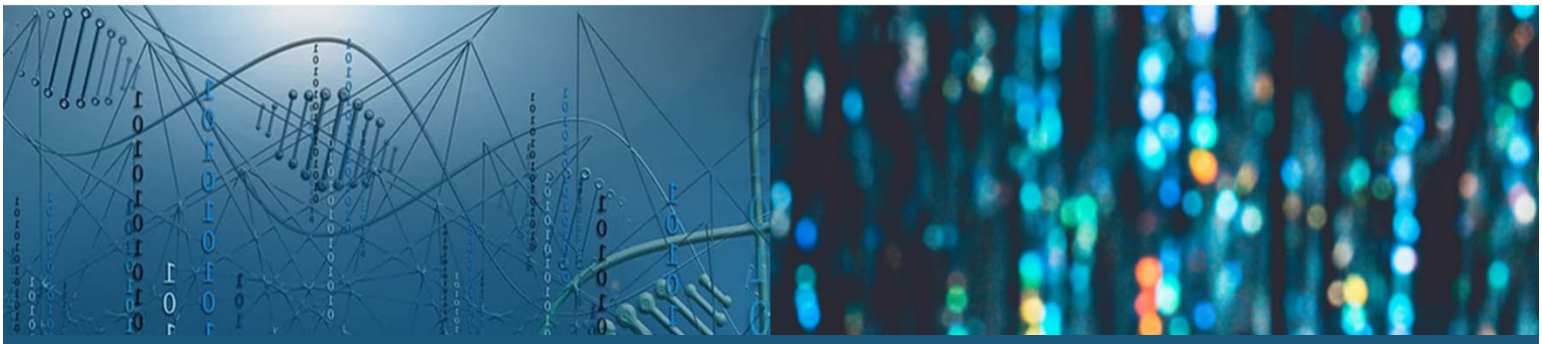
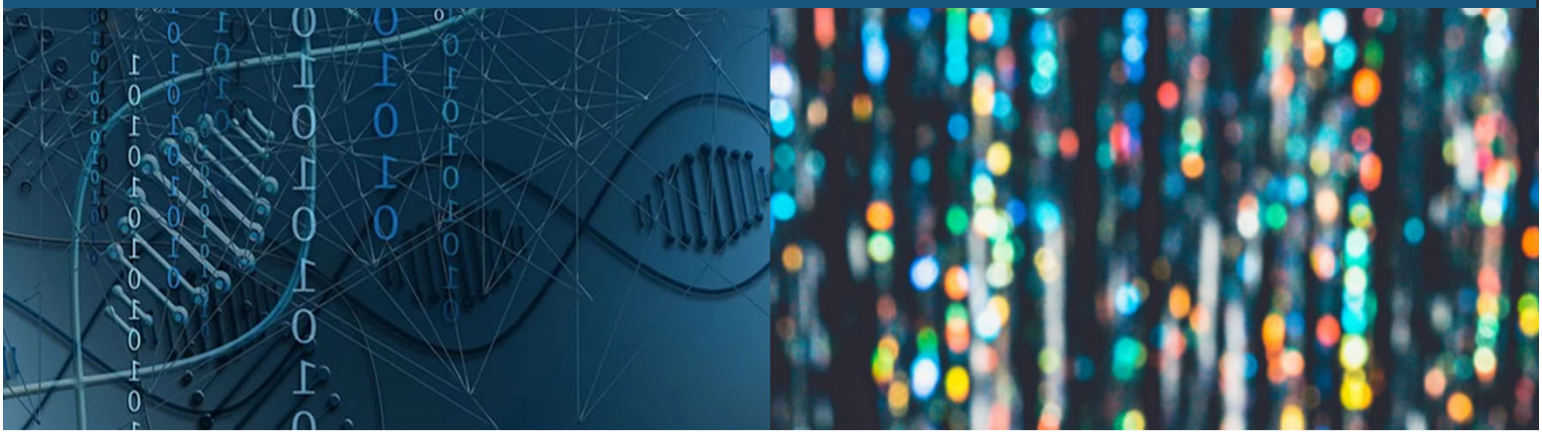
21st Annual CCR and DCEG Staff Scientists and Staff Clinicians Retreat



Bridging the Gap Between Basic and Clinical Cancer Research in the Era of Digital Transformation

April18th, 2025, NCI Shady Grove, Rockville, MD 20850



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21st Annual CCR and DCEG Staff Scientists and Staff Clinicians Retreat

Welcome Retreat Participants! On behalf of the Organizing Committee, it is our pleasure to welcome you to the 21st Annual Staff Scientist and Staff Clinician (SSSC) Retreat of the National Cancer Institute's Center for Cancer Research (CCR), Division of Cancer Epidemiology and Genetics (DCEG), and SSSC core services.

The theme for this year's SSSC retreat is, **"Bridging the Gap Between Basic and Clinical Cancer Research in The Era of Digital Transformation".** We are living in the era of digital transformation that is changing at an amazing pace while becoming an integral part of basic and translational cancer research. NCI intramural research has been at the forefront of developing and employing modern technologies to alleviate human suffering from cancer, and the SSSC community has been the backbone of such research developments. Digital innovation in combination with traditional and translational biomedical approaches has ushered in a new era of efficient, and cost-effective strategies that have exponential potential to transform future cancer treatments, drug discovery and efficacy, precision medicine, diagnostics, preventive measures, etc. We are gathered today to discuss how we can leverage digital technology to drive new types of data generation and biological analyses, to integrate basic, clinical, and epidemiologic findings to better understand fundamental biological processes and how their defects could lead to cancer, to overcome translational difficulties, standardization and reproducibility issues and thus narrow the gap between basic and clinical research. This could lead to a better understanding of the big picture of cancer and successfully advance NCI's mission and vision.

Today's events emphasize the astonishing research conducted by the resolute Staff Scientists and Staff Clinicians within the CCR/DCEG and core services. We are thrilled that eighty-four posters will be featured and eight short talks selected from the top-ranking abstracts will be presented. This year's retreat features four eminent speakers, whose work embody the retreat's theme. We are fortunate that the Opening Remarks will be delivered by Dr. Douglas Lowy, M. D., the Principal Deputy Director of NCI. As the preeminent expert on basic and translational aspects of human papillomavirus infection and a leader of the nation's efforts against cancer, his presence at the retreat will be inspirational. This year's retreat will include two keynote addresses from world renowned intramural scientists, Dr. Ramaprasad Srinivasan, M.D., Ph.D. and Dr. Eytan Ruppin, M.D., Ph.D., who have significantly overcome gaps between basic and translational sciences in their research accomplishments. Dr. Srinivasan is internationally recognized for novel treatment strategies for patients with kidney cancer and for developing personalized FDA approved medicines like Belzutifan for management of von Hippel Lindau disease. Dr Ruppin is a world leader in the field of AI and machine / deep learning and systems biology. He has pioneered the development and testing of bulk-expression and single cell precision oncology approaches and the computational study of immunotherapy by developing tools for transcriptomics-based cell-type specific characterization of the tumor microenvironment. The Closing Remarks and a talk will be delivered by Dr. Sharon Savage, M. D., Clinical Director, DCEG. Her comprehensive approach combining clinical, genetic, molecular and epidemiologic studies to improve understanding of cancer etiology and the lives of patients with cancer-prone disorders epitomizes the retreat theme. We deeply value the continued support of the NCI/CCR/DCEG leadership to the SSSC community.

The Organizing Committee highly appreciates the guidance and support from Dr. Oliver Bogler, Director of Center for Cancer Training (CCT), Dr. Chanelle Case Borden, Branch Director, Office of Training and Education, and the invaluable help from Ms. Maria Moten, Program Specialist, CCT, Ms. Angela Jones, Lead Program Analyst, CCT, and the web team.

We hope that our 21st Annual Staff Scientist and Staff Clinician Retreat will inspire digital innovation and bridge basic, and clinical studies via collaborative efforts in our fight against cancer.

Best regards and many thanks for your participation,

Dr. Sukhbir Kaur and Dr. Usha Acharya
(Co-Chairs 2025 NCI SSSC Retreat Organizing Committee)

SSSC Retreat Organizing Committee Members 2024-2025

Abdul Waheed, Ph.D.
Acong Yang, Ph.D.
Balamurugan Kuppusamy, Ph.D.
Brajendra Tripathi, Ph.D.
Brian Ko, Ph.D.
Chih-Shia Lee, Ph.D.
Daniël Melters, Ph.D.
Daniel Russ, Ph.D.
Dong Kong, Ph.D.
Duane Hamilton, Ph.D.
Govind Kunduri, Ph.D.
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Jibran Ahmed, Ph.D.
Kellsye Fabian, Ph.D.
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Sofia Gameiro, Ph.D.
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Sukhbir Kaur, Ph.D.
Tiaojiang Xiao, Ph.D.
Usha Acharya, Ph.D.
Yanlin Yu, Ph.D.

BRIDGING THE GAP BETWEEN BASIC AND CLINICAL CANCER RESEARCH IN THE ERA OF DIGITAL TRANSFORMATION

AGENDA

Location: NCI Shady Grove Campus
9609 Medical Center Drive
Rockville, MD 20850

Date: April 18, 2025
Time: 9:00 AM-5:00 PM
Venue *Conf. Rm TE406/408/410*

Agenda Items

8:45-9:00 a.m.	Registration and poster setup	Front of TE406/408
9:00-9:05 a.m.	Welcome by Dr. Sukhbir Kaur	
9.05-9.25 a.m.	Opening Remarks by Dr. Douglas Lowy, NCI Principal Deputy Director	
9:25-10:05 a.m.	Keynote Address I Dr. Ramaprasad Srinivasan (30-minute talk + 10-minute Q&A) "Development of pathway directed targeted therapies - Learning from inherited forms of kidney cancer"	Dr. Sukhbir Kaur (Moderator)
10:05-10:15 a.m.	Coffee Break	
10:15-10:25 a.m.	Introduction of Staff Scientist Organization (Dr. Nicolas Cuburu, Dr. Lisa Jenkins, Dr. Ravi Chalamalasetty, Dr. Xuezhi Zhao, Dr. Esther Mena Gonzales)	SSSC Co-chairs

10:25-11:25 a.m.	Oral Presentation Session I: 4 talks (10-minute talk + 3-minute Q&A) Dr. Dong Kong - Dissecting the dynamics of human centriole assembly by correlative cell cycle-centriole cycle ultrastructural analysis Dr. Sigrid Dubois – Engineering of Natural killer cells to express a tumor-targeting T-cell receptor and membrane-tethered IL-15/IL-21 improves anti-tumor activity in solid tumors Dr. Sabina Kaczanowska – Development of Human Genetically Engineered Myeloid Cells (GEMys) for first-in-human clinical trial Dr. Brajendra K. Tripathi – Identification of new drug combinations that cooperatively target KRAS-dependent nuclear protein export to treat mutant KRAS-driven pancreatic adenocarcinoma	Dr. Balamurugan Kuppusamy (Moderator)
11:25-12:25 p.m.	Poster Session I	Odd numbered abstracts
12:25-1:25 p.m.	Lunch & Networking	Lunch on your own
1:25-2:25 p.m.	Poster Session II	Even numbered abstracts
2:25-3.05 p.m.	Keynote Address II Dr. Eytan Ruppin (30-minute talk + 10-minute Q&A) "Towards fast and accessible precision oncology"	Dr. Daniel Russ (Moderator)
3.05-3:15 p.m.	Coffee Break	

3:15-4.15 p.m.	Oral Presentation Session II 4 talks (10-minute talk + 3-minute Q&A) Dr. Adrian Lita – Raman-based machine-learning platform reveals unique metabolic differences between IDH mutant and IDH wt glioma Dr. Julio C. Valencia – IL27 modulates a dysfunctional molecular IFN signature to drive progression of lupus nephritis Dr. Wei Zhang – Single cell RNA sequencing unveils the altered landscape of the blood-brain barrier in breast cancer brain metastases Dr. Oscar Florez-Vargas – Genetic regulation of TERT splicing affects cancer risk by altering cellular longevity and replicative potential	Dr. Brajendra Tripathi (Moderator)
4:15-4:45 p.m.	Talk & Closing Remarks: Dr. Sharon Savage (25-minute talk + 5-minute closing remarks) "The Long and Short of Telomere Biology in Disease Etiology"	Dr. Monica Sierra (Moderator)
4:45-4:55 p.m.	Presentation of awards by Dr. Sharon Savage	Dr. Lucas Horn (Moderator)
4:55-5:00 p.m.	Acknowledgements by Dr. Usha Acharya	

INVITED SPEAKERS

Biographies of Invited Speakers

Dr. Douglas R. Lowy M. D. Principal Deputy Director, NCI

Dr. Lowy is the Chief of the Laboratory of Cellular Oncology and a NIH distinguished investigator. He has previously served as acting director of NCI. He received his medical degree from the New York University School of Medicine and trained in internal medicine at Stanford University and dermatology at Yale University. He has directed a research laboratory at NCI since 1975, after receiving training as a Research Associate in the National Institute of Allergy and Infectious Diseases. Dr. Lowy is well known for his research on papillomaviruses. In collaboration with Dr. John Schiller, he has identified many aspects of the human papillomavirus (HPV) life cycle, developed technology underlying the FDA-approved HPV vaccines, and elucidated mechanisms for the high efficacy of the vaccines. Dr. Lowy's laboratory has also investigated the function of signaling molecules shown to be influential in the regulation of neoplastic growth, centering on defining the function of Ras oncogenes mutated in more than 25% of human cancers. More recently, he has studied the molecular aspects of the tumor suppressor gene, DLC1, which is frequently deleted or downregulated in a wide variety of cancers. This research has identified important scaffold functions for DLC1, including protein-protein interactions that contribute to the regulation of its activity and its role as a tumor suppressor, and identified kinases that activate and inactivate the functions of DLC1 and the mechanisms by which they do so. As an NCI leader, Dr. Lowy has supported NCI-Designated Cancer Centers, increased investment in health disparities and pediatric oncology research, tirelessly championed investigator-initiated research and led key initiatives including the Precision Medicine Initiative and Cancer Moonshot[SM]. Dr. Lowy is a member of the National Academy of Sciences and the National Academy of Medicine. Dr. Lowy has received several awards including the Samuel J. Heyman Service to America Medal (2007), the Albert B. Sabine Gold Medal (2012), the National Medal of Technology and Innovation (2014), the Lasker-DeBakey Clinical Medical Research Award (2017), Szent Györgyi Prize for Progress in Cancer Research (2018) and the Prince Mahidol Award in the field of Public Health (2022).

Dr. Ramaprasad Srinivasan M. D., Ph.D. Deputy Director, CCR, NCI

Dr. Srinivasan is a senior investigator in the Urologic Oncology Branch, NCI. He obtained his medical degree from the Bangalore Medical College, India. Dr. Srinivasan subsequently obtained a Ph.D. at the University of Texas MD Anderson Cancer Center. He came to the NCI in 1999 after completing his Internal Medicine Residency training at the University of Texas Health Science Center-Houston, to pursue a fellowship in Medical Oncology/Hematology. He subsequently joined the Urologic Oncology Branch, where he is developing precision, targeted treatment strategies and conducting clinical trials for patients with both hereditary and non-hereditary forms of kidney cancer. His research focus includes the preclinical and clinical development of novel targeted agents in clear cell and papillary kidney cancer, as well as hereditary kidney cancer syndromes such as von Hippel-Lindau (VHL), Hereditary Leiomyomatosis and renal cell cancer (HLRCC) and hereditary papillary renal cell cancer (HPRC). Dr. Srinivasan's work is focused on developing and evaluating individualized, mechanism-based proof-of-concept studies based on the recognition that there are inherent genetic and

molecular differences between various subtype of kidney cancer. He has pioneered the evaluation of systemic treatment strategies in patients with VHL and co-led an international study that led to the FDA approval of the HIF2a inhibitor, Belzutifan, heralding a paradigm shift in the management of VHL. His work has also led to the development of a new standard of care in patients with metastatic kidney cancer associated with Hereditary Leiomyomatosis and HLRCC. Dr. Srinivasan serves on numerous NIH and extramural committees and on the editorial board of several journals. He is an investigator on numerous clinical trials and has authored over a hundred scientific papers and book chapters. He is the recipient of two NCI Director's Awards, recognizing his contributions to the development of new treatments for patients with kidney cancer, and the Alan S. Rabson Award (2024) for scientific and medical excellence and the outstanding, compassionate care for patients and those who love them.

Dr. Eytan Ruppin M. D., Ph.D. Chief, Cancer Data Science Laboratory, NCI

Dr. Ruppin received his M.D. and Ph.D. (Computer Science) from Tel-Aviv University. He has served as a professor of Computer Science & Medicine in Tel-Aviv University and as a Computer Science professor and director of the center for bioinformatics and computational biology at the University of Maryland. Dr. Ruppin joined NCI in 2018 where he founded and is chief of its Cancer Data Science laboratory. His research is focused on multi-disciplinary algorithmic computationally driven analysis of large-scale biomedical omics and clinical data, with an emphasis on AI and machine / deep learning approaches. Overall, it is centered on three main aims: (1) development of next generation precision oncology approaches, (2) the computational study of immunotherapy response and (3) collaborative multiomics studies of cancer development and treatment. His group has developed *SELECT* and *ENLIGHT* to identify synthetic lethality vulnerabilities as a new way for stratifying patients to treatment from the transcriptome of a patient's tumor and are in the final approval steps for starting a prospective clinical trial study focused initially on breast cancer. His group has developed a new computational approach called *PERCEPTION* that builds treatment response models based on patients' single-cell (SC) tumor transcriptomics. The group's computational study of immunotherapy has led to the development of *CODEFACS*, a tool for deconvolving cell type–specific gene expression in each individual tumor sample from its bulk expression, and *LIRICS*, a statistical framework prioritizing clinically relevant ligand–receptors. Dr. Ruppin has collaborated with over 40 different national and international groups on multiomics studies of cancer to extend lab and animal model findings via complementary analyses of patients' data.

Dr. Ruppin is a fellow of the International Society for Computational Biology (ISCB), a recipient of the NCI Director Award for his work on precision oncology (2022), the DeLano Award for computational biosciences for his work on synthetic lethality (2023) and the NIH director Award for developing new computational paradigms for precision oncology (2024). He is a member of GSK Oncology, Pangea Biomed, Win Consortium and ProCan scientific advisory boards and a co-founder of several precision medicine and cancer discovery startup companies.

Dr. Sharon A. Savage M. D. Clinical Director, DCEG, NCI

Dr. Savage earned her M.D. from the University of Vermont College of Medicine. She completed residency training in Pediatrics at Children's National Medical Center, in Washington D.C., and a fellowship in Pediatric Hematology/Oncology at the NCI Pediatric Oncology Branch and Johns Hopkins University. She is board-certified in both Pediatrics and Pediatric Hematology-Oncology. Dr. Savage joined the Clinical Genetics Branch, DCEG in 2006 as a tenure-track investigator and was appointed as senior investigator in 2012. In 2013, she was promoted to Director, CGB, and she became the Clinical Director for DCEG in 2018. Dr. Savage leads clinical, genetic, and epidemiologic studies of individuals and families at high risk of cancer. Her comprehensive approach combines genomics with clinical genetics and molecular biology to improve understanding of cancer etiology and the lives of patients with complex cancer-prone disorders. Dr. Savage's internationally recognized research in dyskeratosis congenita (DC) and telomere biology is leading the way in understanding the consequences of aberrations in telomere biology and cancer etiology. Her expertise in the clinical aspects of DC has defined, in detail, its complex dermatologic, neurologic, pulmonary, and vascular complications manifestations. Her multi-disciplinary collaborative approach led to the NCI intramural program's first clinical and genetic study of Li-Fraumeni syndrome (LFS) since the mid-1980s. Dr. Savage and her colleagues have developed a cancer screening program for carriers of *TP53* mutation, the primary cause of LFS and assisted in the creation of an international LFS research consortium. Dr. Savage received an NIH Director's Award (2022), she has been recognized with the Distinguished Academic Achievement Award from UVM Larner College of Medicine and the Robert H. Goddard Alumni Award for Outstanding Professional Achievement from her alma mater, Worcester Polytechnic Institute. She received the Women Scientist Advisors Annual Mentoring Award (2024).

