability in aKS. Gene set enrichment analysis revealed activation of E2F targets, DNA repair pathways, the G2/M checkpoint, and the mitotic spindle program, along with TP53 downregulation and MYC pathway activation. Thus, the transcriptional programming supporting tumor cell survival and rapid proliferation reflects the somatic mutations of aKS tumors. Direct comparison of $LANA^+$ ROIs between aKS and KS confirmed a stark shift from TP53, KRAS, and inflammatory pathways toward E2F, G2/M checkpoint, and DNA repair pathways that promote proliferation in aKS. Finally, the transcriptional landscapes of KS and aKS were preserved in their respective PDX models.

Conclusions

We report the first detailed transcriptional analysis of aKS. The distinct transcriptional signature of LANA ROIs in this aggressive variant is consistent with genetic instability and rapid tumor growth in vivo. We aim to define the roles of viral and host oncogenes that coordinate the aggressive and fatal outcome of aKS. The aKS PDX model provides a powerful system to evaluate molecular interventions aimed at blocking uncontrolled aKS growth.

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"Mechanistic Analysis of the Novel Cereblon-binding Immunomodulators in Herpesvirus-associated Lymphomas"

BACKGROUND AND HYPOTHESIS
Primary effusion lymphoma (PEL) and Burkitt lymphoma (BL) are aggressive non-Hodgkin lymphomas associated with Kaposi sarcoma-associated herpesvirus (KSHV) and Epstein-Barr virus (EBV), respectively. These malignancies have poor survival outcomes and present an urgent need for new therapies. Pomalidomide (Pom) is an FDA-approved cereblon-binding immunomodulator (CBI) currently in clinical trials for PEL and BL, while the next-generation CBIs golcadomide (Golc) and iberdomide (Iber) are in clinical development. Our previous preclinical studies in PEL and BL demonstrated that these newer CBIs are more potent than Pom, with Golc showing the greatest activity. In this study, we further evaluated the efficacy of these CBIs and investigated the mechanisms underlying their anti-tumor effects to support their clinical testing in these lymphomas.

STUDY DESIGN AND METHODS
Proteins and genes differentially regulated by the CBIs were assessed using both proteomics and bulk RNA-sequencing approaches. Pathway analysis was carried out to further identify key pathways altered by the CBIs. Mechanistic studies were carried out by suppressing cereblon using proteolysis targeting chimera (PROTAC) against cereblon.

RESULTS AND CONCLUSIONS
A protein array analysis of PEL cell lines identified the oncoprotein survivin as one of the most strongly downregulated proteins following treatment with CBIs, with Golc showing the greatest effect. Survivin is an anti-apoptotic protein overexpressed in most non-Hodgkin lymphomas, including PEL and BL, where it is essential for tumor cell survival. The extent of survivin downregulation correlated with growth inhibition and increased caspase-3/7 activity across all CBIs, with Golc being the most potent in all cell lines. Degradation of

cereblon using a cereblon-targeting PROTAC abrogated CBI-induced survivin downregulation and growth suppression, indicating that cereblon interaction is required for CBI activity. Interestingly, downregulation in survivin protein was associated with significant decrease in survivin mRNA levels in only a subset of cell lines; the role of post-transcriptional mechanisms in CBI-induced survivin regulation is currently under investigation. Bulk RNA sequencing revealed that Golc induced more extensive transcriptional changes than the other CBIs. Additionally, all three CBIs downregulated latent oncogenic KSHV genes and showed a general trend toward reduced EBV gene expression, suggesting that antiviral effects may partially contribute to CBI activity in these lymphomas. Together, these findings demonstrate that newer CBIs exert anti-tumor effects in PEL and BL cell lines through multiple mechanisms and support further investigation of these drugs in clinical settings.

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“Rapid analysis and response evaluation of combination anti-neoplastic agents in rare tumors (RARE CANCER) trial (RARE 3): Tiragolumab + Atezolizumab”

Background

Rare tumors (incidence <6 per 100,000 per year in the US) have limited therapeutic options. Combined immune checkpoint inhibition (ICI) with antiâ€“PD-L1 (atezolizumab) and anti-TIGIT (tiragolumab) demonstrated activity in early clinical trials and enhanced $CD8^+$ T-cell-mediated tumor rejection in preclinical models. This trial will assess activated intratumoral $CD8^+$ T cells and clinical activity of this combination in rare tumors.

Methods

RARE-3 (NCT05715281) is a phase II pharmacodynamic (PD) trial with a primary objective of assessing the increase in proportion of activated $CD8^+$ T cells in tumor biopsies at baseline and prior to cycle 3, day 1 (±3 days). Secondary objectives included overall response rate (RECIST v1.1), progression-free survival (PFS), and the proportion of patients (pts) with a clinically meaningful increase in $CD8^+$ T -cell infiltration. Eligible pts had

histologically confirmed tumors, age ≥18 years, ECOG ≤2 and adequate organ function. Prior ICI therapy (excluding anti-TIGIT) was permitted. Pts received atezolizumab (1200mg IV) plus tiragolumab (600mg IV) every 21 days until progression or unacceptable toxicity. Ten evaluable paired biopsies would provide 95% probability and 88% power (α=0.05) to detect a >1.25 standard deviation increase from baseline.

Results

Twelve pts were enrolled from 9/26/23 to 8/18/25. Median age was 60.5 years (range 19â€“71); pt population was 75% female, 83.3% White, and median number of prior systemic therapies was 3 (range 0â€“9). As of 11/3/25, the highest treatment-emergent adverse events (AEs), reported in 9 pts, were grade 3. Treatment-related AEs included lymphopenia, Guillain–Barré syndrome, pruritus, maculopapular rash, myositis, and diarrhea. Nine pts were assessed for response including stable disease (SD) in 6 pts (50%) (3 with SD ≥6 cycles; including adrenocortical carcinoma, chromophobe renal carcinoma, perivascular epithelioid cell tumor), and progressive disease in 3 pts. One pt discontinued treatment for clinical progression; 2 were not evaluable due to not starting treatment and consent withdrawal after 1 cycle. Median PFS was 4.29 months (interquartile range, 2.29-8.97). Eight paired biopsies are currently being assessed.

Conclusion

The study closed early due to sponsor decision. There was no unexpected toxicity. Ongoing analyses are assessing the impact of treatment on intratumoral $CD8^+$ T cells and other PD markers, and their association with clinical activity to inform future studies

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"GB OMICS Technology Facility: Wet Lab Support for the Genetics Branch"

Mission of the GB OMICS Technology Facility
The GB OMICS Technology Facility is dedicated to advancing cancer research within the Genetics Branch (GB) by providing state-of-the-art technologies, bioinformatics resources, and customized assay development. Our team offers end-to-end support for GB labs and collaborators throughout the project lifecycle, including hypothesis generation, experimental design, bench work, data acquisition, analysis, interpretation, visualization and archiving and manuscript preparation. We also provide training for GB staff and fellows. To ensure comprehensive documentation and effective communication among team members and collaborators, we utilize the Analysis Management Portal (AMP) to manage and document our wet lab and bioinformatics projects.

Wet Lab Capabilities
We offer a wide array of molecular technologies to study cancer genomes, epigenomes, and transcriptomes and proteomes. Our services include:

- Preparation and characterization of DNA, RNA, single cells/nuclei, and metaphase chromosomes.
- Whole genome, exome, transcriptome, and targeted panel sequencing on Illumina and Oxford Nanopore Technology (ONT) platforms.
- Chromatin interaction assays such as Hi-C, ChIP-seq, Cut&Run, and ATAC-seq.
- Isolation and sequencing of cell-free circulating DNA (cfDNA) for disease prediction and monitoring in patient samples.
- Spectral Karyotyping (SKY) and multiplexed FISH (miFISH) for chromosomal aberrations and tumor heterogeneity analysis.
- Single-cell transcriptional profiling using PIP-seq and 10x Genomics.
- CITE-seq: a single-cell technology that simultaneously measures transcriptomic and surface protein expression.
- Mesoscale protein analysis.
- Mito-seq: a custom-developed assay for detecting low-level mitochondrial DNA mutations.

- Developing other custom assays in collaboration with GB labs.
- We are currently implementing Biomodal evoC, a technique that enables the simultaneous analysis of the genome and methylome.

Over the last two years, the wet lab team has established a wide spectrum of cytogenetic, genomic, epigenetic and protein assays and developed tailored and novel approaches working in close collaboration with the GB labs and the GB OMICS bioinformatics team.

Our goal is to enable GB labs and their collaborators to characterize, compare and monitor their cell lines, mouse models and patient samples from various angles.
Cytogenetic tools like SKY and miFISH are used to identify chromosomal variations (ploidy, copy number, translocations, tandem repeats, HSRs, ecDNA etc.) and specific gene copy numbers on a single cell level giving insights into tumor clonality and heterogeneity. ONT long-read sequencing provides exact chromosomal break points and variant allele frequencies within phased haplotypes allowing further delineation of structural, mutational and copy number aberrations. To check for promoter and/or enhancer hijacking, we apply Hi-C chromatin profiling. Single-cell RNA-Seq, CITE-Seq, and methylation assays provide a comprehensive cell characterization across multiple modalities. This allows for cell phenotyping and clustering based on differential gene expression, methylation, and epitope profiles, potentially identifying disease-driving cells in tumors, or therapeutically successful CAR-T cells in patient blood.

With the GB OMICS team supporting clinical trials, particularly the FGFR4 CAR-T trial (IRB001570), initiated by GB with the Pediatric Oncology Branch, establishing and optimizing multi-modal single cell assays and analytical pipelines has become an important focus for our facility.

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"AI-based machine learning framework for supervised treatment response prediction from tumor transcriptomics: A large-scale pan-cancer study"

Precision oncology aims to guide treatment decisions using biomarkers. While DNA-based panels are increasingly applied, RNA transcriptomics remain underused due to limited datasets and the absence of robust models. We assembled the largest transcriptomic resource for drug response prediction to date, spanning 69 cohorts, 3,729 patients, nine cancer types, and six frontline therapies: anti-PD-1/PD-L1 immune-checkpoint inhibitors, trastuzumab, bevacizumab, BRAF inhibitors, paclitaxel, and FAC/FEC (Fluorouracil-Adriamycin-Cyclophosphamide/Fluorouracil-Epirubicin-Cyclophosphamide) chemotherapy. We developed EXPRESSO (EXpression-Profile-RESponSe-Optimizer), a supervised machine-learning framework that predicts treatment response from pre-treatment transcriptomes by integrating drug targets and context-specific biomarkers. EXPRESSO achieves ROC-AUCs of 0.64-0.73 and odds ratios of 2.4-4.6 across therapies, outperforming 20 published transcriptomic signatures. Robustness analysis reveals that predictive performance plateaued for some therapies with increasing training cohorts but continued to improve for others. These findings suggest inherent limits of supervised brute-force learning for certain treatments, but additional data and deeper mechanistic modeling may further enhance transcriptomics-based predictors.

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"Locoregional heterodimeric IL-15 reprograms tumor vasculature into CD8/NK self-maintained high endothelial venules that generate tertiary lymphoid structures"

Background and Hypotheses

Most tumors harbor immune infiltrates, yet their spatial organization and functional impact vary widely across cancer types and individual lesions. Tertiary lymphoid structures (TLS) are ectopic lymphoid aggregates that arise in chronically inflamed non-lymphoid tissues, including tumors, and are among the strongest predictors of immunotherapy responsiveness and favorable clinical outcomes. Despite their prognostic significance, the mechanisms governing TLS formation in tumors remain incompletely defined, and therapeutic strategies capable of intentionally inducing TLS-like immune architecture are limited. Interleukin-15, a γ-chain cytokine currently under clinical investigation for its capacity to enhance tumor-infiltrating lymphocytes, has not been evaluated for its ability to remodel tumor vasculature and promote structured lymphoid organization. We hypothesized that locoregional administration of heterodimeric IL-15 (hetIL-15) reprograms tumor vasculature inducing high endothelial venules (HEVs) and driving organized TLS development through an adaptive immune-dependent feed-forward mechanism.

Study Design and Methods

We evaluated the effects of locoregional delivery of a native, fully human heterodimeric IL-15 formulation in syngeneic murine breast cancer models. Spatial and cellular remodeling were assessed by multiplex immunofluorescence, histopathology, and flow cytometry. Transcriptional changes were profiled using NanoString gene expression analysis and single-cell RNA sequencing, integrated with multiplex cytokine quantification (Mesoscale diagnostics), Ingenuity pathway analysis, and ligand-receptor modeling (Cell-Chatdb). The requirement for immune subsets was examined using NK cell depletion, Rag1-/- mice, and IFN-γ–deficient mice. Translational relevance was assessed by analyzing publicly available UCSC Xena Pan-Cancer (TCGA) breast cancer datasets to correlate TLS, IL-15, IFN- γ, and IL-10-associated gene signatures with overall survival.

Results and Conclusions

Locoregional hetIL-15 induced complete tumor regression in approximately 60% of treated mice and conferred durable protection against tumor rechallenge. The treatment actively reprogrammed tumor vasculature by driving metaplastic conversion of post-capillary venules into MECA-79^{+} HEVs, thereby further enhancing lymphocyte extravasation and initiating organized TLS formation, as multiplex immunofluorescence analysis of whole mammary tissues revealed. HEV induction occurred exclusively following locoregional, but not systemic IL-15 administration, demonstrating that spatially restricted cytokine exposure is required for vascular remodeling. Transcriptomic analysis revealed that HEV differentiation was sustained through continuous lymphotoxin-β (LTβ)/LTβR signaling from infiltrating CD8^{+} T cells and NK cells. Histopathological evaluation demonstrated that TLS

formation persisted despite NK cell depletion but was completely abrogated in Rag1-deficient mice, establishing that adaptive immune signals are essential for vascular trans-differentiation. Notably, HEV and TLS structures regressed upon cessation of therapy, indicating that this specialized endothelial state is dynamically regulated and therapeutically controllable. TLS induction occurred independently of IFN-γ, identifying an IFN-γ-independent pathway of vascular activation. Across transcriptomic and cytokine analyses, hetIL-15 suppressed IL-10-associated immunoregulatory programs while enhanced IFN-γ-related activation signatures, shifting the tumor microenvironment from immune-restrictive to lymphocyte-permissive. In human TCGA breast cancer cohorts, TLS, IL-15, and IFN-γ associated gene signatures correlated with improved overall survival, whereas IL-10 signatures predicted adverse outcomes. Collectively, these findings demonstrate that locoregional hetIL-15 engineers HEVs and organizes TLS through an adaptive immune-driven feed-forward circuit, providing mechanistic validation for TLS induction as a strategy to enhance durable anti-tumor immunity warranting further evaluation in organoid models or clinical settings.

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"Synergistic activation of innate and adaptive anti-tumor immunity drives the efficacy of CD47-targeted near-infrared photoimmunotherapy in solid tumors"

Background and Hypotheses
Overexpression of CD47 delivers a "don't eat me" signal that inhibits macrophage-mediated phagocytosis of cancer cells. Although anti-CD47 blocking antibody therapies show promise, clinical translation has been limited by on-target, off-tumor toxicity, including severe anemia and thrombocytopenia, due to the ubiquitous expression of CD47. To develop a more tumor-selective CD47-targeting strategy, we developed and evaluated anti-CD47 near-infrared photoimmunotherapy (CD47-NIR-PIT), which selectively kills antibody-bound cells within tumors upon near-infrared light exposure.

