47th Annual
UNC Lineberger Comprehensive Cancer Center
Postdoctoral-Faculty Research Day

Thursday
October 13 ,2022
8 AM – 6 PM

Magnolia C Room & Classroom 109 (Loudermilk Hall)
Rizzo Center
150 Dubose Home Ln, Chapel Hill, NC 27517

Event sponsored by the LCCC Training and Education Program (Cancer Research Training and Education Coordination, CRTEC). Planning and implementation of this event was made possible by the essential support of the Postdoctoral Committee and by the generous assistance of LCCC faculty and staff.

POSTDOCTORAL COMMITTEE MEMBERS

Fernando Diaz, MD
Matthew LeBlanc, PhD, RN
Denis Okumu, PhD
Sherette Godfrey, PhD

FACULTY ADVISOR

Bernard E. Weissman, PhD
Professor of Pathology and Laboratory Medicine, LCCC Associate Co-Director of Education

Session 1 (A): Basic & Clinical/Translational Sciences

(10:45 – 11:45 AM, Magnolia C Room / Zoom Session A)

10:45 – 11:05 AM: **Peter Buttery**, graduate student
(Dr. Stephen Frye and Dr. Lindsey James, Molecular Therapeutics)
Title: Development and characterization of covalent chemical probes for chromodomain proteins MPP8 and CDYL2
Abstract: Histone post-translational modifications are interpreted by specific classes of epigenetic "reader" proteins to help regulate gene expression. Methyllysine (Kme) reader proteins recognize and bind to Kme marks to help convey methylation signals. The specific contexts in which Kme readers bind to chromatin and the resulting biological outcomes are the subject of much study. One way in which we seek to elucidate the functions of these proteins is through the development of chemical probes that serve as antagonists for the reader domains. Two targets for which such chemical tools are lacking are the chromodomain-containing proteins MPP8 and CDYL2. MPP8 participates in the Human Silencing Hub (HUSH) complex to mediate heterochromatin formation through H3K9me3 recognition and recruitment of methyltransferase SETDB1. HUSH mediated silencing has been implicated in a number of cancers including AML, making MPP8 a promising therapeutic target. CDYL2 similarly acts to recruit methyltransferases G9a and EZH2 at H3K9me2 and H3K27me3 marks and has been implicated in triple negative and ER+/HER2- breast cancer. As such, we seek to create chemical probes for these proteins to both elucidate their biological functions and to further evaluate their therapeutic potential. Our lab has successfully developed chemical probes for a number of Kme readers, yet such endeavors are often hampered by potency and selectivity challenges due to shallow binding pockets and the high sequence homology within Kme reader families. To address these issues, we have designed targeted covalent antagonists that contain electrophilic warheads to engage a cysteine residue within the binding pocket of these proteins. Targeted covalent inhibitors afford a number of benefits over traditional non-covalent inhibitors, including prolonged duration of action, resilience to drug resistance mechanisms, and improved potency and selectivity. This strategy represents a novel approach for developing chemical probes for these proteins.

11:05 – 11:25 AM: **Maddy Jenner**, graduate student
(Dr. Jen Jen Yeh, Clinical Research)
Title: Targeting the tumor-stroma in pancreatic cancer
Abstract: Pancreatic cancer remains one of the deadliest cancers with only 10% of patients surviving 5 years post-diagnosis. Cytotoxic therapies are the standard of care, but these only benefit a subset of patients before ultimately encountering resistance. It is widely accepted that the tumor microenvironment plays a critical role in modulating drug response and enabling resistance mechanisms. One of the major contributing cell types to the microenvironment are cancer-associated fibroblasts (CAFs). While it is still unknown which CAF populations are tumor-supportive versus tumor-suppressive, there is established reciprocal signaling between the tumor and CAFs. CAFs can secrete soluble factors, metabolites, and extracellular matrices that have been found to feed tumor cells, thus supporting proliferation, or altering tumor signaling cascades. For these reasons, targeting the tumor stroma (CAFs and connective tissue) is an emerging therapeutic approach. There have been a few stroma-targeting therapies in clinical trials for pancreatic cancer. However, none have been approved to date. This is partially due to CAF heterogeneity; current approaches are not selective against normal tissue or tumor-suppressive CAFs. Therefore, there is an unmet need to understand tumor-CAF biology to harness its therapeutic potential. Our lab has identified differentially expressed kinases in patient pancreatic cancer samples that could be targeted for individualized therapy. My work will identify CAF-induced kinase vulnerabilities in tumor cells. Patient-derived xenograft pancreatic cancer cell lines will

be cocultured with CAF lines to represent the tumor microenvironment and cultivate tumor-CAF cell signaling. The cell viabilities of tumor and CAF cells can be collected simultaneously after drug treatment using a novel, dual luciferase system. This allows for simultaneous cell viability readings of both cell types in the same well. Kinase inhibitor hits will maximize tumor cell sensitivity and minimize CAF sensitivity. Characterized hits will identify specific kinase drug targets that differ in drug sensitivity between tumor and CAF cells. This work will elucidate tumor-stroma vulnerabilities and signaling components and identify kinase inhibitors to treat pancreatic cancer.

11:25 – 11:45 AM: **Justin Sperlazza**, postdoctoral researcher
(Dr. Ian Davis, Cancer Genetics)
Title: Epigenetic modulation of cancer antigens to enhance immunotherapy in pediatric solid tumors
Abstract: Chimeric Antigen Receptor T-cell (CAR-T) therapy has revolutionized the treatment of relapsed/refractory hematologic malignancies by engineering a patient's own immune system to target tumor cells. Patients with solid tumors however have yet to see a meaningful benefit from CAR-T therapy due to, in part, heterogenous expression of targetable cancer antigens. Reversible transcriptional programs control cancer antigen expression, which ultimately implicates chromatin regulation pathways as a target for intervention. Therefore, we hypothesize small molecule inhibitors of chromatin modifying enzymes can enhance the sensitivity of solid tumors to CAR-T cell immunotherapy. The histone methyltransferase EZH2 was recently shown to regulate expression of tumor antigen GD2, a disialoganglioside expressed in a range of solid tumors. The Davis lab has demonstrated tazemetostat, an FDA approved EZH2 inhibitor, can induce a 50-100-fold increase in GD2 expression Ewing sarcoma, osteosarcoma, and neuroblastoma cell lines. In multiple xenograft models of Ewing sarcoma, we also find oral administration of tazemetostat results in a dose-depended induction of GD2. Importantly, in vitro GD2-targeted CAR-T cell lysis of the examined Ewing sarcoma and osteosarcoma cell lines is negligible at baseline and dramatically increases following tazemetostat treatment, corresponding to the increased GD2 expression. To identify other chromatin regulating compounds that modulate GD2 expression, we developed a flow-cytometry based screening assay and evaluated UNC's EpiG Diamond compound library. The screen identified 25 presumptive hits that induced GD2 expression, 21 of which inhibit pathways outside of the EZH2/PRC2 complex. The seven most robust hits were validated and evaluated for synergistic interactions with EZH2 inhibitors. We discovered the class I HDAC inhibitors (entinostat, tacedinaline, vorinostat) and the DOT1L inhibitor pinometostat are capable of synergistically inducing tumor GD2 expression in combination with tazemetostat, both in vitro and in a xenograft model of Ewing sarcoma. In summary, we identified multiple chromatin-targeted molecules that induce GD2 expression in Ewing sarcoma and osteosarcoma cell lines and shown increased GD2 expression sensitizes tumor cells in vitro to lysis by CAR-T cells. Ongoing mouse studies with GD2-targeted CAR-T cells are investigating the identified small molecule effects on tumor response and survival. Results of this project will provide preclinical data to support the addition of chromatin directed inhibitors in UNC's ongoing GD2.CAR-T trial for pediatric solid tumors. However, the principle of augmenting tumor antigen expression with small molecules has broader significance to cancer antigen targeted therapies and potential to benefit any immunotherapy approach with antigen dependence, such as monoclonal antibody-based drugs, natural killer-CAR cells, *etc*.

Session 1 (B): Population Sciences

(10:45 – 11:45 AM, Classroom 109 / Zoom Session B)

10:45 – 11:05 AM**:** **Jessica Islam**, former LCCC postdoctoral researcher; current Assistant Professor of Cancer Epidemiology at Moffitt Cancer Center; 2022 winner of the Michael S. O'Malley Alumni Award for Publication Excellence in Cancer Population Sciences

(Dr. Jennifer Lund and Dr. Jennifer Smith, Cancer Epidemiology)
Title: Impacts of the COVID-19 pandemic on mental health and care among cancer survivors in the US
Abstract: PURPOSE: The COVID-19 pandemic has affected the mental health of adults in the United States because of recommended preventive behaviors such as physical distancing. Our objective was to evaluate mental health symptoms and identify associated determinants among cancer survivors during the COVID-19 pandemic in the United States. METHODS: We used nationally representative data of 10,760 US adults from the COVID-19 Impact Survey. We defined cancer survivors as adults with a self-reported diagnosis of cancer (n = 854, 7.6%). We estimated associations of mental health symptoms among cancer survivors using multinomial logistic regression. We estimated determinants of reporting at least one mental health symptom 3-7 times in the 7 days before survey administration among cancer survivors using multivariable Poisson regression. RESULTS: Cancer survivors were more likely to report feeling nervous, anxious, or on edge (adjusted odds ratio [aOR], 1.42; 95% CI, 1.07 to 1.90); depressed (aOR, 1.57; 95% CI, 1.18 to 2.09); lonely (aOR, 1.42; 95% CI, 1.05 to 1.91); and hopeless (aOR, 1.51; 95% CI, 1.11 to 2.06) 3-7 days per week in the last 7 days when compared with adults without cancer. Among cancer survivors, adults of age 30-44 years (adjusted prevalence ratio [aPR], 1.87; 95% CI, 1.18 to 2.95), females (aPR, 1.55, 95% CI, 1.12 to 2.13), adults without a high school degree (aPR, 1.79; 95% CI, 1.05 to 3.04), and adults with limited social interaction (aPR, 1.40, 95% CI, 1.01 to 1.95) were more likely to report at least one mental health-related symptom in the last 7 days (3-7 days/week). CONCLUSION: Cancer survivors are reporting mental health symptoms during the COVID-19 pandemic, particularly young adults, adults without a high school degree, women, and survivors with limited social support.

11:05 – 11:25 AM: **Claire (I-Hsuan) Su,** graduate student
(Dr. Jennifer Lund,1 Cancer Epidemiology)
Title: Comparing the effects of neoadjuvant versus adjuvant chemotherapy in stage II-III, triple-negative breast cancer: Illustration of trial emulation using the clone-censor-weight method
Abstract: BACKGROUND: The target trial framework can guide the robust design of comparative effectiveness studies using observational data. Emulation conducted with the clone-censor-weight method (CCW) addresses immortal time bias introduced by misalignment of study eligibility, start of follow-up, and treatment initiation to estimate per-protocol treatment effects. OBJETIVE: To apply the CCW method for evaluating effect of timing of chemotherapy in relation to surgery for stage II-III, triple negative breast cancer (TNBC) using the National Cancer Database (NCDB). METHODS: We identified incident female, stage II-III, adult (18 years and older) TNBC patients from 2013-2017 using the American College of Surgeons NCDB. We first cloned each patient and assigned each clone to 1 of the 2 treatment strategies. The neoadjuvant chemotherapy (NACT) strategy was defined as initiating chemotherapy within 9 weeks from diagnosis followed by surgery within 40 weeks from diagnosis. The adjuvant chemotherapy (ACT) strategy was defined as surgery within 9 weeks from diagnosis followed by initiating chemotherapy within 18 weeks from diagnosis. Clones were followed from diagnosis until death or end of data availability. Clones were analytically censored in their treatment arm when their observed treatment pattern deviated from the assigned strategy. For example, clones assigned to NACT were analytically censored at 9 weeks if they did not initiate chemotherapy within 9 weeks of diagnosis. To account for potentially informative censoring, we calculated inverse probability of censoring weights (IPCW) using 2 logistic regression models for each treatment strategy at 9 and 40 weeks for NACT and 9 and 18 weeks for ACT. We estimated 3-year mortality comparing NACT versus ACT using an IPCW-weighted pooled logistic model. The 95% confidence intervals were calculated using bootstrapping with 500 iterations. RESULTS: Our cohort included 32,138 women, resulting in a cloned dataset of 64,276 replicates. We observed 15,040 women who completed the NACT strategy, 7,585 women who completed the ACT strategy, and 9,513 women whose treatment did not follow either strategy in full. Standardized mean differences fall within absolute value of 0.1 after IPCW weighting, indicating

adequate covariate balance. We estimated the difference in 3-year overall survival comparing women who received NACT to women who received ACT to be -3.6% (-4.3%, -2.9%). CONCLUSION: Our study applied the CCW method to avoid immortal-time bias, a major concern in comparative effectiveness studies using observational data. Our results highlight key design and analytic decisions necessary for successful implementation of the CCW method.