Biographies of SSSC Retreat Co-chairs

Dr. Sukhbir Kaur Ph.D. Associate Scientist, HIV and AIDS Malignancy Branch, NCI

Dr. Kaur obtained her Ph.D. from Guru Nanak Dev University (Punjab, India). She then took a post-doctoral fellowship in the Genome Technology Branch at NHGRI where she developed an interest in miRNAs and non-coding RNAs. Later, she joined the Biochemical Pathology Section, eventually becoming a staff scientist to Associate Scientist. During her time at pathology, she made many breakthrough discoveries. She developed a novel method for generating pluripotent and multipotent somatic cells which have utility in both basic research and in disease modeling. She discovered that post translational modification of heparan sulfate proteoglycans of CD47 and blocking of CD47 lead to suppress tumor initiating cells of triple negative breast cancer and she was awarded patents for these findings. Dr. Kaur further discovered that CD47, which is known as the "don't eat me" signal and which is upregulated in many cancers, is also present on exosomes (EVs). Her research has found that donor exosomes can modulate functional role of recipient cells via releasing their internal contents which also carry coding and noncoding RNAs. Her research interests are to explore cell-cell communications via exosomes and their functional role in tumor initiating and benign normal cell biology. She has been recognized as a leader in the extracellular vesicle field as evidenced by being a frequently invited speaker in their national and international meetings. In addition to this, Dr. Kaur is well versed in single, bulk and spatial RNA-seq

analysis, proteomics and the TCGA database to understand coding-non-coding RNAs from cells as well as from extracellular vesicles using a wide variety of bioinformatics software. Upon retirement of her supervisor, she joined HAMB branch where she is extending her research work on CD47 and thrombospondin 1 and EVs role in the context of chronic diseases linked to gamma herpesvirus in cancer progression and metastasis. She is a co-author of 47 scientific publications and has received many awards including the Director Innovation Award, NIH Mentoring Award, and Technology Transfer Awards. Dr. Kaur serves on peer review panels for numerous journals and serves as associate editor for Frontiers Journals. Additionally, she has been an active member of the SSSC Organization, since she became a staff scientist in 2015. She plays an active role in organizing Professional Development Day for the Professional Development Committee and is currently serving as co-chair of the retreat committee.

Usha Acharya Ph.D. Staff Scientist, Cancer and Developmental Biology Laboratory, NCI

Dr. Acharya received her Ph.D. in biochemistry from the Indian Institute of Science, Bangalore India. Her postdoctoral work at the University of California, San Diego was on the assembly of the mammalian Golgi Complex and intracellular transport. She joined the UMass Chan Medical School, Worcester, as an independent investigator where she continued her interest in membrane biology by focusing on sphingolipids. Links between sphingolipid imbalances and human pathologies including sphingolipidoses, neurodegeneration, cancer, and metabolic syndrome underscore the importance of these lipids. Her group has elucidated specialized functions for sphingolipids and enzymes involved in their metabolism in maintaining photoreceptor structure and function. These functions include facilitating endocytic turnover of rhodopsin, a protein central to phototransduction, modulating endo-lysosomal trafficking in photoreceptors and cooperation of sphingolipids with phospholipids to maintain a suitable membrane microenvironment for functioning of the eye. An important outcome of these findings is that the sphingolipid pathway can be modulated to rescue retinal degeneration in a subset of visual mutants. She has been a recipient of both private and NIH funded RO1 grants. Her academic service included being part of graduate student thesis committees, serving on the graduate student admissions committee, participating in faculty recruitment efforts of the university and teaching courses on genetics, signaling and stem cell and regenerative biology as part of the first-year curriculum. After several years at UMass, Dr. Acharya moved to CDBL, NCI Frederick in 2019. Her recent work is centered on inter-organ communication between the gut and brain involved in insulin secretion. As part of her mentoring activities, she continues to guide postdoctoral and postbaccalaureate fellows. She serves as a grant reviewer on NIH study sections and is a member of the CDBL mentoring and SSSC retreat committees.

ABSTRACTS

Oral Presentations

60. Dong Kong, Ph.D., LPDS - Laboratory of Protein Dynamics & Signaling, CCR

Dong Kong and Jadranka Loncarek

"Dissecting the dynamics of human centriole assembly by correlative cell cycle-centriole cycle ultrastructural analysis"

Background and Hypothesis:
Centrioles are small cylinders built of nine triplet microtubules. They scaffold the assembly of centrosomes and cilia, which, in turn, perform a multitude of cellular roles. Structurally aberrant centrioles impede centrosome and cilia function, leading to perturbations in cell signaling, mitosis, and tissue architecture, and can promote invasive properties of cells, among others. Centriole assembly starts in early S phase. However, the precise and systematic dissection of the dynamics of centriole growth in correlation with the cell cycle stages is still lacking. The centriole cycle-cell cycle synchronization operates throughout the lifetime, ensuring centriole, centrosome, and cilia homeostasis. It needs to be understood on fundamental level to understand the mechanisms leading to centriole-related diseases. Here, we dissect the dynamics of procentriole assembly and unravel the regulators of centriole assembly.

Study Design and Methods:
We used transformed and untransformed human cells in culture, expressing Centrin1-GFP as a centriole marker. To associate the cell cycle phases to centriole microtubule morphology and length, some cell lines were engineered to express proliferating cell nuclear antigen (PCNA) fused with mTagRFP. Cells inducibly expressing Plk1, Plk4, and Cyclin A are used to controllably increase the levels of Plk1, Plk4, and CDK2, respectively. Specific inhibitors were also used to inhibit these kinases. Centriole ultrastructure was studied by super-resolution and electron microscopy.

Results and Conclusions:
We found that both longitudinal and lateral incorporation of procentriole microtubules is nonlinear and tied to cell cycle transitions. We demonstrate that centriole assembly is driven by the concerted action of Plk1, Plk4, and CDK2. We show that experimentally imbalanced activity of these kinases decouples procentriole growth from the cell cycle progression, increasing the number of centrioles (hence centrosomes) in cells. Our precise approach unravels novel fundamental principles of centriole formation and suggests how centriole assembly can go awry in cancer cells with perturbed cell cycle regulation.

Cancer Innovation Laboratory, Center for Cancer Research, National Cancer Institute

24. Sigrid Dubois, Ph.D., GMB - Genitourinary Malignancies Branch, CCR
Sigrid Dubois, Kazusa Ishii, Ling Zhang, Yetzali Claudio, Scott M. Norberg, Juergen Mueller, Duane H. Hamilton, James L. Gulley

"Engineering of Natural killer cells to express a tumor-targeting T-cell receptor and membrane-tethered IL-15/IL-21 improves anti-tumor activity in solid tumors"

Background: T- cell receptor (TCR)-engineered T cells have demonstrate clinical activity in a subset of solid tumors. However, tumor immune evasion leading to tumor antigen heterogeneity and downregulation of MHC class I can impede efficacy of this therapy. To recognize and kill tumor cells, NK cells sense MHC class I defects and utilize an array of activating receptors that detect ligands expressed during malignant transformation. Allogeneic NK-cell transfer has been shown to be safe, offering the possibility for an off-the-shelf product. To enhance tumor recognition and the cytotoxic functions of adoptive NK cells in vivo, we have engineered NK cells to express a tumor-targeting TCR and membrane-tethered IL-15/IL-21.
Methods: We have optimized a method to engineer NK cells from the peripheral blood of healthy volunteers to express various TCRs targeting antigens expressed in solid tumors (HLA-A*02:01-restricted TCRs targeting HPV16 E7, NY-ESO-1 and HLA-A*01:01-restricted TCR targeting KK-LC-1) with or without membrane-tethered IL-15 or IL-15/IL-21 using co-transduction with retroviral vectors. The cytotoxic activities of engineered NK cells were compared to non-engineered NK cells and TCR-T cells in vitro and in vivo.

Results: Specific cytotoxicities of TCR-armed NK cells (TCR-NK) were equivalent to those observed with T cells expressing the identical TCR. Similar to non-engineered NK cells, TCR-NK cells also mediated NK cell-inherent killing functions including increase cytotoxicity against tumors with MHC class I defects and tumors coated with antibodies. Importantly, TCR-NK cells could simultaneously kill antigen/MHC class I+ cells and MHC class I deficient tumor variants. Although expression of tethered IL-15 alone drives proliferation and supports the cytotoxicity of TCR-NK cells, co-expression with tethered IL-21 is essential to completely eradicate tumors in a setting where tumor cells outnumbered effector cells by 10 folds. In vivo model, membrane-tethered IL-15/IL-21 TCR-NK cells infiltrated and significantly reduced the growth of antigen/MHC class I+ solid tumors while TCR-NK cells alone could not. Importantly, membrane-tethered IL-15/IL-21 TCR-NK cells were more effective than TCR-T cells at controlling the growth of mixed tumor composed of both MHC class I- and antigen/MHC class I+ cells.

Conclusions: Co-expression of TCR and membrane-tethered IL-15/IL-21 in NK cells enhances their cytotoxicity capacity. Combining TCR-mediated and NK-specific cytotoxic mechanisms allows NK cells to target both antigen-expressing tumor cells and MHC class I-deficient tumor cells, which may prevent tumor immune evasion. This approach, in conjunction with the potential use of an off-the shelf allogeneic cell product, could improve treatments for more patients with solid tumors.

28. Sabina Kaczanowska, Ph.D., POB - Pediatric Oncology Branch, CCR

Sabina Kaczanowska1, Hong Lei2, Jessica Lake1, James Cronk1, Emily San Andres Montalvan1, Zhiya Yu3, Maria Parkhurst3, Steven Highfill2, Rosandra Kaplan1

"Development of Human Genetically Engineered Myeloid Cells (GEMys) for First-in-Human Clinical Trial"

1Pediatric Oncology Branch, CCR, NCI; 2Center for Cellular Engineering, CCR, NCI; 3Surgery Branch, CCR, NCI

14. Brajendra Tripathi, Ph.D., LCO - Laboratory of Cellular Oncology, CCR

Brajendra K. Tripathi1, Sophia M. Shahin1, Xiaolan Qian1, Marian E. Durkin1, Ross Lake2, James H. Doroshow3, Dunrui Wang1 and Douglas R. Lowy1

"Identification of new drug combinations that cooperatively target KRAS-dependent nuclear protein export to treat mutant KRAS-driven pancreatic adenocarcinoma"

Metastatic pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive cancer with a poor prognosis. PDAC is one of the most lethal cancers due to the lack of early diagnosis and its limited response to current therapies. Identifying useful drug combinations for PDAC treatment is an important challenge for molecularly targeted therapy. Oncogenic mutations in the KRAS gene, which are found in over 90% of PDAC patients, play a critical role in PDAC. Although KRAS inhibitors may be found useful in PDAC treatment, it is likely that resistance will develop in most patients given single agent treatment. To overcome this anticipated limitation, effective drug combinations that include KRAS inhibitors will be necessary. We have recently determined in lung cancer models that RAS facilitates the export of nuclear proteins into the cytoplasm via a mechanism independent of canonical RAS signaling pathways. This suggests that the new drug combinations that cooperate with the inhibition of the RAS nuclear export function might be suitable for targeted cancer therapy. Our preclinical PDAC studies suggest the lung cancer mechanisms are relevant to PDAC, with implications for possible new drug combinations. In PDAC lines, we have found that the DLC1 tumor suppressor protein is a critical downstream target of KRAS-dependent nuclear protein export. Perinuclear binding of KRAS-GTP to RanGAP1 promotes the hydrolysis of RAN-GTP to RAN-GDP and the consequent release of nuclear protein cargo into the cytoplasm. Export of the nuclear EZH2 methyltransferase leads to methylation of the DLC1 protein, making it susceptible to proteasomal degradation in PDAC lines. Conversely, KRAS inhibition prevents the nuclear export of EZH2, leading to an increase in DLC1 protein levels. Consistent with these findings, analysis of the Clinical Proteomic Tumor Analysis Consortium (CPTAC) database indicates DLC1 protein levels in PDAC are lower than would be expected from the relatively high DLC1 mRNA expression in these tumors. In the adjacent normal tissues, CPTAC indicates EZH2 protein levels are significantly lower and DLC1 protein levels are significantly higher than in the tumors. Our preclinical studies in PDAC models indicate that a three-drug combination consisting of a KRAS inhibitor plus AKT and SRC inhibitors have significantly more antitumor activity than the KRAS inhibitor alone. It is likely that the cooperation of the three-drug combination is attributable to the KRAS inhibitor increasing DLC1 protein levels, while the inhibitors of AKT kinase and SRC kinase reverse and prevent the

direct phosphorylation and attenuation of DLC1 tumor suppressor functions by these kinases, thereby reactivating the tumor suppressor activity of DLC1 protein, which enhances the antitumor activity against PDAC with mutant KRAS. In addition to this useful three-drug combination, we are currently testing other drug combinations that take advantage of the KRAS-mediated nuclear protein export function.

1Laboratory of Cellular Oncology, 2Laboratory of Genitourinary Cancer Pathogenesis, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA; 3Developmental Therapeutics Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland

26. Adrian Lita, Ph.D., NOB - Neuro-oncology Branch, CCR

Adrian Lita1, Joel Sjöberg2, David Păcioianu 3 , Nicoleta Siminea3, Orieta Celiku1, Tyrone Dowdy1, Andrei Păun 4, Mark R Gilbert1, Houtan Noushmehr5, Ion Petre2, Mioara Larion1

“Raman-based machine-learning platform reveals unique metabolic differences between IDHmut and IDHwt glioma”

BACKGROUND: Formalin-fixed, paraffin-embedded (FFPE) tissue slides are routinely used in cancer diagnosis, clinical decision-making, and stored in biobanks, but their utilization in Raman spectroscopy-based studies has been limited due to the background coming from embedding media.

METHODS: Spontaneous Raman spectroscopy was used for molecular fingerprinting of FFPE tissue from 46 patient samples with known methylation subtypes. Spectra were used to construct tumor/non-tumor, IDH1WT/IDH1mut, and methylation-subtype classifiers. Support vector machine and random forest were used to identify the most discriminatory Raman frequencies. Stimulated Raman spectroscopy was used to validate the frequencies identified. Mass spectrometry of glioma cell lines and TCGA were used to validate the biological findings.

RESULTS: Here we develop APOLLO (rAman-based PathOLogy of maLignant gliOma)- a computational workflow that predicts different subtypes of glioma from spontaneous Raman spectra of FFPE tissue slides. Our novel APOLLO platform distinguishes tumors from nontumor tissue and identifies novel Raman peaks corresponding to DNA and proteins that are more intense in the tumor. APOLLO differentiates isocitrate dehydrogenase 1 mutant (IDH1mut) from wildtype (IDH1WT) tumors and identifies cholesterol ester levels to be highly abundant in IDH1mut glioma. Moreover, APOLLO achieves high discriminative power between finer, clinically relevant glioma methylation subtypes, distinguishing between the CpG island hypermethylated phenotype (G-CIMP)-high and G-CIMP-low molecular phenotypes within the IDH1mut types.

CONCLUSIONS: Our results demonstrate the potential of label-free Raman spectroscopy to classify glioma subtypes from FFPE slides and to extract meaningful biological information thus opening the door for future applications on these archived tissues in other cancers.

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72. Julio Valencia, Ph.D., CIP - Cancer & Inflammation Program, CCR

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"Il27 modulates a dysfunctional molecular IFN signature to drive progression of lupus nephritis"

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38. Wei Zhang, Ph.D., WMB - Women's Malignancies Branch, CCR

Wei Zhang1, Huaitian Liu2, Kristine Isanogle3, Christina Robinson3, Debbie Wei1, Simone Difilippantonio3, Michael Kelly4, Stefan Ambs2 and Patricia Steeg1

"Single-cell RNA sequencing unveils the altered landscape of the blood-brain barrier in breast cancer brain metastases"

Brain metastasis is a common and serious complication in patients with metastatic triple-negative breast cancer (TNBC). There are currently no effective therapies to prevent or treat brain metastases. The primary challenge in treating brain metastases is the presence of the blood-tumor barrier (BTB), an altered blood-brain barrier (BBB) structure that limits the delivery of therapeutic drugs directly to the tumor cells in the brain. A comprehensive, quantitative analysis of the molecular and cellular differences between the BBB and BTB in breast cancer brain metastasis is essential for the development of novel treatment strategies. Here, we performed single-nucleus RNA sequencing to investigate altered gene expression and cell subpopulations within the BTB in two mouse hematogenous models of triple-negative breast cancer brain metastasis (4T1-BR and E0771-BR). For each model, nuclei isolated from brain metastases, normal brain tissue from sham-injected animals, and cancer cell lines were sequenced at the single cell resolution. We then used the R package Seurat to integrate the data sets, followed by clustering the data in UMAP space and identifying differentially expressed genes in the key components of the BBB, including endothelial cells, pericytes, astrocytes, and microglia. We identified extensive molecular changes across all cell types within the BTB. Differential gene expression analysis revealed significant changes in genes associated with maintaining homeostasis in astrocytes and astrocyte-neuron interactions, disease-related genes and immune checkpoint inhibitors in microglia, pro- and anti-angiogenic factors in endothelial cells, and structural and adhesion molecules in pericytes. Notably, dystrophin (Dmd) was significantly decreased in pericytes within the BTB of both 4T1-BR and E0771-BR models, a finding validated by immunofluorescence staining. Further in vivo studies in Dmd-deficient mice revealed that Dmd loss leads to increased leakage in both the uninvolved brain and the BTB compartment. Our single-cell transcriptomics provides valuable insights into the heterogeneity

and complexity of the BTB in breast cancer brain metastases and provides a reference for future functional studies in brain metastasis.

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"*Genetic regulation of TERT splicing affects cancer risk by altering cellular longevity and replicative potential"*

Genome-wide association studies (GWAS) have identified multiple loci within chromosome 5p15.33 associated with reduced risk of some cancers but elevated risk of others. This region encodes the telomerase reverse transcriptase (TERT), which is critical in normal cells and carcinogenesis. We investigated a locus marked by SNPs rs10069690 and rs2242652 within TERT intron 4 and identified a linked variable number tandem repeat within TERT intron 6 (VNTR6-1, 38-bp repeat unit, 24-66.5 repeat copies). In 544 phased long-read genome assemblies from 272 controls of diverse ancestries, we found more VNTR6-1 copies segregating with the rs10069690-T allele (p=2.53E-13) and rs2242652-A allele (p=9.52E-21) than with their alternative alleles. Based on the 1000 Genomes dataset, we constructed a custom imputation reference panel by adding the VNTR6-1 marker (Short allele, 24-27 copies and Long allele, 40.5-66.5 copies) inferred based on short-read whole-genome sequencing and targeted long-read PacBio sequencing. rs10069690-T and VNTR6-1-Long alleles independently reduce TERT levels: rs10069690-T by increasing intron 4 retention and VNTR6-1-Long by expanding a G4-quadruplex in intron 6 (G4Q, 35-113 copies per allele). In two cell lines, UMUC3 (bladder cancer) and A549 (lung cancer), CRISPR/Cas9 deletion of VNTR6-1 increases the ratio of TERT-full-length (FL) to the alternative TERT-beta isoform, promoting apoptosis and limiting cell proliferation. In contrast, treatment with G4-stabilizing ligands shifts splicing from TERT-FL to TERT-beta isoform, implicating VNTR6-1 as a splicing switch. To account for the combined effects of both variants on TERT splicing, we imputed a compound marker (V6.1rs100), with VNTR6-1-Short/Long and rs10069690-C/T alleles. In cancer-free individuals (UK Biobank, European ancestry, n=339,103), we observed a steeper age-related shortening of relative telomere length (rLTL) in peripheral blood leukocytes in those without the Short-C haplotype (marker, 5-years interval age groups interaction, p-int=1.39E-02). In a multi-cancer analysis (PLCO study, European ancestry, 73,085 cancer-free controls, and 29,623 cases), rs10069690-T, VNTR-6.1-Long, and the Long-T haplotype were comparably associated with reduced risk of cancers originating from tissues with low homeostatic proliferation, which maintains tissue self-renewal but high regenerative proliferation in response to environmental exposures and tissue damage (bladder and prostate). The same alleles were associated with elevated risk of cancers from tissues with no/low replicative potential (ovaries, thyroid, and brain). We conclude that the genetic

regulation of TERT splicing by VNTR6-1 and rs10069690 contributes to differences in cancer risk by altering cellular replicative of the normal tissues at homeostasis but also on the types, timing, and intensity of damaging exposures and the ability of the tissue to regenerate after damage.

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BIOCHEMISTRY, BIOPHYSICS AND STRUCTURAL BIOLOGY

1. **High-resolution analysis of human centromeric chromatin**
Daniël P Melters , Minh Bui1, Tatini Rakshit1,2, Sergei A. Grigoryev3, David Sturgill1,4, and Yamini Dalal1

Centromeres are marked by the centromere-specific histone H3 variant CENP-A/CENH3. Throughout the cell cycle, the constitutive centromere-associated network is bound to CENP-A chromatin, but how this protein network modifies CENP-A nucleosome conformations in vivo is unknown. Here, we purify endogenous centromeric chromatin associated with the CENP-C complex across the cell cycle and analyze the structures by single-molecule imaging and biochemical assays. CENP-C complex bound chromatin was refractory to MNase digestion. The CENP-C complex increased in height throughout the cell cycle culminating in mitosis, and the smaller CENP-C complex corresponds to the dimensions of in vitro reconstituted constitutive centromere-associated network. In addition, we found two distinct CENP-A nucleosomal configurations; the taller variant was associated with the CENP-C complex. Finally, CENP-A mutants partially corrected CENP-C overexpression-induced centromeric transcription and mitotic defects. In all, our data support a working model in which CENP-C is critical in regulating centromere homeostasis by supporting a unique higher order structure of centromeric chromatin and altering the accessibility of the centromeric chromatin fiber for transcriptional machinery.