Study Design and Methods
C57BL/6 mice bearing syngeneic colon (MC38), breast (E0771), or fibrosarcoma (MCA-205-OVA-GFP)tumors received an intravenous injection of anti-CD47 monoclonal antibody (50 μg) conjugated with a photoactivatable near-infrared dye IRDye700DX (IR-700). Subsequently at 24 hours later, the tumors were subjected to a local near-infrared light exposure. Effects of CD47-NIR-PIT on tumor growth, systemic toxicity, effects on immune cells and their roles in tumor eradication were examined.

Results and Conclusions
CD47-PIT exhibited significant antitumor effects against MC38 and EO771 tumors without inducing anemia, thrombocytopenia, or body weight loss, suggesting a superior safety profile compared with systemic CD47 blockade. Mechanistically, monocytes/macrophages, CD8 T cells, IFN-gamma production, and cancer cell CD47 expression were required for therapeutic efficacy. Accordingly, CD47-NIR-PIT failed to suppress MCA-205-OVA-GFP tumors with low monocyte/macrophage abundance. CD8 and CD4 T cells, along with NK cells, produced IFN-gamma, which peaked 3 hours after treatment and then persisted at low levels during the 7-day observation period. By 24 hours, proliferation and influx of various immune cells were induced. Intravital imaging demonstrated enhanced tumor cell phagocytosis by monocytes/macrophages at 24 hours. Macrophages transitioned from an anti-tumorigenic M2-type dominant state to more immature phenotype after CD47-NIR-PIT. Collectively, these findings indicate that tumor-selective CD47-expressing cell depletion by CD47-NIR-PIT is a promising and safe therapeutic approach for monocyte/macrophage-rich cancers, exerting antitumor effects through direct cancer cell killing and activation of local innate and adaptive immune responses. Given that anti-CD47 antibodies and NIR-PIT are already in clinical use, CD47-NIR-PIT has strong potential for clinical translation.

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"Prospective Analysis of Mesotheliomas in Subjects with BAP1 Cancer Syndrome: Clinical Characteristics and Epigenetic Correlates of Disease"

Background

Whereas mesotheliomas are the most common malignancies identified in BAP1 Cancer Syndrome (BCS), the prevalence and natural history of the neoplasms have not been elucidated. Protocol NCT04431024 was initiated to prospectively examine if high resolution computed tomographic (CT) imaging and minimally invasive surgical evaluation could facilitate detection and surveillance of mesotheliomas in subjects with germline BAP1 mutations.

Methods

Subjects ≥33 years of age with or without prior malignancies underwent CT imaging followed by bilateral thoracoscopies and laparoscopies. CT imaging and intra-operative findings were objectively scored; surgical biopsies were interpreted by two expert pathologists. Skin, PBMC, plasma, serum, and tumor biopsies were collected for correlative research studies.

Result

50 subjects with 32 germline BAP1 mutations were enrolled between March 2021 and July 2024. Median follow-up was 21.8 months (range: 1.7 - 41.1 months). 16 sites of prior mesothelioma in 15 patients were excluded from analysis. Surgical evaluation identified diffuse mesotheliomas in 39 of 45 (87%) subjects affecting 63 of 81 (78%) hemi-thoraces and 27 of 32 (84%) peritoneal cavities; these mesotheliomas exhibited unique histological features and slow clinical progression without therapeutic interventions. CT scans were unreliable for detecting or ruling out these mesotheliomas. Common as well as mutation-specific, cancer-associated epigenomic alterations were identified in dermal fibroblasts and PBMC which correlated with cancer predilection in subjects with BCS.

Conclusion

Adult subjects with germline BAP1 mutations exhibit a high prevalence of subclinical, multicompartment mesotheliomas which may be manifestations of systemic epigenetic burdens. These findings support evaluation of epigenetic reprogramming regimens for these malignancies and the identification of surrogate epigenetic biomarkers for detection and monitoring of malignancies in subjects with BCS. For which surveillance alone may be a safe alternative to reflexive SOC interventions. Epigenetic burdens in histologically normal, non-target tissues may be surrogates of malignancy in subjects with BCS. The presence of subclinical, multicompartment mesotheliomas with reproducible epigenomic

COMPUTATIONAL BIOLOGY OR SYSTEMS BIOLOGY

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“Optical Pooled Screening at HiTIF: Expanding Pooled Image-Based Functional Genomics for CCR Investigators”

Background and Hypotheses
High-content imaging-based genetic screens provide a powerful way to link genetic perturbations to complex cellular phenotypes, but most approaches remain limited to arrayed formats that constrain scale and experimental flexibility. Optical Pooled Screening (OPS) enables pooled CRISPR/Cas9 knock-out (CRISPR-KO) perturbations to be mapped to single-cell imaging phenotypes via in situ barcode sequencing (ISS). In conventional OPS, perturbations are identified by ISS of integrated barcodes expressed as mRNA. This approach can be incompatible with certain phenotypic assays, is susceptible to barcode degradation or loss, and often exhibits low barcode expression in primary cells and tissue contexts, restricting applicability largely to transformed cell lines. OPS-T7 extends this framework by incorporating a T7 promoter upstream of the integrated barcode, enabling in situ transcription (IVT)-mediated signal amplification prior to ISS and removing dependence on endogenous barcode mRNA expression. We hypothesized that implementing OPS as a full-stack platform at the NCI High-Throughput Imaging Facility (HiTIF), including assay-adaptable image analysis pipelines, would substantially expand pooled image-based CRISPR-KO functional genomics capabilities for Center for Cancer Research (CCR) investigators.

Study Design and Methods
Standardized, end-to-end OPS workflows were established at HiTIF for both conventional and OPS-T7, including assay setup, in situ barcode sequencing, and modular image analysis pipelines for barcode decoding and phenotypic quantification. Assay-specific analysis workflows were developed to support diverse optical phenotyping readouts and are adaptable to different imaging assays and biological questions. To demonstrate the pairing of OPS with specialized optical phenotyping assays in a cancer-relevant context, we developed OPS-nFISH, which integrates conventional OPS with telomeric native fluorescence in situ hybridization (nFISH) to enable pooled, imaging-based interrogation of telomere phenotypes. OPS-nFISH was applied to a pooled CRISPR-KO screen targeting ~6,000 sgRNAs to interrogate regulators of the alternative lengthening of telomeres (ALT) pathway.

Results and Conclusions
OPS has been implemented as a shared screening platform at HiTIF and is actively supporting multiple ongoing CCR projects, enabling pooled image-based CRISPR-KO screens that were previously impractical within the intramural program. The platform supports diverse optical phenotyping assays through adaptable analysis workflows, expanding the range of cellular features that can be quantified in pooled screening formats. Using OPS-nFISH, we performed a 6,000 sgRNA pooled screen targeting regulators of the ALT pathway, validating known ALT regulators and identifying novel candidate factors. These results demonstrate that OPS enables scalable, biologically informative pooled image-based CRISPR-KO screening and provides a broadly applicable framework for interrogating complex cancer-relevant cellular processes at single-cell resolution.

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“Raman-Based Molecular Fingerprinting of Live Cells Using Machine Learning”

Background and Hypothesis
Coherent Raman spectroscopy enables label-free biochemical fingerprinting of live cells with subcellular resolution. We previously developed a machine learning framework capable of classifying glioma FFPE tissues using Raman spectral signatures. To accelerate live cell acquisition, we developed RADAR (Raman Spectral Analysis Using Deep Learning for Artifact Removal), a method that increases imaging speed by an order of magnitude while preserving spectral integrity. By integrating high-speed Raman imaging with supervised machine learning, we aimed to define unique biochemical fingerprints specific to cell type. We hypothesized that intrinsic biochemical composition alone is sufficient to distinguish cellular identity and tumor subtype. To test this, we generated metabolic maps of diverse brain brain-derived cell types – including astrocytoma, oligodendroglioma, and glioblastoma cells – using coherent Raman spectroscopy at single-cell resolution.

Study Design and Methods
Patient-derived brain tumor cell lines representing genetically heterogeneous backgrounds

were analyzed. Samples were stratified by IDH1 mutation status (IDH1-mutant and IDH1-wild-type) and histologically classified as oligodendroglioma or astrocytoma. Raman spectral data were acquired from 286 live single cells across the two principal molecular classes, with further subdivision into two histologic subtypes within the IDH1-mutant group. Classification was performed using an XGBoost model with shallow tree depth (1-3), a 20% held-out test set, and grouped, stratified 5-fold cross-validation to control for sample-level bias.

Results and Conclusions
The machine learning framework distinguished IDH1-mutant from IDH1â€“wild-type cells with a ROC-AUC of 0.78 and further discriminated IDH1-mutant astrocytoma from oligodendroglioma cells with a ROC-AUC of 0.81.
Feature importance analysis demonstrated that separation between IDH1-mutant and IDH1-wild-type cells was driven primarily by Raman peaks associated with protein amide bands, total NADH, unsaturated fatty acids, and heme-related vibrational modes. Within the IDH1-mutant class, discrimination between oligodendroglioma and astrocytoma was driven by lipid-rich vesicle signatures, protein/polyamide amide bands, and lipid-associated spectral features.

Together, these findings support the feasibility of label-free, machine learning-assisted Raman profiling to resolve clinically relevant glioma subtypes at single-cell resolution. This scalable analytical framework provides a translational platform for investigating metabolic heterogeneity, therapeutic response, co-culture systems, and patient-derived organoid models.

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“Loss of Smarca4 Function Reprograms Chromatin Accessibility to Promote Metastatic Progression of Bladder Cancer”

Metastatic bladder cancer (BC) remains a highly lethal disease with a 5-year survival rate of 5-10%, and the epigenetic mechanisms driving its progression are poorly understood. To define chromatin-based drivers of malignancy, we profiled genome-wide chromatin accessibility and transcriptomes across 16 bladder cancer cell lines spanning a wide spectrum of tumorigenic and metastatic potential. Highly aggressive cells exhibited widespread reduced chromatin accessibility. IPA upstream regulator analysis identified suppression of SMARCA4, an ATPase subunit of the SWI/SNF chromatin remodeling complex, as a key event associated with these alterations.

The tumorigenic and metastatic T24T cell line, derived from the same patient as the non-tumorigenic T24 cell line, carries a partial genomic deletion of SMARCA4, while T24 retains an intact locus. Multi-omics analysis revealed that T24T cells display marked loss of accessibility at urothelial-specific open chromatin regions and extensive transcriptomic reprogramming, characterized by suppressed immune response pathways, downregulation of apical junction genes, activation of DNA damage response programs, and features of urothelial cell identity loss and dedifferentiation. Also, the TCGA analysis reveals that, across multiple cancer types, SMARCA4 alterations are associated with significantly reduced patient survival, underscoring its clinical relevance.

To directly assess causality, we generated CRISPR/Cas9-mediated SMARCA4 knockout (KO) T24 cells. SMARCA4 loss was sufficient to induce anchorage-independent growth and highly tumorigenic and metastatic phenotypes in vivo. Notably, SMARCA4 KO cells recapitulated the epigenomic and transcriptomic features observed in T24T cells, including loss of urothelial lineage-specific chromatin accessibility and widespread changes in gene expression. Importantly, SMARCA4-deficient cells demonstrated increased sensitivity to DNA-damaging agents, suggesting that these cells may serve as a valuable model for identifying therapeutic strategies for BC involving SMARCA4 loss.

Together, our findings identify SMARCA4 loss as a critical epigenetic driver of bladder cancer progression, demonstrating the link between reduced global chromatin accessibility, disturbed urothelial lineage, genomic instability, and metastatic competence. These findings have important clinical implications for the understanding and therapeutic management of SMARCA4-deficient bladder cancer.

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"Interactive Exploration of a Large-Scale Multiple Myeloma Drug Screening Dataset"

Background and Hypotheses

Multiple myeloma remains an incurable plasma cell malignancy characterized by marked genetic heterogeneity and variable therapeutic response. To support systematic therapeutic discovery and biomarker identification, we generated a large-scale pharmacologic screening resource comprising 1,912 small molecules tested across 47 genomically characterized human myeloma cell lines (HMCLs) at 11 concentrations, yielding nearly one million dose-response measurements. The dataset includes raw and processed data, curated drug and cell line metadata, and a fully reproducible Snakemake-based computational workflow. While this published resource [Scientific Data, 2025] prioritizes transparency, methodological rigor, and reproducibility, effective exploration of the resulting high-dimensional dataset still requires computational fluency. Our goal was to build upon this rigorously processed dataset by developing an interactive analytic layer that broadens accessibility and accelerates hypothesis generation across the multiple myeloma research community.

Study Design and Methods

Forty-seven HMCLs were screened in 1,536-well format against the NCATS MIPE 4.0 compound library (1,912 compounds), with 11-point dose-response curves measured following 48-hour drug exposure. Raw luminescence measurements were normalized to positive and negative plate controls and adjusted for spatial bias using a background correction strategy to mitigate plate-level artifacts. Outlier plates and cell lines were identified through quantitative and visual quality control procedures prior to downstream analyses. Four-parameter log-logistic dose-response models were fit to each cell line-drug pair to estimate AC50 (half maximal activity concentration) and related response parameters.

To enable interactive exploration of this high-dimensional dataset, we developed a Shiny application that directly interfaces with the processed data and associated genomic and drug metadata. The application provides dynamic visualization of dose-response curves, AC50 matrices, and global summary distributions. Users can subset data by mutation status, canonical translocations, drug annotations, or potency thresholds and evaluate comparative response patterns in real time.

Results and Conclusions

The dataset reveals substantial heterogeneity in both drug efficacy and cellular treatment response. Of the 1,912 compounds screened, 36 demonstrated broad, high potency activity (AC50 <100 nM in over 80% of cell lines), and 60 additional compounds achieved AC50 values below 500 nM across most cell lines. In contrast, 660 compounds exhibited limited efficacy (AC50 >1000 nM in over 80% of cell lines), while the remaining agents displayed intermediate or cell line-restricted activity patterns. These distributions highlight both broadly active compounds and selective vulnerabilities that may reflect underlying molecular features.

In addition to compound-level differences, global viability profiles revealed distinct sensitivity landscapes across cell lines, consistent with underlying biological heterogeneity driven by translocations, oncogenic mutations, and other genomic alterations.

The interactive application transforms this comprehensive screening resource into an accessible analytical environment, enabling rapid identification of genotype-drug associations, selective versus pan-active compounds, and outlier response phenotypes.

By pairing a rigorously processed dataset with an interactive exploration platform, this resource enhances the translational utility of large-scale pharmacologic screening and supports hypothesis-driven therapeutic discovery in multiple myeloma.

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54. Chin-Hsien Tai, taic@nih.gov - Laboratory of Molecular Biology, CCR
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"Can AlphaFold3 predict where Nanobodies bind?"

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"Liquid biopsy diagnostics: The NCI/Bethesda CNS classifier reliably classifies cell-free DNA methylation data from cerebrospinal fluid"

Background
DNA methylation-based classification has transformed the molecular diagnosis of central nervous system (CNS) tumors through machine learning-driven approaches. The Heidelberg (v12.8) and NCI/Bethesda (3.1) classifiers are widely implemented for tissue-based tumor classification. Cerebrospinal fluid (CSF) cell-free DNA (cfDNA) represents a minimally invasive alternative for tumor detection and monitoring; however, existing classifiers were developed using tumor tissue and have not been optimized for CSF-derived cfDNA.