11:25 – 11:45 AM: **Jiona Mills**, graduate student
(Dr. Shakira Grant, Clinical Research)
Title: "If [multiple myeloma] is just for Black people, they don't care to study it, maybe that's why it is no cure": Dyadic perspectives on the legacy of Tuskegee and trust in medical care for multiple myeloma.
Abstract: BACKGROUND: Older Black adults are disproportionately burdened by multiple myeloma (MM) yet continue to face significant challenges in accessing high-quality cancer care, including opportunities to engage in research. Knowledge of experiments such as the Tuskegee Study, where Black persons experienced deliberate harm from research, has created mistrust of the healthcare system, especially in the Black community. We sought to examine racial differences in dyadic (patient-informal caregiver) knowledge of the Tuskegee study and understand their perceptions of care received for MM. METHODS: We conducted 21 in-depth semi-structured interviews with racially concordant patient-informal caregiver dyads living in North Carolina. Dyads were asked open-ended questions about the Tuskegee Study, mistrust, and their healthcare experiences. We used the Sort and Sift, Think and Shift approach for qualitative data analysis. RESULTS: Between November 2021 and April 2022, we enrolled 44 participants [(mean age, patients: 70 years (range=57-90), caregivers: 68 years (range=37-88)], and interviewed 42 (11 Black and 10 White dyads). Fourteen (67%) dyads (6 White, 8 Black) reported knowledge of the Tuskegee Study. We identified Black-White differences in how this knowledge influenced perceptions about the care received for MM, including provider and healthcare system interactions, where Black dyads reported mistrust because of this knowledge ("if [MM] is just for Black people, they don't care to study it, maybe that's why it is no cure"). Conversely, most White dyads reported no impact of this knowledge on their current level of trust in the healthcare system and expressed their discomfort with discussing the Tuskegee study and other events that led to the deliberate harm of Black persons. Black dyads emphasized the persistent nature of racial injustice in the healthcare system, creating a shared consciousness within the Black community that "Black patients don't get the attention... the care, that [their] counterpart does." Black dyads stressed the need for self-advocacy when interacting with providers and proactively sought to gain knowledge about their disease. Black and White dyads highlighted the importance of having a caregiver as an advocate, but Black dyads perceived caregiver presence as a potential mitigator of discrimination ("seeing a husband [and] wife, together. I think that makes a difference"). CONCLUSION: Black dyads often expressed knowledge of the Tuskegee Study, the related legacy of mistrust in the healthcare system, the need for self-advocacy, and knowledge of the disease when interacting with providers. These factors, including transparent communication with providers and acknowledgment of drivers of mistrust, are critical for enhancing the care experiences of older dyads affected by MM.

Session 2 (A): Basic & Clinical/Translational Sciences

(1:00 – 2:00 PM, Magnolia C Room / Zoom Session A)

1:00 – 1:20 PM: **Sophie Roush,** graduate student
(Dr. Yuri Fedoriw, Pathology and Laboratory Medicine)
Title: Increased Tumor T-Cell Receptor Repertoire Clonality Associates with HIV/ART Status and Improved Outcome in a Cohort of Diffuse Large B-Cell Lymphoma Patients

Abstract: BACKGROUND: Highly associated with HIV, diffuse large B-cell lymphoma (DLBCL) is the most common lymphoma worldwide and likely differs biologically based on HIV status and antiretroviral therapy (ART) exposure. Recent studies suggest increased tumor T-cell receptor (TCR) repertoire clonality associates with improved response to immune checkpoint inhibitors (ICI). HIV decreases CD4+ and naïve T-cell counts and leads to a clonal TCR repertoire due to T cells targeting HIV-specific epitopes. We therefore hypothesized HIV+ DLBCL would have more clonal TCR repertoires compared to HIV-. METHODS: The Kamuzu Central Hospital Lymphoma Study has prospectively enrolled patients with newly diagnosed lymphomas in Malawi since 2013. All patients receive standardized treatment and follow-up. We extracted DNA from 68 pre-treatment formalin-fixed paraffin-embedded (FFPE) DLBCLs from this cohort (QIAmp DNA FFPE Advanced) and performed TCR sequencing (immunoSEQ, Adaptive Biotechnologies). ART-experienced was defined as greater than 6 months of ART prior to DLBCL diagnosis. 36 FFPE tumors (n=12 HIV-, n=8 HIV+/ART-naïve, n=16 HIV+/ART-experienced) had >100 productive templates and passed quality control, meeting inclusion criteria for analysis. Of these, 2 tumors were EBV+ by EBER-ISH (n=1 HIV+/ART-experienced, n=1 HIV-). We used random downsampling to 100 productive templates due to template count variation. To test associations with clinical/demographic variables, we used ANOVA with Bonferroni correction, Pearson's correlation or Wilcoxon signed-rank test. For survival analysis, we generated binary variables from median cut-off, then calculated hazard ratios (HR) by Cox regression and produced Kaplan-Meier curves. RESULTS: TCR repertories from HIV+/ART-naïve tumors were more clonal than those from HIV- (productive Simpson clonality: 1.4 fold-change, adj. p=0.023; max productive frequency: 3.3 fold-change, adj. p=0.027) and HIV+/ART-experienced patients (productive Simpson clonality: 1.4 fold-change, adj. p=0.052; max productive frequency: 2.6 fold-change, adj. p=0.05). There were no differences between HIV- and HIV+/ART-experienced tumor clonality or in total productive template count by HIV/ART status. When analyzing HIV+ and HIV- tumors together, high clonality correlated with improved event-free (productive Simpson clonality: HR 0.26, p=0.011) and overall survival (productive Simpson clonality: HR 0.32, p=0.031). This trend was maintained when analyzing HIV+ and HIV- tumors separately. Age was not associated with tumor TCR clonality or outcome. Non-germinal center tumors trended toward worse event-free survival (HR 2.15, p=0.12), but not overall survival. CONCLUSIONS: The TCR repertoire in HIV+/ART-naïve DLBCL was more clonal than HIV- and HIV+/ART experienced cases. Longer duration of ART exposure prior to DLBCL diagnosis appeared to restore overall TCR repertoire diversity in the developing tumor. Increased tumor TCR clonality associated with improved outcome in our cohort, irrespective of HIV/ART-status. Based on these results, HIV+/ART-naive DLBCL patients may represent a subset of lymphoma patients who would benefit from ICI.

1:20 – 1:40 PM: **Megan Knuth,** postdoctoral researcher
(Dr. Folami Ideraabdullah, Cancer Genetics)
Title: Developmental Vitamin D Deficiency Alters Adult Liver Energy Metabolism Pathways
Abstract: Metabolic homeostasis requires a balance between catabolism and anabolism. Changes in these processes alter liver energy metabolite levels and can lead to diseases such as increased adiposity, non-alcoholic fatty liver disease (NAFLD), and hepatocellular carcinoma (HCC). We previously published a reciprocal cross mouse model on CC001 (1) and CC011 (11) recombinant inbred strain backgrounds [11-dam x 1-sire and 1-dam x 11-sire] that demonstrated parent-of-origin dependent (POD) differences in developmental vitamin D deficiency (DVD)-induced adult adiposity. Adult 1x11 offspring exhibited increased body weight and fat mass while adult 11x1 offspring exhibited no change. Here we defined candidate liver transcriptional and metabolic changes in energy metabolism underlying these observed POD differences in adult adiposity. Male 1x11 and 11x1 offspring mice were exposed to either a vitamin D sufficient (1000 IU/kg) or vitamin D deficient (0 IU/kg) diet during development (gestation–lactation). At weaning, all offspring were placed on standard chow (2,400 IU/kg) until adulthood. To determine

whether transcriptional and metabolic changes in liver energy metabolism underly POD differences in DVD-induced adult adiposity, we performed RNA-Seq and untargeted metabolic profiling on adult liver. We found significant DVD-induced parent-of-origin independent (POI) and POD effects on genetic regulators of energy metabolism and liver energy metabolite profiles consistent with models of NAFLD and HCC. Significant (FDR≤0.1) POI effects of DVD included transcriptional changes in genetic regulators of protein maintenance, transcription, and cell cycling; and overrepresentation of cholesterol biosynthesis, glutathione metabolism, and ER stress response (PANTHER, GSA). Significant (FDR≤0.1) POD effects of DVD in 11x1 offspring included transcriptional changes in genetic regulators of membrane trafficking, transcription, signal transduction, and energy metabolism; and overrepresentation of the electron transport chain, mitochondrial calcium ion transport, NAFLD, oxidative stress, and mitochondrial function. Significant (FDR≤0.1) POD effects of DVD in 1x11 offspring included transcriptional changes in genetic regulators of membrane trafficking and protein maintenance; and overrepresentation of cholesterol biosynthesis pathways. We saw enrichment for liver hyperplasia/hyperproliferation across both POI and POD analyses (IPA). We identified significant (VIP≥1.5, p≤0.05) POI effects of DVD on lipids, nucleotides, amino acids, bile acids, cofactors, vitamins, peptides, and carbohydrates. Interestingly, 11x1 offspring exhibited increased levels of amino acids, nucleotides, peptides, bile acids, cofactors, and vitamins, while 1x11 offspring exhibited decreased levels. These data suggest POD differences in DVD-induced anabolic vs. catabolic processes. Taken together, our data support DVD-induced perturbation of liver energy metabolism processes underlying POD differences in adult adiposity, and suggest a potential role for DVD in adult liver disease susceptibility.

1:40 – 2:00 PM: **Hina Rehmani,** postdoctoral researcher
(Dr. Natalia Isaeva and Dr. Wendell Yarbrough, Virology)
Title: JunB regulates expression of HPV genes in head and neck cancer
Abstract: Approximately 80% of head and neck squamous cell carcinoma (HNSCC) in the oropharynx is caused by the human papillomavirus (HPV). The incidence of HPV+ HNSCC is now being considered an epidemic in the US and Western Europe as rates have surpassed those for cervical cancer. For approximately 14,400 patients per year in the US, initial therapy is aggressive and often results in adverse lifelong side effects such as difficulty swallowing and speaking. Despite having a higher cure rate than HPV- HNSCC, the recurrence rate is approximately 30%. These patients have few therapeutic options since resistance to treatment arises from all known therapeutic modalities. The substantial recurrence rate and high morbidity of primary therapy suggest the need for new treatments. Global genome hypermethylation in HPV+ HNSCC, compared to HPV- cancers, prompted our lab to explore demethylation as a therapeutic option. 5-azacytidine (5-aza) is a synthetic cytidine analog that causes DNA demethylation by trapping methyltransferases to chromatin; it is FDA approved for the treatment of myelodysplasia and acute myeloid leukemia. Recently, we found that HPV+ HNSCC cells are more sensitive than HPV- cells to 5-aza, that low doses of 5-aza delayed HPV+ xenograft tumor growth, and that HPV+ tumor samples from patients treated with 5-aza in a window trial had increased apoptosis. We also observed a marked downregulation of HPV gene expression after 5-aza. Decreased HPV E6 expression, followed by reactivation of tumor suppressor p53, induced p53-dependent apoptosis in HPV+ HNSCC. Thus, decreased expression of HPV genes is an important component of 5-aza-associated toxicity toward HPV+ HNSCC; therefore, we further explored mechanisms through which demethylation negatively regulates the expression of HPV genes. First, we determined that 5-aza regulates the transcription of HPV genes, but not the stability of HPV transcripts. Second, we found that transcriptional regulation of HPV genes after 5-aza depends on the proto-oncogene JunB, a component of the transcription factor Activator Protein 1 (AP-1). Importantly, similar to 5-aza treatment, siRNA-mediated depletion of JunB abolished expression of HPV E6/E7 genes and reactivated tumor suppressor

p53 in HPV+ HNSCC. Moreover, our experiments revealed that clonogenic survival of HPV+ head and neck cancer cells is dependent on JunB expression. In summary, our study provides an improved mechanistic understanding of the regulation of HPV gene transcription in HNSCC and identifies a novel HPV+ HNSCC dependency on JunB, potentially providing a basis for a new rational targeted therapy.

Session 2 (B): Population Sciences

(1:00 – 2:00 PM, Classroom 109 / Zoom Session B)

1:00 – 1:20 PM: **Emilie Duchesneau,** graduate student

(Dr. Jennifer Lund, Cancer Epidemiology)

Title: Changes in a proxy measure for frailty in women with stage I-III breast cancer undergoing adjuvant chemotherapy

Abstract: INTRODUCTION: Frailty is a dynamic age-related syndrome characterized by a reduction in physiological homeostasis. Worsening frailty has been observed in patients with cancer undergoing chemotherapy. The Faurot frailty index is a Medicare claims-based proxy measure that uses demographic and billing information to predict frailty in individuals and has been used to assess frailty in cancer outcomes research using SEER-Medicare data. To date, no one has assessed the suitability of the Faurot frailty index for measuring changes in frailty over time. OBJECTIVE: We described changes in a validated Medicare claims-based proxy for frailty during cancer treatment in women with stage I-III breast cancer. METHODS: We identified women (65+ years) with stage I-III breast cancer undergoing adjuvant chemotherapy in the SEER-Medicare database. Women were required to have continuous enrollment in Medicare fee-for-service for ≥180 days prior to chemotherapy initiation. We excluded women who received neoadjuvant chemotherapy. We estimated the Faurot frailty index using demographic and billing information during the 180 days prior to key time points during breast cancer treatment: at adjuvant chemotherapy initiation, 4 months post-chemotherapy initiation, and 10 months post-chemotherapy initiation. RESULTS: We identified 22,925 women with stage I-III breast cancer who initiated adjuvant therapy (median age 70 years). At chemotherapy initiation, mean predicted probability of frailty was 0.035 (median: 0.022). Mean predicted probability of frailty increased to 0.052 (median: 0.030) 4 months post-chemotherapy initiation and subsequently fell to 0.047 (median: 0.026) 10 months post-chemotherapy initiation. CONCLUSION: We observed meaningful changes in the Faurot frailty index during the breast cancer treatment journey. These results are consistent with prior literature and demonstrate the feasibility of using claims data to detect changes in frailty over time. In future work, we will compare changes observed in women with breast cancer to comparator populations and use modeling approaches to further validate our findings.