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2. **Microscopy and Digital Imaging in the CCR Microscopy Core**
Michael Kruhlak, Andy Tran, Langston Lim

The CCR Microscopy Core provides NCI investigators access to state-of-the-art imaging tools and techniques, including high-resolution confocal, multi-photon, and super-resolution microscopy. The primary mission of the Core is to support the microscopy and digital imaging needs of investigators studying the biological structures and cellular processes involved in the cell biology of cancer. This involves developing specialized microscopy-based assays and imaging techniques needed by Core users, such as photoswitching of specialized fluorescent proteins to monitor the dynamics of sub-cellular structural components by live cell super-resolution microscopy. Confocal microscopy is also valuable for imaging fluorescently labeled specimens and permitting accurate optical sectioning for volumetric studies, such as large extended field of view tile imaging of tumor samples. Techniques available in the Core include: 1) high resolution confocal microscopy, including live cell imaging methods of FRAP, FRET, FLIM, and photoactivation, 2) multi-photon imaging, including second harmonic generation, 3) super-resolution imaging by either structured illumination microscopy (SIM) or Airyscan detection, and 4) super-resolution imaging by Stimulated Emission Depletion (STED) imaging. Advanced image processing and analysis workstations are also available with numerous image analysis software programs including AI-based image analysis modules, 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

3. **Structural insights into the cooperative nucleosome recognition and chromatin opening by FOXA1 and GATA4**

Bing-Rui Zhou1,*, Hanqiao Feng1, Furong Huang2, Iris Zhu3, Stephanie Portillo-Ledesma4,5, Dan Shi6, Kenneth S. Zaret7,8,9, Tamar Schlick4,5,10,11, David Landsman3, Qianben Wang2, Yawen Bai1,*

Pioneer transcription factors can access DNA in closed chromatin and play crucial roles in cell differentiation and reprogramming. FOXA1 and GATA4 are the prototypes of pioneer factors, which initiate liver cell development by binding the N1 nucleosome in the enhancer of the ALB1 gene. However, the structural basis of the N1 nucleosome recognition by FOXA1 and GATA4 remains unknown. Here, using cryo-EM, we determined the structures of the free N1 nucleosome and its complexes with FOXA1 and GATA4 individually, as well as in combination. We found that the DNA binding domains of FOXA1 and GATA4 mainly recognize the linker DNA and an internal site in the nucleosome, respectively, while their intrinsically disordered regions interact with the acidic patch on histone H2A-H2B. FOXA1 repositions the N1 nucleosome to facilitate GATA4 binding. In vivo DNA editing and bioinformatics analyses suggest that the co-binding mode of FOXA1 and GATA4 play important roles in regulating the genes involving liver cell functions. Our results reveal the mechanism whereby FOXA1 and GATA4 cooperatively bind the nucleosome through nucleosome repositioning, opening chromatin through bending linker DNA, weakening H1 binding and obstructing nucleosome packing.

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4. **Development of hRpn13-targeting molecules as an anti-cancer strategy**

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5. **Oncohistone H3 E97K mutation facilitates CENP-A mislocalization and chromosomal instability (CIN) in budding yeast**

Kentaro Ohkuni, Wei-Chun Au, Amira Z. Kazi, Vinutha Balachandra and Munira A. Basrai

Centromeric localization of histone H3 variant, CENP-A (Cse4 in Saccharomyces cerevisiae, Cnp1 in Schizosaccharomyces pombe, CID in Drosophila melanogaster, and CENP-A in humans), is essential for chromosomal stability. Overexpressed CENP-A leads to its mislocalization to non-centromeric chromatin and contributes to chromosomal instability (CIN) in yeasts, flies, and humans. Overexpression and mislocalization of CENP-A is observed in many cancers and this correlates with poor prognosis. Understanding pathways that contribute to mislocalization of CENP-A will help us understand CIN an important hallmark of many cancers. We have defined a role for histone stoichiometry in preventing mislocalization of Cse4 and shown that gene dosage of histone H4 and the interaction of Cse4 with histone H4 facilitates a conformational state of Cse4 in vivo from a â€œclosedâ€□ to an â€œopenâ€□ state facilitating Cse4 mislocalization (Au et al., 2008, Eisenstatt et al.,2021, Ohkuni et al., 2022, 2024). Furthermore, studies with human cells depleted for H3-H4 chaperones such as DNAJC9 and CHAF1B exhibit defects in CENP-A mislocalization and CIN (Shrestha et al., 2023, Balachandra et al., 2024). Since histone H3 is an obligate partner of histone H4, we hypothesized that reduced gene dosage or mutants of histone H3 will facilitate Cse4-H4 interaction and promote mislocalization of Cse4. These studies are clinically relevant because mutations in histone H3 (oncohistone) in the histone hold domain, are frequently seen (hotspots) in many cancers.
In this study, we showed that altered stoichiometry of histone H3 and expression of oncohistone mutation H3 E97K contributes to mislocalization of Cse4 and CIN. We used histone H3 deletion (hht1âˆ† and hht2âˆ†), structurally defective (hht1 Y99A), and oncohistone (hht1 E97A/K) H3 mutants to examine Cse4-H4 interaction, Cse4 structure, and chromosomal localization of Cse4. We demonstrated that strains with reduced gene dosage of histone H3 (hht1âˆ† and hht2âˆ†) or histone H3 mutants (H3 Y99A and oncohistone H3 E97K/A mutants) exhibit enhanced Cse4-H4 interaction, an in vivo change in the conformational state of Cse4 and this contributes to mislocalization of Cse4. Oncohistone H3 E97K/A mutant proteins were unstable and exhibited defects in interaction with histone H4. Notably, mislocalization of Cse4 and CIN phenotypes were observed in hht1âˆ† and oncohistone H3 E97K/A mutants expressing endogenous Cse4. We propose that defects in the interaction of H3 E97K with histone H4 result in increased levels of free histone H4 thereby enhancing the interaction of Cse4 with histone H4 resulting in Cse4 mislocalization and higher incidence of CIN phenotypes. In summary, our studies highlight the importance of histone H3 stoichiometry in preventing mislocalization of Cse4 for chromosomal stability and suggest that oncohistone H3 mutations may contribute to CIN in human cancers.

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6. **Application of a bivalent click approach to target tyrosyl-DNA phosphodiesterase 1**
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Although inhibiting the DNA repair enzyme tyrosyl-DNA phosphodiesterase 1 (TDP1) synergizes with topoisomerase type I (TOP1) inhibitors in anticancer therapy, development of TDP1 inhibitors has been highly challenging. This may be due to the open and shallow nature of the TDP1 catalytic site and the necessity of competing with a large and highly extended substrate. The toolbox available to chemical biologists for studying TDP1 could be significantly enhanced by introducing the ability to selectively eliminate TDP1 using protein degraders. Our current work starts from phenyl imidazopyridine-based TDP1 inhibitors previously developed from small molecule microarrays (SMMs). Using crystal structures of lead inhibitors bound to TDP1, we designed and synthesized a series of bivalent proteolysis-targeting chimeras (PROTACs). The focus of our current work is to explore synthetic approaches that permit installation of E3 ligase-targeting functionality, while retaining the TDP1 binding. We employed copper-catalyzed azide–alkyne cycloaddition (CuAAC) “click” reactions to assemble PROTAC constituents with 1,2,3-triazole-containing linkers. With the addition of the relatively large parts of the linkers and E3-targeting moieties, we retained the ability to inhibit TDP1. The successful development of TDP1-directed PROTACS would yield a new therapeutic class that could potentially enhance the efficacy and selectivity of TOP1 inhibitors including those used as payloads in antibody drug conjugates (ADCs).

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7. **14-3-3 binding maintains the Parkinson's associated kinase LRRK2 in an inactive state**
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8. **Interaction of J-domain proteins with Hsp90 chaperones in yeast and human**
Patrick G. Needham1, Anushka Wickramaratne1, Conner P. Jewel2, Lisa Jenkins2, and Sue Wickner1

Molecular Chaperones are a critical component of the cellular proteosome, involved in the folding of newly synthesized proteins, protection and recovery of proteins from environmental conditions such as heat or chemical stress, and targeting damaged and unfolded proteins to degradation pathways. The Hsp70 chaperone along with co-chaperones of the J-domain protein (JDP) family, are involved in folding and maintenance of cellular proteins. Some proteins require the cooperation of the Hsp70 and Hsp90 chaperones for their folding, remodeling, and activation. Many of these Hsp90 clients regulate cell growth and division and Hsp90 activity is often associated with proliferation of tumor cells. Previous work with E. coli proteins demonstrated that Hsp90 and Hsp70, DnaK in E. coli, directly interact and the interaction is facilitated by a J-domain protein, DnaJ or CbpA in E. coli. Further work showed E. coli Hsp90 forms binary complexes with DnaJ and CbpA, and S. cerevisiae Hsp90, Hsp82, forms binary complexes with

yeast JDP, Ydj1. Additionally, ternary interactions between E. coli Hsp90, DnaK and CbpA were observed.
In the present study we tested if the interaction between Hsp90 and JDP is conserved in higher eukaryotes. Using human proteins, we observed by both pull-down experiments and biolayer interferometry (BLI) that Hsp90alpha, a human Hsp90, directly interacts with DnaJB1, a human JDP. We also observed that yeast Hsp82 interacts with Sis1, a yeast type II JDP, as well as with Ydj1. Crosslinking followed by mass spectrometry was performed to identify the sites of interaction between Hsp82 and Sis1, Hsp82 and Ydj1, and Hsp90alpha and DnaJB1.
Our results showed that there are multiple sites on JDPs that interact with Hsp90 and multiple sites on Hsp90 that interact with JDPs. A series of cysteine mutants in Hsp82 and Sis1, which lack cysteines in the wild type proteins, were constructed and used in crosslinking experiments with a sulfhydryl reactive crosslinker to confirm sites of Hsp90-Sis1 interaction. We also found evidence of a ternary complex between yeast Hsp90-JDP-Hsp70 using the tri-arm sulfhydryl reactive crosslinker TMEA. Altogether our observations show that complex formation between Hsp90s and JDPs is conserved in higher organisms, although the physiological importance of the interaction remains to be understood.

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9. Amplified LZK promotes esophageal squamous cell carcinoma survival via AKT

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

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

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

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10. **RNA Polymerase and CI Regulator Interactions in Gene Regulation in Bacteriophage Lambda Re-visited**

Dale E.A. Lewis1, and Sankar Adhya1

Background: The Lambda paradigm in gene regulation revealed several basic principles of transcription regulation as elegantly established in the Genetic Switch by Mark Ptashne. After infection of its host E. coli, bacteriophage lambda follows its normal lytic growth or a lysogenic lifestyle in which the phage DNA integrates into the host chromosome and represses its lytic promoters by phage encoded protein, CI. Cells containing repressed Lambda in the chromosome can switch to the lytic growth (genetic switch) at a low frequency. The CI expression in the repressed phage (prophage) is highly autoregulated. At the prophage state CI not only represses the lytic promoter PR but activates PRM (at low concentration) and represses PRM (at high concentration) by differentially binding to tripartite, OR1, OR2 & OR3, located between PRM and PR in a defined pattern. Cooperative CI binding to OR1 and OR2 represses PR while biding to OR2 activates PRM through a direct contact between RNA polymerase at PRM and CI at OR2. CI binding to OR3 represses PRM.
Methods: In a purified in vitro transcription system, we analyzed the RNA polymerase and CI interactions in the autoregulation of PRM using DNA templates with altered angular orientations by deleting 1-bp or inserting 5-bp DNA between PRM and OR2 that is expected to disrupt DNA-bound RNA polymerase-CI contacts. We also analyzed CI mutants (E34K and D38N) which are defective in contacting RNA polymerase. Finally, we analyzed RNAP mutant R596H in the presence of CI mutant (D38N).
Results: We obtained unexpected findings. First, both 1-bp deletion and 5-bp insertion did not result in failure to activate PRM as expected but resulted in repression of PRM at the same CI concentration that represses PR & PL. The binding of CI to OR2 is responsible for this PRM

repression. Second, mutating E34K of CI which is involved in forming the activation complex also resulted in the repression of PRM at the same CI concentration for the repression of and PR. R596H in the presence of D38N restored the activation of PRM .
Conclusion: Attempted disruption of the activation complex between RNAP at PRM and CI at OR2 by mutating CI or inserting or deleting base pair to change the angular orientation and distance between RNAP and CI unexpectedly led to CI-dependent repression of PRM. From these unexpected results, we propose that under all three conditions the OR2 bound CI is creating an inhibitory contact with RNA polymerase at PRM preventing the latter to escape the promoter and repressing transcription. Studies are being conducted to test the model.

1NIH, NCI, CCR, LMB

11. Cell cycle dependent methylation of Dam1 regulates kinetochore integrity and faithful chromosome segregation

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The kinetochore, a megadalton structure composed of centromeric (CEN) DNA and protein complexes, is required for faithful chromosome segregation in eukaryotes. The evolutionarily conserved Dam1/DASH complex (Ska1 in metazoans) is one of the essential protein sub-complexes of the budding yeast kinetochore. Methylation of lysine in Dam1 by Set1 is essential for haploid growth as mutation of methylated lysine to alanine causes lethality. In this study, we defined a molecular role for Set1-mediated cell cycle dependent Dam1 lysine methylation in kinetochore function and chromosomal stability. Our results show that Dam1 methylation is cell cycle regulated with the highest levels of methylation in metaphase cells. Consistent with these results, co-immunoprecipitation experiments showed an interaction of Dam1 with Set1 in metaphase cells. Set1 has been shown to colocalize with Jhd2, a histone lysine demethylase which demethylates Set1-methylated histones. Affinity purification-based mass spectroscopy of Jhd2 associated proteins identified seven of the ten subunits of the Dam1 complex; an association of Jhd2 with non-histone proteins, such as Dam1 has not been previously reported. We confirmed the interaction of Jhd2 with Dam1 and showed that cells overexpressing JHD2 exhibit reduced levels of methylated lysine in Dam1 in metaphase, lethality in kinetochore mutants, reduced levels of kinetochore proteins at CEN chromatin, defects in kinetochore biorientation and chromosome missegregation. In summary, we have defined a novel role for cell cycle dependent methylation of Dam1 in kinetochore assembly and showed that the dynamic methylation of Dam1 is essential for faithful chromosome segregation.

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CLINICAL/ TRANSLATIONAL

12. **Robust differentiation of NK cells from MSLN.CAR-IL-15-engineered human iPSCs with enhanced anti-tumor efficacy against solid tumors**

Qun Jiang1, Weiming Yu2,3, James Ma4, Mingming Zhao1, Jizhong Zou5, Sameer Mir1, Jingli Zhang1, Ronald N. Germain2,3, Raffit Hassan1

Human induced pluripotent stem cells (iPSCs) offer a promising source for producing standardized, off-the-shelf CAR-NK products. However, the complex and time-consuming iPSC-NK (iNK) manufacturing challenges clinical use. Here, we identified LiPSC-GR1.1 as a superior iPSC line for iNK production. By engineering LiPSC-GR1.1 with a mesothelin (MSLN)-targeting CAR and IL-15, we achieved efficient robust differentiation of iPSCs into mature and activated iNKs, which demonstrated enhanced tumor-killing efficacy, superior tumor-homing, and vigorous proliferation. Single-cell transcriptomic analysis revealed that TGF-Î²-producing tumor cells upregulated MHC molecules and downregulated MSLN expression post-CAR-IL-15 iNK treatment. Tumor-infiltrating CAR-IL-15 iNKs exhibited high levels of CAR, IL-15, and NK-activating receptors, negligible checkpoint exhaustion markers, and extremely low levels of NK suppressive factors CISH, TGFBR2, and BATF, enabling them to sustain activation, metabolic fitness, and effective tumor killing within the TGF-Î²-rich hypoxic tumor microenvironment. Overall, we developed a MSLN.CAR-IL-15-engineered GR1.1-iNK product with enhanced anti-tumor efficacy for solid tumor treatment.

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13. **Serum analytes as predictors of disease recurrence and the duration of invasive disease-free survival in triple negative breast cancer patients enrolled in the OXEL trial treated with immunotherapy, chemotherapy, or chemoimmunotherapy**

Renee N. Donahue1, Nicole J. Toney1, Megan T. Lynch1, Filipa Lynce2, 3, 4, Candace Mainor5, Claudine Isaacs6, and Jeffrey Schlom1

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5 MedStar Georgetown University Hospital, Washington, DC, USA.
6 Georgetown University, Washington, DC

15. **Three-Dimensional Imaging of Tumor-Bearing Tissue Using the Iterative Bleaching Extends Multiplexity Approach**
Kirsten Remmert1, Yuri Lin1, Ashley Rainey1, Marcial A. Garmendia-Cedillos2, Shruthi R. Perati1, Jeremy L. Davis1, Andrew M. Blakely1, Jonathan M. Hernandez1

The spatial heterogeneity of the tumor microenvironment (TME) is a critical determinant of therapeutic response, particularly for immune-oncology agents, where success depends on the distribution of specific immune cell subpopulations. Over the past decade, multiple sophisticated technologies have been introduced to achieve detailed resolution of the TME using two-dimensional sections from either formalin-fixed, paraffin-embedded (FFPE) or fixed-frozen tissues. While these thin sections are easier to procure and analyze, they lack the three-dimensional architecture needed to reliably and comprehensively characterize a tumor. To address this limitation, a tissue mounting and imaging technique was developed to enable the three-dimensional analysis of tumor lesions in their native in vivo state. This protocol outlines the procurement of human tumor tissue, the mounting of samples on custom-printed platforms, and staining procedures for post-fixation samples. The multiplexed immunofluorescence technique, IBEX (Iterative Bleaching Extends Multiplexity), was adapted to characterize the three-dimensional TME with up to 15 markers for tumor, immune, and stromal cells using commercially available antibodies. Imaging depths of up to 100 µm were achieved using an inverted white-light laser confocal microscope with a custom-printed imaging adaptor and commercial glass-bottom dishes to ensure optimal tissue orientation. This protocol highlights the potential of the IBEX method to expand multiplexed immunofluorescence studies, providing a more comprehensive understanding of TME composition.

1 Surgical Oncology Program, CCR, NCI

16. **Spatial transcriptomics advances the use of canine preclinical models in cancer research: a study of tumor-bearing pet dogs enrolled in an osteosarcoma clinical trial**
Jessica Beck, Anjali Garg, Christina Mazcko, Amy LeBlanc

Comparative Oncology Program, CCR, NCI

17. **Combination of a therapeutic cancer vaccine targeting the endogenous retroviral envelope protein ERVMER34-1 with immune-oncology agents facilitates expansion of neoepitope specific T cells and promotes tumor control**
Duane H. Hamilton, Maria del Mar Maldonado, Maria Gracia-Hernandez, Loc Le, Masafumi Iida, James L. Gulley, Renee N. Donahue, Claudia Palena, Jeffrey Schlom

Center for Immuno-Oncology, CCR, NCI

18. **Non-V600E BRAF Gene Alterations in Hairy Cell Leukemia**
Evgeny Arons1, Chin-Hsien Tai2, Suraj Joshi3, Yuelin Liu3, Chi-ping Day3, Mark Raffeld4, Liqiang Xi4, Hong Zhou1, Mory Gould2, Isaac Shpilman5, Christopher C. Oakes6,7,8, Seema A Bhat9, Michael R. Grever9, Kerry A. Rogers9, Hao-Wei Wang4, Constance M. Yuan4, Cenk Sahinalp10 and Robert J. Kreitman1

Hairy cell leukemia (HCL) is an indolent B-cell malignancy associated with the BRAF V600E mutation which responds well to purine analogs or class I BRAF inhibitors. The poorer prognosis variant (HCLv) has a distinct immunophenotype, lacks BRAF V600E, and responds poorly to

purine analog monotherapy. Some cases of immunophenotypically classic HCL also lack BRAF V600E, and a few cases have been reported with non-V600E BRAF mutations with or without BRAF V600E, but their clinical significance is unknown. Through whole exome sequencing (WES), we found a total of 18 patients with BRAF mutations other than V600E. Surprisingly, all these patients had the classic rather than variant immunophenotype. Twelve patients had only 1 non-V600E BRAF mutation. Six had V600E in addition to 1 (n=5) or 2 (n=1) non-V600E BRAF co-mutations. These non-V600E alterations included missense mutations, deletions, and insertions located in exons 5, 12, and 18. Some of the non-V600E mutations were previously reported in cancers but none in HCL/HCLv. The most common non-V600E BRAF mutation observed was p.N486_P490del (n=5), and 3 other patients lacked 5 amino acids in this region. In patients with non-V600E BRAF mutations, relapse-free survival after purine analog monotherapy (n=8) was poor compared to a similarly treated control group (n=39, $p<0.0001$) but was significantly better in 7 who received cladribine and rituximab ($p=0.0004$). Detection of non-V600E BRAF mutations in HCL may be clinically and therapeutically important and such mutations constitute an opportunity for development of specific inhibitors.

