LANDRY
CANCER BIOLOGY
CONSORTIUM

SPRING SYMPOSIUM

6 May 2019
12:00 – 6:30 PM

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About the Landry Cancer Biology Consortium

The Landry Cancer Biology Consortium provides a unique and essential service to the cancer biology community at Harvard: it brings this community together to realize its full potential.

Harvard University is home to 14 life sciences PhD programs—but no one gets a PhD in cancer. To drive new advances in multidisciplinary cancer research, and to introduce students at all levels to research and training opportunities across Harvard, the Landry Cancer Biology Consortium creates new courses, scientific events, and co-curricular activities, all designed to bring students and faculty together to share recent developments, address current challenges, and promote synergy in cancer research and treatment. In addition, through the Landry Cancer Biology Research Fellowship—a premier fellowship awarded to five exceptional PhD students each year—this program supports emerging leaders within the growing network of cancer biology researchers across Harvard.

This program is made possible by the generous support of the late C. Kevin Landry ’66 and his family, colleagues, and friends. This gift represents a transformative investment in some of the best and brightest young minds in cancer biology.

Symposium Schedule

Starting 11:30 am	Registration *
12:00 - 1:45 pm	Karin Grunebaum Foundation Poster Competition *
2:00 - 2:45 pm	*"Tumor Evolution and Heterogeneity in Lung Cancer" *** **Alice Shaw, MD, PhD**
2:45 - 3:30 pm	*"Evolution of Resistance to Targeted Therapies for Lung Cancer" *** **Aaron Hata, MD, PhD**
3:45 - 4:30 pm	*"Engineering the Cancer Genome" *** **Tyler Jaks, PhD**
4:30 - 6:30 pm	Reception * Poster Prizes Announced

*NRB 2nd Floor Lounge
**NRB 350, 3rd Floor Seminar Room

Tumor Evolution and Heterogeneity in Lung Cancer

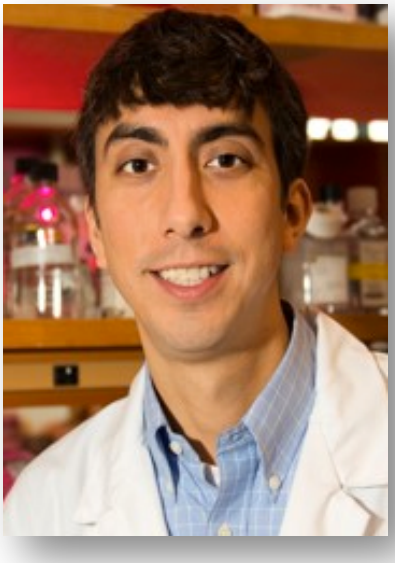
Alice Shaw, MD, PhD
Professor of Medicine, Harvard Medical School
Director of Thoracic Oncology, Massachusetts General Hospital
Paula O'Keefe Endowed Chair in Thoracic Oncology

Alice T. Shaw, M.D., Ph.D., received her B.A. in biochemistry at Harvard University and her M.D. and Ph.D. degrees from Harvard Medical School (where she is currently an Associate Professor). She then completed her residency at Massachusetts General Hospital and her postdoctoral work at Massachusetts Institute of Technology. Dr. Shaw was also a fellow at Massachusetts General Hospital and is currently the Director of Thoracic Oncology and an Attending Physician there.

Dr. Shaw's major research interests include studying anaplastic lymphoma kinase (ALK) translocations in non-small cell lung carcinoma (NSCLS); developing targeted strategies to treat NSCLCs harboring activating KRAS mutations; discovering new targets in NSCLC using both genetic and phosphoproteomic strategies; and developing novel nanoparticle-based siRNA delivery systems to target genetically defined subsets of lung cancer.

Evolution of Resistance to Targeted Therapies for Lung Cancer

Aaron Hata, MD, PhD
Assistant Professor of Medicine, Harvard Medical School
Assistant Physician, Massachusetts General Hospital Cancer Center



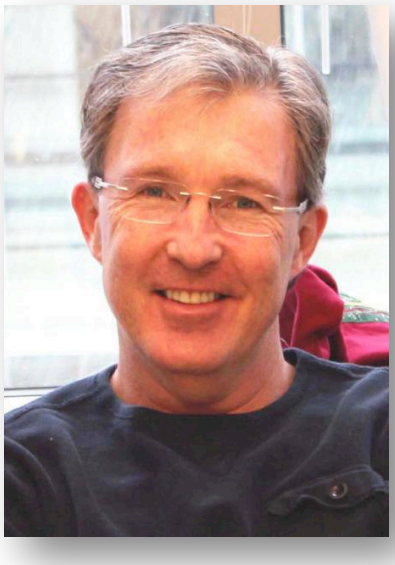
Dr. Hata received his MD/PhD from the Vanderbilt University School of Medicine where he performed his PhD research in the laboratory of Dr. Richard Breyer, studying the structure and function of prostaglandin receptors. Following graduation from medical school in 2007, Dr. Hata completed a internship and residency in Internal Medicine at the Brigham and Women's Hospital in Boston. In 2009 he entered the Dana Farber/Partners CancerCare Oncology Fellowship Program. He performed a post-doctoral research fellowship in the laboratory of Dr. Jeffrey Engelman at the Massachusetts General Hospital, where he focused on understanding the role apoptosis in response and evolution of resistance of lung cancers to targeted therapies.