1:20 – 1:40 PM: **Lauren Bates,** graduate student

(Dr. Shakira Grant, Clinical Research)

Title: "Life like we used to be": Dyadic perspectives on the burden of multiple myeloma.

Abstract: INTRODUCTION: Multiple myeloma (MM) is an incurable cancer that impacts older adults. Over time, MM-therapies become more burdensome, leading to physical function decline, gradual loss of independence, and the need for caregiver support. Due to the influence that patients and caregivers have on each other's health, understanding dyadic (patient-informal caregiver) perspectives about the long-term functional impacts of MM is critical. METHODS: Between November 2021 and April 2022, we conducted 21 dyadic interviews with older adults in North Carolina diagnosed with MM between 2006 and 2021 and treated for at least 6 months. Each older adult was paired with an informal adult caregiver. We used the Sort and Sift, Think and Shift approach for qualitative data analysis. RESULTS: We enrolled 11 Black and 10 White, mostly older dyads. The average age of patients was 70 years (range= 57-90), and for caregivers, 68 years (range = 37-88). Most patients (54%) and caregivers (52%) self-

identified as female. All dyads reported one or more functional impacts of MM (Table 1), with the majority (67%) of caregivers reporting changes to their physical function due to less physical activity engagement and needing to prioritize their care recipients' needs. Factors that promoted dyadic loss of physical function included: 1) disease phase (pre-treatment and within six months of treatment initiation, 71%); 2) treatment intensity (e.g., receipt of autologous stem cell transplantation, 71%), 3) patient symptom burden [e.g., severe pain (76%), peripheral neuropathy (43%), and excessive fatigue (81%)] and 4) adequacy of the social support network [e.g., MM support group, church, friends, (52%)]. Dyads also reported a sense of loss and yearning to return to their lives before the MM diagnosis. CONCLUSION: MM and its treatments adversely impact dyadic physical function. Reports of functional decline are most severe at the onset of treatment but persist throughout later stages of the disease. This research highlights a need for dyadic-level interventions to help maintain or improve the physical activity and function of long-term MM survivors and their caregivers.

1:40 – 2:00 PM: **Matthew LeBlanc**, postdoctoral researcher
(Dr. Katherine Reeder-Hayes, Breast Cancer)
Title: Trends in frontline multiple myeloma therapy in older adults diagnosed 2007 - 2017: A Seer Medicare Study
Abstract: BACKGROUND: Multiple myeloma (MM) is an incurable cancer of the plasma cells that has see dramatic improvements in survival over the past twenty years fueled in part by newer more effective therapies (novel agents) and changing treatment patterns. Innovation as seen in MM, often leads to disparities in treatment and outcomes. This study explores changes over time in frontline MM treatment in older adults and the disparities between frontline treatment in non-Hispanic white and non-Hispanic Black MM patients. METHODS: Patients diagnosed with multiple myeloma between 2007 and 2017 were identified in the SEER-Medicare registry linked claims database. Subjects were excluded if they did not have continuous Medicare Parts A, B and D coverage for at least 12 months prior to and 12 months following diagnosis or until death, had managed care coverage, or were diagnosed with MM at death or on autopsy. Outcomes were described over time for the entire cohort by year. These outcomes included death within 12 months of diagnosis, any treatment within 12 months of diagnosis, and among those treated within 12 months we also explored the use of high-quality frontline therapies defined as any use of newer 'novel' therapies, the use of novel triplet regimens, the use of stem cell transplant and the combination of novel triplets and stem cell transplant. Results were then stratified by race (non-Hispanic White, non-Hispanic Black) and compared across 3 time periods (2007-2010, 2011-2014, 2015-2017). RESULTS: Our cohort includes 11,773 patients diagnosed with myeloma. Those alive at 12 months after MM diagnosis increased from 62.9% in 2007 to 75.1% in 2017. The proportion on MM treated within 12 months of diagnosis increased from 57.1% in 2007 to 72.3% in 2017. Of those treated within 12 months (n=8010) most received novel therapies as part of frontline MM treatment across the study period (95.0% - 99.7%). The use of novel triplet therapy increased from 3.7% in 2007 to 56.2% in 2017, replacing doublets as the most common regimen type. The use of stem cell transplant in the frontline increased from 14.2% in 2007 to 24.6% in 2017. And the combination of novel triplets and stem cell transplant increased from 2.0% in 2007 to 19.3% in 2017. Persistent racial disparities were noted in receipt of stem cell transplant, novel triplet therapies and the combination of stem cell transplant and novel triplet regimens over time. CONCLUSION: Overall movement toward higher quality treatment. More surviving at least 12 months, more receiving frontline treatment. Increasing use of novel triplets over doublets and stem cell transplant. We found persistent though changing disadvantage for non-Hispanic Black Americans in treatment receipt, particularly in the use of stem cell transplant.

Session 3 (A): Basic & Clinical/Translational Sciences

(2:00 – 3:00 PM, Magnolia C Room / Zoom Session A)

2:00-2:20 PM: **Jun Wang**, postdoctoral researcher, 2022 Winner of the Joseph S. Pagano Award
(Dr. Greg Wang, Cancer Cell Biology)
Title: Dissecting and targeting non-canonical and canonical oncogenic activities of EZH2 in cancer
Abstract: Canonically, EZH2 serves as the catalytic subunit of PRC2, which mediates H3K27me3 deposition and transcriptional repression. Our research finds that in acute leukemias, EZH2 has additional noncanonical functions by binding cMyc at non-PRC2 targets and uses a hidden transactivation domain (TAD) for (co)activator recruitment and gene activation. Both canonical (EZH2-PRC2) and noncanonical (EZH2-TAD-cMyc-coactivators) activities of EZH2 promote oncogenesis, which explains the slow and ineffective antitumor effect of inhibitors of the catalytic function of EZH2. To suppress the multifaceted activities of EZH2, we used proteolysis-targeting chimera (PROTAC) to develop a degrader, MS177, which achieved effective, on-target depletion of EZH2 and interacting partners (that is, both canonical EZH2-PRC2 and noncanonical EZH2-cMyc complexes). Compared with inhibitors of the enzymatic function of EZH2, MS177 is fast-acting and more potent in suppressing cancer growth. Our work reveals noncanonical oncogenic roles of EZH2, reports a PROTAC for targeting the multifaceted tumorigenic functions of EZH2 and presents an attractive strategy for treating EZH2-dependent cancers.

2:20-2:40 PM: **Jennifer Klomp**, postdoctoral researcher
(Dr. Channing Der, Molecular Therapeutics)
Title: System-wide determination of the functional ERK-regulated phosphoproteome in KRAS-mutant pancreatic cancer
Abstract: The KRAS oncogene is mutationally activated in 95% of pancreatic ductal adenocarcinoma (PDAC). The recent approval of a clinically effective inhibitor of one KRAS mutant, KRASG12C, marks a milestone in KRAS drug development, and preclinical development of direct inhibitors of other KRAS mutants is ongoing. However, as seen with essentially all targeted anti-cancer therapies, patients who are initially responsive eventually relapse. In approximately 60% of patients, treatment-induced drug resistance was associated with activation/inactivation of signaling components that lead to reactivation of RAS and RAF-MEK-ERK and PI3K-AKT effector signaling. We determined that ectopic expression of constitutively activated MEK1S118/222D or ERK1R84S/S170D, but not myristoylated AKT1, drove near-complete resistance to selective direct inhibitors of KRASG12C or KRASG12D. Thus, we expect that reactivation of ERK but not AKT will be a key limitation for all RAS-targeted therapies, prompting our studies to define the mechanistic basis for ERK-dependent PDAC growth. ERK activation can cause direct/indirect phosphorylation of >1000 proteins, including transcription factors and protein kinases. Thus ERK activation can regulate a complex transcriptome and phosphoproteome. Our recent determination of the ERK-dependent transcriptome in KRAS-mutant PDAC showed that it diverges significantly from the cancer type naïve Gene Set Enrichment Analysis Hallmark KRAS signature, supporting a pancreas-specific function of ERK. Therefore, we performed proteomic analyses to define the ERK-dependent phosphoprotein landscape in KRAS-mutant PDAC. To avoid complications of ERK inhibition-induced compensatory signaling activities and minimize cell line heterogeneity, we evaluated six KRAS-mutant PDAC cell lines after 1- or 24-hour treatment with the ERK1/2-selective inhibitor SCH772984. We first compared our findings with a recent compendium of ERK substrates (2,507 phosphosites; 1,308 phosphoproteins) compiled from 14 different published studies (Ünal et al., 2017). We identified 4,032 phosphosites on 1,884 proteins that were significantly deregulated by ERK inhibition, with 41% and 77%, respectively, not previously been attributed to ERK. Of the 4,032 phosphosites, 60% were components of the p[S/T]-P (48%) or the extended P-X-p[S/T]-P (12%) ERK

phosphorylation consensus motif. Of the 1,884 proteins, 33%, 5%, and 6% contained the ERK substrate recognition D-, DEF-motifs, or both, respectively. We then began to define the ERK substrates that support KRAS-dependent PDAC growth. First, we found that a D-motif association-deficient mutant of activated ERK1 (ERK1R84S/S170D/D338N) failed to drive resistance to KRAS inhibition. Second, KEGG analyses implicated ERK substrates in regulation of cell cycle progression and cancer metabolism. Third, mining genome-wide CRISPR data from the Broad Achilles Project (DepMap), we identified a subset of ERK substrates required for the growth of KRAS-mutant PDAC that were highly enriched in nuclear localized proteins. Finally, using a CRISPR library targeting the compendium of ERK substrates, we identified additional ERK substrates that are synthetic lethal with ERK inhibitor treatment. In summary, our determination of the ERK-regulated phosphoproteome will further elucidate how KRAS drives PDAC growth through activation of the ERK signaling network.

2:40-3:00 PM: **Margarita Dzama,** postdoctoral researcher
(Dr. Jesse Raab, Cancer Genetics)
Title: Identification of new potential targets in liver cancer using focused CRISPR-Cas9 screens
Abstract: Hepatocellular carcinoma (HCC) is a highly deadly disease with few available treatment options for patients with unresectable tumors and not eligible for chemotherapy. Unfortunately, these existing systemic therapies only moderately prolong the lifespan and often result in emerging resistance. It has been previously shown that mutations in the SWI/SNF chromatin remodeling complex can be collectively detected in 40% of HCC cases. Patients carrying these mutations showed worse overall survival. In this project, we focused on the identification of new therapeutic targets as well as their combination with FDA-approved systemic therapy and therapy in the final phase of clinical trials by performing focused CRISPR knockout screens using different HCC models. We constructed a CRISPR library of 6000 guide RNAs targeting 737 genes involved in chromatin-mediated gene regulation. We chose to perform this screen in the presence of the multikinase inhibitor sorafenib, as it remains the most commonly used drug for HCC treatment. We also chose the additional kinase inhibitor donafenib, a deuterated sorafenib derivative that shows lower side toxicity in late-stage clinical trials. The CRISPR screens performed in various hepatoblastoma (HepG2) and hepatocellular carcinoma (HLF, PLC/PRF/5, Huh7) cell lines revealed new targets for HCC, including some members of the menin-MLL1 complex such as MEN1 and ASH2L. Evaluation of potential resistance in hepatoblastoma cells revealed KEAP1 and members of the SWI/SNF family (SMARCC1, ARID1A, and ARID1B) as important modulators of sorafenib-specific resistance. We confirmed this result by generating a mutant HepG2 cell line with a mutated ARID1B gene using CRISPR-Cas9 and performing sensitivity assays. A CRISPR screen on HCC cell lines in the presence of donafenib revealed that a knockout of KEAP1 increases sensitivity to the drug, which is the opposite effect of sorafenib treatment in the hepatoblastoma cell line. We intend to investigate further the striking differences of KEAP1 knockout in HCC cell lines upon treatment with sorafenib and donafenib. Overall, these data suggest differences in drug response could be driven by cell model or genotype. We believe that understanding this is critical for rational drug choice. We anticipate that epigenetic regulators are novel targets and represent an appealing therapeutic strategy for HCC treatment. A better understanding of acquired resistance may also help in the development of new treatment strategies and improve therapy outcomes.