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19. The Genetics Branch OMICS Technology Facility

Kerstin Heselmeyer-Haddad, David Petersen, Zhigang Kang, Yunkai Yu, Danny Wangsa, Jun Wei, Shaoli Das, Erica Pehrsson, Patrick Zhao, Javed Khan

Mission:
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 supports GB PI labs and their collaborators throughout the project lifecycle, from experimental design, bench work, data acquisition, analysis, and interpretation to visualization, archiving, and submission to internal and public databases. We also provide training for staff and fellows. We utilize the Analysis Management Portal (AMP) to efficiently manage all our wet lab and bioinformatics projects, ensuring comprehensive documentation and effective communication among team members and collaborators.

Wet Lab Capabilities:
We offer a wide array of molecular technologies to study cancer genomes, epigenomes, and transcriptomes using cell lines, mouse models, and patient samples. 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 ChIP-seq, Cut-&-Run, Hi-C, and ATAC-seq.
- Isolation and sequencing of cell-free circulating DNA (cfDNA) to monitor 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.
- Custom assay development, including Mito-Seq for detecting low-level mitochondrial DNA mutations.
- Mesoscale protein analysis.
- Developing other custom assays in collaboration with GB PIs and their lab members.

Our highly trained wet lab and bioinformatics team members support projects with their comprehensive and in-depth expertise and skill sets. We work together as an integrated team in a complementary fashion and in close collaboration with the GB labs. This allows us to provide end-to-end support, including hypothesis generation, experimental design, interpretation, final analysis, and manuscript preparation.

The Genetics Branch OMICS Technology Facility

20. **Docetaxel chemotherapy synergizes with signaling inhibition of the chemokine receptors CXCR1 and CXCR2 for effective tumor control and remodeling of the immune microenvironment of HPV-negative head and neck cancer models**
Lucas A. Horn1, Hanne Lind1, Kristen Fousek1, Haiyan Qin1, Nika Rajabian1, Shantel Angstadt1, Nicole Hsiao-Sanchez1, Miriam M. Medina-Enriquez1, Marcus D. Kelly1, Clint T. Allen2, Sarah M. Hammoudeh3, Roberto Weigert3, and Claudia Palena1

Novel approaches are needed to improve the clinical outcome and prolong survival in relapsed head and neck squamous cell carcinoma (HNSCC) cases unrelated to HPV infection which often present with a poor prognosis and have poor long-term responses to immune checkpoint blockade. This study evaluated the chemokine receptors CXCR1 and CXCR2 as potential novel targets for the treatment of HPV-negative HNSCC and combination therapy strategies for more effective treatment of this disease.

Overexpression of CXCR1, CXCR2, and their ligands CXCL1/2/3/5/6/7/8 in several tumor types has been shown to promote mechanisms of tumor progression while subsequently driving resistance to chemotherapy and immunotherapy. Resistance mechanisms related to CXCR1/2 receptor-associated signaling pathways include increasing tumor angiogenesis, inducing tumor cellular plasticity leading to increased tumor cell migration, invasiveness, and resistance to cell death, and chemoattracting polymorphonuclear myeloid-derived suppressor cells (PMN-MDSC) to the tumor microenvironment.

Our initial analysis of HNSCC tissues via RNA in situ hybridization revealed that high levels of IL-8, CXCR1, and CXCR2 expression were present in HPV-negative compared to HPV-positive HNSCC tumors or cell lines. Treatment of HPV-negative HNSCC cell lines in vitro with a clinical stage, small molecule CXCR1/2 inhibitor, sensitized the tumor cells to the cytotoxic activity of docetaxel. In addition, in vivo, treatment of HNSCC xenograft models with the combination of CXCR1/2 inhibition plus docetaxel led to strong anti-tumor control resulting in tumor cures. This phenomenon was associated with an increase of microRNA-200c and a decrease of tubulin beta-3, a protein involved in resistance to microtubule-targeting

chemotherapies. Additionally, in vivo treatment of a murine syngeneic model of HNSCC with CXCR1/2 inhibition plus docetaxel led to potent anti-tumor efficacy through a simultaneous decrease in suppressive CXCR2+ PMN-MDSC and an increase in cytotoxic CD8+ T cells in combination therapy treated tumors compared to the controls.

This study reports, for the first time, mechanistic findings through which the combination of CXCR1/2 inhibition and docetaxel chemotherapy exhibits synergy in models of HPV-negative HNSCC. These findings provide rationale for the use of this novel combination approach to treat HPV-negative HNSCC patients and for future combination studies of CXCR1/2 inhibition, docetaxel, and immune-based therapies.

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21. Modeling rare SDH-deficient gastrointestinal stromal tumors (GIST)

Francesco Tomassoni-Ardori1,2, Karlyne M Reilly2, 3, Colleen Barrick1, Robert Koogle1, Elijah F Edmondson4, Sudhirkumar Yanpallewar1, Dieter Saur5, Brigitte C Widemann2, 3, John W Glod2, 3 and Lino Tessarollo1

Background and Hypothesis:
Succinate Dehydrogenase (SDH) deficient gastrointestinal stromal tumors (SDH-GIST) are rare sarcomas originating from c-KIT/DOG1 positive interstitial cells of Cajal (ICC). Unlike the majority of GIST (about 85%), SDH-GIST belong to the remaining 15% of GIST denominated wild-type GIST (WT-GIST), which is not characterized by activating mutations in the KIT or PDGFRA genes. Loss of the SDH enzyme activity in humans can be due to mutations or epimutations of the genes encoding for the subunits forming the SDH-enzyme complex (SDHA, SDHB, SDHC or SDHD). Additional recurring genetic alterations found in WT-GIST include the BRAF activating mutation V600E, NF1 loss of function mutations and other ultra-rare sporadic mutations. SDH-GIST do not respond to standard therapies with tyrosine-kinase inhibitors and currently there are no effective systemic therapies for SDH-GIST nor genetically engineered animal models to study these tumors in vivo.

Study Design and Methods:
We have generated new mouse models of WT-GIST by mimicking two of the most common genetic mutations found in patients with WT-GIST including loss of succinate dehydrogenase B (SDHB) and/or the BRAFV600E activating mutation. These mutations were conditionally activated by a tamoxifen inducible c-KitCreERT2 system targeting specifically ICC at specific time points.

Results and Conclusions:
Mice with only loss of SdhB developed no neoplastic lesions, suggesting that SDH-deficiency in ICC is insufficient for tumorigenesis. However, the activating BRAFV600E mutation induced GIST-like tumors and moderate to severe hyperplasia along the GI tract. Interestingly, mice with the double BRAFV600E/SdhBko/ko mutation, compared to mice with only BRAFV600E, developed more penetrant GIST-like lesions associated with molecular alterations including high levels of HIF2-alpha and FGF-4, and low levels of 5-hydroxymethylcytosine (5-hmC) closely resembling phenotypes observed in human SDH-deficient GIST. Treating both the BRAFV600 and the BRAFV600E/SdhBko/ko mice with the specific BRAF-inhibitor dabrafenib™ also revealed that loss of SdhB exacerbates tumorigenesis of BRAF-driven GIST. In summary, we

have developed new mouse models that help addressing an unmet need for preclinical models of SDH-GIST. These models develop quick and localized GIST-like lesions with many distinctive molecular features reported in human patients. The close molecular similarities to human SDH-GIST suggest that they can be used to identify unknown molecular pathways of GIST development and progression. Lastly, the successful use of the BRAF inhibitor in these tumors provide proof-of-concept that these GIST mouse models can be employed to develop and test therapeutics for SDH-deficient GIST and that are valid tools for future molecular and preclinical studies.

1 Mouse Cancer Genetics Program (MCGP), NCI, NIH, Frederick, Maryland, USA.

22. Metabolomic profiling identifies molecular modifiers contributing to proteasome inhibitor resistance in multiple myeloma

Snehal M Gaikwad1, Nana Gyabaah-Kessie1 , Keith Hughitt1, Aleksandra Michalowski1, Thorkell Andresson2, Beverly Mock1

Multiple myeloma (MM) is the second most common hematologic cancer with poor prognosis. Novel therapeutic strategies are needed to decrease the high mortality rate.
Survival of patients with multiple myeloma is highly heterogeneous, from periods of a few weeks to more than 10 years. Drug resistance poses a significant challenge to conventional proteasome inhibitor (PI) based therapy. Metabolic reprogramming plays a crucial role in the maintenance and differentiation of various cancers, including multiple myeloma (MM). A body of literature suggests that several metabolites are significantly deregulated in MM patients, however, their role in driving resistance is unclear. This study aims to identify metabolites modulated during acquired resistance to proteasome inhibitors in relapsed refractory (RR)MM. Two clinically relevant cell line models of PI-resistance (PIR) were developed by dose-escalation of the PI over one year. Cell cytotoxicity assays, immunoblotting, immunofluorescence, and flow cytometric analyses were used to assess the resistance phenotype. Mass spectrometry-based metabolomic profiling was performed to identify altered metabolites in resistant compared to sensitive cell lines. PIR cells demonstrated pan-resistance to multiple PIs, including a >10-fold increase in IC50 for oprozomib compared to parental cells. PIR cells had higher bioenergetic and glycolytic activity (Seahorse) while gene expression profiling suggested downregulation of oxidative phosphorylation. Mass spectrometry-based metabolomic profiling identified distinct metabolites deregulated in drug-resistant cells. The metabolomic profiles of the drug-resistant cell lines showed that the lipid synthesis and beta-oxidation of fatty acid pathways were highly modulated. Several pathway components demonstrated a negative association with overall survival in multiple myeloma patient datasets (MMRF, GSE31161 & GSE9782). PI resistance led to increased phosphorylation of ACLY involved in fatty acid synthesis and increased expression of CPT1A indicative of activation of the beta-oxidation pathway. Use of ACLY and FASN tool compounds affected proliferation, survival, and response to treatment in PIR cells. Metabolites modulated during acquired resistance to proteasome inhibition in relapsed refractory (RR)MM were identified and potential mechanisms driving resistance were studied.

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23. Immunogenic modulation with the ATR inhibitor tuvusertib enhances NK-mediated killing of prostate cancer

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25. Characterization of a humanized monoclonal antibody targeting cancer-expressed EGFR

Antonella Antignani1, Tamara G. Fernandes Costa1, Jingyu Zhan2, Di Xia2, Angel Obiorah1, Robert Sarnovsky1 and David FitzGerald1

The epidermal growth factor receptor (EGFR), an established oncology target, is a transmembrane protein highly expressed on the surface of malignant tumors such as glioblastoma, lung, liver, bladder and breast cancers. In addition to overexpression, EGFR mutants are frequently associated with malignancy. For example, EGFR variant III (EGFRvIII), deletion of exons 2-7, is only expressed on cancer cells and is associated with poor prognosis and chemotherapy resistance. To target cancer-expressed EGFR, including EGFRvIII, we generated the 40H3 monoclonal antibody which is reactive with the 287-302 loop that is fully exposed on the extracellular domain of EGFRvIII. The 40H3 antibody also showed strong reactivity for cells expressing gene-amplified EGFR but little or no reactivity for cells expressing normal levels of wild type EGFR. When conjugated with toxic payloads (either tesirine or deruxtecan), the resulting antibody-drug conjugates (ADCs) exhibited antitumor activity in several xenograft models.

To improve its utility as a clinical candidate, we humanized the variable portions of the heavy and light chains of 40H3, without changing the original CDRs. The variable regions of 40H3, heavy and light chains, were compared to the closest human immunoglobulin families and then framework residues were altered to produce candidate humanized antibodies. The humanized variable chains were then fused with a human IgG1 heavy chain and a kappa light chain to generate full-sized humanized antibodies. A total of 15 humanized antibodies were generated by pairing 3 variable humanized heavy chains (VH1, VH2, VH3) with 3 variable humanized light chains (VL1, VL2, VL3). To evaluate the binding activity of humanized antibodies, we used either immobilized EGFRvIII or various cancer cells expressing either EGFR or EGFRvIII. All humanized antibodies retained binding activity to immobilized EGFRvIII but bound with different apparent affinities to cells overexpressing EGFR or cells transfected with EGFRvIII. The â€˜A10â€™ antibody was the best cell-binding candidate. A10 binding was characterized further on a collection of cancer cell lines each harboring EGFR mutations or overexpressed EGFR and where appropriate compared with MoAb-40H3 and Cetuximab. A10 was also incubated with a fibroblast cell line (WI-38) expressing wild type EGFR at normal levels and exhibited little or no binding. A structural study of the A10 Fab was undertaken following co-crystallization with the reactive peptide. Contacts between the CDRs and the EGFR peptide indicated that it was unlikely to react with wild type EGFR, confirming the epitope as cryptic. To produce therapeutic agents from A10, ADCs with MMAE were produced along with the generation of CAR-T cells. Both, ADC and CAR-T cells proved potently cytotoxic in vitro while in vivo antitumor activity is currently being assessed.

Antonella Antignani1, Tamara G. Fernandes Costa1, Jingyu Zhan2, Di Xia2, Angel Obiorah1, Robert Sarnovsky1 and David FitzGerald1

27. Novel Cereblon-binding Immunomodulators Suppress an Oncoprotein Survivin in Lymphomas Associated with Human Gammaherpesviruses

Prabha Shrestha1, Emma N. Treco1, David A. Davis1, and Robert Yarchoan1

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29. Tuberous Sclerosis-associated renal cell carcinoma, an underappreciated form of familial renal cancer, is characterized by activation of the TFEB/TFE3 pathway

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Objective: To describe the genetic, phenotypic, and pathologic manifestations of patients presenting with inherited kidney cancer and germline variants of the Tuberous Sclerosis Complex (TSC) genes.

Materials and Methods: Inherited kidney cancer patients were screened for germline RCC susceptibility gene variants and patient histories and clinical evaluations were performed. Renal tumors were evaluated for somatic genetic alterations by DNA sequencing and mRNA expression analysis by RNAseq and immunohistochemical analyses were performed.

Results: Nine distinct germline TSC1/TSC2 variants were identified in 13 patients, including seven known or likely pathogenic alterations. Five patients presented with a clinical diagnosis of TSC, and eleven patients had a genetic diagnosis of TSC. Nine patients had bilateral RCC and nine had multifocal RCC. The average initial age at diagnosis of RCC was 47 years old. The TSC-associated tumors demonstrated a variety of histologies including ccRCC, RCC with clear cell and papillary features, chromophobe RCC, and oncocytoma; with ccRCC being the most prevalent. Loss of heterozygosity or secondary somatic alteration of TSC1/TSC2 was observed in ~37% of tumors. RNAseq analysis demonstrated specific expression patterns associated within histologically defined tumor clusters and increased expression of CLEAR genes activated by the TFE3/TFEB transcription factors, including GPNMB and NPC1 which were confirmed with immunohistochemistry.

Conclusion: This study confirms the importance of screening individuals with a family history of kidney cancer for TSC1/TSC2 germline variants, even in the absence of canonical TSC manifestations, and indicates a critical role of TFE3 and TFEB as drivers of human TSC-deficient renal cell carcinoma

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30. SCLC TumorMinerCDB a new interactive web-based tool for mining the genomics of Small Cell Lung Cancer patient samples

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Small cell lung cancer (SCLC) is a highly aggressive malignancy that, while accounting for 15% of all lung cancers and over 30,000 new cases annually in the United States. SCLC remains difficult to treat, and current therapies offer limited effectiveness, resulting in a poor prognosis for patients. It is therefore critical to explore and analyze the genomic data of SCLC patients to identify potential prognosis and predictive biomarkers to enhance treatment strategies.

While genomic initiatives led by The Cancer Genome Atlas (TCGA) have significantly advanced cancer research, SCLC was not included in TCGA's initial scope. However, over the past decade, several public SCLC datasets have been made available through publications and tools like cBioPortal and Xena, which facilitate the mining of published data, as well as data visualization and survival analysis.

In this context, we introduce SclcTumorMinerCDB, a novel web application designed to analyze and mine SCLC patient clinical, genomic, and treatment response data. Inspired by the SclcCellMinerCDB (https://discover.nci.nih.gov/rsconnect/SclcCellMinerCDB/), our new platform is dedicated to explore SCLC genomics data of patient treated at the NCI in comparison with public databases.

SclcTumorMinerCDB shares many of the core functionalities of SclcCellMinerCDB and offers key features, including: i) the ability to compare genomic characteristics and signatures across different patients, diseases, and treatments; ii) the construction and evaluation of multivariate linear response prediction models; and iii) the identification of potential biomarkers for treatment. In addition to these core features, we have introduced several new functionalities, such as predictive biomarker queries, the display of mutation variants, multivariate survival analysis, and the generation of prognostic signatures. A major enhancement is the inclusion of a new Patient Module, which allows users to query and assess clinical and genomic features of individual patients, as well as compare a selected patient sample with other patient samples or cell lines.

Our genomics data currently includes 235 samples from 212 SCLC patients, encompassing RNA-seq and whole exome sequencing (WES) data sourced from the National Cancer Institute (NCI), University of Cologne, University of Rochester, and Tongji University. To minimize batch effects, all RNA-seq and WES samples have been processed uniformly using the NCI Center for Cancer Research Collaborative Bioinformatics Resource (CCBR) pipelines on the hg38 human reference genome. Genomic signature scores such as Antigen Presentation Machinery (APM), Neuroendocrine (NE), Replication Stress, and Tumor Mutation Burden (TMB) were computed. Clinical data, survival information, and treatment response data were meticulously curated by NCI clinicians.

SclcTumorMinerCDB is implemented as a Shiny application, hosted on CBIIT servers using RStudio Connect, and accessible via VPN for NCI/NIH staff at https://discovery.nci.nih.gov/rsconnect/SclcTumorMinerCDB/. This interactive tool enables scientists and clinicians to explore patient omics data, formulate hypotheses, and identify potential prognostic and therapeutic options.

Looking ahead, we plan to expand the platform to include additional data types such as DNA methylation, copy number variations, and fusion data. We also aim to incorporate a broader range of internal and external patient datasets. Most important, the SCLCTumorMinerCDB tool can be exported and cloned in other cancer centers and companies.

1 Developmental Therapeutics Branch, CCR, NCI, NIH, USA. 2 Cornell University, USA. 3 Jikei University, Japan

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

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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. Presence of FcRn in 231-BR cells were further explored for its role in T-DXd efficacy observed in 231-BR prevention model. Knockdown of FcRn in 231-BR cells abrogated T-DXd efficacy observed in prevention settings, highlighting the role of FcRn in T-DXd efficacy observed in triple negative model.

Collectively, the data confirm 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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32. XPC splice founder mutation in families with xeroderma pigmentosum from Dominican Republic, Honduras, and Tanzania: WES reveals an ancient origin of this mutation

Sikandar G. Khan, Deborah Tamura, Sharif Hosein, Maxwell Lee, Huaitian Liu, John J. DiGiovanna* and Kenneth H. Kraemer

Background and Hypotheses:
Xeroderma pigmentosum (XP) is a rare autosomal recessive disorder of DNA repair with about a 10,000-fold increased risk of sunlight induced skin cancer. XP has been reported worldwide. In our cohort of 172 XP patients examined at the US National Institutes of Health (NIH) from 1971 to 2021, an XPC splice founder mutation (c.2251-1G>C) was present in 8 XP patients from seven unrelated families (4 from the Dominican Republic, 2 from Honduras and 2 from Tanzania). Seven patients were homozygous for this splice mutation, and one was a compound heterozygote. This XPC splice founder mutation has been hypothesized to originate from the Bantu population living in East Africa.

Study Design and Methods:
We conducted DNA sequencing of the XP causing genes in our patients and performed extensive medical evaluations. To determine if our patients had a common ancestor, we performed whole exome sequencing (WES) on genomic DNA to quantify the degree of homogeneity.

Results and Conclusions:
The XPC splice founder mutation in the Bantu population living in East Africa is thought to have been distributed throughout the world through the African slave trade beginning about 800 years ago. The principal component analysis (PCA) of the WES data, together with homozygosity mapping, revealed heterogeneity among the patients suggesting that they had different ancestors. The homozygosity mapping and the ancestral analysis using UT-AIM250 markers implied a common ancestor about 1000 to 8000 generations ago. If the generation is 20 years this is a minimum of 20,000 years ago. The data indicates that this founder mutation originated much earlier than the African slave trade.