In 2016, Dr. Hata joined the MGH Cancer Center as Principal Investgator. His laboratory is currently focused on unraveling the biological underpinnings of sensitivity and resistance to kinase inhibitor targeted therapies in lung cancers with specific genetic abnormalities (EGFR, ALK, KRAS). In particular, Dr. Hata is interested in understanding how heterogeneous cancer cell populations adapt and evolve during the course of therapy in order to identify vulnerabilities of pre resistant drug tolerant cancer cells that might be exploited to prevent resistance from developing. His research integrates analysis of clinical samples from patients enrolled in targeted therapy clinical trials with development of patient-derived experimental tumor models for functional interrogation. By understanding how oncogenic signaling modulates cell proliferation, endogenous stress responses, and ultimately cell survival, Dr. Hata aims to identify promising new therapeutic strategies that can be quickly moved into clinical trials for altering the evolution of drug resistance in lung cancer.

Engineering the Cancer Genome

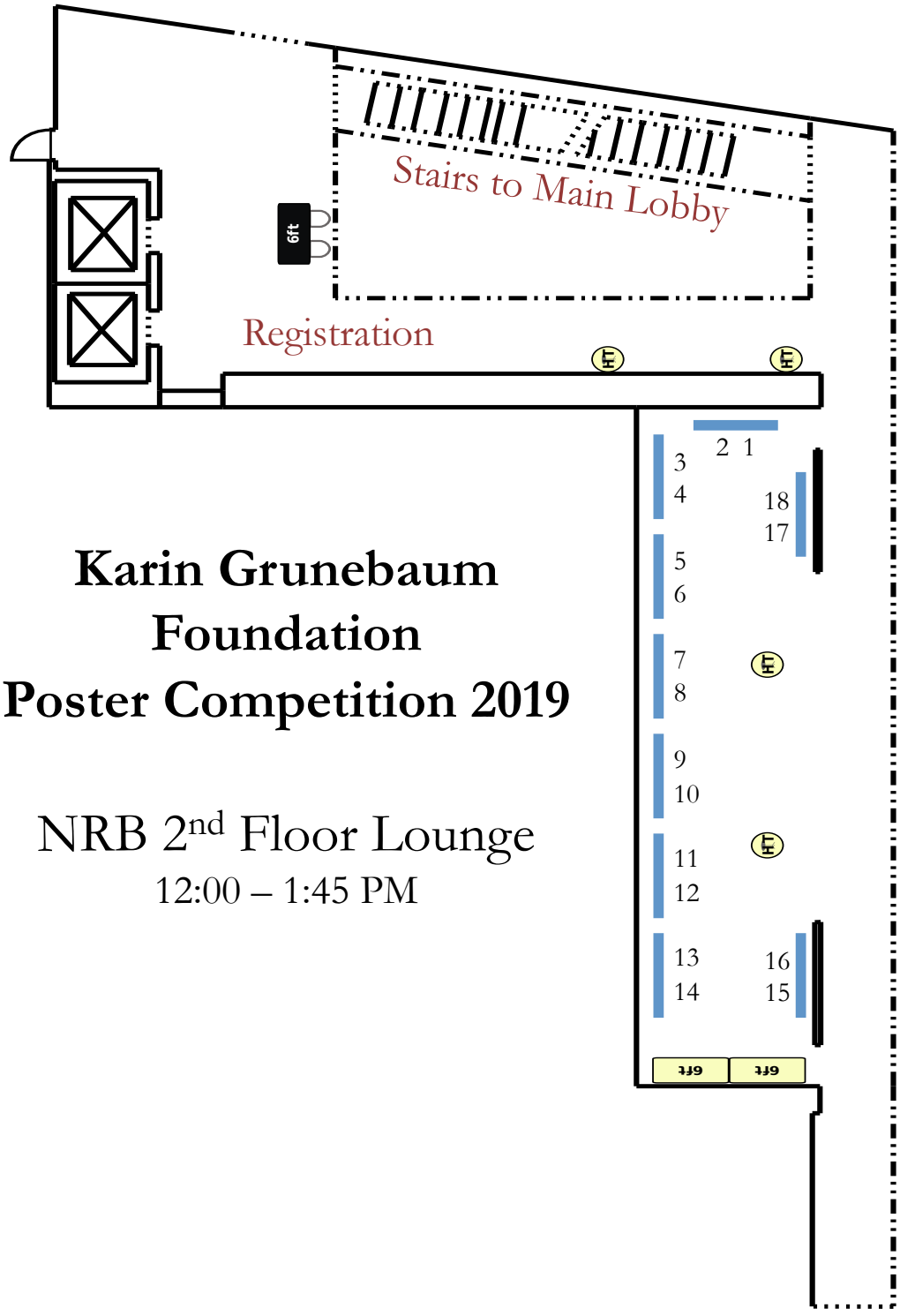
Tyler Jacks, PhD

David H. Koch Professor of Biology, Massachusetts Institute of Technology
Howard Hughes Medical Institute Investigator