Session 3 (B): Population Sciences

(2:00 – 3:00 PM, Classroom 109 / Zoom Session B)

2:00 – 2:20 PM: **Karthik Adapa,** graduate student
(Dr. Lukasz Mazur, Radiation Oncology)

Title: Developing an evidence-based implementation framework for implementing quality assurance checklists in radiation oncology

Abstract: BACKGROUND: Digital health technologies such as quality assurance (QA) checklists could improve patient safety in radiation oncology. However, there is limited evidence and guidelines for the successful implementation of QA checklists in radiation oncology clinical settings. To the best of our knowledge, no previous study has examined the determinants, and strategies for implementing QA checklists in radiation oncology. OBJECTIVE: The study aims to investigate the barriers and facilitators for implementing QA checklist, examine the strategies for implementing QA checklists and propose an implementation framework for implementing QA checklists in radiation oncology clinic METHODS: This study was conducted from January 2022 to August 2022 during pre-implementation, implementation, and post implementation of a dosimetry QA checklist (DQC) in a radiation oncology clinic of a large US academic cancer center. Following an abductive research approach with both inductive and deductive analysis, we used the Consolidated Framework for Implementation Research and the Expert Recommendations for Implementing Change to analyze the transcripts of 12 semi-structured interviews with dosimetrists (n=5) and physicists (n=5) and descriptive questionnaire data. The results of the qualitative analysis and the findings from the daily safety huddles, team meetings, field observations, and informal meetings, provided the basis for the development of the proposed implementation framework. RESULTS: The inductive analysis of the interview transcripts revealed two broad categories – the implementation process of DQC and the suggestions from participants for improving enhanced DQC's implementation in the clinic. Three themes emerged regarding the implementation process of enhanced DQC–QA checklist features, IT infrastructure and user training, and engaging users. The suggestions provided by interviewees were organized into two themes- involvement of users, user training, and support. The deductive coding revealed 4 CFIR constructs and 12 sub-constructs as barriers and 5 CFIR constructs, and 19 sub-constructs as facilitators. The suggestions for improving enhanced DQC's implementation were mapped on 7 clusters and 19 ERIC strategies. The proposed implementation framework includes 14 evidence-based strategies belonging to 4 clusters from the CFIR-ERIC matching tool and organized temporally in an implementation lifecycle. CONCLUSIONS: Implementing digital health technologies such as QA checklists in radiation oncology clinical settings should involve a comprehensive pre-implementation assessment and a continuous evaluation of the implementation conditions. Our proposed framework may guide healthcare system leaders, human factors engineers and implementation science researchers with concrete, evidence-based, and step-by-step recommendations for implementing QA checklists in radiation oncology clinical settings.

2:20 – 2:40 PM: **Austin Waters,** graduate student

(Dr. Erin Kent, Cancer Prevention and Control)

Title: The Impact of Employment Loss on Mentally Unhealthy Days among LGBTQ+ Cancer Survivors During the COVID-19 Pandemic: Findings from the OUT National Survey

Abstract: BACKGROUND: Lesbian, Gay, Bisexual, Transgender, Queer, and all other sexual and gender minority (LGBTQ+) populations made up 7.1% of the US population in 2021. LGBTQ+ cancer survivors face a variety of economic and mental health disparities, however, the determinants of poor mental health among LGBTQ+ cancer survivors is understudied. METHODS: This analysis utilized the OUT National Survey which consists of N=2,233 LGBTQ+ cancer survivors. Bivariate and multivariable regression models were used to assess the association between COVID-19 related employment loss and mentally unhealthy days as well as frequent mental distress among LGBTQ+ cancer survivors. Predicted values were generated with interaction terms to explore the differential impact of employment loss on survivors with intersecting racial and LGBTQ+ identities. RESULTS: In bivariate analyses employment loss was associated with a higher number of mentally unhealthy days (10.3, SD=9.9 vs. 8.4, SD=9.6; p-value<0.001) and frequent mental distress (34% vs 26%; p-value=0.001). In multivariable models'

employment loss (β:0.17, 95%CI: 0.04-0.30) and demographic factors such as cis-female and non-binary gender (β:0.19, 95%CI: 0.06-0.33; β:0.42, 95%CI: 0.16-0.68), and a current cancer diagnosis (β:0.39, 95%CI: 0.25-0.5) were associated with more mentally unhealthy days. Further, cis-female, trans-female, and non-binary genders (OR:1.38, 95%CI: 1.09-1.75; OR:2.70, 95%CI: 1.05-6.95; OR:2.24), American Indian and Alaska Native race (OR:2.02, 95%CI: 1.11-3.65), and a current cancer diagnosis (OR:2.14, 95%CI: 1.67-2.74) were associated with frequent mental distress. CONCLUSIONS: COVID-19 related employment loss negatively impacted the mental health of LGBTQ+ cancer survivors. LGBTQ+ specific supportive services as well as equity-based employment and income interventions are needed.

2:40 - 3:00 PM: **Bethany Ogbenna,** graduate student
(Dr. Cher Dallal, Epidemiology and Biostatistics, University of Maryland School of Public Health)
Title: Association between a healthy lifestyle index and breast cancer risk among post-menopausal women in the Multiethnic Cohort (MEC) Study
Abstract: Consistent evidence supports maintaining a healthy weight, engaging in physical activity, and limiting alcohol consumption as potential strategies for reducing breast cancer risk. Sleep duration and sedentary behavior are also emerging breast cancer risk factors. While these cardiometabolic factors have been associated with an increased risk of breast cancer, prior studies have primarily investigated these factors separately. The association between a healthy lifestyle index (HLI) and breast cancer risk, particularly across racially and ethnically diverse postmenopausal women, has not been well studied. Within the Multiethnic Cohort study, ongoing analyses are examining associations between an HLI and risk of invasive breast cancer among 65,561 eligible postmenopausal women (African American [18.7 %], Native Hawaiian [6.7 %], Japanese American [28.2%], Latina [21.1%], and White [25.3%]). During a median follow-up of 23 years, 4,555 incident invasive breast cancer cases were diagnosed between baseline (1993-1996) and the end of follow-up (2017). Self-reported baseline information on seven lifestyle factors was used to create an HLI based on the sum of scores (max HLI score=23): Healthy Eating Index 2010 (HEI 2010) score divided into quartiles (scored 1-4), metabolic equivalents (METs) of moderate and vigorous physical activity (1-3), time spent sedentary (1-3), smoking status and duration (1-5), alcohol consumption (1-3), body mass index (BMI) (1-3) and sleep duration (1-2). Preliminary analyses included descriptive statistics as well as age- and race and ethnicity-adjusted Cox proportional hazards models of invasive breast cancer to estimate hazard ratios (HR) and 95% confidence intervals (CIs) for the association with HLI score (tertiles, (T)) using age as the timescale. The mean HLI score in the overall population was 15.3 [Standard Deviation (SD): 2.1] with 51.4% of the analytic population in an HLI score tertile of ≥18 to 23 (T3; most healthy) and 13.2% in the lowest HLI score tertile ≥7 to <15 (T1; least healthy). The mean HLI scores by race/ethnicity were the following: African American 16.5 [SD: 2.5], Native Hawaiian 16.7 [SD: 2.6], Japanese American 18.2 [SD: 2.2], Latina 17.3 [SD: 2.2] and White 17.2 [SD: 2.6] women. Preliminary findings suggest that overall, women in HLI score T3 had on average, a BMI of 23.6 [3.7] (mean [SD] kg/m2), dietary intake HEI 2010 scores between 60-78.9, exerted > 6 METs of moderate and strenuous activity, spent < 6 hours a day sedentary, consumed less than 4.9 grams of ethanol per day, slept between 7 to 8 hours and were never smokers. In multivariable adjusted analyses, postmenopausal women with an HLI score in T2 (HR: 0.85 [95% CI:0.78-0.93]) and in T3 (HR: 0.75 [95% CI: 0.69–0.82]) (p-trend <0.001) were at reduced risk of breast cancer compared to women in T1. Ongoing analyses include race and ethnicity stratified multivariable Cox proportional hazards regression.

Poster Session I: Odd-Numbered Posters

(3:00 – 3:30 PM, Magnolia C Room)

Poster #1: Taghreed Al Turki, postdoctoral researcher

(Dr. Jack Griffith, Virology)
Title: Novel Poly Arginine-Valine and Glycine-Leucine repeating dipeptide proteins are encoded by Human Telomeric RNA and expressed at elevated levels in human cancer cells upon Telomere Disruption
Abstract: Telomeres are the nucleoprotein complexes that distinguish the end of each linear chromosome in eukaryotes and protect them from end-to-end fusions. Although telomeres were previously thought to be silent, various lengths of G-rich long non-coding RNAs known as TERRA were found to be transcribed from telomeres. Recent studies of mammalian TERRA have enriched our understanding of the role of this repetitive RNA sequence (UUAGGG) n, as a structural component, in particular their G-quadruplex secondary structures in chromosome end protection. Similar to other long non-coding RNA species, TERRA was not believed to be translated into protein due to the lack of ATG start codons and the simple repeating sequence. However, the Repeat-Associated Non-ATG (RAN) translation mechanism discovered in SCA8, and DM1 diseases, and extended to ALS/FTD revealed that strong secondary structures including hairpins and G-quartets allow ribosomes to load and begin translation along these simple repeating RNAs leading to long homo-or di-peptide repeating proteins. In the disease studies the repeating dipeptide proteins are shown to be toxic and lead to disease pathology. These discoveries led us to propose that the (UUAGGG)n repeats of mammalian telomeric TERRA could be translated via RAN mechanism to produce two repeating dipeptide proteins: poly Arginine-Valine (RV) and glycine- Leucine (GL). Using an antibody to RV we developed I will show the detection of endogenous RV dipeptide proteins in human cancer Osteosarcoma U2OS and ICF cell lines both of which have higher TERRA levels. I will present EM studies that reveal the ability of synthetic RV dipeptide proteins to bind to replication forks and facilitate p53 loading on damaged DNA. Additional evidence will be presented that RV dipeptide proteins in cells form aggregates (misfolded protein structures) that accumulate significantly in nuclei of cancer cells upon telomere disruption. Sarcoma Tumor Micro array slides probed with the RV antibody will be shown and experiments to monitor the activation of innate immune pathways by the GL protein with the Ting laboratory will be described. To date, mammalian telomeric RNA, TERRA, has been considered a non-coding RNA. However, in light of the evidence to be presented, TERRA must be considered as an RNA encoding two dipeptide micro-proteins which most likely have potent activities in the cell when highly expressed. This may include activation of innate immune pathways and induction of genomic instability, changes related to cancer and aging in cells.

<u>Poster #3</u>: Audra Bryan, postdoctoral researcher
(Dr. Jill Dowen, Cancer Genetics)
Title: Elucidating structural regulators of enhancer-promoter loops and determining their roles in the regulation of gene expression
Abstract: 3D (three-dimensional) genome structure affects gene expression and can change dynamically during normal development or disease progression. Knowledge of the proteins and molecular mechanisms that regulate 3D genome structure is of critical importance for understanding how their dysregulation leads to developmental disorders and cancers. Cohesin is a multi-subunit complex which facilitates 3D genome structure by mediating long-range DNA interactions in chromatin. Cohesin-mediated DNA loops are critical for higher order genome structure and proper gene expression. Cohesin mediates two major types of DNA loops: 1) enhancer-promoter loops associated with gene transcription and 2) loops between pairs of CTCF bound sites, known as insulators. While the protein-protein interaction interface between cohesin and CTCF has been identified and characterized, there is little molecular insight into how cohesin associates with enhancers and promoters in the genome. I hypothesize that cohesin recruitment to active enhancers and promoters occurs via interaction with common chromatin regulators and transcriptional activators, functioning as structural regulators. I have elucidated three such proteins: WDR5, SP1, and NFYA, all of which are enriched at cohesin binding sites.

WDR5 is a core scaffolding component of multiple chromatin modifying complexes and transcriptional activator complexes, while SP1 and NFYA are transcription factors. All three proteins are essential for the proper regulation of critical cellular processes. To further investigate these novel structural regulators in mouse embryonic stem (mES) cells, ChIP-seq was performed and revealed that cohesin localizes to many enhancers and promoters bound by WDR5, SP1, and/or NFYA. Co-immunoprecipitation studies showed that cohesin physically interacts with WDR5, SP1, and NFYA in mES cells. Further, serial ChIP studies confirmed that cohesin can co-occupy the same DNA molecule as WDR5, SP1, or NFYA, at the same time, within the same cell. These results demonstrate that WDR5, SP1, and NFYA each interact with cohesin and could therefore act as structural regulators, recruiting cohesin to specific promoters and enhancers and helping cohesin establish DNA loops important for proper gene expression and development. Future studies will interrogate how these proteins function together or in distinct sub-complexes to recruit cohesin to chromatin, facilitating DNA loop formation between specific enhancers and promoters, and selectively regulating the expression of genes that control the maintenance of the embryonic stem cell state or differentiation in mESCs, as well as genes important for tumorigenesis in cancer cells.

Poster #5: Evan Dewey, postdoctoral researcher
(Dr. Jeff Sekelsky, Cancer Genetics)
Title: Defining Mitotic Crossover Mechanisms Using CRISPR/Cas9 and Bloom Syndrome Helicase
Abstract: Genome stability is key to the longevity of multicellular organisms and disease avoidance. Despite being challenged daily by DNA damage threatening this stability, cells regularly repair their DNA and maintain resilience. Sometimes however, improper repair or repair dysregulation causes accumulation of “scars” in the form of detrimental mutations within the genome, eventually leading to loss of heterozygosity, genome instability, cancer, and other disease. Homology directed repair (HDR) of DNA double strand breaks is one DNA repair pathway that, if improperly regulated, leads to accumulation of mutations via mitotic (somatic) crossovers and loss of heterozygosity. Therefore, understanding mitotic crossover mechanisms is critical to the prevention of cancer and other genetic diseases. CRISPR/Cas9 has also become increasingly reliant on accurate HDR to integrate desired mutations or corrections in genome editing, but precise CRISPR/Cas9 HDR mechanisms remain elusive. Through use of total mismatch repair (MMR) knockout only possible in Drosophila, it is now possible to analyze resulting HDR products. I have begun to use this tool to define mitotic crossover mechanisms for the first time in a multicellular organism, with initial results pointing to resolution of non-ligated double Holliday junctions as the predominant means of repair. I will also use this unique MMR knockout to define Bloom Syndrome Helicase (Blm) mutation-induced mitotic crossovers. This work will enhance understanding of how DNA is repaired in both CRISPR and Blm mutant contexts, expanding knowledge of how mitotic crossovers cause genome instability and how to beneficially utilize them in genome editing.