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33. hetIL-15 orchestrated anti-cancer immune response interpreted at a single cell level resolution

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Background and Hypotheses:
The interplay between cancer cells and the immune system is regulated by the secretion of many different cytokines and chemokines, which shape the microenvironment. Interleukin-15 (IL-15), a homeostatic cytokine, has been shown to regulate a wide range of immune functions, including development activation and maintenance of memory T cells and natural killer (NK) cells. We have previously shown that bioactive IL-15 in vivo comprises a complex of the IL-15 polypeptide chain with the so-called IL-15 receptor alpha. This stable complex was named heterodimeric IL-15 (hetIL-15) and is the only form of IL-15 produced in the body. IL-15 promotes tumor infiltration and proliferation of resident and adoptively transferred T cells in an antigen-specific way. IL-15 has shown anti-cancer activity in many preclinical models, is being tested in multiple clinical trials and has been FDA approved for cancer immunotherapy of bladder cancer. Using locoregional delivery of hetIL-15 in breast cancer orthotopic mouse models we investigated the cell interactions in tumor infiltrated immune cells and the corresponding draining lymph node, responsible for the hetIL-15 anti-cancer immune response.

Study Design and Methods:
We used the syngeneic EO771 breast tumor model to understand the mechanism of hetIL-15 function. Tumor infiltrating immune cells and the corresponding draining lymph node cells were collected from mice treated with either hetIL-15 or control PBS respectively. scRNA seq analysis was performed. The cell-cell interactions in the tumors and LNs were further analyzed using Cell-ChatBD, an integrated web-based cell interaction explorer. The observed changes in the activity and the cytotoxicity of CD8+ T cells were confirmed using seahorse metabolic analysis and flow cytometry. New monocytic derived DC populations were verified using in situ RNA hybridization (RNA-scope) and multicolor flow cytometry.

Results and Conclusions:
hetIL-15 excreted its anti-cancer function through a cascade of effects involving different cell types. A major effect of the locoregional delivery of hetIL-15 in the proximity of the tumors was the activation of cytotoxic cells. Transcriptomic analysis revealed increased expression of genes involved in several metabolic pathways such as oxidative phosphorylation, Fatty Acid oxidation and glycolysis, of the tumor infiltrated cytotoxic CD8+T cells and reduction of the genes involved in exhaustion phenotypes. The direct effect of hetIL-15 signaling on lymphocytes was further enhanced by signaling generated from infiltrated Antigen Presenting Cells (APCs). One of the most prominent responses, as visualized by Cell ChatDB, observed only in the hetIL-15 treated group, was mediated through the direct interaction of conventional type 1 dendritic cells cDC1 with CD8+ T cells via the PVR/TIGIT axis. This signaling further enhanced their cytotoxic abilities. Importantly, hetIL-15 signaling resulted in increased tumor infiltration of monocytic derived DCs expressing both CD103 and CD11b markers with enhanced antigen cross presenting phenotype. The effects of hetIL-15 in the LNs include enhanced Th1 signaling, which further strengthened the observed anti-tumoral responses. Overall, hetIL-15 re-shaped the tumor microenvironment by promoting the intratumoral accumulation of cytotoxic lymphocytes and APCs, resulting in complete tumor eradication in 40% of treated mice, reduction of metastasis, and induction of long-term immunological memory.

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34. Predicting ADC Map Quality from T2-Weighted MRI: A Deep Learning Approach for Early Quality Assessment to Assist Point-of-Care

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Purpose: Accurate diagnosis of localized prostate cancer relies heavily on high-quality multi-parametric MRI (mpMRI). mpMRI is usually the first, crucial step in screening positive patients. Since MRI is commonly used to guide prostate biopsies, poor quality images heavily influence all downstream events. Suboptimal mpMRI image quality can lead to delayed diagnosis, unnecessary biopsies, and misclassification of tumors, ultimately compromising patient care. Within mpMRI, the ADC maps from diffusion weighted imaging (DWI) have been particularly valuable in identifying cancer lesions. However, the EPI sequence used in DWI is susceptible to magnetic susceptibility distortion which can distort the posterior region of the prostate, where majority of clinically significant lesions are expected to be located. Given that acquiring the ADC map is the most time-consuming step of mpMRI and occurs late in the imaging procedure, an interventional method capable of predicting the quality of the ADC map before DWI acquisition occurs would have obvious benefits.

Materials and Methods: A paired multi-site image corpus of T2-weighted images and ADC maps was constructed from 486 patients imaged in-house and at 62 external clinics. A senior radiologist assigned 1-3 quality ratings to each image set, later converted to a binary "non-diagnostic" or "diagnostic" scale. A deep learning model and a rectal cross-sectional area measurement approach were developed to predict ADC image quality from T2 images. Model performance was evaluated retrospectively by accuracy, sensitivity, negative and positive predictive value, and AUC.

Results: Image quality was highly variable. Adherence to PI-RADSv2 Minimum Technical Standards had a minimal effect in reducing this variability for T2 imaging sequences and no effect for DWI sequences. Distortion from rectal gas emerged as the most obvious sources of problems in most scans. No single acquisition parameter in the metadata was statistically associated with image quality for either T2 or ADC maps. These results suggest that simply prescribing a set of sequence specifications is unlikely to solve the problem of image quality, as additional physiological factors that are situational and patient specific also exist.

In contrast to standardization of acquisition parameters, our neural network showed both strong predictive power and broad generalization. In the challenging task of predicting quality of future ADC maps from prior T2 images, our multi-site model achieved performance comparable to the best models directly using ADC maps, with 83% sensitivity and 90% negative predictive value. The model showed even stronger performance on in-house data (94Â±2% accuracy) despite being trained exclusively on external data from other sites. Rectal cross-sectional area on T2 images provided a simple, interpretable quality metric for predicting the quality of future ADC maps (AUC 0.65).

Conclusion: The probability of low quality, uninterpretable ADC maps can be inferred early in the imaging process by a neural network approach, allowing corrective action to be employed. Given that low-quality images are over three times more likely to be upgraded to a higher-grade, more dangerous status after biopsy than high quality images, the adoption of a neural network approach could both reduce cost and enhance patient safety.

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35. Vaccine-induced responses persisted in patients with prostate cancer several years after a series of vaccination for biochemical recurrence

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Background and Hypotheses

TCR gamma alternate reading frame protein (TARP) is a tumor antigen. It is most commonly in prostate cancer, but the transcript has been identified in breast cancer and blood cancers as well. Previously, our group investigated two types of vaccines targeting two overlapping epitopes of TARP (TARP Study, NCT00972309) and showed safety and immunogenicity in HLA-A2 participants with stage D0 prostate cancer (Wood LV et al. OncoImmunology 2016). In this report, we present the results of the multi-epitope (ME)-TARP Study, which administered the updated ME-TARP dendritic cell (DC) vaccine in patients who were previous vaccinated with TARP vaccines.

Study Design and Methods

Previous TARP Study participants were invited for a participation in the ME-TARP study. Those who are willing and with adequate ECOG performance and organ function were enrolled. Patients underwent with apheresis to collect peripheral blood mononuclear cells. Monocyte derived dendritic cell (DC) vaccines were manufactured at the Center for Cellular Engineering/NIH for each dose. Two million DCs pulsed with five overlapping synthetic long peptides (SLPs) of TARP and two HLA-A2 restricted peptides of TARP were administered at Weeks 3, 6, 9, 12, 15, and 24. Patients were assessed every 3 weeks during the treatment and every 12 weeks for a total of 24 months. PSA Doubling Time (PSADT) and the slope of PSA change were assessed in patients who were not exposed to androgen deprivation therapy (ADT). Restaging scans were obtained every 12 months or when clinically necessary. Peptide stimulated PBMCs were analyzed for immune responses by FluoroSpot and tetramer assays.

Results

Among 41 TARP Study participants, 14 participants were available and eligible for the ME-TARP study. The interval between the two studies was 95 (median; range 46-122) months. Thirteen participants were evaluable. Six had remained androgen deprivation treatment (ADT)-naive and four of 6 (ADT)-naive patients showed no increase in the PSA slope after the vaccination. Adverse events were mainly self-limited grade 1-2 injection site reactions. Paired baseline and post-vaccination peripheral blood samples of the ME-TARP study showed baseline tetramer responses (N=4) that increased in post vaccination samples (N=11). Multifunctional T cell responses in combinations of IFN-gamma, granzyme B, or TNF-alpha were detected by Fluorospot assay. TARP 29-37EE tetramer + cells also recognized TARP 29-38WT. TARP 27-35

and TARP 29-37 are only two amino acids apart, but they were recognized by distinct T cell populations.

Conclusions

Re-vaccination targeting the same tumor antigen several years after the initial vaccination in HLA-A2 patients with prostate cancer was safe and immunogenic. Vaccine immune responses detected at the time of enrollment in the ME-TARP study several years after the initial vaccination might suggest vaccine-induced immune memory.

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COMPUTATIONAL BIOLOGY OR SYSTEMS BIOLOGY

36. Single-nucleus sequencing of breast tumors reveals a distinct tumor microenvironment by ancestral group with important implications for biology and disease progression

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Women of African descent are at higher risk of developing lethal breast cancer. While a connection between Western Sub-Saharan African ancestry and aggressive disease has been tentatively established, it remains unclear to what extent breast cancer in Sub-Saharan Africa reflects the disease seen in US African American women. In this study, we used single-nucleus (sn) ATAC- and RNA-sequencing (snMultiome) to analyze chromatin accessibility and gene expression patterns in breast tumors from African American (n=33. Luminal A=17, Luminal B=4, Her2-like=2, Triple-negative=10), Kenyan (n=25. Luminal A=11, Luminal B=3, Her2-like=2, Triple-negative=9), and European American (n=24. Luminal A=13, Luminal B=2, Her2-like=2, Triple-negative=7) women. A total of 292,458 nuclei were analyzed, with 163,419 from cancerous cells and 129,039 from non-cancerous cells. Thirteen major immune, epithelial, and stromal cell types in the tumor microenvironment were identified, revealing distinct patterns across population groups. US African American tumors exhibited an elevated presence of myeloid and T-cells whereas Kenyan tumors had a higher fraction of functionally distinct pericytes and fibroblasts. Moreover, we identified 24 additional immune subpopulations with ancestry-specific gene expression patterns.

Our study represents one of the largest single-nucleus breast cancer datasets and describes ancestry-related differences in tumor biology beyond current knowledge. Besides the aforementioned differences in immune cell profiles, we identified CART targets for precision immunotherapy that are patient group-specific and developed a single cell-derived tumor microenvironmental gene signature that predicts breast cancer survival, with validation in external populations (Bulk RNA-seq data comprising 112 adjacent normal samples and 1,092 tumor samples from The Cancer Genome Atlas Breast Cancer cohort; Microarray-based expression data including 1,992 breast tumor samples from the Molecular Taxonomy of Breast Cancer International Consortium). Lastly, we identified pro-tumorigenic subpopulations of pericytes and endothelial cells that are enriched in women of African descent that may promote a more immunosuppressive tumor microenvironment. In summary, these findings point to population differences in breast cancer biology that may modulate disease aggressiveness and response to therapy.

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37. The Optical Microscopy and Analysis Laboratory

Stephen Lockett, David Scheiblin, Valentin Magidson, Will Heinz

The Optical Microscopy and Analysis Laboratory (OMAL) provides the Center for Cancer Research (CCR) without recharge cutting-edge expertise in fluorescence microscopy and atomic force microscopy. Our expertise includes handling of live samples, sample labeling (including multi-cycle immunofluorescence staining of scores of protein targets in tissue sections), optical clearing of thick tissue and tissue expansion for enhanced spatial resolution. Multiple fluorescence microscopes are available for confocal 3D image acquisition, super-resolution microscopy, high throughput screening of multi-well plates and fluorescence life-time measurement. Image analysis software is provided for 3D visualization, 2D and 3D cell segmentation, deconvolution, cell and molecule tracking and co-localization analysis.
OMAL collaborates with CCR labs to understand how chemical gradients in solid tumors drive disease progression via cellular reorganization of the tumor microenvironment. We take three approaches to understand these mechanisms: (1) Multi-cycle immunofluorescence labeling to investigate the expression of scores of proteins in thin tissue sections. Working with Dr David Wink, we have shown that high levels of nitric oxide synthase 2 (NOS2) and cyclooxygenase-2 (COX2) combined with a lack of CD8 T cells infiltrating into the tumor are associated with poor survival in patients with ER-negative breast cancer. Recently, spatial analysis revealed distinct cellular niches associated with immune desert regions and cancer stem cells and are suggestive of metastatic hotspots (DOI: 10.1158/2767-9764.CRC-24-0235). (2) Fluorescence labeling and 3D imaging of thick tissues to understand the 3D context of each cell. Working with Dr. Wink, The NCI Division of Cancer Treatment and Diagnostics and Oxford University (UK), we have developed a protocol to enumerate and measure the proximity of CD8 T cells and DNA damaged tumor cells (detected by gammaH2AX staining) in 200 Î¼m thick tumor sections (DOI:10.1007/978-3-031-16961-8_9). (3) Utilization of a restricted exchange environment chamber where cell metabolism leads to spatial gradients of oxygen and nutrients forming across the cell culture. We characterized the chamber with Dr. Wink and utilized it to understand NOS2 / COX2 signaling mechanisms (DOI:10.1038/s42003-021-01954-0). Recently, working with Dr. Lalage Wakefield, we developed analytical methods to determine if hypoxia induces cancer stem cell phenotypes and with Dr. Kedar Narayan we are understanding how hypoxia leads to changes in mitochondria and nuclear morphology.

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39. A large-scale WGS and WES study identifies risk-associated genetic variants in Mediterranean families with melanoma history

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DEVELOPMENTAL AND CELL BIOLOGY

40. Non-stochastic mechanism enhances phenotypic heterogeneity during differentiating

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Phenotypic heterogeneity, which underlies myriad phenomena, such as antibiotic tolerance in bacteria, differences in tumor onset and progression rate, and task allocation in unicellular population, can be characterized as genetically identical populations of cells exhibiting different phenotypes during growth in a uniform environment. The most common mechanism for this type of heterogeneity is the slight and stochastic differential expression of a gene (or a subset of genes) across the clonal population that can result in vastly different phenotypes. Sporulation is a relatively simple developmental pathway in bacteria where a progenitor cell differentiates into a dormant cell type when it encounters starvation conditions. Cells in a population, though, enter the sporulation pathway non-uniformly to secure against the possibility that favorable growth conditions, which puts sporulation-committed cells at a disadvantage, may resume. This non-uniform entry was thought to exclusively be initiated by the stochastic activation of a master transcriptional regulator. We identified a genetically encoded mechanism involving cell-cell communication, wherein a small subpopulation of bacteria start sporulating earlier and secrete glycerol (through the coordinated action of two genes of previously unknown function). The extracellular glycerol acts both as a signaling molecule and a nutrient to delay non-sporulating cells from entering sporulation, thus increasing the heterogeneity of cells entering sporulation. We showed that the genetic advantage of this pathway is that it produced a population that was better poised to take advantage of a sudden influx of nutrients, compared to populations that lacked the genes required to produce this heterogeneity. To our knowledge this is the first example of a system used exclusively to enhance phenotypic heterogeneity to boost the fitness level of a population of clonal cells and suggests that phenotypic heterogeneity in other systems may also result from active, not simply stochastic, mechanisms.

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

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Intron retention (IR) is a form of alternative splicing in which an intron that is typically spliced out, is retained in the mature RNA. Although there is emerging evidence of widespread IR in protein-coding genes and long noncoding RNAs (lncRNAs), the underlying molecular mechanisms remain largely unclear. Here, we developed a genome-wide CRISPR-based screening approach to investigate the mechanisms underlying IR in the lncRNA PURPL. 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. 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. 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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42. Delta133p53alpha-mediated inhibition of astrocyte senescence and neurotoxicity as a possible therapeutic approach for neurodegenerative diseases

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[Background and Hypotheses] Non-neuronal glial cells in the brain, such as astrocytes, play essential roles in maintaining the functional integrity of neuronal cells. A growing body of evidence suggests that cellular senescence of astrocytes, characterized by loss of proliferative potential and secretion of neurotoxic cytokines, makes significant contribution to neurotoxicity in Alzheimers disease (AD) and other neurodegenerative diseases such as amyotrophic lateral sclerosis (ALS), Parkinsons disease (PD), radiotherapy-associated cognitive impairment in cancer survivors, and chronic traumatic encephalopathy (CTE) in ex-contact sport athletes and military veterans. Our series of previous studies have linked the regulation of cellular senescence to delta133p53alpha (d133p53a hereafter), a natural p53 protein isoform that inhibits p53-mediated cellular senescence, in various types of human cells. We thus hypothesize that d133p53a also inhibits astrocyte senescence and neurotoxicity.

[Study Design and Methods] Brain tissues with increased senescent astrocytes from patients with AD, ALS, PD, radiotherapy-associated cognitive impairment and CTE were examined for d133p53a expression and senescence-associated factors and cytokines. These senescence conditions in vivo were reproduced in vitro in primary human astrocytes in culture, which were induced to be senescent by replicative cell exhaustion, irradiation or exposure to amyloid-Î².
Effects of the lentiviral expression of d133p53a on astrocyte senescence, secretion of neurotoxic cytokines, and neuronal death in astrocyte-neuron co-culture were examined. For animal studies, d133p53a transgenic mice were generated.

[Results and Conclusions] The expression levels of endogenous d133p53a protein were consistently and significantly reduced in the disease brain tissues as well as in the senescent human astrocytes in vitro. The lentiviral expression of d133p53a protected human astrocytes from cellular senescence and neurotoxic secretory phenotype, leading to their cellular reprogramming to a neuroprotective state associated with neurotrophic growth factors in astrocyte-neuron co-culture experiments. We propose that d133p53a is worth testing as a therapeutic target that can be enhanced in a wide range of neurodegenerative diseases with accumulated senescent astrocytes. We hypothesize that a d133p53a-mediated cellular reprogramming approach and a senolytic or senomorphic approach, both targeting non-neuronal cells, may be complementary with each other, and may cooperate with neuron-protecting or amyloid-Î²-targeting therapies currently in use. The d133p53a transgenic mice are ready to use in preclinical studies to test these hypotheses.

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43. Limitations of Existing Proximity Labeling Methods and the Development of a New Approach

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44. PARP3 Suppresses the Alternative Lengthening of Telomeres (ALT) pathway

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45. Predictive Prioritization of Enhancers Associated with Pancreas Disease Risk

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Genetic and epigenetic variations in regulatory enhancer elements increase susceptibility to a range of pathologies. Despite recent advances, linking enhancer elements to target genes and predicting transcriptional outcomes of enhancer dysfunction remain significant challenges. Using 3D chromatin conformation assays, we generated an extensive enhancer interaction dataset for the human pancreas, encompassing more than 20 donors and five major cell types, including both exocrine and endocrine compartments. We employed a network approach to parse chromatin interactions into enhancer-promoter tree models, facilitating a quantitative, genome-wide analysis of enhancer connectivity. With these tree models, we developed a machine learning algorithm to estimate the impact of enhancer perturbations on cell type-specific gene expression in the human pancreas. Orthogonal to our computational approach, we perturbed enhancer function in primary human pancreas cells using CRISPR interference and quantified the effects at the single-cell level through RNA FISH coupled with high-throughput imaging. Our enhancer tree models enabled the annotation of common germline risk variants associated with pancreas diseases, linking them to

putative target genes in specific cell types. For pancreatic ductal adenocarcinoma, we found a stronger enrichment of disease susceptibility variants within acinar cell regulatory elements, despite ductal cells historically being assumed as the primary cell-of-origin. Our integrative approach combining cell type-specific enhancer-promoter interaction mapping, computational models, and single-cell enhancer perturbation assays produced a robust resource for studying the genetic basis of pancreas disorders.

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46. The senolytic agent (ABT-263) attenuates radiation-induced skin fibrosis by targeting CD206 positive macrophages

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47. ZMIZ1 modulates cohesin function and cGAS-STING pathway activation to drive osteosarcoma progression

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Background and Hypothesis: Osteosarcoma (OS) is the most common primary bone cancer, primarily affecting children and adolescents. Despite decades of research, treatment options for OS have remained largely unchanged for over 30 years, resulting in a dismal 5-year survival rate of only 25% for patients with metastasis. A major obstacle in developing effective therapies is the lack of FDA-approved targeted drugs. The underlying causes of OS remain elusive, necessitating a deeper understanding of its molecular mechanisms to identify novel therapeutic targets. Previous whole-genome sequencing (WGS) studies have failed to identify actionable genetic mutations, suggesting that OS oncogenesis may be driven by non-genetic factors such as transcriptomic and epigenomic dysregulation.

Study design and Methods: To investigate these mechanisms, we utilized chromatin immunoprecipitation sequencing (ChIP-Seq) to identify specific protein binding partners. Cell proliferation, apoptosis, and DNA replication rates were assessed using 5-ethynyl-2-deoxyuridine (EdU) incorporation combined with DNA content staining. A xenograft mouse model was employed to evaluate the functional role of specific genes in tumor progression. Additionally, BioID-based proximity labeling and mass spectrometry were performed to identify the ZMIZ1 protein interactome. Finally, in situ Hi-C sequencing was conducted to comprehensively map chromatin interactions in OS cells.