Tyler Jacks, PhD is the Director of the Koch Institute for Integrative Cancer Research at MIT, the David H. Koch Professor of Biology, and an Investigator of the Howard Hughes Medical Institute. Over the course of his career at MIT, Dr. Jacks has pioneered the use of gene targeting technology to study cancer-associated genes and to construct models of many human cancer types, including cancers of the lung, brain, and ovary. His laboratory has made seminal contributions to the understanding of the effects of mutations of several common cancer-associated genes. This research has led to novel insights into tumor development, normal development and other cellular processes, as well as new strategies for cancer detection and treatment. Dr. Jacks has published more than 300 scientific papers.

Dr. Jacks has served on the Board of Scientific Advisors of the National Cancer Institute, is the immediate past chair of the National Cancer Advisory Board, and served as co-chair of Vice President Biden's Cancer Moonshot's Blue Ribbon Panel. He is an advisor to several biotechnology and pharmaceutical companies, and is a member of the Board of Directors of Amgen and Thermo Fisher Scientific. He is a founder of T2 Biosciences and Dragonfly Therapeutics, where he serves as chair of the Scientific Advisory Board. Among many honors, Dr. Jacks is a member of the National Academy of Sciences, the National Academy of Medicine, the American Academy of Arts and Sciences, and the Fellows of the American Association of Cancer Research Academy. In 2015, he received the Killian Award, the highest honor MIT bestow upon a member of its faculty.



Karin Grunebaum Foundation Poster Competition 2019

NRB 2nd Floor Lounge

12:00 – 1:45 PM

Karin Grunebaum Foundation Poster Competition

1) **Sherry Chao**
   Towards generalizing classification of whole-genome doubling across cancers

2) **Antonella Dost**
   Modeling lung cancer progression with tumor organoids

3) **Jefte Drijvers**
   Diet-induced obesity causes metabolic reprogramming and suppresses immunity in the tumor microenvironment

4) **Samuel Freeman**
   TBX3 expression, T cell abundance and mutational burden predict outcomes of melanoma to checkpoint blockade therapy

5) **Manav Gupta**
   The mammalian SWI/SNF complex regulates origin firing in lung cancer

6) **Yixuan He**
   Genetic Ancestry Versus Environmental Differences in Oncogenic Mutations and Driver Genes in Lung Adenocarcinoma

7) **Jennifer Hsiao**
   The role of methylthioadenosine phosphorylase in melanoma

8) **Mitchell Leibowitz**
   Therapeutic CRISPR strategies induce on-target genome catastrophe

9) **Adrija Navarro**
   Investigating the role of ALG3 in the regulation of N-glycosylation by the PI3K/AKT signaling pathway

10) **Nishita Parnandi**
    TIRR regulates 53BP1 in both DNA repair and P53-mediated cellular fate

11) **Alexandra Pourzia**
    Cancer cell defects in apoptosis attenuate killing by CAR T cells

12) **Carmen Sivukumaren**
    Targeting the PI5P4K and PIKfyve Lipid Kinases Using Novel Covalent Inhibitors in Cancer

13) **Devon Stork**
    Genetic Code Expansion in Bacillus subtilis

14) **Alfredo Valencia**
Cancer and intellectual disability-associated mutations alter mSWI/SNF nucleosome interactions and transcriptional regulation

15) **Michael Vinyard**
CRISPR-suppressor scanning reveals a nonenzymatic role of LSD1 in AML

16) **Marina Watanabe**
Developing a novel combination therapy using EZH2i for HER2+ breast cancer

17) **Golnaz Morad***
Nanocourse: Extracellular vesicles in human pathologies

Student Poster Abstracts

1) **Sherry Chao**
G2 BIG Program

Towards generalizing classification of whole-genome doubling across cancers
Cancer remains an intractable disease. The presence of certain cancer-specific characteristics, such as whole-genome doubling (WGD), have important implications that serve to inform clinical care. To date, classifying these characteristics from readily-available data (i.e., histopathology images) is impaired by variability between cancer types. In order to classify WGD across cancers, we take a multi-task view of the problem by treating cancer types as separate tasks. This meta-learning approach is able to outperform the baseline on sample-level accuracy and generalize well to held-out cancers.