Poster #7: Minguk Jo, postdoctoral researcher
(Dr. Gaorav Gupta, Breast Cancer, Clinical Research)
Title: Mre11 liberates cGAS from nucleosome sequestration during tumorigenesis
Abstract: Oncogene-induced replication stress generates endogenous DNA damage that activates cGAS/STING-mediated innate immune signaling and tumor suppression. However, the mechanism for cGAS activation by endogenous DNA damage remains enigmatic, particularly given the constitutive inhibition of cGAS by high-affinity histone acidic patch (AP) binding. Here we report an *in vivo* CRISPR screen that identified the DNA double strand break sensor Mre11 as a suppressor of mammary tumorigenesis induced by Myc overexpression and p53 deficiency. Mre11 antagonizes Myc-induced proliferation through cGAS/STING activation. Direct binding of the Mre11-Rad50-Nbn (MRN) complex to

nucleosomes displaces cGAS from AP sequestration, which is required for DNA damage-induced cGAS mobilization and activation by cytosolic DNA. Mre11 is thereby essential for cGAS activation in response to oncogenic stress, cytosolic DNA transfection, and ionizing radiation. Furthermore, we show Mre11-dependent cGAS activation suppresses Myc-induced proliferation through ZBP1/RIPK3/MLKL-mediated necroptosis. In human triple-negative breast cancer, ZBP1 downregulation correlates with increased genome instability, decreased immune infiltration, and poor patient prognosis. These findings establish Mre11 as a critical link between DNA damage and cGAS activation that regulates tumorigenesis through ZBP1-dependent necroptosis.

Poster #9: Sirui Li, postdoctoral researcher
(Dr. Jenny Ting, Immunology)
Title: STING-induced B regulatory cells compromise NK function in cancer immunity
Abstract: An immunosuppressive tumor microenvironment is a major obstacle in the control of pancreatic and other solid cancers. STING (stimulator of interferon genes) agonists trigger inflammatory innate immune responses to potentially overcome tumor immunosuppression. Although these agonists hold promise as potential cancer therapies, tumor resistance to STING monotherapy has emerged in clinical trials and the mechanism(s) are unclear. We show that the administration of five distinct STING agonists, including cGAMP, results in an expansion of human and mouse IL-35+ regulatory B lymphocytes in pancreatic cancer. Mechanistically, cGAMP drives B cell IL-35 expression in an IRF3-dependent but type I interferon-independent manner. In multiple preclinical cancer models, the loss of STING signaling in B cells increases tumor control. Furthermore, IL-35 blockade or genetic ablation of IL-35 in B cells also reduces tumor growth. Unexpectedly, the STING-IL-35 axis in B cells reduces NK proliferation and attenuates NK-driven anti-tumor response. These findings reveal an intrinsic barrier to systemic STING agonist monotherapy and provide a novel combinatorial strategy to overcome immunosuppression in tumors.

Poster #11: Jenna Perry, postdoctoral researcher
(Dr. Amy Maddox, Cancer Cell Biology)
Title: Engineering septin disease alleles in C. elegans
Abstract: Cell function and tissue integrity rely on the maintenance of cell shape and organization. The cytoskeleton, consisting of polymers and motor proteins, serves to structure and remodel the cell and determines tissue architecture. A highly conserved family of GTP-binding proteins called septins forms filaments and other higher-order structures, including gauzes and rings, from the non-polar arrangement of core proteins (ex. ABCDDCBA). Many cellular functions, including cell division and cell motility, are thought to involve the septin higher-order structures, where they serve to scaffold and recruit or sequester other proteins at the plasma membrane. Septin dysregulation by mutation or abnormally high or low expression occurs in many human diseases, including cancer, fibrosis, and neuropathies. Yet, how septin perturbation influences disease is unknown. A significant obstacle to defining septins' role in human health and pathology is the number of septin genes encoded in the human genome (13, some of which also have splice isoforms). Additional complexity arises from the functional substitution of septins within each of the four subfamilies. By contrast, the simple model animal Caenorhabditis elegans has only two septins. C. elegans septins, like septins in other species, can form filaments and localize to the cytokinetic ring during cell division. This project aims to understand how septins contribute to cell and tissue behavior and organization through the creation of transgenic C. elegans "avatars" containing septin mutations in conserved residues and mutations derived from human disease septin alleles. The phenotype of the C. elegans avatars will be compared to that of septin-null worms and previously characterized septin loss-of function alleles. Preliminarily, we found that septins are required for normal development and function of the C. elegans germline, which is a simple single-

cell model for stem cell biology, cell migration, and the regulation of cell cycle progression and differentiation. C. elegans bearing septin mutations have reduced brood size and oocyte production. Mutant septin germlines also develop more slowly than control, perhaps indicating a defect in cell proliferation. Using C. elegans to study septin mutation will elucidate how septin dysfunction perturbs cell and tissue organization, with the long-term goal of providing insights into human development and disease.

Poster #13: Hina Rehmani, postdoctoral researcher
(Dr. Natalia Isaeva and Dr. Wendell Yarbrough, Virology)
Title: JunB regulates expression of HPV genes in head and neck cancer
Abstract: Approximately 80% of head and neck squamous cell carcinoma (HNSCC) in the oropharynx is caused by the human papillomavirus (HPV). The incidence of HPV+ HNSCC is now being considered an epidemic in the US and Western Europe as rates have surpassed those for cervical cancer. For approximately 14,400 patients per year in the US, initial therapy is aggressive and often results in adverse lifelong side effects such as difficulty swallowing and speaking. Despite having a higher cure rate than HPV- HNSCC, the recurrence rate is approximately 30%. These patients have few therapeutic options since resistance to treatment arises from all known therapeutic modalities. The substantial recurrence rate and high morbidity of primary therapy suggest the need for new treatments. Global genome hypermethylation in HPV+ HNSCC, compared to HPV- cancers, prompted our lab to explore demethylation as a therapeutic option. 5-azacytidine (5-aza) is a synthetic cytidine analog that causes DNA demethylation by trapping methyltransferases to chromatin; it is FDA approved for the treatment of myelodysplasia and acute myeloid leukemia. Recently, we found that HPV+ HNSCC cells are more sensitive than HPV- cells to 5-aza, that low doses of 5-aza delayed HPV+ xenograft tumor growth, and that HPV+ tumor samples from patients treated with 5-aza in a window trial had increased apoptosis. We also observed a marked downregulation of HPV gene expression after 5-aza. Decreased HPV E6 expression, followed by reactivation of tumor suppressor p53, induced p53-dependent apoptosis in HPV+ HNSCC. Thus, decreased expression of HPV genes is an important component of 5-aza-associated toxicity toward HPV+ HNSCC; therefore, we further explored mechanisms through which demethylation negatively regulates the expression of HPV genes. First, we determined that 5-aza regulates the transcription of HPV genes, but not the stability of HPV transcripts. Second, we found that transcriptional regulation of HPV genes after 5-aza depends on the proto-oncogene JunB, a component of the transcription factor Activator Protein 1 (AP-1). Importantly, similar to 5-aza treatment, siRNA-mediated depletion of JunB abolished expression of HPV E6/E7 genes and reactivated tumor suppressor p53 in HPV+ HNSCC. Moreover, our experiments revealed that clonogenic survival of HPV+ head and neck cancer cells is dependent on JunB expression. In summary, our study provides an improved mechanistic understanding of the regulation of HPV gene transcription in HNSCC and identifies a novel HPV+ HNSCC dependency on JunB, potentially providing a basis for a new rational targeted therapy.

Poster #15: Justin Sperlazza, postdoctoral researcher
(Dr. Ian Davis, Cancer Genetics)
Title: Epigenetic modulation of cancer antigens to enhance immunotherapy in pediatric solid tumors
Abstract: Chimeric Antigen Receptor T-cell (CAR-T) therapy has revolutionized the treatment of relapsed/refractory hematologic malignancies by engineering a patient's own immune system to target tumor cells. Patients with solid tumors however have yet to see a meaningful benefit from CAR-T therapy due to, in part, heterogenous expression of targetable cancer antigens. Reversible transcriptional programs control cancer antigen expression, which ultimately implicates chromatin regulation pathways as a target for intervention. Therefore, we hypothesize small molecule inhibitors of chromatin modifying enzymes can enhance the sensitivity of solid tumors to CAR-T cell immunotherapy.

The histone methyltransferase EZH2 was recently shown to regulate expression of tumor antigen GD2, a disialoganglioside expressed in a range of solid tumors. The Davis lab has demonstrated tazemetostat, an FDA approved EZH2 inhibitor, can induce a 50-100-fold increase in GD2 expression Ewing sarcoma, osteosarcoma, and neuroblastoma cell lines. In multiple xenograft models of Ewing sarcoma, we also find oral administration of tazemetostat results in a dose-depended induction of GD2. Importantly, in vitro GD2-targeted CAR-T cell lysis of the examined Ewing sarcoma and osteosarcoma cell lines is negligible at baseline and dramatically increases following tazemetostat treatment, corresponding to the increased GD2 expression. To identify other chromatin regulating compounds that modulate GD2 expression, we developed a flow-cytometry based screening assay and evaluated UNC's EpiG Diamond compound library. The screen identified 25 presumptive hits that induced GD2 expression, 21 of which inhibit pathways outside of the EZH2/PRC2 complex. The seven most robust hits were validated and evaluated for synergistic interactions with EZH2 inhibitors. We discovered the class I HDAC inhibitors (entinostat, tacedinaline, vorinostat) and the DOT1L inhibitor pinometostat are capable of synergistically inducing tumor GD2 expression in combination with tazemetostat, both in vitro and in a xenograft model of Ewing sarcoma. In summary, we identified multiple chromatin-targeted molecules that induce GD2 expression in Ewing sarcoma and osteosarcoma cell lines and shown increased GD2 expression sensitizes tumor cells in vitro to lysis by CAR-T cells. Ongoing mouse studies with GD2-targeted CAR-T cells are investigating the identified small molecule effects on tumor response and survival. Results of this project will provide preclinical data to support the addition of chromatin directed inhibitors in UNC's ongoing GD2.CAR-T trial for pediatric solid tumors. However, the principle of augmenting tumor antigen expression with small molecules has broader significance to cancer antigen targeted therapies and potential to benefit any immunotherapy approach with antigen dependence, such as monoclonal antibody-based drugs, natural killer-CAR cells, *etc*.

Poster #17: Dimitris Theofilatos, postdoctoral researcher
(Dr. Ageliki Tsagaratou, Immunology)
Title: TET proteins regulate T cell and iNKT cell lineage specification in a TET2 catalytic-dependent manner
Abstract: The precise regulation of cell-type specific expression programs is required to establish cell fate and function. Aberrant lineage specification of immune cell populations can result in disease emergence such as cancer, autoimmunity, or inflammation. The Ten Eleven Translocation (TET) proteins have a critical role in orchestrating the lineage-specific expression programs since they mediate the DNA demethylation by oxidizing 5-methylcytosine to 5-hydroxymethylcytosine (5hmC) and other oxidative derivatives. The TET family of proteins consists of three members, TET1, TET2, and TET3 that all share a conserved catalytic function. In addition, it has been reported that TET proteins can exert additional, catalytic independent roles that can either prohibit or enhance gene expression. Interestingly, Tet2 is one of the most frequently mutated genes in hematological malignancies, whereas studies in mouse models have revealed that all TET proteins can act as tumor suppressors. In the present study, we will focus on TET2 and TET3 as these are more highly expressed in conventional and unconventional T cells. We have previously demonstrated a dynamic enrichment of 5hmC during T and invariant natural killer T (iNKT) cell lineage specification. Here, we investigate shared signatures in gene expression of Tet2/3 DKO CD4 single positive (SP) and iNKT cells in the thymus. We discover that TET proteins exert a fundamental role in regulating the expression of the lineage specifying factor Th-POK, which is encoded by Zbtb7b. We demonstrate that TET proteins mediate DNA demethylation - surrounding a proximal enhancer, critical for the intensity of Th-POK expression. In addition, TET proteins drive the DNA demethylation of site A at the Zbtb7b locus to facilitate GATA3 binding. GATA3 induces Th-POK expression in CD4 SP cells. By introducing a novel mouse model that lacks TET3 and expresses full-length, catalytically inactive TET2, we establish a causal link between TET2 catalytic activity and lineage

specification of both conventional and unconventional T cells. Finally, we demonstrate that the observed aberrant expansion of iNKT cells that lack concomitantly TET2 and TET3 occurs in a TET2-dependent catalytic manner. Our findings agree with a recently discovered TET2 catalytic-dependent role in clonal hematopoiesis and myeloid transformation by Yeaton *et al.,* 2022. The results of this study are included in a recently published paper by Aijo *et al*., 2022.