Methods
We evaluated the feasibility of applying current methylation classifiers directly to CSF samples and assessed their potential for adaptation to liquid biopsy-based tumor classification. We analyzed primary brain tumor tissues (n = 157) and cerebrospinal fluid (CSF) samples (n = 23) profiled using the Illumina methylation array from a recent study (GSE292312). All CSF samples had matched primary tumor tissues, enabling direct concordance assessment between tissue- and CSF-based classifications. Both sample types were evaluated using the Heidelberg and NCI/Bethesda classifiers. Tissue performance was assessed using the recommended calibrated match score threshold (≥0.9). For CSF samples, classification was evaluated based on the highest predicted class probability.

Results

In primary tumor tissues, both classifiers demonstrated strong performance. The Heidelberg classifier showed 78.3% high-confidence matches (123/157; calibrated score ≥0.9), while the NCI/Bethesda classifier achieved 88.5% high-confidence matches (139/157; calibrated score ≥0.9).

When applied to the paired CSF samples, the Heidelberg classifier correctly identified tumor class in 35% of cases (8/23). In contrast, the NCI/Bethesda classifier achieved 70% concordance (16/23), including multiple high-confidence predictions (n=7; ≥0.9 probability).

Conclusions

Tumor-specific methylation signals are detectable as well as classifiable from CSF-derived cfDNA. These findings support the feasibility of adapting tissue-based methylation classifiers, particularly the NCI/Bethesda framework, for non-invasive CNS tumor classification using CSF. Further optimization and CSF-specific model development may enhance the clinical utility of liquid biopsy approaches in neuro-oncology.

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DEVELOPMENTAL AND CELL BIOLOGY

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"The NCI-CCR Intravital Microscopy Core"

Intravital microscopy (IVM) is a powerful imaging approach that enables visualization of tissue dynamics at cellular and subcellular resolutions directly in live animals under physiological and pathological conditions. The integration of transgenic mouse models, advanced molecular labeling strategies and access to multiple tissues continues to provide an unprecedented real-time insight into tissue behavior in multiple disease models. Established in 2021, the NCI-CCR IVM Core offers a complete suite of services to intramural investigators - from experimental design, animal study proposals, and microsurgery to intravital imaging, data visualization, quantitative analysis, interpretation and manuscript preparation. The core specializes in enabling investigators to: i) investigate cellular mechanisms underlying disease progression ii) study pathological tissue microenvironment (e.g., vasculature, extracellular matrix, immune response), and iii) evaluate drugs and therapy efficacy at the tissue, cellular and subcellular levels in live animals. The breadth of projects IVM core investigates includes, but not limited to, - tumor progression, tumor-immune interactions, cell migration and apoptosis, intravascular leukocyte dynamics etc. using multiphoton microscopy across various mice models. This diversity is reflected in the wide range of tissues and organs routinely imaged including - skin, tongue, mammary gland, liver, pancreas, spleen, lymph node, intestine, and bone marrow. We will present representative examples from past and ongoing studies, as well as provide future directions of the IVM core. We look forward to collaborating with additional intramural investigators and apply cutting-edge IVM approach to provide transformative insights into their research programs.

Intravital Microscopy Core, CCR, NCI

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"Senescence-inhibitory delta133p53alpha counteracts accelerated aging and mortality"

Research on progeria not only contributes to treatments for the disease but also enhances our understanding of physiological aging. Mouse models of progeria recapitulate pathological aging phenotypes seen in patients, including cardiovascular defects, increased cellular senescence, systemic inflammation, DNA damage accumulation, and shortened lifespan. In cultured cells from Hutchinson-Gilford progeria syndrome (HGPS) patients, the human p53 isoform delta133p53alpha (d133p53a) was previously shown to inhibit p53-mediated cellular senescence, proinflammatory IL-6 production, and DNA damage accumulation, and to extend cellular replicative lifespan. Here we show that, in a heterozygous HGPS mouse model, transgenic expression of d133p53a reproduces these in vitro-observed effects across multiple organs in vivo and extends median lifespan by 11% (387 versus 349 days, P = 0.0379). In the aorta and skin, d133p53a abrogates progeria-characteristic pathological changes and preserves tissue integrity. Our data further suggest that d133p53a may promote a broad spectrum of aging-counteracting mechanisms, including bone homeostasis, metabolic fitness, antioxidant defense, youthful epigenome, and tissue stemness. Together with the anti-inflammatory and tissue-preserving effects of d133p53a in naturally aged mice and its age-associated downregulation in human tissues, this study suggests that d133p53a-based therapeutic strategies may be applicable not only to HGPS but also as broader interventions for preventing or delaying aging.

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“Non-canonical function of the splicing activator U2AF2 in promoting intron retention in the lncRNAs PURPL and MALAT1”

Intron retention (IR) is a form of alternative splicing in which an intron that is typically spliced out is retained in the mature RNA. Despite emerging evidence of widespread IR in protein-coding genes and lncRNAs, the underlying molecular mechanisms remain unclear. Here, we developed a genome-wide screen termed CRASP-seq to investigate the mechanisms underlying IR in the lncRNA PURPL, utilizing a genome-wide guide RNA library

expressing a reporter minigene containing the sequence of PURPL intron 2. Unexpectedly, we discovered that the top hit in the screen in two cell lines was the essential splicing activator U2AF2 that we found to promote IR in PURPL by directly binding to a weak polypyrimidine tract. U2AF2 showed the highest number of binding sites and strongest signals in eCLIP-seq datasets from ENCODE. Furthermore, at a transcriptome-wide level we found that U2AF2 promotes IR in several additional transcripts, including the very abundant and nuclear speckle-enriched MALAT1. U2AF2 depletion resulted in enhanced splicing of two MALAT1 introns and disrupted its localization to nuclear speckles. Reintroduction of MALAT1 isoforms in MALAT1-knockout cells revealed that retention of one of these introns is essential for MALAT1 localization to nuclear speckles. Moreover, the same intron contributes, at least partially, to the role of MALAT1 in cell migration. Overall, these findings reveal a previously unrecognized non-canonical function of U2AF2 in promoting IR and provide insights into the subcellular distribution of PURPL and MALAT1 lncRNAs through the control of an IR-driven splicing regulatory program.

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"Allelic Variants of mTOR Associated with Tumor Susceptibility and Cancer Development via DNA Damage Response Pathways"

Plasmacytomas (PCTs) are malignancies derived from mature, terminally differentiated B cells and can be induced at high frequency in genetically susceptible mouse strains, such as BALB/cAn and NZB/BINJ, by intraperitoneal pristane administration. In contrast, most inbred strains, including DBA/2 and C57BL/6 (B6), are resistant to PCT development. Our previous genetic studies identified mTOR as a determinant of plasmacytoma

susceptibility. In vitro kinase assays revealed that the BALB-derived mTOR allele, encoding cysteine at amino acid 628 (628C), exhibits reduced kinase activity compared to the common or wild-type allele encoding arginine (R628), present in DBA/2 and B6 mice. To investigate the functional impact of this allelic variation, we generated homozygous knock-in (KI) mice carrying the 628C allele on a >99% B6 genetic background. Following exposure to 8 Gy irradiation, male 628C KI mice exhibited significantly reduced survival compared to R628 wild-type controls. Consistently, mouse embryonic fibroblasts (MEFs) derived from 628C KI mice showed decreased sensitivity to rapamycin and irradiation-induced growth inhibition relative to R628 MEFs. To define allele-specific mechanisms, we established doxycycline-inducible HEK293Trex cell lines expressing Flag-tagged R628 or 628C mTOR. Upon irradiation, 628C-expressing cells displayed reduced G2 cell cycle arrest and more rapid recovery compared to R628-expressing cells. Immunoprecipitation-Western blot analyses demonstrated weaker RAPTOR binding to 628C mTOR, accompanied by reduced mTORC1 signaling, as indicated by decreased phosphorylation of 4E-BP1 and p70 S6 kinase. Transcriptomic profiling using RNA-seq revealed global, genotype-dependent differences in the irradiation response. Pathway analysis identified significant enrichment of DNA damage repair and replication pathways, including the G2/M checkpoint, which was upregulated in R628 cells but downregulated in 628C cells. Ongoing integrative analyses of RNA-seq, ATAC-seq, and public cancer datasets aim to define how mTOR allelic variation influences DNA damage responses and promotes cancer susceptibility.

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61. Tiao Jiang Xiao, xiaotiao@mail.nih.gov - Laboratory of Cellular & Molecular Biology, CCR

Tiaojiang Xiao and Ying E. Zhang

“SMAD3-CTCF Interaction Regulates Higher-order Chromatin Organization During TGF-β-Induced Epithelial-Mesenchymal Transition”

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62. Michael Kruhlak, kruhlakm@mail.nih.gov - Laboratory of Cancer Biology & Genetics, CCR

Michael Kruhlak, Ph.D., Langston Lim, M.Sc. and Andy Tran, Ph.D.

“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.

CCR Microscopy Core, Laboratory of Cancer Biology and Genetics, CCR, NCI

63. Dale Lewis, Lewisdal@nih.gov, Laboratory of Molecular Biology, CCR
Dale E. A. Lewis, Phuoc Le, and Sankar Adhya

“The Interplay of Transcription Factors in Keeping with Physiological Requirements in the Amphibolic galactose Operon of E. coli”

Background
In E. coli, the gal operon genes encoding enzymes for D-galactose metabolism are transcribed from P1 and P2. The cAMP-CRP complex (CCC) binds to an activating site (AS),

activating P1 and repressing P2. The galactose repressor, GalR, binds to an operator, OE, repressing P1 and activating P2. When GalR and CCC are present, the regulation of P1 and P2 is nullified.

Hypothesis
We proposed that when GalR and CCC are present, the alpha-CTD of RNA polymerase cannot bind adjacent to OE or AS, thus making GalR and CCC function incomplete. We investigate the mechanisms of the neutralization effect.

Methods: In vitro transcription assays measure transcripts from promoters on supercoiled plasmid DNA with GalR and CCC. DNase I foot printing assays localize GalR, CCC, and RNAP on these templates.

Results
When the DNA distance between OE and AS was increased by 10-bp and 20-bp, the GalR and CCC neutralization decreased. DNase I assays confirmed the binding of alpha-CTD to DNA with GalR and CCC in 10-bp and 20-bp DNA but not in wild-type DNA.
Discussion: We demonstrated that the binding of alpha-CTD to the DNA and regulators between OE and AS determines P1 and P2 regulation by GalR and CCC.

64. Sarah Clatterbuck Soper, sarah.clatterbucksoper@nih.gov - Cancer Genetics Branch, CCR
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"Organized nuclear f-actin is dispensable for ALT"

Proliferating cells must enact a program of telomere maintenance to counteract the end-replication problem. While most cells rely on telomerase for telomere lengthening, a subset of tumors use a recombination-based mechanism termed Alternative Lengthening of Telomeres or ALT. Recent studies have shown that nuclear f-actin facilitates the recruitment of telomerase to telomeres. What role nuclear f-actin may play in enabling the ALT mechanism remains undetermined. We speculated that f-actin may help to bring together telomeres and PML bodies, facilitating ALT. In U2OS osteosarcoma cells, we find that PML protein associates with nuclear actin fibers. PML interactions with nuclear f-actin are not exclusive to ALT but are also observed in diploid RPE-1 cells. Strikingly, U2OS cells lacking PML, which are defective for ALT, seldom form detectable nuclear f-actin. In rare U2OS-PMLKO cells with nuclear f-actin the network appears disordered, suggesting that PML promotes nuclear f-actin organization. PML is a scaffold protein, so we predicted that

PML would not organize actin directly but would localize the proximal effector. A top PML interactor is plectin, a cytoskeletal crosslinker that can bind both actin and intermediate filaments such as the nuclear lamins. We hypothesized that plectin may be the direct organizer of nuclear f-actin. We found that in plectin KD cells that nuclear f-actin is rarely detectable, phenocopying the PML KO condition. Unlike PML KO cells, however, plectin KD cells remain competent for ALT. Thus, plectin KD reveals that bundling of nuclear f-actin is dispensable for ALT activity in U2OS osteosarcoma cells.

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"The HP1 chromo shadow domain restrains heterochromatin propagation"

Background and hypothesis
Although it is well established that the formation of H3 lysine-9 methylated (H3K9me) heterochromatin suppresses transcription across extensive regions of eukaryotic genomes, the initiation of heterochromatin assembly often requires transcription. How heterochromatin manages transcription during its various assembly stages, and how it balances silencing with anti-silencing activities, remains incompletely understood. HP1 proteins are key regulators of heterochromatin assembly, as they recruit both silencing and anti-silencing factors. In the model organism Schizosaccharomyces pombe, the anti-silencing JmjC protein Epe1 associates with heterochromatin through its interaction with the HP1 homolog Swi6. Epe1 promotes transcription of repetitive DNA by RNA polymerase II and prevents the uncontrolled spread of heterochromatin domains. Its activity is opposed by histone deacetylases, particularly the class II HDAC Clr3, which is recruited to heterochromatin via HP1 proteins to enhance heterochromatin stability and transcriptional repression.

In this study, we investigated the role of HP1 in preserving heterochromatin stability by screening for mutations in Swi6HP1 that shift the balance of Swi6HP1-associated activities to favor heterochromatin propagation.

Study Design and Methods
We performed targeted mutagenesis on the swi6+ gene in cells with heterochromatin that is sensitive to impaired propagation due to the lack of locally acting repressive elements. We identified mutations that compensated for the missing repressive elements by enhancing heterochromatin propagation and analyzed how they function.

Results and Conclusions
Our results identified a substitution in the chromo shadow domain of Swi6HP1 that restored heterochromatin propagation compromised by the absence of local repressive DNA elements. This mutated Swi6HP1 maintained heterochromatin propagation in cells with reduced H3K9 methylation density and effectively preserved heterochromatin in the absence of initiating activity. The mutation disrupted the interaction between Swi6HP1 and Epe1, leading to the removal of the anti-silencing factor from heterochromatin. This study identified an HP1 interface essential for recruiting factor that restricts heterochromatin propagation and protects gene expression programs from inappropriate heterochromatin interference.

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“HSP90 inhibition disrupts telomere maintenance and promotes chromosomal instability (CIN) in cancer cells”

Background
Heat shock protein 90 (HSP90) stabilizes numerous oncogenic client proteins, including factors required for telomere maintenance. Telomere dysfunction triggers chromosomal instability (CIN). To quantify telomere-directed activity and separate it from general mitotic effects, we utilized a human artificial chromosome (HAC) assay with isogenic lines carrying a linear, telomere-containing EGFP HAC or a circular, telomere-lacking EGFP HAC.

Methods
Four HSP90 inhibitors (TAS-116, XL-888, SNX-2112, 17-AAG) were tested at each compound’s cell-specific LC50 in HT1080 (linear and circular HACs) and HEK293 (linear

HAC) cells, with GRN163L as a positive control. HAC loss was quantified by flow cytometry. Telomere length was measured by Southern blot and qPCR in parental HT1080 and HEK293 cells, and telomere signal intensity by FISH in HEK293. Telomere dysfunction (TIFs; γH2AX/TRF2) and micronuclei (MNi) were also scored.