2) **Antonella Dost**
Kim Lab

Modeling lung cancer progression with tumor organoids

Lung cancer is the leading cause of cancer related deaths worldwide and adenocarcinoma (ADC) is the most common subtype. Despite tremendous research efforts, there is no targeted therapy for patients that harbour a mutation in the most commonly mutated oncogene in ADC, Kras. Organoids are a promising new way to study tumor progression without the limitations of cancer cell lines or lengthy mouse models. To study ADC progression in vitro, we established a tumor organoid system using primary alveolar type 2 (AT2) cells from uninduced KrasLSL-G12D;YFPfl/fl (KY) and KrasLSL-G12D;p53fl/fl;YFPfl/fl (KPY) mice. Cells were induced in vitro and resulting oncogenic Kras expressing cells were plated in a 3D air-liquid-interphase co-culturing system together with supporting stromal cells. Tumor organoid progression recapitulated in vivo tumor progression histologically. We detected different types of tumor organoids that differed in size, morphology, and gene expression. Single cell RNA-sequencing revealed that the various types of tumor organoids represented different differentiation stages. Some organoids mostly contained cells that expressed AT2 markers such as SftpC and Lyz2, while others were more undifferentiated and expressed developmental markers such as Sox9 and Hmga2. Loss of differentiation markers and upregulation of developmental markers is a sign of advanced ADC in vivo. Therefore, in our system different tumor organoids represented different ADC stages in vivo. Furthermore, the tumor organoids had various degrees of stromal cell interaction. Immunofluorescence staining for the mesenchymal marker vimentin revealed that some organoids did not interact with stromal cells directly, while others were closely associated with stromal cells. Some organoids were even completely surrounded by stromal cells and showed a distinct morphology. Future studies will aim to understand the tumor propagating potential of the different types of organoids observed and how this translates to tumor progression in vivo.

3) **Jefte Drijvers**
Sharpe Lab

Diet-induced obesity causes metabolic reprogramming and suppresses immunity in the tumor microenvironment

Obesity is a well-known risk factor for many cancers. Various systemic alterations, including nutrient availability and signaling changes, are associated with obesity. However, how these changes impact the anti-cancer immune response is not yet clear. Tumors maintain an immunosuppressive, nutrient-poor microenvironment, and it is unknown whether the systemic metabolic changes associated with obesity are reflected in the tumor microenvironment (TME) locally. We tested the hypothesis that obesity impacts anti-tumor immune function in the TME using the murine obesity model of feeding a high-fat diet (HFD). Our studies show that HFD accelerates tumor growth by inhibiting the anti-cancer immune response. Moreover, HFD induces different metabolic adaptations in tumor cells and intratumoral immune cells, resulting in an altered nutrient composition in the TME. Genetic manipulations that alter metabolic reprogramming in tumor cells normalize the metabolic milieu in the TME and reduce tumor growth in an immune system-dependent manner. These findings demonstrate how cell-type specific rewiring of metabolism in the TME in response to systemic metabolic changes resulting from diet-induced obesity inhibits the anti-tumor immune response locally. Analysis of publicly available transcriptional data of human cancers from The Human Genome Atlas suggest that similar metabolic reprogramming events correlate with obesity and decreased anti-tumor immune function in human cancers as well. Thus, our studies reveal a novel mechanism linking obesity to cancer through decreased anti-tumor immune function and may inform the development of cancer therapies targeting cancer metabolism and anti-tumor immunity.

4) **Samuel Freeman**
Getz Lab

TBX3 expression, T cell abundance and mutational burden predict outcomes of melanoma to checkpoint blockade therapy

Cancer immunotherapy with checkpoint blockade has improved survival and outcomes in melanoma and other tumor types, but a majority of patients do not respond. Both high tumor mutation burden (TMB) and high levels of tumor-infiltrating T cells have consistently been associated with response to immunotherapy, but integrative models to predict clinical benefit have not been as comprehensively explored. We sequenced tumors from patients receiving checkpoint blockade and aggregated datasets with whole exome sequencing (n = 189) and bulk RNA sequencing (n = 154) to derive genomic and transcriptomic factors that predict survival and response to immunotherapy. We calculated T cell burden (TCB) and B cell burden (BCB) based on rearranged TCR/Ig sequences, respectively, and combined them with TMB to create a DNA-based predictor of survival and response to checkpoint inhibition. Clustering of tumor transcriptomes identified 5 tumor subtypes based on melanocyte differentiation, immune infiltration and keratin levels. These subtypes were associated with distinct survival outcomes after immunotherapy, and patients whose tumors had high immune infiltrate and low expression of TBX3 prior to checkpoint blockade had longer survival times. Thus, both RNA-based (TBX3 and tumor subtype) and DNA-based metrics (TMB/TCB or TMB/BCB) can be used as pre-treatment predictors of survival after checkpoint blockade in melanoma patients.