Poster #19: Whitney Bell, graduate student
(Dr. Yuliya Pylayeva-Gupta, Immunology)
Title: Cytokine modulation can suppress an EMT-like phenotype in pancreatic cancer
Abstract: Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest cancers with a 5-year survival rate of only 11% and approximately half the patients present with distant metastasis at the time of diagnosis. PDAC is accompanied by pronounced alterations in stromal responses and immune surveillance programs, which are now recognized as some of the major drivers in PDAC tumor evolution. The role of immune modulators in driving metastatic potential in PDAC is poorly understood. We find that low expression levels of cytokine IL-23 in patients with PDAC correlate with poor differentiation status of these tumors, and thus predict poor outcomes. Consistent with this observation, we have found that a loss of host-derived IL-23 in a mouse model of pancreatic cancer promotes and EMT-like phenotype. The tumor morphology is altered and appears to be less differentiated. Additionally, we see a significant increase in the number of tumor cells expressing Zeb1 and a decrease in E-cadherin expression. We also find an increase in metastatic capacity in the absence of IL-23 in the tumor stroma. Single cell RNA sequencing of tumors injected orthotopically into IL-23 knockout mice reveals clusters of tumor cells with increases in EMT-associated genes such as Zeb1 and Vimentin and concurrent decreases in gastric identity genes. Overall, our findings indicate that IL-23 plays a role in suppressing an EMT-like phenotype in pancreatic cancer with one mechanism being through regulation of gastric lineage genes.

Poster #21: Kevin Field, graduate student
(Dr. Ian Davis, Cancer Genetics)
Title: Uncovering the Mechanism of Induced Immunotherapy Target Antigen Expression
Abstract: Chimeric Antigen Receptor (CAR)-T-cell immunotherapy has been a major treatment breakthrough in pediatric oncology, enabling the treatment of relapsed B-cell acute lymphoblastic leukemia. However, the use of CAR-T-cells is limited to a small set of cancers that express target antigens. Recent research has explored inducing expression of CAR-T target antigens in currently non-targetable tumors, thus enabling the treatment of a wide variety of tumors with existing CAR constructs. In pediatric cancers, our lab and others have focused on the epigenetic regulation of these target antigens. Of particular interest are cancers which lack efficacious treatment for metastatic disease, such as Ewing sarcoma and osteosarcoma. Representative cell lines exhibit heterogeneous and low expression of disialoganglioside GD2, the target antigen of dinutuximab treatment of GD2-positive neuroblastoma. Pharmacologic inhibition of EZH2, the catalytic component of the PRC2 chromatin modulating complex, has been shown by our lab and others to induce GD2 expression in previously GD2-negative cell lines. We then used the UNC EpiG Diamond small molecule library to screen for other compounds capable of inducing GD2 expression. Interestingly, several compounds identified as capable of inducing GD2 expression are highly synergistic with EZH2 inhibition, leading to questions as to the mechanism of GD2 induction by epigenetic modulation. To further investigate these findings, I have used gene expression analysis of several enzymes in the GD2 synthetic pathway to identify GD3 synthase as the key differentially regulated step in the pathway. Surprisingly, combination therapy with EZH2 inhibitors and other compounds increase levels of GD3 synthase, but do not act on other synthetic

enzymes. This work adds important mechanistic information to the *in vivo* arms of this study and has the potential to improve tumor-directed therapy in pediatric solid tumors.

Poster #23: Merrill Froney, graduate student
(Dr. Samantha Pattenden, Molecular Therapeutics)
Title: Development of a Platform for Therapeutic Target Discovery of Alternative Lengthening of Telomeres (ALT) Cancers
Abstract: Telomeric DNA acts as a protective cap at the ends of chromosomes by preventing DNA replication machinery from recognizing chromosome ends as double stranded breaks. In somatic cells, telomeres are shortened with each cell division due to the "end-replication problem", which eventually leads to senescence. Tumor cells avoid senescence triggered by telomere shortening by activating one of two telomere maintenance mechanisms (TMMs): telomerase reactivation or alternative lengthening of telomeres (ALT). TMMs are a viable target for cancer treatment, as they are only activated in tumor and not somatic cells. While there is a telomerase inhibitor currently undergoing clinical trials, there are no effective inhibitors of the ALT pathway. Many cancers with high prevalence of ALT activation do not have effective treatment options such as neuroblastoma which is the most common and lethal solid tumor type in children. Thus, discovery of an ALT-specific chemical probe would provide important insight into ALT biology and assess the potential of targeting ALT for cancer treatment. To fulfil these unmet needs, we have developed a first-in-class high throughput screen to identify inhibitors of ALT activity. Our screen uses an ALT-specific phenotypic biomarker, C-circles, as a readout to detect ALT inhibition induced by compound treatment. Hit compounds from this screen will potentially enhance our understanding of ALT biology through the identification of novel therapeutic targets.

Poster #25: Sierra McDonald, graduate student
(Dr. Angela Murphy, Pathology, Microbiology, and Immunology, University of South Carolina School of Medicine)
Title: Panaxynol Reduces Murine Colitis Associated Colorectal Cancer by Targeting Macrophages
Abstract: Colon cancer is the second leading cause of cancer death in the US for men and women combined. Panaxynol (PAX), a bioactive component in American Ginseng, has been shown to possess anti-cancer properties in vitro and was recently shown to suppress DSS-induced colitis in mice. We investigated whether PAX (2.5mg/kg, p.o., 3x/wk for 12 wks) has any promise in alleviating AOM/DSS induced colitis-associated colorectal cancer (CAC) using C57BL/6 female (n=20) and male (n=20) mice. PAX significantly suppressed clinical colitis symptoms including diarrhea and fecal occult blood, indicative of reduced inflammation and tumorigenesis. Moreover, PAX significantly reduced colon polyp number and size compared to vehicle. Reduced tumor burden displayed in panaxynol treated mice was confirmed by histopathological analysis, which assessed the degree of colonic injury. Given that macrophages play a substantial role in the pathogenesis of CAC, we examined the role of macrophages and other immune cells as a mechanism by which panaxynol suppresses CAC. We demonstrate that PAX reduced total macrophages and pro-tumoral M2-like macrophages in the lamina propria via flow cytometry. These results were confirmed by RT-PCR in which PAX reduced overall, pro-tumoral M2-like, and pro-inflammatory M1-like macrophage markers. In addition, PAX increased colonic gene expression of occludin which is necessary to maintain intestinal barrier function and regulate colonic permeability. Our results suggest that PAX is effective in reducing colitis and tumor burden in murine CAC possibly through its inhibition of overall and M2 macrophages.

Poster #27: Michelle Thomas, graduate student
(Dr. Samantha Pattenden, Molecular Therapeutics)
Title: Interrogating chromatin accessibility in archived tissues

Abstract: Epigenetics is the alteration in expressed cell phenotypes without changes to the underlining DNA sequence. An important component of the epigenome is chromatin, which contains repeating units called nucleosomes. Each nucleosome consists of an octamer of histone proteins wrapped with 147 base pairs of DNA. Nucleosomes pose a barrier to DNA-templated processes such as transcription or DNA repair, so their position is dynamic. For DNA to become accessible, nucleosomes must be moved to expose the underlying DNA sequence. Therefore, "accessible" chromatin is nucleosome-depleted and nucleosome- rich regions are "inaccessible". Specifically, we are interested in studying chromatin accessibility because chromatin architecture is indicative of the "how" and "where" gene expression can happen. Thus, mapping the chromatin landscape can be more predictive of cell fate than examining gene expression alone. The most common method for preservation of patient biopsy tissues is formalin fixation followed by paraffin embedding (FFPE). Large repositories of FFPE tissues are available, however, there are currently no practical methods for extracting high-quality chromatin from FFPE tissues. Assessing genomic DNA from FFPE tissue for genetic research and therapeutic diagnostic purposes is routine, however, the use of these samples for epigenetic research such as chromatin accessibility assays is extremely challenging. We have developed a novel nanodroplet technology that consists of a perfluorocarbon liquid core and lipid monolayer shell. Nanodroplets are added during sonication of FFPE tissues to promote rapid and consistent chromatin fragmentation for use in epigenetic assays. We have demonstrated that nanodroplets extract chromatin from FFPE rodent xenograft tissue and mouse organ tissue. We found that extraction of high-quality chromatin from mouse organ tissue required additional steps in processing compared to rodent xenograft tissues. These results indicate that our method will be influenced by cellular composition. Overall, the nanodroplet reagent is a first-in-class technology, providing a fast and simple resource for extracting high-quality chromatin from FFPE tissue for epigenetic research.

Poster #29: Travis Nelson, postdoctoral researcher
(Dr. Nate Hathaway, Molecular Therapeutics)
Title: Targeted TP53 expression with a small molecule epigenetic modifier & CRISPR/Cas9
Abstract: Epigenetic dysregulation of gene expression is a common driver of a variety of human diseases, including cancer. Post-translational modifications of chromatin can result in abnormal regulation of key genes, leading to pathogenesis and a suppression of normal function. Of particular concern is the gene TP53 and the tumor suppressing protein it encodes, p53. This transcription factor regulates signaling pathways that are associated with the maintenance of cellular homeostasis, response to cellular stresses, and tumor suppression. As such, TP53 is mutated or epigenetically downregulated in many cancers and therefore makes for an attractive target for therapeutic upregulation. Current approaches towards epigenetic regulation largely rely small molecule drugs or large CRISPR/Cas9-based fusion proteins, which provide either a dose-dependent response or a gene-specific response, but not both. Recent work in our lab has focused on combining these two features to utilize the best of both worlds. We have engineered a system that combines a nuclease-deficient of CRISPR/Cas9 (dCas9), a guide RNA (gRNA), and a fusion protein containing the FK506 binding protein (FKBP), which links to a small molecule chemical epigenetic modifier (CEM). This final CEM component is a two-headed molecule containing FK506 linked to a bromodomain inhibitor, and is thus designed to recruit a cell's endogenous epigenetic activators to a specific gene of interest. Here, we present preliminary work demonstrating that this dCas9-FKBP-CEM system to is capable of epigenetically upregulating TP53 mRNA expression in cancer cell lines known to be wild-type and epigenetically downregulated, as well as P53 protein expression in colorectal cancer cells.

Poster #31: Priya Hibshman, graduate student
(Dr. Channing Der, Molecular Therapeutics)

Title: MYC is a Major Driver of KRAS-Dependent Metabolic Abnormalities in Pancreatic Cancer
Abstract: Mutationally activated KRAS drives pancreatic ductal adenocarcinoma (PDAC) growth predominantly through activation of the ERK mitogen-activated protein kinase (MAPK) cascade. However, how ERK supports KRAS-dependent cancer growth remains to be established. While ERK phosphorylates and regulates a complex phosphoproteome (>1000 direct/indirect substrates), we hypothesized that the MYC transcription factor is a key ERK substrate critical for supporting KRAS-dependent PDAC growth. To delineate the contribution of MYC to KRAS-driven PDAC, we first applied RNA-Seq analyses to establish a system-wide profile of the MYC-dependent transcriptome. We determined gene transcription changes at 24 h after treatment with siRNA targeting MYC. We identified ~2000 significantly downregulated and ~2400 significantly upregulated genes. GO/KEGG/Reactome pathway analyses determined that MYC-deregulated genes control diverse cellular processes. Next, we validated key MYC-dependent processes using acute genetic suppression of KRAS or MYC expression. We found that MYC is a major driver of KRAS-dependent cell proliferation and metabolism. In particular, MYC depletion suppressed KRAS-regulated processes such as nucleotide synthesis, glucose metabolism, mitochondrial function, and nutrient scavenging. Furthermore, consistent with MYC-dependent regulation of genes involved in autophagy and lysosomal function, we observed compensatory upregulation of autophagic flux. KRAS or ERK inhibition similarly altered the metabolic landscape, supporting a critical role for KRAS-regulated MYC function in PDAC. For example, both KRAS and MYC suppression promoted mitochondrial fusion. In contrast, we determined that other KRAS-dependent processes, such as steroid biosynthesis and fatty acid metabolism, may be regulated independently of MYC. In summary, our studies have revealed that MYC facilitates diverse KRAS-driven cellular activities, and have also identified KRAS-dependent, MYC-independent mechanisms of PDAC growth. Our studies support the provocative concept that inhibiting MYC function may be an effective strategy for targeting KRAS for PDAC treatment.

Poster #33: Rebecca Johnson, graduate student
(Dr. Stephen Frye and Dr. Lindsey James, Molecular Therapeutics)
Title: Development of a chemical probe for Chromodomain-Helicase DNA-Binding Protein 1 (CHD1)
Abstract: Targeting the histone tail binding pockets of chromatin readers is a highly advantageous strategy in elucidating the functions of these proteins in both healthy and malignant biology. Chromodomain-Helicase DNA-Binding Protein 1 (CHD1) preferentially recognizes and interprets di- and tri- methylated lysine 4 on histone H3 (H3K4me2/3) through tandem chromodomains and is associated with active chromatin. Characterization of full-length CHD1 has revealed roles in chromatin remodeling and transcription with implications in prostate cancer oncogenesis and influenza. Although previous research has explored the role of CHD1 silencing on influenza viral replication and prostate cancer growth, studies have fallen short of teasing apart the specific role and contributions of the tandem chromodomains. We report the discovery of a small molecule inhibitor that binds the tandem chromodomains of CHD1 with low micromolar affinity. Current inhibitor optimization, structural, and phenotypic studies are ongoing in attempts to achieve chemical probe status.