Results
TAS-116 showed the strongest telomere-specific activity among HSP90 inhibitors, significantly increasing linear HAC loss in both HT1080 and HEK293 while circular HACs showed only minimal instability. TAS-116 shortened telomeres in both lines (qPCR: ~1.8 fold in HT1080; ~2 fold in HEK293) and, in HEK293, reduced FISH telomere signal to 59.66% of control. TAS-116 also increased telomere-associated damage (~1.8 TIFs per TIF positive nucleus), DNA double-strand breaks, and micronuclei relative to the other HSP90 inhibitors.

Conclusions
TAS-116 consistently disrupts telomere maintenance driving linear HAC loss, telomere shortening, reduced FISH signal, elevated TIFs, and increased MNi, thereby validating the HAC-based framework for discriminating telomere-directed activity. These findings support the therapeutic promise of HSP90 inhibition against telomere maintenance in cancer.

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"TONSL-MMS22L ensures completion of DNA replication in S-phase"

Background & Hypotheses
TONSL and its heterodimeric partner MMS22L are evolutionarily conserved factors in metazoans with presumed functions in S-phase. Familial and somatic mutations in TONSL have been identified in human developmental abnormalities and a variety of cancers, though their biological significance have not been clearly defined. Previous studies using RNA interference (RNAi) have repeatedly implicated the TONSL-MMS22L complex as a facilitator of homologous recombination (HR) whose function may be especially important

under conditions where the canonical RAD51 loader BRCA2 is defective. Yet, can a backup role in HR really be the main function of the TONSL-MMS22L complex, when loss of either factor imparts cellular lethality even in a BRCA2-proficient background? Study Design &

Methods

To address this question, and to circumvent potential issues associated with traditional RNAi methodology such as lack of temporal resolution and/or insufficient knockdown efficiency, we developed a series of PROTAC-based cellular models in which endogenous TONSL or MMS22L can be rapidly degraded in an inducible manner. We then proceeded to investigate systematically how controlled loss of the TONSL-MMS22L complex impacts cell fate using a series of cutting-edge imaging- and sequencing-based techniques.

Results & Conclusions

Our analyses reveal that acute degradation of either TONSL or MMS22L in otherwise unchallenged cells leads to immediate and profound perturbations in replication fork progression that prevent complete duplication of chromosomes in the first S-phase. Consistent with previous studies implicating TONSL as a putative histone chaperone, preliminary evidence suggests that aberrant replication-coupled histone deposition could be a primary driver of these S-phase defects. Interestingly, TONSL- or MMS22L-degraded cells then undergo a protracted arrest in a state resembling G2, as evidenced by strong enrichment of the G2 marker CENPF. In this G2-like state, TONSL- or MMS22L-degraded cells attempt to slowly fill in their under-replicated genomes, which are also fragile and prone to breakage. TONSL- or MMS22L-degraded cells eventually escape G2, possibly through checkpoint adaptation, with still incompletely replicated genomes and unrepaired DNA double strand breaks, which in turn trigger prolonged mitotic arrest culminating in cohesion fatigue, catastrophic chromatid separation and cell death. Though certain mechanistic details remain to be elucidated, our discovery of TONSL-MMS22L as a key regulator of DNA replication provides a likely explanation for why both are highly essential proteins. We propose that TONSL-MMS22L promotes genome stability by coupling DNA synthesis to histone deposition, thereby ensuring timely execution of the replication program within S-phase.

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"CRISPR Screen Revealed Novel Role of TOP3B in RNA splicing"

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"Presence of a transitional intermediate phase from RNA processing bodies to stress granules during cellular stress response"

RNA processing bodies (PB) and stress granules (SG) are dynamic cytoplasmic ribonucleoprotein (RNP) condensates that coordinate the fate of untranslated mRNAs. PBs are enriched in mRNA decay machinery and are present in all normal cells, whereas SGs are induced in response to cellular stress and contain stalled pre-initiation complexes, of which both function as host innate immunities. It has been well documented that PBs and SGs frequently dock and exchange components under stress conditions. The mechanistic relationship between these condensates during SG biogenesis has remained unclear. We recently observed that SG assembly proceeds through a previously unrecognized intermediate transition phase characterized by the formation of hybrid PB/SG bodies of which each SG is formed likely via the multiple sticked PBs. Using arsenite-induced oxidative stress and high-resolution imaging, we observe that early SG components accumulate adjacent to, and partially overlap with, PBs prior to the emergence of fully mature SG. Several core PB factors were found to play essential roles in this process. At an

early stage, the PB scaffold protein GW182 facilitates initial SG condensation by recruiting SG proteins to PB interfaces, promoting the formation of transient hybrid PB/SG assemblies. Critically, co-recruitment of the DEAD-box RNA helicase DDX6, an essential PB component, triggers their remodeling and resolves the hybrids into distinct SGs and PBs. This intermediate transient phase happens quickly, is unstable, and would not be visible in cells under stress conditions. However, the loss of DDX6 in the cells leads to the hybrids becoming stable and persistent at the transient stage and can be caught by high-resolution microscopy, indicating a failure in PB/SG separation required for SG maturation. The ability of wild-type DDX6, but not its helicase-deficient mutant, in rescuing PB/SG separation defect in DDX6 knockout cells implies this is an active, energy-dependent process. DDX6 resolution of the PB/SG hybrids can be regulated by its functional co-factors CNOT1 and 4E-T. Together, our unexpected findings reframe SG formation as a continuum of RNP condensation events rather than an independent assembly pathway and highlight DDX6 activity as a critical determinant of condensate identity. Our findings also indicate the presence of a stepwise, transient intermediate phase in SG biogenesis, which is actively executed by the PB machinery.

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“PARP3 suppresses the Alternative Lengthening of Telomeres pathway by promoting G4 quadruplex resolution”

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“DNA replication profiles and large genomic structural variations detected by molecular combing on single DNA fibers”

The notorious characteristic of cancer is out of control cell proliferating. Precise mapping of DNA replication patterns in cancer cells during proliferation and in response to agents

that perturb DNA synthesis might help us understand cancer progression and the response of cancers to chemotherapy.

To provide a detailed view of the DNA replication process , we monitor the progression of DNA synthesis at a single-molecule resolution using molecular combing, a technique that facilitate uniform, parallelly stretching very long single DNA fibers on silanized coverslips (like combed hairs). We can trace single replicating DNA on the combed DNA molecules by incorporating thymidine analogs in replicating cells and detecting the incorporated molecules using specific antibodies against distinct analogs combined with single DNA fiber detection by an anti-single stranded DNA antibody. We routinely use this technique in the lab to study the DNA replication dynamics with/without exogenous replication stress, allowing us to measure replication fork speed, replication origin initiation frequency, replication fork asymmetry and stalling, replication fork recovery, et cetera. By comparing DNA replication patterns in untreated and drug treated cells, the protocol can characterize the cellular response to replication stress in cancers with specific vulnerability to chemotherapeutic drugs. We can also monitor replication fork progression at specific chromosomal regions by combining the molecular combing method with fluorescent in situ hybridization (FISH). We were also able to employ FISH on single DNA fibers to detect structural variations in large stretches of repetitive DNA, which are difficult to detect by whole genome sequencing, including the integration patterns of multiple copies of Human papillomaviruses (HPVs), large genome DNA fragment deletions/amplifications/rearrangements, rDNA arrays, centromere patterns and telomere length. We will show some representative examples of the above applications and discuss additional potential applications of this method.

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“Prime Editing Based In Vivo Saturation Mutagenesis Maps Functional DICER1 Variants”

DICER1 is a highly conserved RNase III endonuclease that is instrumental in the maturation of microRNAs, which are master regulators of gene expression. Mutations in the DICER1 gene are associated with many diseases, including cancer. DICER1 syndrome is an autosomal dominant tumor predisposition disorder that develops when individuals inherit a loss-of-function germline DICER1 variant. A second, trans-somatic missense mutation at

key hotspots in DICER1 can lead to tumorigenesis. To date, a plethora of mutations have been recorded in DICER1 syndrome patients who developed cancer; however, the majority remain poorly characterized and are classified as variants of unknown significance (VUS). Here, we aim to decipher the functional significance of DICER1 mutations by conducting a CRISPR prime editor screen, in which we perform saturated mutagenesis of DICER1 in vivo in a human cell line and evaluate the impact of these variants on miRNA biogenesis in parallel in a high-throughput manner.

To this end, we developed a robust functional reporter system that quantitatively monitors DICER1 activity through changes in the cellular miRNA profile. The test reporters carry defined miRNA binding sites in the 3' UTR of a fluorescent protein, while a GFP reporter lacking miRNA target sites serves as an internal control. In this system, Dicer-processed miRNAs downregulate reporter expression, and alterations in miRNA levels caused by DICER1 variants are readily detected as changes in reporter fluorescence. We stably integrated the reporters into a DICER1 heterozygous 293T cell line generated by specifically deleting one DICER1 allele using CRISPR-mediated genome editing. Furthermore, the PEmax prime editor was stably incorporated into this cell line using the PiggyBac transposon system. A prime editing guide RNA (pegRNA) library comprising 83,235 guides was constructed to engineer all theoretically possible variants in the DICER1 coding sequence. The library also includes 1,061 clinically relevant intronic mutations in DICER1. At the end of the screen, cells were sorted into different bins based on reporter fluorescence intensity, followed by genomic DNA isolation and deep sequencing of pegRNA cassettes. Preliminary data showing strong enrichment of positive controls and depletion of negative controls in the DICER1 loss-of-function (LOF) phenotype highlight the high specificity of the screen. A substantial number of previously uncharacterized missense mutations were identified that lead to partial or complete loss of DICER1 function.

Taken together, the outputs of this screen, along with further validation of individual hits, will not only be invaluable for interpreting clinical DICER1 variants but will also provide new mechanistic insights into Dicer function and regulation.

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“Characterization of Ionizing Radiation-Dependent Substrates of PPM1D Phosphatase”

Protein phosphatase 1D (PPM1D, Wip1) is a member of the metal-dependent protein phosphatase (PPM) family of serine/threonine protein phosphatases that is upregulated by p53 in response to DNA damage signaling. PPM1D functions to negatively regulate several tumor suppressor proteins, including p53, mitogen-activated protein kinase γ ± (p38Î± MAPK), and ataxia-telangiectasia mutated (ATM) kinase, thereby dampening the stress response. The PPM1D gene is amplified and PPM1D protein overexpressed in several human cancers, with increased expression associated with worse prognoses. Thus, PPM1D is an oncoprotein and potential target for therapeutic development. Full characterization of the substrates of PPM1D, though, is still needed to understand the full activity of this phosphatase and the effects of its inhibition.

To identify novel substrates of PPM1D, U2OS wildtype or PPM1D-/- cells were treated with 8 Gy ionizing radiation, recovered for 6 h, and then pelleted. Quantitative global phosphoproteomics was then used to identify changes in sites of phosphorylation upon PPM1D knockout; simultaneously, total protein quantitation was performed to characterize effects on protein abundance.

The mass spectrometry analysis identified and quantified 7900 proteins and 13100 phosphopeptides. At the total protein level, 542 protein groups showed significantly lower level in the PPM1D-/- cells (FC ≤ -1.25, adj. p-value < 0.05) and 650 protein groups had a significantly higher level (FC ≥ 1.25, adj. p-value < 0.05). Negatively regulated proteins were significantly enriched in phosphoproteins and proteins with cell cycle functions. Positively regulated proteins were significantly enriched in phospho- and mitochondrial proteins, suggesting a role for PPM1D in regulation of cellular metabolism.

Of the phosphopeptides, 1182 phosphopeptides, representing 622 proteins and 1039 phosphosites, had significantly increased phosphorylation in the PPM1D-/- cells (FC ≥ 1.25, adj. p-value < 0.05), suggesting a potential role for PPM1D in their dephosphorylation. Nearly all are annotated as phosphoproteins, and there was a significant enrichment in nuclear proteins, consistent with the known functions of PPM1D.

Among the increased phosphorylation were known substrates of PPM1D, such as S139 of γ-H2AX. In total, 56 phosphosites from 48 proteins were pS/T-Q sites, which has been defined as part of the PPM1D substrate motif. These proteins are predominantly nuclear and have important roles in DNA repair. Motif and kinase analysis of the increased phosphosites provided better understanding of PPMD specificity and expansion of the signaling pathways regulated by PPM1D and its potential oncogenic roles. Overall, this phosphoproteomic analysis of the effects of PPM1D knockout following ionizing radiation provides new insights into phosphatase substrate specificity and signaling roles for the enzyme.

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"Single-cell transcriptomic analysis identifies LRRC15 as a critical mediator of adhesion, invasion, and osteogenic signaling in osteosarcoma"

Background
Osteosarcoma (OSA) is the most common primary bone cancer in children and adolescents, typically arising during rapid skeletal growth. A frequent feature of OSA is an osteoblastic phenotype with abnormal bone formation. Leucine-rich repeat containing 15 (LRRC15) is a cell-surface protein highly expressed in several solid tumors, particularly OSA. LRRC15 is upregulated during TGFβ-induced osteogenic differentiation of mesenchymal stem cells and is highly expressed across multiple solid malignancies, including osteosarcoma. Antibody-drug conjugates and radio-immunotheranostic approaches targeting LRRC15 have demonstrated encouraging therapeutic activity in osteosarcoma, underscoring its potential clinical relevance. The current study is focused on the functional roles of LRRC15.

Study designs
We established inducible shRNA-mediated knockdown (KD), CRISPR-Cas9 knockouts (KO) and rescue of LRRC15 osteosarcoma cell models to investigate its functional roles. We implemented time-resolved single cell transcriptomics, 3-D spheroid cultures and mass spectrometry to identify differentially regulated pathways.

Results: LRRC15 depletion significantly reduced cellular adhesion to extracellular matrix (ECM) components and impaired migratory capacity. Three-dimensional spheroid assays further revealed that LRRC15 knockdown markedly disrupted scaffold-free spheroid compaction and diminished invasive capacity in Matrigel-embedded spheroids. Single-cell RNA sequencing performed across the knockdown time course identified progressive dysregulation of collagen-mediated ECM pathways in LRRC15-deficient populations. Key osteogenic transcriptional regulators, including RUNX2, β-catenin, and SP7, exhibited sustained downregulation within LRRC15-low clusters. Additionally, genes associated with osteoid matrix production were significantly altered over time. Notably, transcripts involved in cell-cell communication networks were substantially reduced in clusters characterized by low LRRC15 expression.

Conclusion
Collectively, these findings define LRRC15 as a critical cell-adhesion molecule that sustains osteogenic intercellular communication, invasive behavior, and differentiation programs in osteosarcoma. This work provides insights into LRRC15-driven tumor biology with implications for further mechanistic and translational studies.