Results and Conclusions: Our analysis of H3K27ac ChIP-Seq data revealed 53 chromatin regulators having super-enhancers in OS cells. By correlating the expression levels of these candidates with patient prognosis, we identified ZMIZ1 as the only significant candidate. Loss of ZMIZ1 induced massive apoptosis in OS cells and significantly reduced tumor size in xenograft models, highlighting its critical role in OS cell survival. To elucidate the molecular mechanism

underlying ZMIZ1s interaction with super-enhancers, we found that ZMIZ1 regulates cohesin binding to chromatin, maintaining proper chromatin topology and promoting timely DNA replication. Loss of ZMIZ1 disrupts cohesin function, leading to enhanced cohesin binding, which induces topological stress and DNA damage. This DNA damage activates the cGAS-STING pathway, triggering a type I interferon response and cellular senescence. Additionally, our proteomic analysis identified cyclin-dependent kinases CDK12 and CDK13 as key interactors of ZMIZ1. Since CDK12/CDK13 have emerged as promising therapeutic targets in various cancers, we tested the efficacy of their inhibition in OS. Notably, treatment with the small-molecule inhibitor SR-4835 selectively killed OS cells while sparing normal osteoblasts, indicating a potential therapeutic avenue. Our study unveils a novel epigenetic mechanism governing OS progression and identifies ZMIZ1 as a critical factor in OS oncogenesis. By targeting ZMIZ1s binding partners, particularly CDK12/CDK13, we propose a feasible therapeutic strategy for OS, paving the way for the development of targeted treatments for this aggressive cancer.

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48. Decoding telomerase: High-throughput system to decipher the enzymes secrets

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Telomeres, the protective caps found at the ends of chromosomes, gradually shorten with each cell division, ultimately leading to cellular senescencea key aspect of tissue aging. However, cancer cells can bypass this limitation by activating telomerase, an enzyme that prevents telomere shortening, enabling unlimited cell division and tumor formation. Despite the pivotal role of telomerase in cell proliferation and telomere maintenance, fundamental questions regarding its regulation remain unanswered, such as how telomere binding proteins influence its activity and recruitment. Additionally, although telomerase has been an attractive target for drug development, progress has been limited, with only one candidate drug, Imetelstat or GRN163L, advancing to clinical trials after decades of research. To address these challenges, we have developed an in vivo telomerase processivity assay (iTAP) utilizing targeted mutation in telomerase RNA template. This assay enables precise quantification of telomerase activity at the single-cell level, facilitating a deeper understanding of telomerase biology and the discovery of telomere length modulators in a high-throughput manner. Through iTAP, we have elucidated the role of telomere binding proteins in telomerase regulation and conducted genome-wide screens to identify novel regulators of telomerase activity. Moreover, we are utilizing iTAP for high-throughput imaging-based screens to identify potential telomerase modulators. While most somatic cells do not express telomerase, over 90% of cancer cells rely on its activity for telomere maintenance, presenting telomerase as a promising target for anticancer therapy. However, the development of effective telomerase inhibitors has been hindered by the lack of high-throughput screening assays. Our iTAP assay addresses this challenge directly and holds great promise for the discovery of novel therapeutic interventions against telomerase.

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49. Comparative analysis and classification of highly divergent mouse rDNA units based on their intergenic spacer (IGS) variability

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Ribosomal DNA (rDNA) repeat units are organized into tandem clusters in eukaryotic cells. In mice, these clusters are located on at least eight chromosomes and show extensive variation in the number of repeats between mouse genomes. To analyze intra- and inter-genomic variation of mouse rDNA repeats, we selectively isolated 25 individual rDNA units using Transformation-Associated Recombination (TAR) cloning. Long- read sequencing and subsequent comparative sequence analysis revealed that each full-length unit comprises an intergenic spacer (IGS) and a âˆ¼13.4 kb long transcribed region encoding the three rRNAs, but with substantial variability in rDNA unit size, ranging from âˆ¼35 to âˆ¼46 kb. Within the transcribed regions of rDNA units, we found 209 variants, 70 of which are in external transcribed spacers (ETSs); but the rDNA size differences are driven primarily by IGS size heterogeneity, due to indels containing repetitive elements and some functional signals such as enhancers. Further evolutionary analysis categorized rDNA units into distinct clusters with characteristic IGS lengths; numbers of enhancers; and presence / absence of two common SNPs in promoter regions, one of which is located within promoter (p)RNA and may influence pRNA folding stability. These characteristic features of IGSs also correlated significantly with 5 ETS variant patterns described previously and associated with differential expression of rDNA units. Our results suggest that variant rDNA units are differentially regulated and open a route to investigate the role of rDNA variation on nucleolar formation and possible associations with pathology.

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50. Investigation of the Targets of Zinc-Finger Inhibitors for Development of Novel Chemotherapeutics

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Background and Hypothesis
Covalent modification of target proteins is a well-established mechanism for therapeutics. It allows inhibition through modulation of structural elements rather than blocking enzymatic activity. SAMT-247 was developed as a covalent inhibitor of HIV Gag polyprotein and shows potent activity against the zinc-binding domains (ZBD) of the nucleocapsid protein. It modifies cysteine and lysine residues in the C-terminal ZBD, disrupting zinc coordination and structure. In the nucleocapsid ZBDs, zinc is coordinated in the motif CCHC, which is also found in mammalian zinc-finger proteins. Further, other zinc-coordinating motifs can also be targeted in vitro. We hypothesize that the activity of SAMT-247 can be exploited to target mammalian zinc-finger proteins, which are frequently involved in RNA-binding functions that are mis-regulated in cancer.

Study Design and Methods
Thermal proteome profiling of SAMT-247 effects was performed as a preliminary un-biased screen for affected proteins in THP-1 monocyte cells untreated or stimulated with PMA. Post TMT-labeling, samples underwent C18 clean-up and offline, high pH fractionation, followed by LC-MS/MS analysis using a Thermo Exploris 480. Simultaneously, a candidate list of ZBDs with sequence characteristics similar to nucleocapsid was compiled for direct testing. Potential targets from approaches were tested for reaction using zinc-refolded peptides and recombinant proteins by LC-MS on a SCIEX X500B QTOF and LC-MS/MS on a Thermo Exploris 480.

Results and Conclusions
Thermal proteome profiling of THP-1 cells showed minimal effects in untreated cells but more widespread effects in cells stimulated with PMA. Of those proteins that were affected by SAMT-247 in PMA-treated THP-1 cells, seventeen are annotated has containing at least one zinc-coordinating domain. These span different coordination motifs, including C3H1, C2H2, and RING-type domains, as well as biological functions.
Among the zinc-finger proteins affected by SAMT-247 in the thermal proteome profiling analysis, ZC3H7A was also identified as a candidate containing zinc-coordination sequences similar to the ZBDs of nucleocapsid. For example, both have lysine residues proximal to zinc-coordinating cysteine residues as well as aromatic residues within the finger, both of which are known to be important for SAMT-247 reactivity. Analysis of potential covalent modification of peptides representing the four ZBDs of ZC3H7A demonstrated that three were modified, with ZBD-3 (C2H2) having the most adducts and ZBD-1 (C3H1) being unreacted. Further, when recombinant ZC3H7A(440-971) was incubated with SAMT-247, multiple sites of reaction were observed on both cysteine and lysine residues. Additional candidates from both the thermal proteome profiling results and the sequence screen were also tested for reactivity. Comparison of the ZBD sequences of reactive fingers suggests structural elements, at the primary and secondary structure level, that promote SAMT-247 reactivity.

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51. CAR-NK cells against AXL for treating BRAF inhibitor drug-resistant and metastatic melanoma

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Background and Hypotheses
Melanoma has a high mutational burden and is a highly metastatic and difficult-to-treat form of human cancer. The characteristics of more than 50% with BRAF mutation and immunogenicity in melanoma have translated to targeted and immune therapies with remarkable responses and significantly improved patient survival. However, most patients with melanoma will develop a tumor relapse and acquire resistance within several months. Understanding the molecular mechanism of recurrence and acquired resistance, as well as the potential of new therapeutic strategies in BRAF mutant melanoma, could significantly improve patient outcomes. We previously have shown that resistance to BRAF kinase inhibitors (BRAFi) frequently occurs through the reactivation of AXL signaling. AXL was also shown to be expressed in the majority of metastatic melanoma, suggesting AXL is a viable target for treating patients with BRAFi-resistant and metastatic melanoma. Targeting AXL has shown promising anti-tumorigenic potential by the small-molecule inhibitors; however, the side effects of small-molecule inhibitors have hindered the clinical application. Recently, engineered immune cells with chimeric antigen receptors (CAR) allow them to recognize specific targets (antigens) on the surface of cancer cells

from "invisible to visible" and eliminate them. CAR natural killer (NK) cell therapies with favorable safety records have been shown to be an attractive approach for cancer therapy, especially for solid tumors, offering a valuable alternative to genetically modified cell therapies. We hypothesize that engineered NK cells with chimeric anti-AXL receptors (CAR) allow them to recognize specific target AXL on the surface of BRAFi-resistant melanoma to kill the BRAFi-resistant melanoma.

Study Design and Methods
To generate NK cells with chimeric anti-AXL receptors (CAR) for targeting BRAFi-resistant melanoma, we have designed the following studies for testing this hypothesis: 1) develop the nanobody against AXL from the library; 2) design and generate AXL CAR structure; 3) develop AXL CAR NK cells; 4) test the CAR-NK kill resistant melanoma efficacity and specificity; 5) CAR-NK for treating BRAFi resistant and metastatic melanoma in preclinical study using mouse models.

Results and Conclusions
We found that BRAFi-resistant melanomas could resist NK cell lysis while exhibiting significant sensitivity to anti-AXL CAR-NK cells. The cytotoxicity assay indicated that the cytotoxicity of CAR-NK cells against BRAFi-resistant melanoma cell lines was significantly more potent (up to a 10-fold difference) than the corresponding parental cells. Moreover, the anti-AXL CAR-NK cells appeared to have a significantly higher ability of cytotoxicity to AXL-positive melanoma compared to low AXL BRAFi-sensitive melanoma, suggesting that our CAR NK could specifically target the BRAFi-resistant melanoma with AXL expression. Notably, we found the anti-AXL CAR-NK cells could inhibit the BRAFi-resistant and AXL-positive melanoma growth and metastasis in vivo preclinical mouse models. Conclusions: Our findings propose that anti-AXL CAR-NK cell immunotherapy is a promising approach to target BRAF inhibitor drug-resistant and metastatic melanoma.

LMB, LCBG, CCR, NCI

52. TGF-beta induces alternative mRNA splicing in human colon cancer cells
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LCMB, CCR, NCI

53. Optical Pooled Screening for the Discovery of Regulators of the Alternative Lengthening of Telomeres Pathway
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Background and hypotheses: The proliferation of cancer cells depends on the telomere elongation, which is achieved either through the reactivation of the telomerase enzyme, or by engaging the recombination-based Alternative Lengthening of Telomeres (ALT) pathway. Although approximately 10-15% of human cancers utilize ALT, the full complement of cellular regulators required for this pathway remains largely unknown, hindering the discovery of novel therapeutic targets. The recently developed Optical Pooled Screen (OPS) platform combines the use of pooled lentiviral libraries within situ sequencing (ISS) to link genetic perturbations with imaging-based phenotypic readouts at the single-cell level and on a large scale. In this study, we developed and validated OPS-nFISH, a pooled imaging assay that measures ALT activity using

telomeric native DNA FISH (nFISH) as its optical quantitative readout, enabling the identification of ALT regulators.

Methods: To develop OPS-nFISH, we used the ALT-positive U2OS cell line and followed standard OPS protocols for library preparation and ISS. The integration of the nFISH and OPS protocols was optimized to maximize their efficiency. For image analysis, we implemented a multi-step pipeline. We first segmented nuclei using DAPI channel images, aligned ISS images across all sequencing cycles and performed cytoplasm segmentation using all the non-DAPI channels. For base calling, the intensity variance was calculated at the pixel level across sequencing cycles to detect the position of spot-like ISS signals, at which fluorescence intensities were measured for each cycle. Base calls were chained into sgRNA sequence reads and the sgRNA sequence with the highest number of reads for every given cell was selected and matched to its corresponding target genes. Finally, a global coordinate-based approach was employed to align the ISS images acquired at 10X magnification with phenotype images acquired at 40X magnification.

Results and Conclusions: In contrast to other OPS assays, we integrated nFISH at the end of ISS library preparation within the OPS protocol to prevent ISS signal loss. Moreover, we applied changes to the nFISH phenotyping assay by replacing the gold standard PNA-probe with a telomeric ssDNA oligo probe, enhancing the signal-to-noise ratio and detection efficiency in combination with ISS. The sensitivity of the modified nFISH protocol was demonstrated by the faithful detection of changes in ALT activity upon CRISPR knock-out of the ALT-associated genes FANCM and BLM. Finally, we used OPS-nFISH to perform a mini-screen targeting 100 genes in which we effectively identified genetic perturbations that have a range of effects on ALT biology. Altogether, the OPS-nFISH assay is a high-throughput method that can help gain a deeper understanding of the ALT pathway and holds promise for identifying novel therapeutic targets in ALT cancers.

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54. p53b, a p53 isoform, as a potential therapeutic target of glioblastoma

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Background and Hypothesis: The human TP53 gene is the most mutated in human cancer and these mutations are generally associated with poor prognosis and resistance to therapy. Gain of function (GOF) missense mutations are one of the most oncogenic drivers of cancer, therefore, novel therapeutic targets are needed. We previously reported that p53beta (p53b), a naturally occurring p53 isoform which expression is negatively regulated by the alternative splicing factor SRSF3, enhances cellular senescence, a known tumor suppressive pathway, in normal human cells. Notably, SRSF3 is a well-described oncogene, and its high expression is associated with poor prognosis and higher malignancy of many cancer types, including glioblastoma (GBM), the most aggressive type of brain cancer. However, the role of p53b in cancer was unknown. In this study, we hypothesized that a) upregulation of p53b may have a tumor suppressive role in glioblastoma; and b) conversion of mutant p53 to tumor suppressive mutant p53b may represent a novel therapeutic strategy to inhibit or delay tumor growth in the hard-to-treat mutant p53 cancers.

Study Design and Methods: We used GBM cells harboring wildtype (U87 and A172) or mutant TP53 (SF268 and SNB19, R273H TP53; SF295, R248Q TP53). We manipulated p53b expression via siRNA-mediated SRSF3 depletion; or p53b vector-driven doxycycline-inducible overexpression. For three-dimensional (3D) validation, we used Incucyte 3D spheroid assays to assess tumor spheroid growth and invasive potential. We performed intracranial injections of GBM cells expressing a doxycycline-inducible p53b vector into the brains of immunosuppressed mice for in vivo validation.

Results and Conclusions: Our data showed that upregulation of p53b expression in GBM cells, either by siRNA-mediated SRSF3 depletion or vector-driven overexpression, resulted in induction of the tumor suppressive pathways, cellular senescence and apoptosis, a type of cell death. Notably, these tumor suppressive effects were observed both in wildtype TP53 and in GBM cells harboring GOF missense hotspot mutations in TP53- i.e. R273H and R248Q. Using 3D spheroid assays to mimic in vivo tumor growth conditions, we found that activation of p53b expression reduced the growth and invasive potential of spheroids derived from GBM cells harboring wildtype or mutant TP53. These results are supported by our in vivo studies, which showed that induction of wildtype p53b expression delayed the tumor growth of GBM cells intracranially injected into the brain of immunosuppressed mice. In vivo studies with mutant p53b isoform are pending. Overall, our in vitro, 3D and in vivo data suggest that upregulation of p53b induces tumor suppressive activities thereby providing a potential therapeutic strategy to delay or reduce tumor progression via induction of tumor cellular senescence and apoptosis in both wildtype and mutant TP53 GBM cells. We are currently analyzing RNAseq data to better understand the underlying mechanisms of both wildtype and mutant p53b tumor suppressive activities. We are also collaborating with Dr. Drew Weissmann (UPenn) developing a lipid nanoparticle (LNP) strategy to selectively delivery SRSF3 siRNA into GBM tumor cells to convert oncogenic mutant p53 into tumor suppressive p53b activities, which would represent a novel strategy to treat mutant p53 cancers.

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55. IL-1 beta/ IL-6 Co-stimulation Synergistically Controls DUOX2/DUOXA2 complex up-regulation via JAK/STAT Signaling Events

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Background and Hypothesis: Chronic inflammation increases the susceptibility of the colon to neoplasia and cancer through establishment of an inflammatory microenvironment and persistent release of reactive oxygen species (ROS), inducing genome damage. Interleukin -1 beta (IL-1 beta), a pro-inflammatory cytokine, plays a pivotal role in the pathogenesis of acute and chronic intestinal inflammation, regulating both innate and adaptive immune responses. IL-1beta has been shown to promote accumulation of IL-17A through lymphoid and Th17 infiltration in the colon tumor microenvironment, supporting increased inflammation and correlating directly with poor patient prognosis. New insights into the signaling pathways supported by IL-1 beta in the pathogenesis of colon cancer, including angiogenesis and metastasis, may provide downstream targets for treatments that minimally perturb intestinal immunity.

Study Design and Methods: Induction of dual oxidase 2 (DUOX2)/DUOXA2 mRNA and protein expression by IL-4/IL-17A was previously reported to increase oxidative stress and DNA damage in colon cancer cell lines by our laboratory. To build on these results, here we report the treatment of Caco2, HT29, Ls513, T84 or Colo205 human colon cancer cells with IL-1 beta, in cooperation with IL-6, affords dramatic up-regulation of a hydrogen peroxide producing (Amplex Red oxidizing) DUOX2 enzyme complex. Crucial signaling pathway components mediating up-regulation were identified and related through monoclonal antibody antagonists, (Anakinra, Tocilizumab), siRNA and CRISPR techniques. Pathway elements for IL-1 beta and IL-6 signaling were perturbed via siRNA knockdown (MYD88, IRAK1, JAK1, STAT1, STAT3, and SOCS3). Chromatin precipitation experiments were designed and optimized to verify the transcription factor binding site(s) for STAT1/STAT3 in the DUOX2 promoter.

Results and Conclusions: IL-1 beta (+ IL-6) stimulation resulted in oxidant production through significant, synergistic up-regulation of DUOX2 protein and enzymatic activity. Up-regulation of the hydrogen peroxide generating DUOX2/DUOXA2 enzyme complex was associated with enhanced histone H2AX phosphorylation (Î³H2AX), a marker of DNA double strand breaks. Investigations with the interleukin-1 receptor antagonist anakinra have established that signaling, both for IL-1 beta / IL-6 co-treatments, proceeded through the IL-1 receptor for all cell lines, though minimal receptor is present in T84 and Ls513. Similarly, Tocilizumab, an IL-6R antagonist, has verified that the IL-1 beta + IL-6 synergistic up-regulation of DUOX2/DUOXA2 is also IL-6 receptor dependent. Perturbation of IL-1 and IL-6 signaling pathway elements MYD88, IRAK1, JAK1, STAT1 and STAT3 by siRNA knockdown demonstrated a significant contribution to DUOX2 up-regulation, while dependence on RELA was absent. In support of these in vivo observations, studies from our group have demonstrated significant up-regulation of DUOX2 and DUOXA2, as well as IL-1, in surgically resected colon cancer specimens compared with adjacent normal colonic epithelium. Recently, SOCS3 has been pursued as a mediator of the synergy noted for IL-1 beta and IL-6 DUOX2 up-regulation. IL-6 stimulation alone causes a significant increase in SOCS3, a suppressor of cytokine signaling, which is attenuated in the combined presence with IL-1 beta. Current in vivo investigations are focused in multiple colon cancer cell lines on establishing a relationship between SOCS3 and DUOX2 up-regulation.

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56. A pivotal role for Wnt antagonists in constraining Wnt activity to de-stabilize pSmad1,5 during joint formation

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Background and Hypotheses
Bmps and Wnts play opposing roles in several contexts during chondrogenesis and joint formation. In long bone, Wnt activates beta-Catenin by which antagonizes Sox9 activity and promotes joint formation. During limb development, the interplay of Wnts and Fgfs maintains mesenchymal progenitors undifferentiated but poised to assume a chondrogenic fate. However, exposure of the mesenchymal progenitors to Wnts in the absence of Fgfs results in stable epigenic changes that suppresses Sox9 activation and chondrogenic fate. In Hoxd mutants, aberrant high level of pSmad1/5 accumulation causes digit joint loss. In digits, phalanx forming region (PFR)

cells at digit tips can be specified to become joint cells (low Bmp response) or chondrocytes (high Bmp response). However, expressing stabilized beta-Catenin (bCatCA) in cartilage could not restore joint formation in Hoxd mutants, instead expressing it in interdigit tissues restores joints. We hypothesize that bCatCA induced target(s) non-autonomously normalize pSmad1/5 level in PFR cells, which results in restoration of PFR cell fate specification program.