5) **Manav Gupta**
Kim Lab

The mammalian SWI/SNF complex regulates origin firing in lung cancer

The SMARCA4 gene encodes the ATP-dependent helicase component of the SWI/SNF complex, BRG1, is involved in chromatin modulation and either mutated or lost in up to 20% of human non-small cell lung cancers. To investigate how loss-of-function mutations in SMARCA4 contributes to lung tumorigenesis, we generated murine and human BRG1 knockout cell lines from tumor cells derived from a KrasG12D/+; p53Î”/Î” (KP) mouse model of lung adenocarcinoma, and KRAS/p53 mutant human lung cancer cell lines H460, H2009, and Calu6. RNA-sequencing of murine Brg1 null cells and human SMARCA4-mutant patient data taken from the TCGA cancer datasets revealed the upregulation of the ATR-mediated response to replication stress and activation of the pre-replicative complex as the top cancer pathways in these cancers. We hypothesized that loss of BRG1 contributed to increased replication stress associated DNA damage and genome instability. BRG1-deficient cells had significant more gamma-H2Ax foci and RPA-bound single-stranded DNA compared to isogenic controls. Western blot analysis confirmed activation of the ATR pathway through increased phospho-CHK1 activity in BRG1-deficient cells. Mechanistically, we observed that loss of BRG1 expression leads to increased number of fired origins of replication as measured by DNA fiber assays. The correlation between replication stress and DNA damage was assessed using comet analysis and we observed increased olive tail moments in BRG1-deficient cells, indicative of more DNA damage and genome instability. We then treated our cells with inhibitors that target key DNA damage response kinases, ATM and ATR. While ATM inhibition resulted in no observable change between BRG1 wildtype and mutant cells, BRG1-deficient cells were more sensitive (3-5 fold) to ATR inhibition compared to isogenic wildtype cells. ATR inhibition was also able to significantly increase the total amount of DNA damage in BRG1-deficient cells, suggesting that loss of BRG1 leads to dependence on the ATR pathway to prevent further genome instability. We re-expressed human BRG1in murine/human Brg1/BRG1 knockout cells and observed a reversal in response to ATR inhibition. We also found that combinatorial treatment with replication stress inducing reagents such as topoisomerase I inhibitor irinotecan or hydroxyurea further sensitized BRG1-deficient cells to ATR inhibition in some models. To address the role of the SWI/SNF complex in origin firing and DNA replication, we examined levels of the early DNA origin licensing proteins and found that loss of BRG1 expression strongly correlated with increased CDC6 presence across all our models of BRG1 loss. To further study the role of Brg1 as a tumor suppressor gene in the lung, we compared KP mice versus KP mice harboring floxed Brg1 (KPB) alleles and found that KPB mice had a significantly higher number of tumor lesions and higher-grade tumors after 13-15 weeks of tumor induction. Interestingly, there was a significant correlation in loss of Brg1 and presence of key immune evasion ligand Pd-l1 by immunohistochemistry in Brg1 null tumors. Subcutaneous injections of Brg1 null murine isogenic lines into flanks of immunocompetent mice further showed the increase in Pd-l1 expression only in tumors derived from Brg1 null cells. Taken together, our data suggests that BRG1 or the SW/SNF complex may have a role in regulating DNA replication in lung cancer cells, and it does so by mediating CDC6 expression and controlling origin firing.

6) **Yixuan He**
Gusev Lab

Genetic Ancestry Versus Environmental Differences in Oncogenic Mutations and Driver Genes in Lung Adenocarcinoma

Cancers differ significantly between ethnic backgrounds. We estimated genetic ancestry of over 1000 individuals with lung adenocarcinoma (LUAD) cancer in the Dana Farber Profile database and performed analysis on the influence of genetic ancestry on genomic alterations. From roughly 700 mutation phenotypes, we identify 31 mutations significantly associated with at least one of five major ancestry groups. To better characterize the mutations that drive ancestry differences, we also identify sets of mutations independently associated with each ancestry. Our analysis reveals many novel significant associations between mutations and ancestry. For example, while Asian ancestry has been known to be significantly associated with EGFR mutations in LUAD, we also find that many non-Asian ancestries, such as Jewish and African ancestry, are significantly associated with EGFR. Lastly, to better understand genetic versus environmental contributions, we compare discrepancies between self-reported ethnicity and estimated ancestry in mutation phenotypes and find that most differences are explained by genetic ancestry.

7) **Jennifer Hsiao**
Fisher Lab

The role of methylthioadenosine phosphorylase in melanoma

Methylthioadenosine phosphorylase (MTAP) is an enzyme that is expressed in virtually all normal tissues but lost in many cancers. MTAP deficiency can be due to either deletion of the MTAP gene or methylation of the MTAP promoter. In normal cells, MTAP catalyzes the conversion of methylthioadenosine (MTA), produced during polyamine biosynthesis, to adenine and 5-methylthioribose-1-phosphate, which is subsequently converted to methionine. Although recent genome wide association studies (GWAS) have associated the MTAP locus with melanoma risk, the molecular mechanisms linking MTAP loss to increased tumorigenesis are not yet fully understood. In this study, we hypothesized that loss of MTAP and the resulting accumulation of MTA would have an effect on microphthalmia transcription factor (MITF), the master regulator of melanocytes that has been shown to be an oncogene in melanoma. We present a novel signaling mechanism in which loss of MTAP and subsequent accumulation of MTA induces expression of MITF via inhibition of the phosphodiesterase PDE4D3 by MTA. Inhibition of PKA abolishes the induction of MITF, suggesting that the PKA pathway is hyperactivated when MTAP levels are low. We show that downregulation of MTAP expression leads to increased proliferation of melanoma cells. Using a melanoma xenograft mouse model, we observed that downregulation of MTAP expression leads to increased tumor growth in vivo. These data all point to MTAP as a tumor suppressor in melanoma, and indicate that MTAP loss is an alternative mechanism of dysregulating MITF, a known oncogene in melanoma.