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"Locally specialized cortex glia differentially regulates seizure behavior in Drosophila central nervous system"

Glial cells permeate the mammalian nervous system. Conventionally glial cells have been studied as homogenous groups of cells that are structurally and functionally identical across brain regions. However, recent studies have shown that depending on the anatomical region in the brain, glia exhibit heterogeneity in their development, molecular profiles, and functions within glial subtypes. Existence of glial subtypes within the same class have also been identified in Drosophila, however, their functional significance remains unknown. In this study, we have taken a genetic approach to assess functional

differences among cortical glial subtypes across brain regions. Towards this goal, we have developed transgenic tools to identify and manipulate cortex glial subpopulations in the larval, pupal and adult brain. We optimized cortex glial subtype specific expression pattern across brain regions in the optic lobe (OL), central brain (CB), and ventral nerve cord (VNC) using Gal4, splitGal4, Gal80, LexA and killer zipper systems. We have used these tools to explore the role of regional cortex glia in epilepsies. Previous studies have shown that Ceramide phosphoethanolamine synthase (cpes) mutants exhibit light inducible seizures while Na+/Ca2+, K+ exchanger (zyedeco/zyd/NCKX) mutants show temperature inducible seizures due to aberrant cortex glial function. Do cortex glia located in different parts of the brain regulate these seizure types differentially? To answer this question, we have performed distinct brain region specific rescue experiments in cpes and zyd mutants using the cortex glial subtype specific drivers developed in this study. We found that OL and CB, but not VNC specific cortex glial expression of UAS CPES, was able to significantly suppress light inducible seizures in cpes mutants. In contrast, VNC but not OL or CB specific cortex glial expression of UAS NCKX was able to suppress temperature sensitive seizures. Further, in a third model, expression and activation of transient receptor potential (TrpA1) just in the VNC specific cortex glia was sufficient to induce temperature sensitive seizures in wild type flies. Taken together our findings show that regionally specialized cortex glial subtypes differentially regulate seizure susceptibility, suggesting glial subtype heterogeneity has local functional implications.

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"Nuclear Receptors Ligand Dynamics Govern Timely Responses to Hormone Fluctuations"

Most transcription studies using hormonal signaling are performed under continuous stimulation, even though the secretion of many hormones follows a circadian pattern, comprised of periodic (ultradian) pulses of short duration. We have previously demonstrated that the dynamic, short-lived interactions of glucocorticoid receptor (GR) with its natural ligand (cortisol) allow the receptor to detect ultradian hormone level fluctuations and modify the responses of GR- regulated genes resulting in gene pulsing, cyclic release of nascent RNA by GR-regulated genes (Stavreva et al., 2009, 2015). On a

single promoter level, ultradian pulses lead to restricted RNA bursting, occurring only during the hormone pulses (Stavreva et al., 2019).

The release of other steroid hormones is also ultradian - for example, estradiol (E2) shows infradian cycle composed of ultradian pulses (1993; Bäckström et al., 1982). Yet, the transcriptional consequences of these patterns, if any, remain unexplored.
Using single molecule tracking (SMT) and analysis methods combined with other microscopy and biochemical approaches we demonstrated that in contrast to GR, the strong estrogen receptor alpha (ERα) interactions with E2 result in continuous gene response of the ERα-regulated genes, even under discontinuous (ultradian) E2 stimulation. Similar results were observed for the androgen receptor (AR) and progesterone receptor (PR) treated with testosterone and progesterone, respectively.
Importantly, the less stable interaction of ERα and estriol (E3) or bisphenol A (BPA) allow the ERα to detect E3 and BPA fluctuations, albeit with a delay. The changes in gene responses under these different treatments is accompanied with corresponding changes in the ERα' bound fraction, as measured by SMT. Understanding how the pattern of hormone stimulation impacts the activity of nuclear receptors (NRs) and the transcription of NRs-regulated genes has important implications for human health.

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"Decoding Telomerase: Harnessing Telomerase RNA to Decipher the Enzyme's Secrets"

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"The Dishevelled C-terminus interacts with the centrosomal protein Kizuna to regulate microtubule organization during ciliogenesis."

Microtubules (MTs) serve as dynamic scaffolds that support diverse cellular processes, including cell division, intracellular transport, and epithelial morphogenesis. In ciliated cells, MTs not only form the axonemal backbone of cilia but also work in tandem with actin to build an apical meshwork that anchors and orients basal bodies. However, the mechanisms regulating apical MT organization during ciliogenesis remain poorly understood. Here, we identify the centrosomal protein Kizuna (Kiz) as a critical regulator of apical MT architecture. Kiz interacts with the C-terminus of Dishevelled 2 (Dvl-C), inducing an open conformation and enabling the recruitment of Protein Kinase C delta (PKCδ) to stabilize the apical MT meshwork. This Dvl2-Kizuna-PKCδ signaling axis is essential for ciliogenesis across multiple contexts, including the formation of primary cilia in the eye, multicilia in the mucociliary epidermis (MCE), and mono-motile cilia in the left-right organizer, the gastrocoel roof plate (GRP). These findings reveal a conserved molecular mechanism linking Dvl2 conformational dynamics to MT organization and highlight Kizuna as a key mediator coupling non-canonical Wnt signaling to ciliogenesis, left-right patterning, and the organization of the apical actin microtubule meshwork in ciliated cells of embryos.

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“Small Non-Coding RNAs Buffer Proteotoxic Stress To Preserve Proteostasis”

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“Co-targeting mutant KRAS and autophagy in KRAS-driven cancers”

Background and Hypotheses
of initial response to KRAS inhibition and rapid relapse in a large portion of patients with

mutant KRAS-driven cancers remains a clinical challenge and suggests the needs of combination therapies. To target the vulnerability of KRAS-driven cancer cells with higher specificity, we propose to co-target two cellular components that are more essential for KRAS-driven cancer cells and dispensable in normal cells: the mutant KRAS oncoprotein and the autophagy pathway. Genetic evidence has shown that the core essential components of the autophagosome formation pathway are required for KRAS-driven tumor progression. However, small molecular inhibitors that are selectively inhibiting the autophagy core components are not available, making it difficult to clinically translate this combination strategy.

Study Design and Methods
In this study, we evaluated the anti-tumor effects of combining AMG-510 (Sotorasib, an FDA-approved KRAS_G12C inhibitor) and Compound 19, a recently disclosed small molecule that inhibits LC3 lipidation (which is essential for autophagosome formation) on the fitness of KRAS_G12C non-small cell lung cancer (NSCLC) cells. In these cells we analyzed the single agent and combination effects on the fitness and transformation phenotypes including 2D cell proliferation, 3D spheroid growth, anchorage-dependent and -independent colony formation, and xenograft growth.

Results and Conclusions
Biochemically, Compound 19 treatment effectively inhibited LC3 lipidation and resulted in accumulations of autophagosome-associated adaptor proteins in KRAS mutant cells, indicating a strong inhibition of autophagosome formation. Phenotypically, Compound 19 alone had no effects on cancer cell fitness, including cell proliferation, colony formation, and spheroid growth in both 2D and 3D cultures. However, when combined with AMG-510, Compound 19 weakly increased the effects of KRAS inhibition on cell viability in H2122 NSCLC cells in vitro. Moreover, Compound 19 strongly synergized with AMG-510 to inhibit H2122 xenograft tumor growth in vivo. Further investigation is underway to reveal the mechanisms of the synergistic effects.

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molecule that inhibits LC3 lipidation (which is essential for autophagosome formation) on the fitness of KRAS_G12C non-small cell lung cancer (NSCLC) cells. In these cells we analyzed the single agent and combination effects on the fitness and transformation phenotypes including 2D cell proliferation, 3D spheroid growth, anchorage-dependent and -independent colony formation, and xenograft growth.

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EPIDEMIOLOGY

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"Bladder cancer meta-analysis identifies novel loci and highlights genetic regulation of smoking-related risk"

Urinary bladder cancer (BC) is the ninth most common malignancy worldwide. We conducted a meta-analysis of genome-wide association studies for BC risk in 32,470 cases and 1,753,462 controls, identifying 70 independent genome-wide significant loci, including many novel signals. A 70-marker polygenic risk score was strongly associated with BC risk (hazard ratio=1.61 (1.50-1.73) per standard deviation), substantially improving the area under the curve (AUC) when added to a model with age, sex, and smoking status (AUC=0.75 vs. 0.70, p=4.49E-20). BC risk variants (n=4,196 at p<5.0E-8) were enriched in regions of open chromatin in bladder tissue. Integrated germline, transcriptomic, and proteomic analyses nominated additional susceptibility genes and pathways, particularly related to xenobiotic metabolism.

We detected a novel BC signal within a known smoking-related locus at 15q25.1 (rs7173514-C, OR=1.07, p=9.64E-10 for BC risk overall, OR Never-Smoker=1.00 and OR Ever-Smoker=1.14). This signal was primarily driven by an indel variant within CHRNA3-3'UTR rs71581744/rs10637216 (A/ACCCC, r2=0.78 with rs7173514 in Europeans) linked with smoking cessation, with an additional contribution from a known lead variant for smoking intensity (rs16969968-G/A, D398N within CHRNA5). Further analyses revealed significant heterogeneity for rs71581744 by BC subtype (muscle-invasiveness), particularly among current smokers (ORMuscle Invasive=1.42 vs. ORNon-muscle Invasive=1.11, pheterogeneity=1.84E-02), consistent with epidemiologic observations that current smokers have a higher risk of muscle-invasive bladder cancer.

Our in-vitro reporter assays for rs71581744-A/ACCCC demonstrated allele-specific effects on mRNA stability in several cell lines. Since neuronally expressed CHRNA5 and CHRNA3 encode subunits of the nicotinic acetylcholine receptors (nAChR) that regulate smoking

behavior, we investigated their expression in normal brain tissues in GTEx. The BC risk signal colocalized with a top CHRNA3 eQTL in one brain area, with variable allelic expression imbalance for CHRNA3 in several brain areas from the same donors. Our results implicate rs71581744-A/ACCCC as a functional variant contributing to BC risk via regulation of CHRNA3 mRNA stability in specific brain areas, affecting nicotine reward/aversion circuits and possibly bladder function. Overall, our study provides new insights into BC genetics and etiology with relevant clinical implications.

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“The SOCcer ecosystem: Tools based on natural language models to assist coding occupations and industries from free-text job descriptions”

Exposures that occur at work have been associated with a wide-range of health effects; however, it remains difficult to study occupational risk factors because occupational histories often rely on free-text job descriptions. We built the SOCcer (Standardized Occupation Coding for Computer-Assisted Epidemiologic Research) ecosystem to aid researchers in coding their studiesfree-text job descriptions (freely available at https://soccer.nci.nih.gov/). SOCcerNET codes occupations to the US Standardized Occupation Classification 2010 (SOC2010) system. CLIPS codes industry information to the North American Industry Classification 2022 (NAICS2022) system. Both use small language models that the user runs within a browser to eliminate the need to upload data thereby maintaining data privacy. They provide a ranked order of suggested codes, with a score indicating the probability that the code would agree with an expert-assigned code. The SOCcer ecosystem also includes tools for processing the SOCcer output (socR). Because these tools do not fully replace the need for expert coding, we also provide a platform (SOCAssignWeb) for the coder to see the code suggestions provided by SOCcerNET and CLIPS. In addition, SOCcerNET and CLIPS can be incorporated into online questionnaires to assist the participants in self-coding their own occupation from a list populated in real-time based on their previous free-text responses to questions on job title, job tasks, and the products made or services provided by their employer. These tools and our validation efforts will be presented. These tools can be used to reduce the resources needed to obtain accurately coded job descriptions

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“A novel intergenic 9p21 structural variant confers susceptibility in melanoma”

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"Dietary Assessment in the Connect for Cancer Prevention Study"

Objectives

The Connect for Cancer Prevention Study (Connect) is a new, modern cohort, aiming to recruit 200,000 participants and focusing on cancer etiology, risk prediction, and early detection. Connect infrastructure also enables the study of a broad range of other health outcomes. Given diets role in disease etiologies, dietary assessment is scientifically critical to Connects aims.

Methods

Connect is a prospective cohort, recruiting cancer-free adults (30-70 years) through 10 integrated healthcare systems across the U.S. Recruitment began in 2021 and will continue through at least 2027. Connect integrates electronic health records with longitudinal digital surveys and serial biospecimen collection over 20+ years. Blood, urine, and saliva are currently collected; fecal collections are planned. The Diet History Questionnaire (DHQIII) was released in May 2025 to participants who reached six months of follow-up. The DHQIII includes 135 food and beverage and 26 dietary supplement items, is offered in English and Spanish, and provides back a Health Eating Index (HEI) report. The Automated Self-Administered 24-Hour (ASA24) Dietary Assessment Tool and the Activities Completed over Time in 24-hours (ACT24) tool will be released in late 2026 and will be offered four times each, interspersed, over a 12-month period.

Results

As of March 2026, 9,590 of 25,588 eligible participants (37%) completed the DHQ and 77% of those viewed their HEI report. The completion rate increased with age and was highest for white participants. The median time from survey invitation to completion was 5.6 days; 54% of participants required over an hour to complete the DHQIII. Of note, the response rate to the subsequent follow-up survey was 17% lower among those who first did the 6-month DHQIII (30%) compared to those who did not (47%), even after accounting for a comparable amount of time on study (9-12 months).

Conclusions: Because DHQ-III completion is low and reduces subsequent survey response, a 15-item diet screener will be offered alongside the DHQIII to capture dietary data from all participants. The screener supports rigorous confounder adjustment in non-dietary analyses. These tools, together with the ASA24, support an in-depth framework for dietary assessment in Connect.

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VIROLOGY, IMMUNOLOGY AND MICROBIOLOGY

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"A subpopulation of sporulating Bacillus subtilis cells employs extracellular glycerol as a morphogen to trigger biofilm formation"

Biofilm formation by the bacterium Bacillus subtilis is a model for understanding the diversification of cellular labor in the transition from unicellular to multicellular growth. Historically, B. subtilis biofilm formation is described as preceding the initiation of spore formation, along a linear pathway that involves increasing levels of an activated master transcriptional regulator during stationary phase. Here, we demonstrate that biofilm formation and sporulation lie instead on a branched pathway wherein a subpopulation of cells first enters the sporulation pathway and generates millimolar quantities of glycerol produced by the degradation of lipoteichoic acid via the successive action of the extracellular matrix enzymes ShfP and PhoA. This glycerol is evidently toxic to sporulating cells due to the accumulation of phosphorylated glycolytic intermediates, which reduces intracellular pH and blocks peptidoglycan assembly. To prevent this toxicity, an antidote protein is required. The extracellular glycerol acts as a morphogen to trigger a separate subpopulation of cells to initiate biofilm production, which we visualize directly in growing biofilms. Thus, the formation of a heterogeneous biofilm community first requires the stochastic entry of a subpopulation of cells directly into the sporulation pathway, followed by the directed assignment of other cells via cell-cell communication to commit to biofilm production.

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

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.

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"Metabolic Reprogramming of Tumor Microenvironment by Nitric Oxide"

Background and Hypotheses

Expression of Nitric oxide synthase-2 (NOS2) in breast cancer predicts reduced patient survival. We have shown that endogenous nitric oxide (NO), product of NOS2, reprograms macrophage mitochondrial metabolism by limiting aconitase (ACO)-2 and pyruvate dehydrogenase (PDH) activities. NO controls indeed the levels of immunomodulatory metabolites and uses the PDH-cofactor lipoate to generate nitroxyl, a radical that forms irreversible modifications on proteins. Despite this knowledge, the actual concentration of

NO within the tumor microenvironment (TME) remains unclear; it is unknown if the levels of NO present are sufficient to induce metabolic reprogramming. Therefore we want to understand the impact of NO on tumor growth.

Study Design and Methods

EO771 breast cancer murine cell line was propagated in vitro and CRISPR-Cas9 system was used to generate knockout lines for Nos2. Tumors were formed in mice by injecting cells in 4th inguinal mammary fat pad.