Poster #35: Brittany Rickard, graduate student
(Dr. Imran Rizvi, Molecular Therapeutics)
Title: Photochemical Targeting of Mitochondria to Overcome PFAS-Induced Platinum Resistance in Ovarian Cancer
Abstract: Per- and polyfluoroalkyl substances (PFAS) are endocrine-disrupting compounds that frequently pollute drinking water supplies worldwide. As such, certain PFAS are linked to adverse female reproductive outcomes, including increased ovarian cancer risk. Ovarian cancer is the most lethal gynecologic cancer with a mortality rate of ~65%. A major contributor to the high lethality is resistance

to platinum-based chemotherapy, highlighting the need for an improved understanding of the sources of chemoresistance and the development of mechanism-based treatments. Since environmental exposures have been linked to therapy resistance in other cancers, the effect of PFAS on the response of ovarian cancer to chemotherapy was evaluated. Interestingly, two emerging PFAS, perfluoroheptanoic acid and perfluoropentanoic acid, induced platinum resistance in ovarian cancer cells. We hypothesized that the observed platinum resistance was due to PFAS-induced mitochondrial dysfunction. Studies have shown that PFAS induce adverse ovarian effects through disruption of mitochondrial respiration, and that platinum-resistant ovarian cancer cells demonstrate increased flexibility in using glycolysis and oxidative phosphorylation for energy production compared to platinum-sensitive cells. In the present study, changes in mitochondrial membrane potential were measured following PFAS exposure and after treatment with platinum-based chemotherapy. An increase in mitochondrial membrane potential was observed in ovarian cancer cells post-PFAS exposure and after treatment with carboplatin, suggesting improved cellular health. Since these data implicate mitochondria in PFAS-induced resistance, the ability of photodynamic priming (PDP), a light-based treatment modality that can target specific organelles, including mitochondria, was evaluated. PDP re-sensitizes platinum-resistant tumor cells to subsequent chemotherapy treatment by producing reactive molecular species, causing organelle damage, and modulating apoptotic regulatory proteins. PDP using a clinically approved photosensitizer (benzoporphyrin derivative) or pro-drug (5-aminolevulinic acid-induced protoporphyrin IX) was compared and performed on PFAS-exposed ovarian cancer cells prior to treatment with platinum therapy. Data showed that mitochondrial-targeted PDP successfully overcame PFAS-induced platinum resistance and induced photodamage to the mitochondrial membrane, indicated by decreased mitochondrial membrane potential. Taken together, these findings suggest PFAS contribute to platinum resistance in ovarian cancer by altering mitochondrial function. Targeting mitochondria using PDP may prove effective for the treatment of platinum-resistant ovarian cancer.

Poster #37: Sophie Roush, graduate student
(Dr. Yuri Fedoriw, Pathology and Laboratory Medicine)
Title: Increased Tumor T-Cell Receptor Repertoire Clonality Associates with HIV/ART Status and Improved Outcome in a Cohort of Diffuse Large B-Cell Lymphoma Patients
Abstract: BACKGROUND: Highly associated with HIV, diffuse large B-cell lymphoma (DLBCL) is the most common lymphoma worldwide and likely differs biologically based on HIV status and antiretroviral therapy (ART) exposure. Recent studies suggest increased tumor T-cell receptor (TCR) repertoire clonality associates with improved response to immune checkpoint inhibitors (ICI). HIV decreases CD4+ and naïve T-cell counts and leads to a clonal TCR repertoire due to T cells targeting HIV-specific epitopes. We therefore hypothesized HIV+ DLBCL would have more clonal TCR repertoires compared to HIV-. METHODS: The Kamuzu Central Hospital Lymphoma Study has prospectively enrolled patients with newly diagnosed lymphomas in Malawi since 2013. All patients receive standardized treatment and follow-up. We extracted DNA from 68 pre-treatment formalin-fixed paraffin-embedded (FFPE) DLBCLs from this cohort (QIAmp DNA FFPE Advanced) and performed TCR sequencing (immunoSEQ, Adaptive Biotechnologies). ART-experienced was defined as greater than 6 months of ART prior to DLBCL diagnosis. 36 FFPE tumors (n=12 HIV-, n=8 HIV+/ART-naïve, n=16 HIV+/ART-experienced) had >100 productive templates and passed quality control, meeting inclusion criteria for analysis. Of these, 2 tumors were EBV+ by EBER-ISH (n=1 HIV+/ART-experienced, n=1 HIV-). We used random downsampling to 100 productive templates due to template count variation. To test associations with clinical/demographic variables, we used ANOVA with Bonferroni correction, Pearson's correlation or Wilcoxon signed-rank test. For survival analysis, we generated binary variables from median cut-off, then calculated hazard ratios (HR) by Cox regression and produced Kaplan-Meier curves. RESULTS: TCR repertories from HIV+/ART-naïve tumors were more clonal than those from HIV- (productive Simpson

clonality: 1.4 fold-change, adj. p=0.023; max productive frequency: 3.3 fold-change, adj. p=0.027) and HIV+/ART-experienced patients (productive Simpson clonality: 1.4 fold-change, adj. p=0.052; max productive frequency: 2.6 fold-change, adj. p=0.05). There were no differences between HIV- and HIV+/ART-experienced tumor clonality or in total productive template count by HIV/ART status. When analyzing HIV+ and HIV- tumors together, high clonality correlated with improved event-free (productive Simpson clonality: HR 0.26, p=0.011) and overall survival (productive Simpson clonality: HR 0.32, p=0.031). This trend was maintained when analyzing HIV+ and HIV- tumors separately. Age was not associated with tumor TCR clonality or outcome. Non-germinal center tumors trended toward worse event-free survival (HR 2.15, p=0.12), but not overall survival. CONCLUSIONS: The TCR repertoire in HIV+/ART-naïve DLBCL was more clonal than HIV- and HIV+/ART experienced cases. Longer duration of ART exposure prior to DLBCL diagnosis appeared to restore overall TCR repertoire diversity in the developing tumor. Increased tumor TCR clonality associated with improved outcome in our cohort, irrespective of HIV/ART-status. Based on these results, HIV+/ART-naive DLBCL patients may represent a subset of lymphoma patients who would benefit from ICI.

Poster #39: Lauren Bates, graduate student
(Dr. Shakira Grant, Clinical Research)
Title: "Life like we used to be": Dyadic perspectives on the burden of multiple myeloma.
Abstract: INTRODUCTION: Multiple myeloma (MM) is an incurable cancer that impacts older adults. Over time, MM-therapies become more burdensome, leading to physical function decline, gradual loss of independence, and the need for caregiver support. Due to the influence that patients and caregivers have on each other's health, understanding dyadic (patient-informal caregiver) perspectives about the long-term functional impacts of MM is critical. METHODS: Between November 2021 and April 2022, we conducted 21 dyadic interviews with older adults in North Carolina diagnosed with MM between 2006 and 2021 and treated for at least 6 months. Each older adult was paired with an informal adult caregiver. We used the Sort and Sift, Think and Shift approach for qualitative data analysis. RESULTS: We enrolled 11 Black and 10 White, mostly older dyads. The average age of patients was 70 years (range= 57-90), and for caregivers, 68 years (range = 37-88). Most patients (54%) and caregivers (52%) self-identified as female. All dyads reported one or more functional impacts of MM (Table 1), with the majority (67%) of caregivers reporting changes to their physical function due to less physical activity engagement and needing to prioritize their care recipients' needs. Factors that promoted dyadic loss of physical function included: 1) disease phase (pre-treatment and within six months of treatment initiation, 71%); 2) treatment intensity (e.g., receipt of autologous stem cell transplantation, 71%), 3) patient symptom burden [e.g., severe pain (76%), peripheral neuropathy (43%), and excessive fatigue (81%)] and 4) adequacy of the social support network [e.g., MM support group, church, friends, (52%)]. Dyads also reported a sense of loss and yearning to return to their lives before the MM diagnosis. CONCLUSION: MM and its treatments adversely impact dyadic physical function. Reports of functional decline are most severe at the onset of treatment but persist throughout later stages of the disease. This research highlights a need for dyadic-level interventions to help maintain or improve the physical activity and function of long-term MM survivors and their caregivers.

Poster #41: Emilie Duchesneau, graduate student
(Dr. Jennifer Lund, Cancer Epidemiology)
Title: One-year mortality and varying ascertainment windows for a Medicare claims-based proxy for frailty in women with stage I-III breast cancer
Abstract: BACKGROUND: Frail cancer patients are more likely to die or experience treatment toxicity than non-frail patients. The Faurot frailty index (FFI) is a validated Medicare claims-based proxy that predicts frailty in individuals. Frailty is dynamic and can change over time. We assessed the distribution

of the FFI and associations with 1-year mortality using varying durations of frailty ascertainment windows in breast cancer patients. METHODS: We identified women (66+ yrs) with stage I-III breast cancer in the SEER-Medicare database who had ≥1 year of continuous enrollment in Medicare Parts A and B prior to diagnosis. The FFI-predicted probability of frailty was assessed using demographic and diagnostic and procedural billing information during varying pre-diagnosis ascertainment windows: 3, 6, 8, or 12 months or using all available pre-diagnosis claims. We described the distributions of the claims-based frailty measures using each of the five ascertainment windows and reported 1-year mortality for individuals with high (≥0.20), medium (0.05 - <0.20), or low (<0.05) claims-based frailty using Kaplan-Meier analysis. Follow-up began on the date of cancer diagnosis. RESULTS: We identified 235,145 women with breast cancer. Using a 3-month ascertainment window, 76%, 20%, and 4% were classified as having low, medium, and high claims-based frailty. Although the distributions of claims-based frailty were similar using 3, 6, 8, and 12-month frailty ascertainment windows, the use of all available lookback led a wider interquartile range (IQR) for claims-based frailty (Table). Overall, 4% of women died within 1 year of diagnosis. Higher claims-based frailty was strongly associated with increased 1-year mortality risk, using each ascertainment window. Mortality associations were strongest with a 3-month ascertainment window: risk difference (RD) high vs. low frailty 20% (95% CI, 19-20%). Associations were weakest with an all available lookback window: RD high vs. low frailty 11% (95% CI, 11-12%). CONCLUSIONS: Varying frailty ascertainment window durations did not lead to substantial differences in the distribution of the FFI in women with stage I-III breast cancer. Associations between the FFI and 1-year mortality monotonically decreased with increasing ascertainment window durations. This may be due to claims in the distant past having less etiological relevance for predicting mortality during the year following cancer diagnosis.

Poster #43: Nathaniel Woodard, graduate student
(Dr. Cheryl Knott, Behavioral and Community Health, University of Maryland School of Public Health)
Title: Self-Esteem Moderates the Association between Neighborhood-Level Household Income and Depressive Symptoms
Abstract: Recent research suggests that disadvantaged neighborhood environments can increase health disparities among inhabitants, including disparities of mental health and depression. Prior studies suggest high self-esteem and social support are inversely associated with one's risk of depression, however, whether self-esteem and social support can mitigate the adverse effects of the neighborhood environment on depressive symptoms is understudied. The current study investigates the moderating roles of self-esteem and social support in the association between neighborhood-level median household income and individual-level depressive symptoms in a national sample of 3,115 African Americans. We hypothesized that in addition to the direct effects of self-esteem and social support on depressive symptoms, self-esteem and social support would each serve as protective factors against the inverse association between neighborhood median household income and depressive symptoms. Moderating effects were tested with latent moderated structural equations using maximum likelihood with robust estimation. Analyses indicated inverse direct associations between neighborhood median household income, self-esteem, and social support with depressive symptoms, controlling for participant age, gender, education, and self-reported health status. Interaction terms for neighborhood median household income and self-esteem indicated that self-esteem modestly attenuates the inverse association between neighborhood median household income and depressive symptoms. No moderating effect was detected for social support. Findings suggest that interventions to address depressive symptoms may consider interventional components to bolster self-esteem, particularly in low-income communities of color.

Poster Session II: Even-Numbered Posters

(3:30 – 4:00 PM, Magnolia C Room)

Poster #2: Katherine Barnett, postdoctoral researcher
(Dr. Jenny Ting, Immunology)
Title: An epithelial-immune circuit amplifies inflammasome and IL-6 responses to SARS-CoV-2
Abstract: Elevated IL-1β and IL-6 typify the cytokine storm in severe COVID-19. Here, we investigate how SARS-CoV-2 promotes these cytokines and if this process is enhanced in severe disease. SARS-CoV- 2 replicates in primary human airway epithelial cells (HAE), which have inflammasomes, but infection does not stimulate IL-1β release. In leukocytes, the SARS-CoV-2 E protein upregulates inflammasome gene transcription through TLR2 but does not promote inflammasome activation. IL-1β secretion from leukocytes requires a second signal supplied by infected HAE, including extracellular mitochondrial and genomic dsDNA. Patient autopsy lungs and single cell RNA sequencing (scRNAseq) data show aggravated myeloid cell inflammasome signatures (IL1B/IL18, CASP1/4/5, AIM2, NLRP3/6) in severe COVID-19. Upon IL-1β release, it signals back to stimulate robust IL-6 secretion from HAE. Therefore, infection alone does not increase IL-1β secretion by either cell type. Rather, bi-directional interaction between the SARS-CoV-2-infected epithelium and immune bystanders promotes production of both IL-1β and IL-6, revealing a proinflammatory cytokine circuit.