Study Design and Methods
Genetic approaches were conducted to cross different allele combination and to exam Bmp and Wnt crosstalk in joint restoration by bCatCA. Bmp activity in PFR cells were evaluated by immunohistochemistry staining of pSmad1/5 and RNA in situ hybridization of Bmp target gene, Msx2.
RNAseq analysis of FACS-sorted interdigit cells was performed to identify bCatCA induced targets through DE comparison between control, Hoxd mutants and Hoxd;bCatCA samples. Pharmacological reagent treated short-term limb bud organ culture was performed to test the mechanism by which the pSmad1/5 stability is regulated by Gsk3beta and Erk phosphorylation in its linker region.

Results and Conclusions
Through lineage trancing analysis, we confirmed that no bCatCA descend cells contributed to joint restoration in Hoxd;bCatCA rescued mice after interdigital expressing bCatCA. Stained pSmad1/5 protein was reduced in PFR cells in digit tips in Hoxd;bCatCA rescued limbs. However, removing a copy of Noggin allele in Hoxd;bCatCA blocked joint restoration with increased pSmad1/5 level, indicating that non-autonomous bCatCA restored joint formation through modulating pSmad1/5 in PFR cells. DE comparison from RNAseq analysis showed that the expression of Dkk2, Wnt antagonists, was reduced in Hoxd mutants but was upregulated in Hoxd;bCatCA samples. After removing Dkk2 allele in Hoxd;bCatCA rescued mice, joint restoration was blocked, suggesting that induced Dkk2 in Hoxd;bCatCA limb represses Wnt activity and consequently normalize pSmad1/5 level in PFR cells. It is known that Gsk3beta and Erk phosphorylate linker region of pSmad1/5, which leads to Smurf1 mediated protein degradation. In the limb organ culture, the expression of Msx2 and pSmad1/5 level were increased after adding antagonists to block protein degradation process and Gsk3beta activity in the culture. Further, digit joint loss was observed in Wnt3a transgenic mice, suggesting that excess Wnt activity accelerated chondrogenic commitment and impeded fate switch of PFR cells to joint cells, preventing joint formation. We conclude that, before mesenchymal cells transit into PFR cells, Wnt antagonists play key roles in both cooperating with Fgfs to prevent precocious pSmad1/5 accumulation and curtailing excess Wnt activity to prevent Sox9 silencing as Fgfs decline.

57. The Sp1-related transcription factors Sp5 and Sp5l regulate posterior development in Xenopus

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The Wnt/Î²-catenin signaling pathways are crucial for embryonic development and tissue homeostasis by regulating gene expression. Specificity protein (Sp) 5 is a downstream target of the Wnt/Î²-catenin pathway, and along with Sp8, it plays a vital role in regulating the fate of neuromesodermal progenitors (NMPs) and trunk and tail formation in mice.

However, since there have been no reports describing NMP-type cells in Xenopus embryo, it remains unclear whether Sp5 contributes to posterior development, including somitogenesis and spinal cord formation. To investigate the role of Sp5 in posterior development in Xenopus, we conducted loss-of-function assays using morpholino-mediated knockdown and Cas12a-mediated knockout targeting Sp5 and Sp5l, a Sp5 paralog. The results showed that depletion of Sp5 and Sp5l resulted in a shorter tail length compared to control, indicating that Sp5 and Sp5l is essential for developing the posterior structure in Xenopus. Interestingly, Sp5 and Sp5l morphants also exhibited disrupted somite segmentation, including fused somites and abnormal somite boundary formation. Moreover, the spatial expression of Notch target genes was disrupted in Sp5 and Sp5l morphants, implying that deregulation of the Notch signaling pathway due to compromised Sp5 and Sp5l expression may contribute to impaired somite development. Lastly, comparing to wild-type mRNAs, Sp5 and Sp5l mutants lacking zinc finger domains failed to restore somite segmentation defects in Sp5 and Sp5l morphant embryos, suggesting the gene regulatory role of Sp5 and Sp5l is required for proper somite formation. Thus, Sp5 and Sp5l may regulate posterior development by modulating somite segmentation, boundary formation and subsequent differentiation during early embryonic development of Xenopus laevis.

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58. Mapping Metabolic Vulnerabilities Reveals PRDX1 as a Key Mediator of Tumor Cell Sensitivity to DNA Damage Response Inhibitors

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59. HP1 Chromo Shadow Domain Targets Anti-Silencing JMJC Protein to Limit Heterochromatin Propagation

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EPIDEMIOLOGY

61. DNA methylation-based inflammation risk scores and lung cancer in women who never smoked: A prospective study

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Introduction: Inflammation plays a key role in cancer development, and C-reactive protein (CRP) is often studied as a biomarker, though its association with lung cancer remains inconsistent. We previously reported an inverse association between plasma CRP levels and lung cancer development in never-smoking women in the Shanghai Women's Health Study (SWHS). However, as CRP levels fluctuate with active inflammation, a single measurement may not accurately capture the inflammatory responses relevant to cancer development. DNA methylation-based inflammation risk scores (DNAm-IRS) provide a more stable, integrative measure of long-term inflammation. To date, only one prospective study, primarily in smokers, has examined DNAm-IRS and lung cancer risk, showing an inverse association after adjusting for DNAm-derived smoking patterns. In this study, we expand our previous research by more than doubling the number of lung cancer cases and controls and incorporating CRP-derived DNAm-IRS to investigate associations with lung cancer in never smokers.

Methods: This nested case-control study, conducted within the prospective SWHS, included 683 never-smoking women with incident lung cancer and 683 never-smoking controls, matched on date of birth (Â±2 years) and sample collection time (Â±3 months). Four DNAm-IRS were calculated as the weighted sum of CRP-related CpG sites (52 for IRSLigthart, 1,334 for IRSElnet, 1,333 for IRSWielscher, and 32,196 for IRSHillary) using established algorithms. Conditional logistic regression models were used to estimate odds ratios (OR) and 95% confidence intervals (CI), adjusting for age and body mass index.

Results: Among controls with available plasma CRP levels (n = 214), the Spearman correlation coefficients between biochemical CRP and DNAm-IRS derived from CRP were as follows: 0.20 (p=3.4Ã—10â??Â³) for IRSLigthart, 0.31 (p=4.6Ã—10â??»â??¶) for IRSElnet, 0.13 (p=6.7Ã—10â??»Â²) for IRSWielscher, and -0.02 (p=7.4Ã—10â??»Â¹) for IRSHillary. Higher DNAm-IRS scores were associated with a reduced risk of lung cancer among never-smoking women. Specifically, each standard deviation (SD) increase in IRSWielscher was associated with a 13% lower risk of overall lung cancer (95% CI: 0.77â€“0.97; p=0.017) and a 17% lower risk of lung adenocarcinoma (95% CI: 0.71-0.97; p=0.018), the most common histologic subtype. Additionally, IRSLigthart was associated with a 15% lower risk of overall lung cancer (95% CI: 0.78â€“0.97; p=0.003).

Conclusion: Our findings, along with prior research, suggest that certain aspects of CRP are paradoxically associated with a reduced risk of lung cancer in never-smokers or when analysis accounts for DNAm-derived smoking patterns. Further studies are warranted to replicate these findings and investigate the underlying biological mechanisms.

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62. Determinants of Carcinogenic HPV Incident Detection Among Unvaccinated Monogamous Women in the Costa Rica HPV Vaccine Trial

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Introduction: Women in monogamous relationships may be at relatively low risk of new HPV infections. We evaluated determinants of incident detection of carcinogenic HPV infection among HPV-unvaccinated women who reported having only one lifetime sex partner (i.e., monogamous) in Costa Rica.

Methods: We included 1032 HPV-unvaccinated women (18-31 years) participating in the Costa Rica HPV vaccine trial who reported who reported only one-lifetime sex partner (i.e., monogamous) at the enrollment visit and during the follow-up. Interviewer-based questionnaires were used to record participants sexual behavior (age at first sexual intercourse, monthly frequency of sex, married/living as married), smoking status, contraceptive use, and reproductive history at enrollment and each study follow-up visit. Only at the enrollment visit, women provided information about their male sex partner (age, education, lifetime number of sexual partners, smoking, circumcision, living with the participant). At all visits, cervical samples were collected for cytology and HPV-DNA testing. Incident detection was defined as a cervical HPV infection not present/detected at the previously scheduled visit. We used GEE methods to account for correlated observations.

Results: We detected 332 new carcinogenic HPV infections among 21% (220/1032) of the monogamous women in 3331 visits (median follow-up 4.9-years, IQR: 4.3-6.3). Positive associations of women-related factors with the risk of incident detection were HPV-positivity at enrollment [adjusted Relative Risk, aRR=1.8 (95%CI: 1.3-2.6) vs. HPV-negative] and ever smoking (aRR=2.5, 95%CI: 1.5-4.3 vs. never). Risk of incident detection decreased with increasing age of the male sex partner (p-trend=0.004). No statistically significant associations were observed for other factors evaluated (women-related, or their male sex partner-related). HPV status at enrollment did not modify the effect of the variables evaluated on incident detection.

Conclusion: Monogamous women remain at risk for carcinogenic HPV infections. Incident detection of carcinogenic HPV infections may reflect the complexities of HPV infection dynamics or changes in the (unmeasured) sexual behavior of the male sex partner. These women could benefit from routine screening and HPV vaccination to prevent new vaccine-protected carcinogenic infections.

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63. Implementation of biospecimen collection in the NCI Connect for Cancer Prevention Study

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Background and Hypotheses

The Connect for Cancer Prevention Study is a new prospective cohort aiming to recruit 200,000 participants from 10 integrated healthcare systems across the US. At baseline, participants complete online surveys and donate biospecimens. Intended to be a resource for the wider research community, Connect will focus on studies of cancer etiology, risk prediction, and early detection.

Study Design and Methods

Blood (SST, K2-EDTA, Li-Hep), urine, and mouthwash are collected at baseline in dedicated study research centers or using clinical phlebotomy laboratories combined with mailed home mouthwash kits. A liquid biospsy collection tube (currently Streck) is being collected at one healthcare system, with plans to expand this collection to other systems. In addition to the baseline survey modules, participants are asked to complete surveys regarding factors specific to the time of sample collection. Biospecimens are shipped from across the US (CO, GA, HI, IL, MI, MN, ND, OR, SD, TX, WI) in temperature-controlled coolers to the central NCI laboratory in Frederick, Maryland, for processing and long-term storage. Process metrics to ascertain biospecimen quality include sample completeness, needle-to-receipt time, and specimen deviations. Biospecimen survey completion is also monitored.

Results and Conclusions

As of February 2025, 39,914 of 58,178 Connect participants (average 69%, range 54% to 80% across sites) donated any biospecimens, with baseline collections still ongoing. Among collections, 42% were from research centers and 58% from clinical laboratories, with complete collections (all expected blood, urine, and mouthwash) received from 96% and 99%, respectively. Over 80% of biospecimens were received at the NCI laboratory within 2 days of collection and 97% were received within 4 days. The return of home-collected mouthwash samples was 77%. Among all biospecimens collected, 87% had no protocol or sample deviations. Over 93% of participants submitted the biospecimen survey.

We successfully implemented a robust and efficient biospecimen collection at 10 integrated healthcare systems that complements our survey data collection. In the future, connect data and biospecimens will be available to the research community.

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65. A prospective study of excess weight and weight change in adulthood and risk of colorectal cancer incidence and mortality

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Obesity is a rising public health problem in the U.S. and worldwide and excess body mass index (BMI, kg/m2) is a recognized risk factor for colorectal cancer (CRC). However, the role of weight change over the adult life course and timing of the excess BMI in relation to the incidence and mortality of CRC is not well understood.

With >15 years of follow-up, we prospectively evaluated the risk of CRC incidence (n=3,092) and mortality (n=931) in association with adult weight change among men and women (n=131,814) recruited in the Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial at ages 55 to 74 between 1993 and 2001. At baseline, participants filled out a questionnaire reporting their weight and height at ages 20 years, 50 years, and baseline, allowing us to assess weight change from early to middle, middle to late, and early to late adulthood. Hazard ratios (HRs) and 95% confidence intervals (CI) were computed using multivariable-adjusted proportional hazard regression models.

Higher BMI at all ages was associated with an increased risk of CRC incidence (p-trend=0.05, 0.0003 & 0.0001 for ages 20, 50 and baseline, respectively). Compared with individuals whose BMI never exceeded 25 kg/m2 over their lifetime, those who first exceeded a BMI of 25 kg/m2 in early adulthood had a slightly higher risk of CRC (HR=1.3, CI:1.1-1.4) than those whose BMI did not exceed 25 kg/m2 until middle age (HR=1.2, CI:1.1-1.3) or later (HR=1.2, CI: 1.0-1.3). Moreover, compared with stable weight, weight gain >4 kg per 5 years from early adulthood to middle or late adulthood was associated with an increased risk of CRC (HR=1.2, CI: 1.1-1.4 for both periods). The increased risks for weight gain >4 kg/5 years between early to middle adulthood appeared more pronounced for those with normal BMI at age 20 (HR=1.3, CI: 1.1-1.5). For CRC mortality, higher BMI at age 50 and baseline, but not age 20, was associated with increased risk (p-trend = 0.0002, 0.0002, and 0.3, respectively). Compared to those whose BMI never exceeded 25 kg/m2, those who first exceeded a BMI of 25 kg/m2 in early adulthood had an increased risk of mortality (HR=1.2, CI: 1.0-1.5), but no association was found for those whose BMI did not exceed 25 kg/m2 until middle adulthood or later. A higher risk of CRC mortality was observed for weight gain >4 kg/5 years from early adulthood to middle or late adulthood (HR=1.5, CI: 1.1-1.9 and HR=1.7, CI:1.4-2.1, respectively), especially among those with normal BMI at age 20 (HR=1.5, CI: 1.1-2.0 and HR=1.7, CI:1.3-2.2, respectively).

Exceeding normal weight in early adulthood and weight gain from early to middle or late adulthood were associated with increased risks of both CRC incidence and mortality. These findings highlight the harms of excess BMI in early adulthood, the importance of maintaining healthy weight throughout the adulthood, and the potential benefit of weight control programs for CRC prevention.

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VIROLOGY, IMMUNOLOGY AND MICROBIOLOGY

66. Defective removal of Invariant chain peptides from MHC-II suppresses tumor-antigen presentation and promotes tumor growth

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An effective anti-tumor response requires activation of both CD4 helper T cells (through MHC-II) and CD8 cytotoxic T cells (through MHC-I), but tumors have adapted many different mechanisms to avoid detection by effector cells of the acquired immune system. Tumor-draining lymph node dendritic cells are poor stimulators of tumor antigen-specific CD4 T cells, and we reported for the first time that in tumor-draining dendritic cells a large proportion of MHC-II molecules retain the CLIP fragment of the Invariant chain bound to the MHC-II peptide binding groove, due to reduced expression of peptide-editor H2-M and enhanced proteolytic activity of cathepsin S. The net effect of this is that MHC-II molecules in tumor-draining dendritic cells are unable to efficiently bind antigenic peptides. By generating mice with a mutation in the Invariant chain sequence that results in enhanced accumulation of MHC-II-CLIP we confirmed that defective peptide exchange from MHC-II molecules impaired the CD4 T cell priming by dendritic cells, skewed T cell responses towards pro-tumorigenic Th2 type, and hampered anti-tumor responses, leading to enhanced tumor growth. Our data reveal a novel mechanism of immune evasion induced by tumor cells in which enhanced expression of MHC-II-CLIP complexes limits the MHC-II availability for tumor peptides presentation by lymph node dendritic cells.

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67. Fungal Infection Promotes Upper GI Tract Tumorigenesis via the EGFR Signaling Pathway

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Previously, we reported that fungal infection promotes the progression of esophageal squamous cell carcinoma (ESCC) using an autoimmune mouse model. To further explore the role of fungal infection in upper GI tract tumorigenesis in a broader, non-autoimmune context, we investigated whether fungal pathogens are associated with human ESCC and stomach adenocarcinoma (SADC). We found that fungal DNA is widely present in patients with either ESCC or SADC, as demonstrated by fluorescence in situ hybridization (FISH) using a pan-fungal probe that detects multiple fungi, including Candida, Aspergillus, and Saccharomyces, among others. Interestingly, we observed that fungal foci colocalize with EGFR phosphorylation, showing a strong positive correlation. This suggests that fungal infection may promote tumor development in patients by activating the EGFR signaling pathway. We further validated this hypothesis using our mouse model, where infection with wild-type C. albicans, but not mutant Candida strains that do not activate EGFR, significantly increased EGFR target activation. This activation was associated with enhanced cell survival, proliferation, angiogenesis, and immune escape. As a result, only infection with wild-type C. albicans, and not the mutant strains, promoted upper GI tract tumor progression in mice. Thus, for the first time, our findings demonstrate that fungal infection promotes upper GI tract tumorigenesis via the EGFR signaling pathway.

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68. Repurposing anti-viral subunit and mRNA vaccine T cell immunity for intratumoral immunotherapy against solid tumors

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Intratumoral (IT) immunotherapy can reshape the tumor microenvironment (TME) and induce epitope spreading. Many human tumors harbor T cells recognizing non-tumor antigens, including viral epitopes. We previously demonstrated that IT injection of minimal peptides from murine cytomegalovirus (CMV) with a TLR3/RLR agonist could locally reactivate CMV-specific CD8+ T cells, leading to immune activation, epitope spreading, and long-term tumor eradication.

Here, we hypothesized that preexisting T cells from routine vaccinations could be repurposed for cancer immunotherapy, enabling the use of licensed vaccines while focusing on a defined set of antigens. We investigated IT delivery of three approved viral vaccines Shingrix (VZV shingles), Gardasil-9 (HPV), and Spikevax (SARS-CoV-2) in previously vaccinated mice using the TC-1 tumor model, which expresses the HPV16 oncogenes E6 and E7.

Shingrix IT injection induced tumor regression and resistance to rechallenge. Injecting a VZV glycoprotein E (gE)-derived MHC-II-restricted peptide with polyI:C also led to durable remission, highlighting the role of gE-specific CD4+ T cells. Gardasil-9 IT injection alone was ineffective but combining an HPV L1-derived MHC-I-restricted peptide with polyI:C or Shingrix enhanced tumor regression, eliciting CD8+ T cells against the E7 viral oncoprotein. In vivo antibody-mediated depletion confirmed that Gardasil-specific CD8+ T cells were required for tumor control. Furthermore, unimmunized mice did not respond, demonstrating that pre-vaccination was critical for the antitumor effect.

TME analysis using Nanostring gene expression profiling, multiplex cytokine assays, confocal microscopy, and high-parameter flow cytometry showed that Shingrix IT treatment induced tumor cell killing and a tumor stress response characterized by expression of Fas, PD-L1 and calreticulin, further amplified by the addition of an HPV-derived MHC-I-restricted epitope. This was accompanied by TME remodeling, characterized by increased infiltration of CD8+ T cells expressing CD39 and PD-1, reduced tumor-associated macrophages, and increased neutrophils infiltration, a feature recently associated with tumor cell killing. Additionally, we observed significant upregulation of IFN-Î³, TNF-Î±, and CXCL9, alongside broad transcriptional reprogramming associated with cytotoxicity. In a dual-flank model, IT injection of Shingrix with an MHC-I-restricted E7 tumor-associated peptide led to regression of both injected and contralateral tumors.

Finally, IT injection of SARS-CoV-2 mRNA vaccine resulted in modest tumor growth delay, whereas combining a SARS-CoV-2 spike-derived MHC-I-restricted peptide with polyI:C improved tumor control.

Our study demonstrates that preexisting T cell immunity from routine vaccinations can be harnessed for IT immunotherapy. Both full vaccines and selected epitopes from vaccine antigens induced potent TME activation and tumor killing. This strategy offers a broadly applicable, low-resource alternative for solid tumor immunotherapy, circumventing the need for personalized identification of tumor-associated or patient-specific T cell reactivities while benefiting from tumor-specific antigen combinations when available.

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69. A prospective study of excess weight and weight change in adulthood and risk of colorectal cancer incidence and mortality

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70. Induction of translation-suppressive G3BP1+ stress granules and interferon-signaling cGAS condensates by transfected plasmid DNA

Vladimir Majerciak and Zhi-Ming Zheng

Background and Hypotheses

Plasmid DNA transfection is a fundamental technique in biomedical research however, the cellular responses to incoming DNA are not yet fully understood. In this study, we investigated the activation of two cellular innate immunity pathways in response to lipid-mediated plasmid transfection in several widely used cell lines. This provides us an opportunity to study the underlying molecular mechanisms of activation and regulation of these pathways, including the role of viral proteins.

Study Design and Methods

We employed immunofluorescent staining of intracellular condensates to detect the activation of these pathways in plasmid-transfected cells. As a confirmatory approach, we further used Western blot analysis to assess the phosphorylation status of key pathway regulators. The siRNA-mediated knockdowns and small-molecule treatments were used to identify the molecular drivers of these pathways. Special attention was given to evaluating the role of viral proteins in regulating these two pathways.