Tumor interstitial fluid (TIF) and tumor-associated macrophages (TAMs) were isolated from both wild type (WT) and Nos2 knockout tumors at day 14 from inoculation. Metabolomic analyses were performed on TIF and TAM. Flow cytometry was utilized to comprehensively analyze the cellular infiltrate within dissociated tumors. TMRE staining was used for assessment of membrane potential and Seahorse assays were conducted to measure energy phenotype of TAMs. RNA was extracted from either single cell suspensions of dissociated tumors, isolated cell populations, or in vitro generated TAMs from BMDMs cultures, and analyzed via scRNA sequencing or qPCR for gene expression.

Results and Conclusions

Mice bearing Nos2 knockout tumors exhibited slower tumor growth and resistance to necrosis. Interestingly, Nos2 knockout tumors were characterized by lower infiltrating monocytes but higher F4/80 positive, fully differentiated macrophages, compared to WT tumors. Metabolomics analysis identified a clear NO and HNO imprint within the TIF; the found metabolic shifts hint at a role for NO in invasion and aggressiveness. Metabolomics on TAMs highlighted the significant metabolic differences attributed to NO exposure. TAMs from WT tumors demonstrated profound dysregulation in oxidative metabolism, with clear arginine-pathways related signatures; this was coupled with a rewiring of metabolic pathways mediated by the presence of ROS and subsequent changes in compounds associated with ER stress. Corroborating this data, we observed that TAMs from knockout tumors possess higher mitochondrial bioenergetics. Furthermore, this effect was shown to be independent of any intrinsic NO produced by macrophages. This indicates a profound metabolic reprogramming directly influenced by the tumors NO production.

Our study reveals that the metabolic traits observed in murine macrophages producing large quantities of NO are recapitulated in tumor settings where tumor-derived NO crucially shapes the microenvironment. This study makes a critical finding: the tumor microenvironment is a central orchestrator of cancer progression. We demonstrate that tumor-derived NO fundamentally alters the metabolic profile of the interstitial fluid, directly shaping the function of TAMs. This discovery reveals a new mechanism where

metabolic signaling controls immune cell behavior and promotes tumor growth. Our insights into the metabolic dynamics within the tumor microenvironment suggest potential avenues for targeted metabolic interventions in cancer therapy.

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"Peptide-mediated in situ activation of anti-viral bystander T cells drives tumor control independently of MHC-I expression by cancer cells"

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"Live-Cell Imaging Reveals How, When, and Where Gag Selects HIV-1 RNA for Packaging"

During virus assembly, Gag must selectively package HIV-1 unspliced RNA from an abundant pool of cellular RNAs to serve as the virion genome. How, when, and where Gag selects HIV-1 RNA for packaging is currently unclear. Using neighboring transcription start sites, HIV-1 generates two major transcripts that differ at the 5' end, containing either three guanosines (3G RNA) or one guanosine (1G RNA). Despite only differing by 2 nucleotides, 1G RNA is selectively packaged into virions over 3G RNA. To investigate how HIV-1 selects its genomic RNA, we generated cell lines infected with two modified HIV-1 viruses, one predominantly expressing 3G RNA and the other primarily expressing 1G RNA. These RNA species were differentially labeled using fluorescent proteins or detected with RNAscope probes. We examined their distribution in the cytoplasm and at the plasma membrane using spinning-disk confocal microscopy and total internal reflection fluorescence microscopy, respectively. Regardless of Gag expression, both RNA species were randomly distributed throughout the cytoplasm. Live-cell imaging showed that, in the absence of Gag, both 3G and 1G RNA trafficked to the plasma membrane without preference. However, upon Gag expression, 1G RNA accumulated at the plasma membrane even

before detectable Gag puncta formed and preferentially associated with assembling Gag particles once puncta became visible. Additionally, quantification of 1G-to-3G ratios at different stages of assembly indicated that Gag multimerization leads to further enrichment 1G RNA. Together, these findings demonstrate that although both 1G and 3G RNA can traffic to the assembly site, selective packaging of 1G RNA begins rapidly upon its initial anchoring at the plasma membrane, where it nucleates HIV-1 particle assembly.

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“Immunomodulatory Tr1 CD4 T cells induced by engineered deltaV1 HIV vaccine candidate decrease the risk of SIV acquisition in macaques”

Introduction/rationale
The development of an effective anti-HIV vaccine remains a critical tool to halt the HIV epidemic, particularly due to the limits of the PrEP strategies. CD4+ T lymphocytes play a crucial role in vaccine efficacy; however, they are also the primary target cells for HIV infection. Given these opposing roles, we hypothesized that an in-depth characterization of CD4+ T cell responses to vaccination will elucidate mechanisms of protection.

Methods
To test this hypothesis, we immunized rhesus macaques using a prime-boost strategy that included a DV1-DNA prime and boosts with ALVAC alone or in combination with DV1-gp120 protein, followed by intravaginal exposures to SIVmac251. We then integrated data obtained by flow cytometry and plasma proteome analyses, as well as transcriptome and chromatin accessibility analyses of CD3+ cells, to investigate how the vaccine shapes the CD4+ T cell immunity and how these responses cooperate in reducing the acquisition.

Results
We found that DV1 DNA/ALVAC/gp120 vaccine elicited envelope-specific immunomodulatory Tr1 CD4+ T cells, and that total Tr1 responses were associated with a reduced risk of viral acquisition. Transcriptome analyses of CD3+ cells identified vaccine-induced Tr1, as well as IL-27, gene signatures that were associated with a reduced risk,

confirming the protective role of these cells. Furthermore, the study of the epigenetic landscape of CD3+ cells revealed that the epigenetic reprogramming of enhancer region upstream of the transcription factors BATF and IRF-1, which are involved in the development of Tr1 cells, correlated with lower viral acquisition.

Conclusion

These data suggest that immunoinhibitory Tr1 cells contribute to vaccine efficacy by decreasing inflammation and potentially counteracting the development and recruitment of HIV target cells.

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“Increased avidity of antibodies to V2 mediating ADCC in macaques boosted with recombinant ALVAC rather than DNA Vaccines”

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98. Julio Valencia, valencij@mail.nih.gov - Cancer & Inflammation Program, CCR
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"Engineering A Neutrophil-Activating Therapy Against Prostate Cancer"

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"Transcriptome Signatures of Overexpressed KRAB-Zinc Finger Proteins ZNF544, ZNF567 and ZNF721"

Background and Hypotheses: Intact HIV-1 proviruses have recently been shown to persist near or in a subset of Krüppel-associated (KRAB) zinc finger (ZNF) genes in people on long-term antiretroviral therapy (ART). Given that the function of these KRAB-ZNF genes is largely undetermined, the role that they play in influencing the establishment and/or maintenance of the HIV-1 latent reservoir remains unknown. We hypothesize that overexpression of KRAB-ZNF genes as a result of intact proviral insertion in or near the genes may promote changes in cellular gene expression that contribute to latency by enhancing cell survival and replication. In this study, three KRAB-ZNF genes previously identified in people with HIV-1 (PWH) to harbor intact proviruses, ZNF544, ZNF567 and ZNF721, were selected for further analysis. Study Design and Methods: Doxycyline-inducible T-cell lines were established that expressed codon-optimized ZNF544, ZNF567 and ZNF721 for high-throughput RNA sequencing (RNAseq) analysis. Differential gene expression was analyzed using limma voom and patterns of transcriptional shifts were assessed via principal component analysis (PCA), gene set enrichment analysis (GSEA) and gene ontology (GO). Results and Conclusions: Despite the fact that genes of the KRAB-ZNF family largely act as transcriptional repressors, many genes were found to be upregulated in response to the overexpression of the selected genes, compared to their baseline levels. Common overlapping functional pathways included cytokine and cytokine receptor interactions and

chemokine signaling pathways. These results provide new insights into the role of KRAB-ZNF expression in regulating host cellular gene expression. Understanding how these cellular genes provide a survival advantage to HIV-1 infected cells and contribute to the latent reservoir may lead to new avenues for HIV-1 cure strategies.

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"Negative Regulation of RpoS translation by LrhA and RbsD"

The RpoS sigma factor helps E. coli cells respond to stress or starvation by activating a set of genes whose products mitigate the stress condition. Expression of rpoS is regulated at multiple levels particularly at the levels of translation and protein stability. rpoS translation is inhibited by a stem loop structure in the mRNA that occludes ribosome entry. Small RNAs (ArcZ, DsrA and RprA) bind to the hairpin, loosening it up and allowing for translation to proceed. RpoS is also subject to rapid degradation; it is delivered by an adaptor protein to the ClpXP protease but is stabilized during stress by anti-adaptors. Previous screens in our lab have focused on positive regulators of rpoS expression but little was known about negative regulators. Using a multicopy library screen and a reporter fusion, we identified two multicopy genes, lrhA and rbsD, that appear to down-regulate rpoS expression.
LrhA belongs to the LysR family of transcription regulators. Peterson et al. (2006) had isolated multicopy lrhA as a negative regulator of RpoS translation. Our results agree with this finding and suggest that the LrhA protein, likely indirectly, interferes with DsrA and ArcZ. The other regulator, rbsD, encodes a ribose pyranase from the rbsDACBK operon. The rbsDACBK operon is negatively regulated by the transcriptional regulator rbsR and induced in the presence of ribose. A multicopy plasmid expressing the rbsD mRNA, independent of the protein, sponges the sRNAs that bind to the hairpin, causing down-regulation of RpoS

expression. Induction of the chromosomally encoded rbs operon using ribose is sufficient to reduce rpoS expression, leading us to conclude that the rbsD RNA defines a new level of The physiological implications of this process remain unclear.
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"Conditional immortalized lymphatic endothelial cells support long-term infection of Kaposi Sarcoma herpesvirus"

Kaposi Sarcoma (KS) is characterized by aberrant angiogenesis and proliferative, spindle cells that express lymphatic endothelial markers and are infected with Kaposi sarcoma herpesvirus (KSHV). One barrier to studying KSHV latency in KS is the rapid loss of the KSHV genome upon explant culture of patient-derived KS spindle cells. In addition, KSHV undergoes lytic replication upon de novo infection of primary lymphatic endothelial cells. The lack of a lymphatic endothelial system that supports KSHV latency has hindered research that aims to define the molecular determinants of KSHV as a driver of KS. We developed conditionally immortalized lymphatic endothelial cells (ciLEC) by transducing primary lymphatic endothelial cells with SV40 large T antigen (LTA) under a doxycycline (Dox)-inducible promoter. The growth of ciLEC depended upon Dox as withdrawal induced growth arrest. ciLEC retained lymphatic endothelial markers VEGFR3 and CD34, and preserved permissiveness to KSHV infection. In the absence of Dox, growth-arrested ciLEC supported KSHV lytic replication with most of viral genes expressed; while in proliferating ciLEC supplemented with Dox, KSHV gene expression was largely suppressed. Whereas latency associated transcript and LANA protein were detectable up to 30 days post-infection, KSHV genome was stably maintained in proliferating ciLEC for up to 60 days and lytically reactivated upon treatment with chemical inducer. Thus, the long-term maintenance of the KSHV genome in ciLEC cells cultured with Dox provides a novel model for investigating the viral and host factors that promote KSHV latency, regulated KSHV reactivation in LEC cells and by extension, KS development.

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102. Mohammad Arif Rahman, mohammadarif.rahman@nih.gov - Vaccine Branch, CCR
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"Intravaginal Ring Delivery of SAMT-247 Provides Long-Lasting Protection Against SIVmac251 in Female Macaques"

Despite substantial advances in prevention strategies, HIV acquisition remains a major global health challenge. An effective HIV vaccine would provide significant advantages, including lower cost, minimal need for ongoing follow-up, and durable protection. Mathematical modeling indicates that a vaccine with 70% efficacy could reduce HIV incidence by 44% within the first decade, while a vaccine with 50% efficacy could lower transmission rates by 34% over 15 years. To enhance vaccine-mediated protection, we previously combined the ΔV1DNA/ALVAC/gp120/alum vaccine regimen with the SAMT-247 microbicide gel and observed improved efficacy. To further increase user convenience compared with vaginal gels, we developed an intravaginal ring capable of continuously releasing the SAMT-247 microbicide (S-IVR) and evaluated its protective efficacy when used in combination with the ΔV1DNA/ALVAC/gp120/alum vaccine.

Twelve female macaques were immunized with ΔV1DNA/ALVAC/gp120/alum vaccine and received S-IVR insertion one week after the final vaccination, with rings replaced every four weeks. Five weeks after the final vaccination, the animals underwent up to 14 weekly intravaginal SIVmac251 challenges, followed by an additional 11 challenges at 49 weeks post-initial vaccination.

The combined vaccine and S-IVR regimen produced an 82.8% reduction in SIV acquisition compared with naive controls ($p<0.0001$), was significantly more effective than S-IVR alone ($p=0.04$), and the S-IVR only group showed a trend toward protection ($p=0.08$) during the first round of SIVmac251 challenges. During the second round of challenges, the vaccine+S-IVR group demonstrated a trend toward greater protection relative to the vaccine-only group ($p=0.06$), and both immunization strategies provided significant protection compared with naive controls ($p=0.0005$ and $p=0.02$, respectively). Mucosal analysis revealed that NKp44+ ILCs, gp120-reactive IL-17+ NKp44+ ILCs, DC10, CD73+ mDCs, and gp120-reactive IL-10+ non-classical monocytes were correlates of protection during both rounds of challenge. In addition, S-IVR enhanced NK cell mediated ADCC responses, which correlated with a reduced risk of SIV acquisition.Our ex vivo and in vitro

findings indicate that S-IVR enhances immune responses elicited by the ΔV1DNA/ALVAC/gp120/alum vaccine, contributing to a significant reduction in SIVmac251 acquisition and supporting durable protection. Together, these results suggest that this combined platform could serve as a promising strategy for preventing HIV acquisition in women.

Our ex vivo and in vitro findings indicate that S-IVR enhances immune responses elicited by the ΔV1DNA/ALVAC/gp120/alum vaccine, contributing to a significant reduction in SIVmac251 acquisition and supporting durable protection. Together, these results suggest that this combined platform could serve as a promising strategy for preventing HIV acquisition in women

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"Advancing MAIT Cell-Targeted Therapy: Stable Agonists with Enhanced Anti-Tumor Activity"

Mucosal-associated invariant T (MAIT) cells are innate-like unconventional T cells enriched in the human liver that rapidly produce effector cytokines and exert cytotoxic activity, positioning them as potent responders in anti-tumor immunity. We previously demonstrated that activation of MAIT cells with the prototypic, T cell receptor (TCR) ligand 5-OP-RU (5-(2-oxopropylideneamino)-6-D-ribitylaminouracil) combined with the Toll-like

receptor 9 (TLR9) agonist CpG, induces robust MAIT cell expansion and activation, effectively promoting anti-tumor immunity in various murine tumor models in mice. However, despite its potency, 5-OP-RU is highly unstable, limiting its clinical potential. To address this limitation, in collaboration with CCR's Medicinal Chemistry Accelerator (MCA), we developed a series of novel MAIT agonists with improved chemical stability. These next-generation compounds induce robust MAIT cell activation and cytotoxic reprogramming in vitro and show potent anti-tumor efficacy in vivo in orthotopic HCC models. Importantly, these MAIT agonists exhibit superior chemical stability compared to 5-OP-RU, including prolonged half-life in PBS and human plasma as well as favorable microsomal stability, supporting their potential for therapeutic development.
Together, our findings establish stable next-generation MAIT activators as a promising strategy for harnessing innate-like T cell immunity in cancer and provide a foundation for translational advancement of MAIT-targeted immunotherapy.