8) **Mitchell Leibowitz**
Pellman Lab

Therapeutic CRISPR strategies induce on-target genome catastrophe

CRISPR/Cas9 is emerging as a major tool for correction of genetic disorders. While much focus has been on finding off-target effects of CRISPR/Cas9 cutting, little focus has been placed on negative effects of on-target cutting. In this poster we show that CRISPR/Cas9 is highly effective at inducing chromosome missegregation and micronucleus formation. These missegregation events involve DNA from the cut-site to the telomere of the on-target cut chromosome. Single-cell sequencing after micronucleus formation revealed chromothripsis, massive numbers of local rearrangements, contained on the missegregated DNA segment. Furthermore, cutting was frequently accompanied by aneuploidies and other genomic events including chromosome bridge-formation. As a validation of these results for the clinical application of CRISPR/Cas9 we tested micronucleus formation and missegregation in therapeutically relevant CD34+ hematopoietic stem and progenitor cells using gRNAs currently under investigation for treatment of sickle cell anemia. Although micronuclei were formed, engrafted cells never showed chromosome abnormalities at karyotypic resoltion. Finally, we also demonstrated frequent on-target chromosome missegregation and aneuploidy resulting from use of non-cutting C to T base editors. These results highlight a new caution that must be considered in all cases where CRISPR/Cas9 is utilized.

9) **Adrija Navarro**
Toker Lab

Investigating the role of ALG3 in the regulation of N-glycosylation by the PI3K/AKT signaling pathway

The PI3K/AKT signaling pathway, which is frequently dysregulated in cancer, controls key cellular processes such as survival, proliferation, metabolism, and growth. Protein glycosylation, the process by which carbohydrates are added to amino acids, is essential for proper protein folding and is deregulated in cancer. High proliferation rates in cancer require amplified protein folding. The glycosyltransferase ALG3 catalyzes the addition of a mannose to a glycan precursor once it is flipped into the endoplasmic reticulum lumen during glycan production. ALG3 is required for proper glycan formation and is implicated as a putative AKT substrate. ALG3 resides proximal to *PIK3CA* in the 3q26 amplicon. Consequently, *PIK3CA* and *ALG3* are co-amplified in 89%, 28% and 76% of lung (SCC), breast and ovarian carcinomas, respectively. Notably, we find that in both lung and breast cancer cells, ALG3 is also phosphorylated downstream of PI3K. This represents, to our knowledge, the first identified link between PI3K oncogene signaling and protein glycosylation in the context of cancer. I hypothesize that ALG3 plays a role in the regulation of protein N-glycosylation by PI3K/AKT signaling, and that aberrant PI3K/AKT signaling alters glycosylation, leading to functional consequences in cancer. Specifically, I postulate that cells that harbor *PIK3CA* amplification and ALG3 up-regulation increase glycosylation and protein folding rates, allowing cells to cope with increased protein translation in response to hyperactive PI3K/AKT signaling. This project will advance our understanding of the regulation of glycosylation metabolism by PI3K/AKT signaling and its role in cancer progression; future studies may determine the extent to which combination therapies targeting the PI3K/AKT pathway and protein-glycosylation are effective.

10) **Nishita Parnandi**
Chowdhury Lab

TIRR regulates 53BP1 in both DNA repair and P53-mediated cellular fate

Launching DNA repair and making appropriate cellular fate decisions are both equally crucial to maintain genomic integrity. However, whether these two pathways are co-regulated or happen independently of each other has been a longstanding question. Recently 53BP1, a well-known DSB repair protein, was shown to also have a role in mediating cellular fate decisions by stabilizing TP53, indicating that these two pathways could be co-regulated. In this study we ask if TIRR, a negative regulator of 53BP1 in DSB repair, can also regulate the 53BP1-USP28-P53 axis that controls cellular fate. Indeed, we see that TIRR deficiency results in an increase in p53-mediated gene transactivation and repression events. TIRR-deficient cells have reduced survival and greater sensitivity to the G1/S cell cycle checkpoint upon p53 activation. Furthermore, we reveal that the loss of TIRR results in an *altered* 53BP1 that interacts more strongly with p53 and USP28. Through our study, we demonstrate that TIRR functions as a crucial link between DNA repair and p53-mediated cellular fate by its regulation of 53BP1.