Results and Conclusions

We found that plasmid DNA transfection leads to the formation of stress granules (SG) and cGAS-DNA condensates (cGC) in a majority of tested cell lines in dose-dependent manners, indicating the activation of double-stranded RNA (dsRNA) and DNA sensing pathways. The activation of these pathways was confirmed by elevated phosphorylation levels of eIF2Î±, required for SG formation, and IRF3 and STAT1, a downstream target of cGAS activation. While SG formation required active transcription from transfected plasmid DNA, resulting in the generation of dsRNA in transfected cells, cGC formation was transcription-independent. Notably, we observed for the first time that activation of these two pathways was mutually exclusive at a single cell level, with each transfected cell containing only one type of condensate, SG or cGC, suggesting a mutually suppressive effect. However, we also showed independent activation of these pathways, with each requiring a specific intracellular sensor.

Specifically, siRNA-mediated knockdown of PKR, a dsRNA sensor, led to selective loss of SG but not cGC in plasmid DNA transfected cells. Conversely, the knockdown of the DNA sensor, cGAS, prior to transfection prevented cGC formation without any effect on SG. This independence was further confirmed by co-expression of the Kaposi's sarcoma-associated herpesvirus (KSHV) proteins ORF57 (an SG inhibitor) and ORF52 (a cGAS inhibitor), which selectively inhibited SG or cGC formation in a manner consistent with their assigned roles during viral infection. In conclusion, our findings indicate that plasmid DNA induces cellular innate immune responses collectively resulting in significant perturbation of cells physiology and gene expression. These include suppression of protein translation by SG formation and activation of the interferon pathway by cGAS. These changes should be carefully considered in the design and data interpretation of any experiment utilizing plasmid DNA transfection.

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71. Negative Regulation of RpoS translation by LrhA and RbsD

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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 already 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 RpoS regulation. The physiological implications of this process remain unclear.

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73. Conditional immortalized lymphatic endothelial cells support long-term infection of Kaposi Sarcoma herpesvirus

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Background:
Kaposi Sarcoma (KS) is a major cause of morbidity and mortality in HIV-infected individuals. KS is characterized by aberrant angiogenesis and proliferative, spindle cells that express lymphatic endothelial markers. KS spindle cells are infected with Kaposi Sarcoma Herpesvirus (KSHV). Most tumor cells express the latency associated nuclear antigen and other viral transcripts associated with latency. One barrier to studying KSHV latency is the rapid loss of the KSHV genome upon explant culture of patient-derived KS spindle cells. 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.

Methods:
We developed conditional immortalized lymphatic endothelial cells (ciLEC) by transducing primary lymphatic endothelial cells with lentivirus encoding the SV40 large T antigen (LTA) under a doxycycline (Dox)-regulated promoter. The growth of ciLEC was dependent upon Dox such that withdrawal induced growth arrest. KSHV gene expression was analyzed using qRT-PCR, RNA sequencing, and immunofluorescence.

Results:
ciLEC retained lymphatic endothelial markers VEGFR3 and CD31. They were highly permissive to KSHV infection. In the absence of Dox, ciLEC underwent growth arrest and KSHV lytic gene expression was detected. In proliferating ciLEC that were supplemented with Dox, KSHV gene expression was largely suppressed. Interestingly the latency-associated nuclear antigen was detectable up to 30 days post-infection. Importantly, KSHV genome was stably maintained in proliferating ciLEC for up to 60 days.

Conclusion:
Doxycycline-induced control of ciLEC growth allowed modulates KSHV replication. The long-term maintenance of the KSHV genome in a proliferating ciLEC cells provides a novel model for investigating the viral and host factors that promote KSHV latency, and by extension KS development**.**

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LATE-BREAKING ABSTRACTS

74. Co-targeting mutant KRAS and autophagy in KRAS-driven cancers

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Background and Hypotheses: Despite the great efforts and success of developing mutant KRAS-selective inhibitors, lack 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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75. Histone deacetylase inhibition synergizes with tumor-targeted IL-12 to overcome tumors refractory to PD-1/-L1-targeted therapies

Sofia R Gameiro, Christine M Minnar, and Jeffrey Schlom

Clinical benefit from immune checkpoint blockade (ICB) targeting the PD-1/PD-L1 axis remains elusive for most patients with solid malignancies that are poorly inflamed or display tumor-intrinsic loss of MHC class I (MHC-I) and aberrations in antigen processing machinery (APM) and interferon gamma (IFNg) pathways. Here we demonstrate, by using comprehensive single-cell transcriptome, proteome, and immune cell analysis, that the epigenetic modulator Entinostat, a class I histone deacetylase inhibitor, facilitates accumulation of the necrosis-targeted immune-cytokine NHS-IL12 in ICB-refractory murine colon tumors (CT26) and poorly immunogenic breast carcinomas (EMT6) in which the therapy was curative. Combination therapy reprograms the tumor innate and adaptive immune milieu to an inflamed landscape, where the concerted

action of highly functional CD8+ T cells and activated neutrophils drive a dramatic macrophage M1-like polarization, leading to complete tumor eradication in 41.7%-100% of cases. Further, combination therapy overcame multiple anti-PD1/PDL1-resistant murine tumor models harboring MHC-I, APM, and IFNg response deficiencies [TC-1/a9 (lung, HPV16 E6/E7+), lung CMT.64, and RVP3 sarcoma]. Potent anti-tumor activity and survival benefit observed with combination therapy were driven by prolonged activation and tumor infiltration of cytotoxic CD8+ T cells. Combination therapy promoted M1-like macrophages and activated antigen presenting cells (APCs) while decreasing M2-like macrophages and Tregs in a tumor-dependent manner. This was associated with increased levels of IFNg, IL-12, CXCL9 and CXCL13 in the TME. Combination therapy synergized to promote MHC-I and APM upregulation, and enrichment of Jak/STAT, IFNg-response and antigen processing-associated pathways. A biomarker signature of the mechanism involved in these studies is associated with patientsâ€™ overall survival across multiple tumor types. Conclusions: Our findings provide a rationale for combining the tumor targeting NHS-IL12 with the HDACi entinostat in the clinical setting for patients unresponsive to anti-PD-1/-L1 and/or with innate deficiencies in tumor inflammation, MHC-I, APM expression, and IFNg signaling.

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76. Subcellular Distribution of Unspliced HIV-1 RNA

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77. Association of polygenic risk score for habitual alcohol intake with all-cause mortality

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

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Sphingomyelinases (SMases) are key enzymes that hydrolyze sphingomyelin (SM) to generate phosphorylcholine and ceramide. Recently, we 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). Notably, treatment of HIV-1-infected humanized mice with the small-molecule nSMase2 inhibitor PDDC results in sustained suppression of plasma HIV-1 levels, even after treatment discontinuation.

To elucidate the mechanism by which nSMase2 disruption blocks lentiviral particle maturation, we analyzed chimeras between viruses sensitive (HIV-1), or insensitive (MLV), to nSMase2 disruption. This analysis revealed that the determinants of sensitivity map to 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 also identified resistance mutations in the MA and CA domains of HIV-1 Gag that accelerated the kinetics of Gag processing, as determined by pulse-chase radiolabeling assays. Furthermore, studies using a Gag-PR-leucine zipper construct suggest that nSMase2 disruption, either via PDDC or nSMase2-specific siRNA, impairs PR activity. Lipidomics analysis indicates that PDDC treatment of virus-producing cells increases levels of sphingomyelin and reduces levels of ceramide in the viral membrane and lipid order measurements using the laurdan fluorescent dye show that nSMase2 disruption significantly reduces lipid order in HIV-1 virions. We have developed a FRET assay to directly measure GagPol dimerization in virions produced from cells in which nSMase2 has, or has not, been disrupted and are testing the effect of nSMase2 disruption on the dynamics of the Gag lattice using a crosslinking approach. 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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79. Transcription factors form a ternary complex with NIPBL/MAU2 to localize cohesin at Enhancers

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Synthetic RNA devices are engineered to control gene expression and offer great potential in both biotechnology and clinical applications. Here, we present multidisciplinary structural and biochemical data for a tetracycline (Tc)-responsive RNA device (D43) in both ligand-free and bound states, providing a structure-dynamical basis for signal transmission. Activation of self-cleavage is achieved via ligand-induced conformational and dynamical changes that stabilize the elongated bridging helix harboring the communication module, which drives proper coordination of the catalytic residues. We then show the utility of CRISPR-integrated D43 in EL4 lymphocytes to regulate programmed cell death protein 1 (PD-1), a key receptor of immune checkpoints. Treatment of these cells with Tc showed a dose-dependent reduction in PD-1 by immunostaining and a decrease in messenger RNA levels by quantitative PCR as compared with wild type. PD-1 expression was recoverable upon removal of Tc. These results provide mechanistic insight into RNA devices with potential for cancer immunotherapy or other applications.

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80. Drug-regulatable, inducible, and membrane-bound interleukin 12 for controlled expression in combination with adoptive cell therapies

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Background: Interleukin-12 (IL-12) is a potent proinflammatory cytokine that can enhance adoptive cell therapies (ACT) for cancer, but systemic toxicities have limited its clinical application. Here, we present drug-regulatable, inducible, and membrane-bound IL-12 (DRIM-IL-12), designed to improve ACT efficacy while minimizing toxicity.

Methods: We engineered DRIM-IL-12 by integrating a lenalidomide-responsive degron with an NFAT-inducible system to achieve tight regulation of IL-12 expression. A transmembrane domain was incorporated to anchor IL-12 to the cell membrane, limiting systemic release. The regulation of IL-12 expression was assessed through in vitro and in vivo assays using a p53 neoantigen model. Antitumor efficacy was evaluated across multiple tumor models, including the p53 and KRAS neoantigen model and the CD19-chimeric antigen receptor (CAR).

Results: DRIM-IL-12 expression was effectively induced in a TCR-dependent manner via the NFAT-inducible system, and the lenalidomide-responsive degron enabled its rapid degradation both in vitro and in vivo. In multiple settings including p53- or RAS-targeting TCRs, melanoma-infiltrating lymphocytes, and CD19-CAR therapyâ€”DRIM-IL-12 enhanced ACT efficacy. In mice, ACT with DRIM-IL-12 and the mutant p53 or KRAS-targeting TCRs caused significant tumor regression and improved survival. Notably, uncontrolled DRIM-IL-12 expression upregulated TIGIT and other exhaustion-related genes, but low-dose lenalidomide (20-40% maximal expression) reversed T-cell exhaustion, enhancing efficacy.

Conclusions: DRIM-IL-12 potentiates ACT while enabling precise IL-12 regulation and mitigating exhaustion. These findings support further clinical evaluation of DRIM-IL-12 to assess its therapeutic potential.

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81. Characterization of membrane organization regulating primary ciliogenesis by quantitative isotropic ultrastructure imaging

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The trafficking, docking, and fusion of membrane vesicles at the mother centriole (MC) are required to construct the primary cilium. Here, we determined the three-dimensional (3D) membrane ultrastructures, and associated proteins, involved in primary cilium assembly upstream of axoneme growth. Our work reveals that the enlargement of small vesicles docked to the MC is a key trigger for ciliogenesis progression, a process requiring the MC distal appendage protein CEP164. We show these vesicles subsequently fuse to form tubular C-shaped and an unprecedented toroidal membrane intermediates, which ultimately organize into the ciliary vesicle covering the MC distal end. The formation of these previously uncharacterized tubular membrane ciliogenesis intermediates is orchestrated by the membrane trafficking regulators EHD1 and RAB8, and requires the IFT-B complex protein IFT88. Remarkably, we show that EHD1, through its membrane tubulation function, regulates ciliogenesis progression by directly promoting CP110/CEP97 removal from the MC cap. The establishment of these tubular membrane structures is also associated with the recruitment of the ciliary gate transition zone proteins. This study changes the architectural framework for understanding ciliogenesis mechanisms and highlights the application of isotropic ultrastructure imaging and three-dimensional quantitative analysis in understanding membrane trafficking and organelle biogenesis mechanisms.

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82. Transcription factors form a ternary complex with NIPBL/MAU2 to localize cohesin at Enhancers

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While the cohesin complex is a key player in genome architecture, how it localizes to specific chromatin sites is not understood. Recently, we and others have proposed that direct interactions with transcription factors lead to the localization of the cohesin-loader complex (NIPBL/MAU2) within enhancers. Here, we identify two clusters of LxxLL motifs within the NIPBL sequence that regulate NIPBL dynamics, interactome, and NIPBL-dependent transcriptional programs. One of these clusters interacts with MAU2 and is necessary for the maintenance of the NIPBL-MAU2 heterodimer.

The second cluster binds specifically to the ligand-binding domains of steroid receptors. For the glucocorticoid receptor (GR), we examine in detail its interaction surfaces with NIPBL and MAU2. Using AlphaFold2 and molecular docking algorithms, we uncover a GR-NIPBL-MAU2 ternary complex and describe its importance in GR-dependent gene regulation. Finally, we show that multiple transcription factors interact with NIPBL-MAU2, likely using interfaces other than those characterized for GR.

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83. Novopath BioGRID with Integrated Gene Set Enrichment

Richard Finney and Margaret Cam

Novopath BioGRID is a de novo pathway visualization tool that extends the capabilities of the original Novopath, which utilized interaction data from Pathway Commons. The updated Novopath BioGRID leverages comprehensive biomedical interaction data provided by BioGRID (https://thebiogrid.org/), comprising 1,194,827 interactions across 25,829 genes. A significant enhancement in Novopath BioGRID is the addition of an interactive feature enabling users to select genes dynamically and perform instantaneous gene set enrichment analysis using the L2 framework. This modernized version is implemented as a user-friendly web-based application built with JavaScript and WebAssembly, allowing efficient exploration and visualization of biological pathways.

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

84. Activation of the ER stress response in a premature aging disease

Sandra Vidak1, Leonid A. Serebryannyy1, Gianluca Pegoraro1, Tom Misteli1

Background and Hypothesis: Naturally occurring premature aging disorders are powerful model systems to study human aging and aging-related pathologies. One of the most prominent premature aging diseases is Hutchinson-Gilford Progeria Syndrome (HGPS), an extremely rare genetic condition caused by a de novo heterozygous mutation in the LMNA gene encoding for the nuclear architectural proteins lamin A and C. The disease-causing mutation activates a cryptic splice site resulting in the expression of a mutant form of lamin A termed progerin. Progerin

forms extensive protein aggregates in the nuclei of patient-derived fibroblasts and induces numerous cellular defects, including post-transcriptional reduction of select cellular proteins, pointing to an effect of progerin on protein homeostasis. We hypothesize that progerin exerts its deleterious function in part through its accumulation and aggregation in the nucleus and we propose a mechanism by which progerin-containing protein aggregates recruit and sequester molecular chaperones, affecting protein homeostasis in the rest of the cell.

Study Design and Methods: To assess the effect of progerin on the expression and localization of major molecular chaperones, we performed high-throughput imaging to quantitatively determine protein levels and the cellular distribution of several chaperones in a well-characterized cellular HGPS model system of hTERT-immortalized skin fibroblasts expressing doxycycline-inducible GFP-progerin and in HGPS patient-derived fibroblasts. Immunofluorescence analysis and super resolution microscopy was used to assess the localization of several chaperones in HGPS cells, and immunoprecipitation and western blotting were used to probe the interaction between progerin and chaperones. Real-time quantitative PCR was used to measure the presence of stress-induced pathways in both HGPS cellular models, as well as in two progeria mouse models. In addition, we developed an optimized optogenetic clustering approach based on the Arabidopsis thaliana CRY2-derived optogenetic module CRY2olig, which induces rapid and robust protein oligomerization upon exposure to blue light to uncover how progerin aggregates cause a change in the localization of the chaperones and activate stress pathways.

Results and Conclusions: We find that progerin aggregates promote sequestration of chaperones from the endoplasmic reticulum (ER) lumen to the nuclear periphery, which is accompanied by the activation of an adaptive response to ER stress, including an increase in various ER chaperones and transcriptional activation of Unfolded Protein Response (UPR).

The progerin interactor and INM transmembrane protein SUN2 is necessary and sufficient to trigger progerin-mediated ER stress and contributes to the ER stress phenotype via clustering of its luminal domain, leading to induction of stress pathways. Prolonged ER stress coupled with an inadequate UPR response has been linked to apoptosis of vascular smooth muscle cells, atherosclerosis and cardiovascular diseases, phenotypes commonly observed in HGPS patients. In line with the observed upregulation of the UPR pathway in cultured cells, we find significant upregulation of several UPR genes in the heart and aorta of progeria mice. Taken together our results identify an unanticipated mechanism of communication between the nucleus and the ER and provide novel insight into the molecular disease mechanisms of HGPS.

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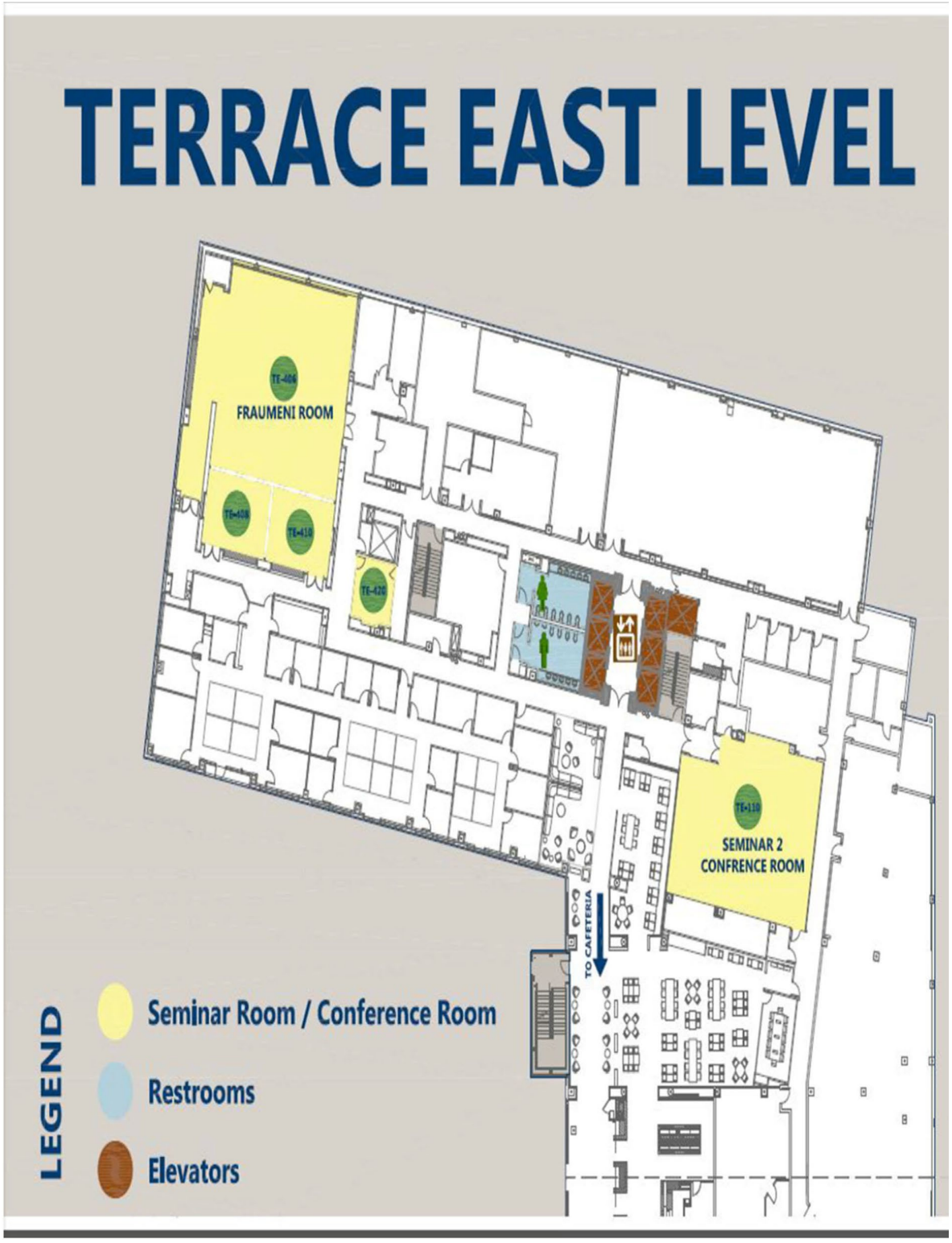
TERRACE EAST LEVEL
TE-406
FRAUMENI ROOM
TE-408
TE-410
TE-420
TE-110
SEMINAR 2
CONFRENCE ROOM
TO CAFETERIA
LEGEND
Seminar Room / Conference Room
Restrooms
Elevators

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

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

21st Annual CCR and DCEG Staff Scientists and Staff Clinicians Retreat

April 18, 2025

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