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"Neutral Sphingomyelinase 2 Is Required for Activation of the HIV-1 Protease in Virions"

Background
Sphingomyelinases (SMases) are key enzymes that hydrolyze sphingomyelin to generate phosphorylcholine and ceramide. We previously demonstrated (Waheed et al., PNAS 2023;

Yoo et al., PNAS 2023) that disrupting nSMase2 in virus-producing cells blocks HIV-1 Gag and GagPol processing, leading to defects in particle maturation and infectivity. nSMase2 disruption severely impairs the maturation and infectivity of other primate lentiviruses but has little to no effect on non-primate lentiviruses or the gammaretrovirus murine leukemia virus (MLV).

Methods

To define the mechanism by which nSMase2 disruption blocks lentiviral particle maturation, we analyzed chimeras between viruses that are sensitive (HIV-1), or insensitive (MLV), to nSMase2 disruption. We monitored virus assembly, release, maturation, and infectivity using multiple assays. In vitro selection experiments were performed to generate and characterize PDDC-resistant HIV-1. Viral lipid composition was analyzed by lipidomics of gradient-purified particles.

Results and Conclusions

Our analysis revealed that the determinants of sensitivity map to HIV-1 Pol. Consistent with this finding, long-term propagation of HIV-1 in the presence of PDDC in T-cell lines led to resistance mutations in the protease (PR) domain of Pol. We found that when PR was incorporated into HIV-1 virions not as part of GagPol but as a Vpr-PR fusion protein nSMase2 disruption did not block Gag processing. This result indicates that nSMase2 disruption does not alter the Gag lattice in a way that renders it inherently impervious to PR cleavage, but rather that PR must be expressed as part of GagPol for PR activity to be blocked. Furthermore, studies using a Gag-PR-leucine zipper construct suggest that nSMase2 disruption, either via nSMase2 inhibitors or nSMase2-specific siRNA, impairs PR activity. We also identified resistance mutations in the MA and CA domains of HIV-1 Gag. Lipidomics analysis indicates that PDDC treatment of virus-producing cells increases levels of sphingomyelin and reduces levels of ceramide in the viral membrane. Notably, lipid order measurements using the laurdan dye show that nSMase2 disruption significantly reduces lipid order in HIV-1 virions, whereas membrane tension assessed with FlipperTR remains unchanged. Together, these findings demonstrate a critical role for the virion lipid composition in the activation of the HIV-1 protease, providing new insights into the process of retroviral maturation.

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"SP5 Couples Posterior Growth with Extraembryonic Restriction in Human Gastruloids"

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"Peroxiredoxin 1 safeguards the nucleolar genome from oxidative damage"

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STAFF SCIENTISTS AND CLINICIANS IN CAREER TRANSITION

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"Development of Tyrosyl-DNA Phosphodiesterase 1 (TDP1) Inhibitors Using Small Molecule Microarray, Oxime Diversification and Virtual Screening"

Human tyrosyl-DNA phosphodiesterase 1 (TDP1) is a 608 amino acid, 68 kDa polypeptide. Its C-terminal domain belongs to a member of the phospholipase D (PLD) family, which has two conserved HKN motifs (H263/K265/N283 and H493/K495/N516) in close proximity that form its catalytic site within a substrate-binding channel. TDP1 confers resistance to inhibitors of anticancer type I topoisomerase (TOP1) by repairing stalled TOP1-DNA covalent complexes through hydrolysis of the phosphodiester bond between the Y723 residue of TOP1 and a 3'-phosphate of DNA. Although inhibition of TDP1 is known to synergize with TOP1 inhibitors and alkylating agents in cancer therapy, developing potent TDP1 inhibitors has been proven challenging. This difficulty arises from the open, shallow nature of the TDP1 catalytic site, coupled with the need to compete with a large and highly extended substrate. To address these challenges, we combined X-ray crystallographic screening, small molecule microarrays (SMM), and oxime-based diversification to develop a family of phenylimidazopyridine-based TDP1 inhibitors. Crystal structures of our lead inhibitors bound to TDP1 guided the design of bivalent proteolysis-targeting chimera (PROTAC) constituents that incorporate 1,2,3-triazole-containing linkers. By executing a virtual screen of the online DrugBank database using X-ray crystal structures of TDP1-bound phenylimidazopyridine as models, we identified the beta-lactam antibiotic cephalosporin C as a TDP1 inhibitor.

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"Targeting DHX9 helicase exposes a mitotic vulnerability that enhances paclitaxel response in ovarian and endometrial cancers"

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The full abstract can be found in the oral presentation section

17. Yang Jo Chung (chungya@mail.nih.gov), Cancer Genetics Branch, CCR
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"HSC exhaustion and GMP self-renewal leads to MDS"

Myelodysplastic syndrome (MDS) is characterized by ineffective hematopoiesis, suggesting the presence of defective hematopoietic stem and progenitor cells (HSPC). NUP98::HOXD13 (NHD13) transgenic mice recapitulate all the key features of human MDS, including ineffective hematopoiesis, peripheral blood cytopenias, dysplasia, and transformation to acute myeloid leukemia (AML), and have been used as a pre-clinical model for human MDS. NHD13 HSPC, as conventionally defined by a Lineage marker negative, Kit positive, Sca-1 positive (LSK) immunophenotype, are significantly decreased compared with wild-type (WT) mice. Moreover, in contrast to WT bone marrow (BM) cells, lineage positive (Lin+) cells from NHD13 mice have self-renewal potential, as specific subsets of NHD13 Lin+ cells were able to self-renew and generate MDS in WT hematopoietic stem cell transplant (HSCT) recipients. This self-renewing subset was marked by the presence of B220 and Kit cell surface markers could be found in WT as well as NHD13 BM; however, the population was markedly increased in NHD13 BM. Further characterization of the B220+Kit+ (BK) BM cells using myeloid cell surface markers (Mac1 and Gr1) revealed that both Mac1+Gr1+B220+Kit+ and Mac1-Gr1- B220+Kit+ populations showed self-renewal and led to an MDS phenotype in HSCT recipients. Interestingly, single-cell transcriptomic analysis (PIP-Seq) shows most of NHD13 BK cells express genes related to granulocyte-monocyte progenitor (GMP) and stem cells. Taken together, these findings demonstrate that in NHD13 mice normal hematopoiesis becomes extinguished

and committed hematopoietic progenitor cells begin to proliferate and self-renew, leading to initiation of MDS.

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18. Yongzhong Wu (wuy@mail.nih.gov), Developmental Therapeutics Branch, CCR
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"Heat Shock Protein Inhibitors Suppress Cytokine-induced DUOX2 mRNA and Protein Expression in Human Pancreatic Cancer Cells in a JAK-STAT Dependent Manner"

Dual oxidase 2 (DUOX2), one of the seven NADPH oxidases family members, plays a critical role in both host defense and chronic inflammation-associated cancer in the gastrointestinal system. In vitro, pro-inflammatory cytokines, such as IFN- y, IFNβ, IL-4 and IL-17A, enhance DUOX2/DUOXA2 expression through activation of STATs and NF-kB signaling pathway proteins; in vivo, DUOX protein and mRNA levels are substantially upregulated in chronic pancreatitis, pre-malignant pancreatic intraepithelial neoplasms and the early stages of pancreatic cancer patients compared to histologically normal pancreatic tissues. In pancreatic adenocarcinoma, increased DUOX2 expression is adversely correlated with overall patient survival. Heat-Shock Protein 90 (Hsp90), an important molecular chaperone involved in tumorigenesis, invasion and metastasis of cancer cells, is critical in folding, maturation and stability of many oncogenic client proteins, including kinases such as AKT and JAK1/2, and transcription factors, such as STAT3 and HIF-1α. Several STAT family members, along with Hsp90, are overexpressed in human pancreatic carcinomas. Using a panel of human pancreatic cancer cell lines (BxPC-3, AsPC-1 and CFPAC-1), we found that two different Hsp90 inhibitors, Tanespimycin (17-AAG) and Ganetespib (STA-9090), inhibit JAK1 and JAK2 kinases, blocking cytokine-induced, JAK-regulated STAT phosphorylation. Additionally, these Hsp90 inhibitors suppress cytokine-induced DUOX2, VEGF-A, MMP-7 and PD-L1 expression in human pancreatic cancer cell lines with varying sensitivity. Furthermore, the JAK1/2 inhibitor Ruxolitinib inhibits IL-4 induced and JAK-mediated STAT6 phosphorylation, and DUOX2 mRNA and protein expression in BxPC-3 cells. Similar results were observed with JAK1, STAT1, 2 and STAT6 specific siRNA knockdown. However, simultaneous knockdown of both isoforms of Hsp90, Hsp90α. and Hsp9β, with specific siRNA did not inhibit JAK1/2 inhibitor Ruxolitinib inhibits IL-4 induced and JAK-mediated STAT6 phosphorylation, and DUOX2 mRNA and protein expression in BxPC-3 cells. Similar results were observed with

JAK1, STAT1, 2 and STAT6 specific siRNA knockdown. However, simultaneous knockdown of both isoforms of Hsp90, Hsp90α. and Hsp9β, with specific siRNA did not inhibit JAK1 activity, cytokine-induced DUOX2 mRNA or protein expression. Either remaining Hsp90 protein or other isoforms of Hsp90 in cells may compensate decreased Hsp90 function after siRNA knockdown. Our data suggests that Hsp90 inhibitors, through blocking the cytokine-activated JAK-STATs oncogenic signaling pathway and their downstream genes

such as DUOX2, VEGF-A, MMP-7 and PD-L1expression, may be a valuable therapeutic approach for inflammation-associated pancreatic cancer.

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“Mutant p53beta restores tumor suppressive functions to mutant p53 and delays glioblastoma tumor growth in vitro and in vivo”

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69. Dan Cheng (dan.cheng@nih.gov), Laboratory of Genome Integrity, CCR
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“TAF7 Accumulates in the Cytoplasm During Cellular Transformation and Engages STAT3, WASH, and CCT”

Background & Hypotheses
TONSL and its heterodimeric partner MMS22L are evolutionarily conserved factors in metazoans with presumed functions in S-phase. Familial and somatic mutations in TONSL have been identified in human developmental abnormalities and a variety of cancers, though their biological significance have not been clearly defined. Previous studies using RNA interference (RNAi) have repeatedly implicated the TONSL-MMS22L complex as a facilitator of homologous recombination (HR) whose function may be especially important under conditions where the canonical RAD51 loader BRCA2 is defective. Yet, can a backup role in HR really be the main function of the TONSL-MMS22L complex, when loss of either

factor imparts cellular lethality even in a BRCA2-proficient background? Study Design & Methods: To address this question, and to circumvent potential issues associated with traditional RNAi methodology such as lack of temporal resolution and/or insufficient knockdown efficiency, we developed a series of PROTAC-based cellular models in which endogenous TONSL or MMS22L can be rapidly degraded in an inducible manner. We then proceeded to investigate systematically how controlled loss of the TONSL-MMS22L complex impacts cell fate using a series of cutting-edge imaging- and sequencing-based techniques.

Results & Conclusions
Our analyses reveal that acute degradation of either TONSL or MMS22L in otherwise unchallenged cells leads to immediate and profound perturbations in replication fork progression that prevent complete duplication of chromosomes in the first S-phase. Consistent with previous studies implicating TONSL as a putative histone chaperone, preliminary evidence suggests that aberrant replication-coupled histone deposition could be a primary driver of these S-phase defects. Interestingly, TONSL- or MMS22L-degraded cells then undergo a protracted arrest in a state resembling G2, as evidenced by strong enrichment of the G2 marker CENPF. In this G2-like state, TONSL- or MMS22L-degraded cells attempt to slowly fill in their under-replicated genomes, which are also fragile and prone to breakage. TONSL- or MMS22L-degraded cells eventually escape G2, possibly through checkpoint adaptation, with still incompletely replicated genomes and unrepaired DNA double strand breaks, which in turn trigger prolonged mitotic arrest culminating in cohesion fatigue, catastrophic chromatid separation and cell death. Though certain mechanistic details remain to be elucidated, our discovery of TONSL-MMS22L as a key regulator of DNA replication provides a likely explanation for why both are highly essential proteins. We propose that TONSL-MMS22L promotes genome stability by coupling DNA synthesis to histone deposition, thereby ensuring timely execution of the replication program within S-phase.

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"A Multi-Omics Approach to Understanding Wip1/p53 Mediated Effects in Cancer"

The tumor suppressor p53 is a master regulator of cellular stress responses, orchestrating DNA repair, apoptosis, cell cycle arrest, and metabolic adaptation in both normal physiology and cancer. In contrast, Wip1 (PPM1D), a serine/threonine phosphatase identified nearly three decades ago, is primarily characterized as a negative regulator of p53 through dephosphorylation. Despite this well-established interaction, the broader biological functions of Wip1 remain poorly defined. Increasing evidence suggests that Wip1 exerts p53-independent effects, including roles in neurodevelopment and inflammatory signaling, indicating that it may function as a broader regulatory node in cellular homeostasis.

We hypothesize that Wip1 controls distinct regulatory programs beyond its canonical suppression of p53 and that simultaneous loss of p53 and Wip1 produces non-linear, compensatory rewiring of stress-response pathways. We further propose that integrated multi-omics profiling will uncover genotype-driven regulatory networks that are not apparent from single-pathway analyses.

We generated a panel of CRISPR-engineered knockout U2OS cell lines comprising p53 knockout, Wip1 knockout, double knockout, and wild-type controls. Cells were analyzed under basal conditions and following irradiation-induced stress to activate canonical DNA damage pathways.

To define regulatory programs at multiple molecular levels, we implemented an integrated multi-omics approach combining global proteomics, RNA sequencing (RNA-seq), and metabolomics, complemented by functional assays assessing proliferation, cell cycle progression, apoptosis, metabolic flux, lactate secretion, and pathway-specific protein expression by Western blot. Principal component analysis (PCA) and pathway enrichment analyses were used to delineate genotype- versus stress-dependent effects.

Transcriptomic and proteomic analyses revealed a striking and reproducible finding: cellular genotype exerted a greater influence on global molecular profiles than irradiation-induced stress. PCA demonstrated robust clustering by genotype, with the presence or absence of Wip1 emerging as a dominant determinant of separation. This pattern was especially pronounced in RNA-seq data and consistently observed in proteomic datasets. Functional assays mirrored these molecular trends. Single knockouts displayed amplified responses to stress relative to wild-type cells, whereas double-knockout cells partially reverted toward wild-type phenotypes across multiple readouts. These results suggest compensatory network rewiring when both regulators are absent, supporting a model in which Wip1 functions as more than a simple p53 antagonist.
Collectively, our findings position Wip1 as a critical determinant of genotype-driven cellular identity and reveal previously unappreciated complexity in p53â€"Wip1 regulatory interplay. Ongoing metabolomic integration will further define how these molecular shifts

translate into metabolic adaptation and may expose novel therapeutic vulnerabilities in cancers characterized by dysregulation of this axis.