11) **Alexandra Pourzia**
Letai Lab

Cancer cell defects in apoptosis attenuate killing by CAR T cells

CAR T therapy is now an FDA approved treatment for several hematologic malignancies, yet not all patients respond to this treatment. While some resistance mechanisms have been identified, the possibility of cell death pathways modifying response to CAR T therapy remains unexplored. To assess whether cell death pathways in target cancer cells could impact response to CAR T therapy, we utilized a HeLa in vitro model system. HeLa cells with intact (HeLa-19) and deficient Bak/Bax (HeLa-DKO-19) expressing CD19 were co-cultured with CD19 CAR T cells. We observed that Bak/Bax deficiency, which blocks the intrinsic pathway of apoptosis, conferred resistance to CAR T killing. However, this resistance could be overcome at high E:T ratios. To confirm that the intrinsic pathway of apoptosis contributes to CAR T killing, we forced the expression of Bcl-2 and Bcl-XL in HeLa-19 cells, and observed that both of these anti-apoptotic proteins conferred protection from CAR-T killing in a similar manner to Bak/Bax knockout. Additionally, we wanted to assess whether caspases may be required for CAR T killing, given the role of intrinsic apoptosis in our model system. The caspase inhibitor Z-VAD-FMK protected both HeLa-19 and HeLa-DKO-19 cells from CD19 CAR T effector cells in our *in vitro* model system. Lastly, we wanted to ascertain the precise mechanism by which CAR T cells eliminate target cancer cells. CAR T cells were co-cultured with HeLa-19 and HeLa-DKO-19 targets in the presence of blocking antibodies against Fas ligand and TRAIL, along with 3,4-dichloroisocoumarin, a granzyme inhibitor. We observed that granzyme inhibition, but not death ligand blocking antibodies, provided protection from CAR T cells. Additionally, a soluble factor contributed to CAR T killing, as demonstrated by conditioned media experiments in which target cancer cells were exposed to filtered supernatant from CAR T co-culture experiments. In conclusion, intrinsic apoptosis allows for efficient elimination of target cancer cells by CD19 CAR T cells. This process also requires downstream caspases, and seems to be mediated in part by granzymes. This work implies that agents that promote tumor cell intrinsic apoptosis may be candidates for combination treatment with CAR T therapy; and suggests that tumor cells that are resistant to intrinsic or downstream apoptosis may resist CAR T therapy. Future work will explore whether the intrinsic apoptotic pathway also modifies response to CAR T cells in a mouse model, and identify soluble factors that contribute to CAR T killing of target cells.

12) **Carmen Sivakumaren**
Gray Lab

Targeting the PI5P4K and PIKfyve Lipid Kinases Using Novel Covalent Inhibitors in Cancer
The phosphatidylinositol 5-phosphate 4-kinases (PI5P4Ks) have been demonstrated to be important for cancer cell proliferation and other diseases. However, the therapeutic potential of targeting these kinases is understudied due to a lack of potent, specific small molecules available. Here we present the discovery and characterization of a novel pan-PI5P4K inhibitor, THZ-P1-2, that covalently targets cysteines on a disordered loop in PI5P4Ka/b/g. THZ-P1-2 demonstrates cellular on-target engagement with limited off-targets across the kinome. AML/ALL cell lines were sensitive to THZ-P1-2, consistent with PI5P4K's reported role in leukemogenesis. THZ-P1-2 causes autophagosome clearance defects and upregulation in TFEB nuclear localization and target genes, disrupting autophagy in a covalent-dependent manner and phenocopying the effects of PI5P4K genetic deletion. PIKfyve, a related understudied lipid kinase, has also been validated as a target in B-cell Non-Hodgkin lymphoma. We observed that THZ-P1-2 was also capable of covalently binding to PIKfyve, albeit at a much lower preference than PI5P4K. We reengineered compound selectivity towards PIKfyve and identified a class of compounds from the THZ-P1-2 scaffold exhibiting picomolar-to-nanomolar affinity. These inhibitors exhibit cellular on-target engagement and vacuolar enlargement, a well-established PIKfyve inhibitory phenotype. Docking/modeling studies and mass spectrometry revealed a similar distant cysteine, Cys1970, to be covalently labeled by these compounds. Lastly, this THZ-family of inhibitors, particularly two top compounds MFH-5-3 and MFH-5-4, exhibit potent antiproliferative activity in lymphoma cell lines dependent on covalent binding. Taken together, our studies demonstrate that the PI5P4Ks and PIKfyve are tractable targets, with inhibitors serving as useful tools to further interrogate the therapeutic potential of these noncanonical lipid kinases and inform drug discovery campaigns in the context of cancer and potentially other autophagy-dependent diseases.