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"Optimizing Potent SARS-CoV-2 Mpro Inhibitors: Effects of Halogen Substitution on Binding Affinity and Cellular Uptake"

The SARS-CoV-2 main protease (Mpro) remains a key antiviral target because its inhibition blocks viral replication. To understand how subtle atomic changes influence drug performance, we performed a systematic halogen scan on a potent ketoamide scaffold, replacing fluorine (F) with chlorine (Cl), bromine (Br), or iodine (I). Biochemical assays showed that the F- and Cl-substituted analogues inhibit Mpro with nanomolar potency, whereas Br- and I-containing compounds are 10-20-fold less active. Cell-based antiviral assays followed the same trend, while uptake studies revealed increased intracellular accumulation with I substitution. High-resolution X-ray crystal structures (1.6-1.8 Ã) explain this dichotomy: smaller halogens preserve optimal binding interactions in the S1' pocket. In contrast, larger halogens perturb local interactions but enhance lipophilicity and cellular permeability. Together, these findings reveal a fluorine-to-iodine structure-activity relationship for SARS-CoV-2 Mpro inhibitors and inform the rational design of next-generation antiviral therapeutics.

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"Synergistic IL-1 beta/IL-6 Signaling Drives DUOX2/DUOXA2 Complex Upregulation through JAK1/STAT1/STAT3 Activation"

Background and Hypothesis
Chronic inflammation increases colonic susceptibility to neoplasia by establishing a pro-tumorigenic inflammatory microenvironment characterized by persistent production of reactive oxygen species (ROS) and consequent genomic instability. Interleukin-1 beta (IL-1 beta), a key pro-inflammatory cytokine, plays a central role in both acute and chronic intestinal inflammation through regulation of innate and adaptive immune responses. In the colon tumor microenvironment, IL-1 beta promotes IL-17A accumulation via recruitment and expansion of lymphoid and Th17 cells, thereby amplifying inflammation and correlating with poor clinical outcomes. Elucidating the IL-1 beta-mediated signaling pathways that drive colon tumorigenesis, including mechanisms underlying angiogenesis and metastasis, may identify therapeutic targets that limit tumor progression while preserving intestinal immune homeostasis.

Study Design and Methods
Our laboratory previously demonstrated that IL-4 and IL-17A induce DUOX2/DUOXA2 mRNA and protein expression, resulting in increased oxidative stress and DNA damage in colon cancer cell lines. Extending these findings, we show that IL-1 beta, in cooperation with IL-6, markedly upregulates a hydrogen peroxide-producing DUOX2 enzyme complex (as measured by Amplex Red oxidation) in HT-29, Ls513, T84, and Colo205 human colon cancer cells. Key signaling intermediates mediating this induction were delineated using monoclonal antibody antagonists (anakinra and tocilizumab), as well as siRNA-based approaches. Targeted knockdown of IL-1 beta and IL-6 pathway components, including MYD88, IRAK1, JAK1, STAT1, STAT3, and SOCS3, identified critical regulators of DUOX2 expression. Chromatin immunoprecipitation assays were optimized to confirm STAT1 and STAT3 binding to putative regulatory elements within the DUOX2 promoter.

Results and Conclusions

IL-1 beta, in combination with IL-6, drives robust oxidant production through synergistic

upregulation of DUOX2 protein expression. Increased expression of the hydrogen peroxide-generating DUOX2/DUOXA2 complex was associated with enhanced Î^{3}H2AX formation, indicating accumulation of DNA double-strand breaks and linking inflammatory cytokine signaling directly to genomic instability in colon cancer cells. Pharmacologic blockade of IL-1R with anakinra and IL-6R with tocilizumab confirmed that this pro-oxidant program is dependent on coordinated IL-1 and IL-6 receptor signaling, even in cell lines with relatively low receptor expression. Disruption of key downstream mediators (MYD88, IRAK1, JAK1, STAT1, and STAT3) significantly attenuated DUOX2 induction, identifying actionable signaling nodes within this inflammatory axis. Importantly, analysis of surgically resected human colon cancers revealed elevated DUOX2, DUOXA2, and IL-1 expression compared with matched normal epithelium, supporting the clinical relevance of this pathway. Ongoing studies aimed at defining cytokine-responsive regulatory elements within the DUOX2 promoter may further inform strategies to therapeutically target inflammation-driven oxidative stress while preserving essential mucosal immune function.

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"Generation of novel anti-tumor chimeric antigen receptors incorporating downstream T-cell signaling proteins targeting GPC3 on Hepatocellular Carcinoma Cells"

Chimeric antigen receptors (CAR) are molecules with an antibody-derived extracellular domain combined with intracellular domains from T cell receptor (TCR) signaling proteins. Although the use of CAR-T cells has been a clinical breakthrough, several challenges persist: adverse effects from cytokine secretion, CAR-T cell exhaustion, and limited sensitivity in tumor microenvironments due to low density of target antigen. While many efforts have focused on identifying new cell surface targets, we designed novel Chimeric

Adapter Proteins (CAPs) to trigger signaling downstream of the TCR-zeta chain which might bypass kinetic proofreading steps defining the signaling threshold at the TCR and the inhibitory regulation of upstream molecules. CAPs replaced the TCR-zeta in CAR with adapter proteins fused to ZAP70 intracellularly and are combined with an extracellular targeting domain. Importantly, CAP-Ts expressing adapter moieties promoted high levels of antigen-independent signaling. CAPs that exclusively contained ZAP70 domains exhibited antigen-dependent signaling and were further developed. These constructs were coined CAR-tyrosine kinases (CAR-TKs). Indeed, CAR-TKs exhibited high anti-tumor efficacy, and significantly enhanced long-term in vivo persistence of tumor clearance in leukemia-bearing NSG mice as compared with conventional CD19-28-zeta CARs. These findings are promising, and we are now interested in further understanding the mechanism of superior CAP performance and evaluating their efficacy in clearing solid tumors.

We next evaluated CAR-TKs in a Hepatocellular Carcinoma (HCC) CAR model that targets the glypican GPC3 on the cell surface since it is overexpressed on HCC cells and has little to no expression on normal tissues. CAR-Ts with a hYP7 antibody targeting GPC3 have been shown to have excellent efficacy in clearing HCC in murine models and are currently being tested in the clinic. We designed a panel of CAR-TKs targeting GPC3 using extracellular hYP7 and intracellular ZAP70. Our panel was based on CAR-TKs that showed best efficacy in the leukemia model, as well as modeling predictions that would produce optimal signaling. From in vitro screening we picked the most promising CAR-TKs for in vivo evaluation. In an HCC xenograft model, two out of three CAR-TKs tested cleared tumors, albeit with delayed kinetics, while exhibiting reduced toxicity. This balance of safety and efficacy suggests clinical potential. Ongoing studies are assessing the mechanistic basis of CAR-TK activity and expanding evaluation of CAR-TK efficacy in additional solid tumor types.

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"Preclinical strategies in enhancing sensitivity to KIF18A inhibitors in ovarian cancer"

High-grade serous ovarian cancer (HGSOC) is the most common (~80%) and lethal subtype of ovarian cancer in the United States. HGSOC is characterized by universal TP53

mutations, genomic instability, and defects in DNA damage repair (DDR) pathways, which often lead to chromosomal instability (CIN). The cytoskeletal motor protein KIF18A is essential for chromosomal congression and segregation during metaphase in CIN+ cancer cells but not necessary in normal cells. We recently demonstrated that a novel KIF18A inhibitor, ATX020 (tool compound), specifically inhibits cell growth in HGSOC cells with higher ploidy and aneuploidy scores (AS), which are markers of CIN (Nair et al, Cancers, 2025). It does this by blocking the plus-end movement of KIF18A from spindle poles and disrupting chromosomal congression and segregation. In that study, we also observed increased WEE1 activity in resistant cells treated with ATX020, as indicated by higher levels of its phosphorylated substrate CDK1 (pCDK1-Y15), a key component of the G2/M cell cycle checkpoint. WEE1 inhibitors can make cells more sensitive to DNA-damaging agents by overriding the G2/M checkpoint and forcing cells with unrepaired DNA damage into premature mitosis, leading to increased DNA damage and CIN. Furthermore, recent RNAseq analysis shows enrichment of pathways involved in regulating the G2/M checkpoint and DNA damage repair in cells treated with ATX020 cells. We hypothesized that targeting WEE1 could enhance sensitivity of both resistant and sensitive cells to ATX020.

We used ATX020 resistant (A2780, PEO4, and OVCAR5) and sensitive (OVCAR3, OVCAR8, and PEO1) cell lines to examine the effects of an WEE1 inhibitor, AZD1775 (Adavosertib), on inducing CIN and thus sensitivity to ATX020. Using multi-well growth inhibition assays, immunofluorescent microscopy, live cell imaging, westerns and flow cytometry, we show that WEE1 inhibition made both resistant and sensitive HGSOC cells more responsive to ATX020, while also increasing DNA damage, prolonging/disrupting mitotic process and causing proliferative arrest. Transcriptomic analysis (bulk RNAseq) and differential gene expression (DEG) analysis show consistent upregulation of target genes and associated pro-inflammatory pathways, TNF signaling, and epithelial-mesenchymal transition in both sensitive and resistant cells when treated with ATX020. This uncovers potential targets that could improve the efficacy of ATX020 in preclinical models. Furthermore, murine models of both resistant and sensitive HGSOC cells treated with ATX020 exhibit tumor growth inhibition that is consistent with the in vitro results. These findings suggest new research approaches that could potentially enhance the impact of KIF18A inhibition in a clinical setting.

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“Development of Naphthyridine-Based HIV-1 Integrase Strand Transfer Inhibitors”

HIV-1 integrase (IN) is a key retroviral enzyme that inserts reverse-transcribed viral DNA (vDNA) into the host genome via two sequential steps. The initial 3'-processing (3'-P) step removes two nucleotides from the 3' ends of vDNA and requires two Mg^{2+} cations coordinated by a conserved DDE motif (Asp64, Asp116, and Glu152). This reaction generates a 3'-hydroxyl group on the terminal deoxyadenosine (dA), which is essential for strand transfer. During this step, the 3'-hydroxyl of vDNA attacks the phosphate backbone of target DNA (tDNA), resulting in covalent integration. Integrase strand transfer inhibitors (INSTIs) block this process by targeting the intasome, a nucleoprotein complex of IN and vDNA. Our multi-year effort to develop INSTIs effective against resistant IN variants has focused on a metal-chelating naphthyridine scaffold. These compounds show potent inhibition in biochemical assays and strong antiviral activity in cell culture against major drug-resistant mutants. Structural studies, including PFV intasome crystal structures and HIV-1 intasome cryo-EM structures, reveal that 6-substituents mimic interactions of viral and target DNA in the strand transfer complex. Notably, functionalization at the 4- and 5-positions enhances π–π stacking with the 3' nucleobase, improving antiviral activity. These insights inform the design of next-generation INSTIs to overcome HIV-1 resistance.

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“*Extrachromosomal DNA (ecDNA) is linked to chromosomal de-regulation of transposon stress responses, particularly LINE-1 (L1) elements”*

Cancer cells progress from normal to precancerous and ultimately to malignant states, raising the question of whether chromosomal status can be monitored across these stages.

We developed a method to map DNA breaks using next-generation sequencing. This is the only method of sensitivity and precision, enabling detection of both single-strand DNA nicks and double-strand breaks with recessed 3'-OH or blunt ends.

Using this method, we evaluated the effects of etoposide, a topoisomerase II inhibitor, in Colo320DM and Colo320HSR cells. We observed significant changes in the responses of multiple L1M and L1P elements. When HEK293 and HCT116 cells were included for comparison, a clear trend emerged: HEK293 → HCT116 → Colo320HSR → Colo320DM

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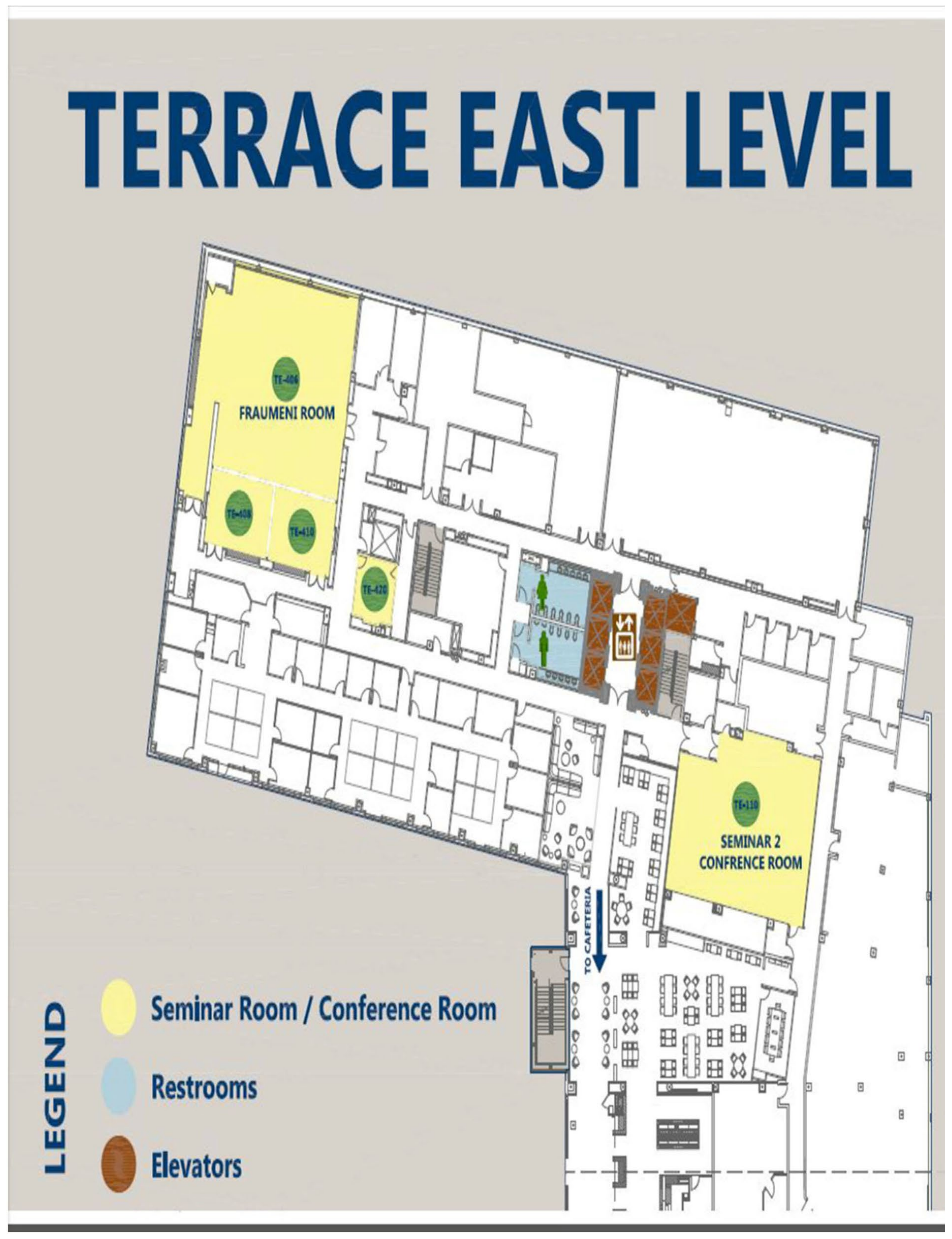
TERRACE EAST LEVEL
TE-406
FRAUMENI ROOM
TE-408
TE-410
TE-420
TE-110
SEMINAR 2
CONFRENCE ROOM
TO CAFETERIA
LEGEND
Seminar Room / Conference Room
Restrooms
Elevators

Food Options Inside the Shady Grove NCI Building:

First Floor

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

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

22nd Annual CCR and DCEG
Staff Scientists and Staff Clinicians
Retreat
May 8, 2026