13) **Devon Stork**
Garner and Church Labs

Genetic code expansion in Bacillus subtilis

Encoding nonstandard amino acids (nsAAs) into proteins allows for expansion of the genetic code beyond the standard 20 amino acids for probing, labelling, or controlling proteins in a minimally disruptive manner. However, these tools have been mostly unavailable in many bacterial systems, such as the primary gram-positive microbiome-relevant model organism, Bacillus subtilis. Here we describe the use of several classes of genome-integrated synthetases to incorporate many different nsAAs into proteins in B. subtilis, including nsAAs used for biorthogonal labelling, fluorescence imaging and photo-crosslinking. We also demonstrate a nsAA-dialable protein expression system in this bacterium. The expression of a target gene can be enhanced >50-fold when nsAAs are added and up to 1000s-fold when combined with a transcriptional inducer. The general and effective expansion of nsAA technology to B. subtilis can facilitate our understanding of cell biology in this bacterium and industrial protein production of nsAA-containing proteins such as biologic therapeutics.

14) **Alfredo Valencia**
Kadoch Lab

Cancer and intellectual disability-associated mutations alter mSWI/SNF nucleosome interactions and transcriptional regulation

Mammalian SWI/SNF (mSWI/SNF or BAF) complexes are multi-component machines that remodel chromatin architecture, however the mechanisms by which this is achieved remain incompletely defined. Recent studies have begun to characterize the effects of deletion of the SMARCB1 (BAF47) mSWI/SNF subunit, which is a defining feature of malignant rhabdoid tumor, one of the most aggressive and lethal pediatric cancers. Through a variety of biochemical, structural, and chromatin mapping experiments we examine the molecular and genome-wide regulatory consequences of recurrent single-residue mutations in the coiled coil (CC) domain of SMARCB1, which are found in several cancers and implicated in the development in the intellectual disability disorder, Coffin-Siris syndrome (CSS). Intriguingly, we find that the SMARCB1 CC domain binds the nucleosome acidic patch and that all cancer and CSS-associated mutations disrupt this binding. Furthermore, SMARCB1 CC point mutations significantly abrogate mSWI/SNF complex-mediated nucleosome remodeling activity in vitro and de novo enhancer DNA accessibility in cells, without changes in genome-wide complex localization. These studies unmask a major, evolutionarily conserved function of SMARCB1 that is perturbed in both intellectual disability as well as cancer.

15) **Michael Vinyard**
Liau Lab

CRISPR-suppressor scanning reveals a nonenzymatic role of LSD1 in AML
We investigated the role of Lysine-specific histone demethylase 1 (LSD1) in acute myeloid leukemia (AML) through an in situ tiling mutagenesis approach. We demonstrate that this approach can identify drug resistant mutations in the LSD1 catalytic site that impair drug binding and simultaneously inactivate enzyme activity. These results indicate that LSD1 demethylase activity is not required for AML survival. We further demonstrate that drug-mediated disruption of a LSD1-GFI1B complex is necessary and sufficient to block AML growth. Through our unbiased approach, we discovered mutations in distal domains of LSD1 that suppress the anti-proliferative activity of LSD1 inhibitors. Moreover, our studies demonstrate that in situ tiling mutagenesis in the presence of a chemical suppressor can identify functional hotspots beyond the binding site of the small molecule. These data provide critical information on molecular mechanism of action of both drug and target biology.

16) **Marina Watanabe**
Cichowski Lab

Developing a novel combination therapy using EZH2i for HER2+ breast cancer

The receptor tyrosine kinase (RTK) human epidermal growth factor receptor 2 (HER2) is overexpressed in 15-25% of breast cancers and acts as a driver of tumor growth. Though there are four FDA-approved agents in the clinic that specifically target HER2, metastatic HER2+ breast cancer remains incurable with a median overall survival of 56.5 months. In addition, the response rates of patients with metastatic disease vary considerably, with 20-50% and 60-80% of patients unresponsive to first- and second-line therapies respectively. Therefore, it is necessary to discover a new therapy able to overcome the therapeutic resistance of HER2+ breast cancer that currently leads to patient relapse, and ultimately, death.

One approach that our lab has successfully used to develop more effective therapies for other tumor types is to co-target different oncogenic pathways for a more potent effect. A target of interest is the histone methyltransferase enhancer of zeste homolog 2 (EZH2), which is overexpressed in many cancer types including breast, prostate, bladder, gastric, lung, and hepatocellular carcinoma. Interestingly, in breast cancer, EZH2 is overexpressed in the more aggressive subtypes including HER2+. EZH2 expression levels correlate with a worse prognosis and parallels cancer progression. We chose to investigate the therapeutic potential of combining EZH2 inhibitors (EZH2i) with lapatinib, an FDA-approved HER2-targeted therapy for late stage HER2+ breast cancer. In vitro assays show that the combination has a potent cytotoxic effect and renders lapatinib-sensitive HER2+ breast lines more sensitive, and causes lapatinib-resistant lines to become sensitive. In addition, in vivo testing of both lapatinib-sensitive and resistant HER2+ xenografts showed potent regression with the combination, confirming the extremely promising results. We will identify the biological settings in which this combination may be most effective and elucidate its molecular mechanism of action. This work will not only validate a promising new therapeutic combination, but will also provide insight into additional vulnerabilities of HER2+ breast cancer, possibly leading to additional therapeutic strategies.

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