Poster #4: Leiah Carey, postdoctoral researcher
(Dr. Sharon Campbell, Molecular Therapeutics)
Title: Combinatory Structural Biology of NRAS Q61 Melanomagenic Mutants
Abstract: RAS proteins are membrane-associated small GTPases that transduce extracellular signals into cells to regulate pathways that control cellular growth. They are proto-oncogenes that are highly mutated in human cancers. Recent studies suggest that certain oncogenic RAS mutants display distinct biochemical and structural properties. Functional knock-in mouse model studies have recently shown that NRAS Q61 mutants exhibit distinct profiles in human melanoma. Specifically, rare melanoma mutants (NRAS G12D, G13D, G13R, Q61H, and Q61P) have weak tendency to induce spontaneous melanoma formation whereas common melanoma mutants (NRAS Q61R, Q61K, and Q61L) drive tumor growth. However, the molecular basis for NRAS Q61 mutant prevalence and melanomagenic capacity is unclear. Here we present integrative NMR, biochemical and computational studies to identify populated conformers in the solution state and profile differences in biochemical properties. Additionally, we present comparative analysis of NRAS Q61 mutants with their KRAS Q61 counterparts. We also generated structural ensembles of individual NRAS Q61 mutants using Replica Exchange Molecular Dynamics (REMD) simulations and examined their interactions with BRAF RBD-CRD RAS binding regions using molecular docking. Highly melanomagenic NRAS Q61R and Q61K were found to stabilize the Switch I region through side chain contacts and associate with RAF with higher affinity. In contrast, low melanomagenic NRAS Q61 mutants do not induce conformational changes in Switch I but rather create backbone interactions with the Switch II region. Based on our findings, we propose experimentally-driven computational models for NRAS Q61 mutants and their differential ability to engage BRAF RBD-CRD, showcasing our ability to conduct multi-faceted, comprehensive structural biology investigations valuable for oncogenic RAS mutants that possess dynamic Switch regions.

Poster #6: Kristina Drizyte-Miller, postdoctoral researcher
(Dr. Channing Der, Molecular Therapeutics)

Title: Targeting Mitochondrial Activity Using Small Molecule Activators of Mitochondrial Protease ClpP for Pancreatic Cancer Treatment

Abstract: The KRAS oncogene is activated in ~95% of pancreatic ductal adenocarcinoma (PDAC) patients and reprograms tumor metabolism to support the bioenergetic and biosynthetic demands of cancer cells. Emerging evidence suggests that PDAC cells rely on altered mitochondrial function for their survival and that inhibiting mitochondrial activity could be a viable therapeutic option. However, there is a lack of effective mitochondrial inhibitors to assess for PDAC treatment. Here, we evaluated ONC201, a clinical candidate agonist of the mitochondrial matrix protease ClpP, and a more potent, next generation analog, TR-107, as potential therapeutics for PDAC. Both ONC201 and TR-107 hyperactivate ClpP, causing proteolytic degradation of a spectrum of mitochondrial proteins such as those involved in oxidative phosphorylation (OXPHOS), the tricarboxylic acid cycle, heme biosynthesis, and mitochondrial translation in a ClpP-dependent manner. We found that ONC201-induced hyperactivation of ClpP inhibited the growth of PDAC cell lines and organoids and impaired mitochondrial respiration and mitochondrial ATP production. However, treatment also resulted in a compensatory increase in glycolysis to offset the deleterious consequences of impaired OXPHOS. We found that concurrent treatment with a selective KRASG12D inhibitor (MRTX1133) or a selective ERK1/2 inhibitor (SCH772984), both of which suppressed glycolysis, further enhanced ONC201 inhibition of PDAC cell growth. KRAS-ERK inhibition additionally promoted mitochondrial fusion and suppressed expression of mitochondrial biogenesis genes. Our ongoing studies are evaluating the consequences of ONC201 treatment on other metabolic activities and cancer cell signaling pathways in PDAC. Additionally, we are using our custom-designed mitochondrial-focused CRISPR/Cas9 library to perform a loss-of-function CRISPR screen to identify metabolic genes that modulate PDAC sensitivity to ONC201 and to identify combination treatment strategies that would enhance the long-term efficacy of ONC201. In summary, our results support a therapeutic value in targeting mitochondrial function using ClpP agonists for KRAS-mutant PDAC treatment.

Poster #8: Jennifer Klomp, postdoctoral researcher
(Dr. Channing Der, Molecular Therapeutics)

Title: System-wide determination of the functional ERK-regulated phosphoproteome in KRAS-mutant pancreatic cancer

Abstract: The KRAS oncogene is mutationally activated in 95% of pancreatic ductal adenocarcinoma (PDAC). The recent approval of a clinically effective inhibitor of one KRAS mutant, KRASG12C, marks a milestone in KRAS drug development, and preclinical development of direct inhibitors of other KRAS mutants is ongoing. However, as seen with essentially all targeted anti-cancer therapies, patients who are initially responsive eventually relapse. In approximately 60% of patients, treatment-induced drug resistance was associated with activation/inactivation of signaling components that lead to reactivation of RAS and RAF-MEK-ERK and PI3K-AKT effector signaling. We determined that ectopic expression of constitutively activated MEK1S118/222D or ERK1R84S/S170D, but not myristoylated AKT1, drove near-complete resistance to selective direct inhibitors of KRASG12C or KRASG12D. Thus, we expect that reactivation of ERK but not AKT will be a key limitation for all RAS-targeted therapies, prompting our studies to define the mechanistic basis for ERK-dependent PDAC growth. ERK activation can cause direct/indirect phosphorylation of >1000 proteins, including transcription factors and protein kinases. Thus ERK activation can regulate a complex transcriptome and phosphoproteome. Our recent determination of the ERK-dependent transcriptome in KRAS-mutant PDAC showed that it diverges significantly from the cancer type naïve Gene Set Enrichment Analysis Hallmark KRAS signature, supporting a pancreas-specific function of ERK. Therefore, we performed proteomic analyses to define the ERK-dependent phosphoprotein landscape in KRAS-mutant PDAC. To avoid complications of ERK inhibition-induced compensatory signaling activities and minimize cell line heterogeneity, we evaluated

six KRAS-mutant PDAC cell lines after 1- or 24-hour treatment with the ERK1/2-selective inhibitor SCH772984. We first compared our findings with a recent compendium of ERK substrates (2,507 phosphosites; 1,308 phosphoproteins) compiled from 14 different published studies (Ünal et al., 2017). We identified 4,032 phosphosites on 1,884 proteins that were significantly deregulated by ERK inhibition, with 41% and 77%, respectively, not previously been attributed to ERK. Of the 4,032 phosphosites, 60% were components of the p[S/T]-P (48%) or the extended P-X-p[S/T]-P (12%) ERK phosphorylation consensus motif. Of the 1,884 proteins, 33%, 5%, and 6% contained the ERK substrate recognition D-, DEF-motifs, or both, respectively. We then began to define the ERK substrates that support KRAS-dependent PDAC growth. First, we found that a D-motif association-deficient mutant of activated ERK1 (ERK1R84S/S170D/D338N) failed to drive resistance to KRAS inhibition. Second, KEGG analyses implicated ERK substrates in regulation of cell cycle progression and cancer metabolism. Third, mining genome-wide CRISPR data from the Broad Achilles Project (DepMap), we identified a subset of ERK substrates required for the growth of KRAS-mutant PDAC that were highly enriched in nuclear localized proteins. Finally, using a CRISPR library targeting the compendium of ERK substrates, we identified additional ERK substrates that are synthetic lethal with ERK inhibitor treatment. In summary, our determination of the ERK-regulated phosphoproteome will further elucidate how KRAS drives PDAC growth through activation of the ERK signaling network.

Poster #10: Marta Overchuk, postdoctoral researcher
(Dr. Imran Rizvi, Molecular Therapeutics)
Title: Targeted PDT Combinations to Overcome Fluid Shear Stress-induced Platinum Resistance in Ovarian Cancer
Abstract: ABSTRACT: Ovarian cancer is the deadliest gynecologic malignancy — in 2020 alone, ovarian cancer claimed lives of 13,940 patients in the United States and 29,000 in Europe. Such high ovarian cancer mortality can be explained by the fact that most patients are diagnosed with advanced-stage disease and 70% of them develop resistance to platinum-based therapies within the first 5 years. One of the potential contributing factors to treatment failure in ovarian cancer is malignant ascites, or excessive fluid buildup in the peritoneal cavity. Ascites creates a unique molecular and biophysical environment, providing cancer cells with a nutrient- and growth factor-rich media and exposing them to abnormal physical stress. Our research group has been studying the effects of fluid shear stress (FSS) on ovarian cancer cell phenotypes and treatment responsiveness. It was found that FSS confers resistance to carboplatin, activates the epidermal growth factor receptor (EGFR) as well as the downstream signaling cascades, and promotes epithelial-mesenchymal transition. These findings revealed the need for strategies that would remain effective under flow conditions and aid the effectiveness of standard of care treatments. Photodynamic therapy (PDT), which utilizes photosensitizers and light to generate cytotoxic reactive molecular species, provides a mechanistically distinct way of targeting chemoresistant cell populations. It was demonstrated that low-dose PDT with EGFR-targeted benzoporphyrin derivative photoimmunoconjugates remains effective in a 3D perfusion model for ovarian cancer, under conditions that induce resistance to carboplatin and EGFR overexpression and activation [2]. Encouraged by these findings, we continue exploring benzoporphyrin derivative-enabled PDT as a stand-alone therapy or in combination with cisplatin under static and flow conditions. Overall, we believe that photodynamic therapy has a potential to become an indispensable tool in late-stage ovarian cancer treatment, effectively destroying and/or sensitizing chemoresistant cell populations, decreasing the required chemotherapy dose and expanding the therapeutic window.

Poster #12: Rosemary Plagens, postdoctoral researcher
(Dr. Hector Franco, Breast Cancer, Cancer Genetics)
Title: Identifying FOXA1 Binding Partners using Proximity Labeling

Abstract: Approximately 75% of breast cancers are driven by the estrogen receptor alpha (ER), and despite the advent of endocrine therapy to block ER signaling pathways, a significant portion of women develop resistance to these drugs. The pioneer factor FOXA1 has been shown to facilitate nearly all DNA-binding events of ER in response to estrogen in ER+ breast cancer (ER+BC). Notably, up-regulation of FOXA1 is a hallmark of endocrine-resistant phenotypes and has been shown to reprogram enhancer elements, leading to an altered transcriptome. However, FOXA1 is a critical pioneer factor for multiple nuclear hormone receptors, aside from ER, and is implicated in regulation of important factors such as HER2 and the androgen receptor (AR). With the diverse array of breast cancer molecular subtypes displaying complex interplay between ER, HER2, AR, PR, and other hormone receptors, describing the complete ensemble of FOXA1 binding partners in various contexts, such as endocrine-resistant tumors, is of increasing importance. To define a comprehensive catalog of FOXA1 binding partners under basal conditions, we generated MCF-7 cell lines stably expressing constructs of FOXA1 fused at its N- or C-terminus to the biotin ligase miniTurbo. Using proximity labeling coupled with mass-spectrometry, we have comprehensively cataloged binding partners of FOXA1, including many expected proteins such as ER, AR, MLL3, YAP1, and GATA-3. Moreover, we have discovered more than 150 previously unidentified binding partners of FOXA1, which may exert profound effects on FOXA1 function. Importantly, high hazard ratios and significant dependencies are associated with several of these new binding partners, such as subunits of a previously described histone deacetylase (HDAC) complex containing genetic suppressor element 1 (GSE1) and lysine-specific histone demethylase 1A (KDM1A). Genomic approaches are currently underway to characterize where in the genome FOXA1 is interacting with these novel proteins and to guide future exploration into the physiological significance of these interactions. Integrating biochemical, molecular, and genomic approaches, we have potentially highlighted new mechanisms of FOXA1, which could have significant clinical impact in the future.

Poster #14: Caleb Studstill, postdoctoral researcher
(Dr. Cary Moody, Virology)

Title: Regulation of the Innate Immune Response During the Productive Phase of the HPV Life Cycle

Abstract: Human papillomaviruses (HPV) are small, double-stranded DNA viruses that infect cutaneous or mucosal epithelial cells. Some HPV types constitute low risk infections that are easily resolved by the host immune response. However, other HPV types are considered high risk (*e.g.* HPV16, 18, 31, 45) and can cause human cancers, including cervical cancer as well as an increasing number of head and neck cancers. HPV has evolved multiple ways to promote its life cycle, which occurs in the stratified epithelium. Previous work from our lab demonstrated that HPV31 appropriates cellular apoptotic caspases to block type I and type III interferon (IFN) responses during productive viral replication in differentiating epithelial cells. In the absence of caspase activity, these IFN responses were induced via the melanoma differentiation-associated gene 5 (MDA5)– mitochondrial antiviral-signaling protein (MAVS)–TBK1 (TANK-binding kinase 1) pathway, signifying a response to double-stranded RNA (dsRNA). Here, we study the role of RNA polymerase III (RNA Pol III) in generating dsRNA from endogenous retrovirus (ERV) loci in HPV infected cells following treatment with a pancaspase inhibitor. ERVs are methylated under normal conditions, which represses transcription. However, cellular stress can downregulate DNA methyltransferase 1 (DNMT1), resulting in hypomethylation of ERVs. In response to demethylation, ERVs can be transcribed by RNA polymerase III (RNA pol III), resulting in the production of dsRNAs that are sensed by MDA5 to stimulate innate immune signaling. We have found that differentiating HPV+ cells exhibit decreased protein levels of DNMT1 during productive viral replication. Upon pancaspase inhibition, we observed a significant increase in the transcription of several ERVs in HPV+ cells. Treatment with an RNA Pol III inhibitor abrogated the increased ERV expression and blocked immune signaling induced by pancaspase inhibition. Overall, these results suggest a possible mechanism by which HPV utilizes apoptotic caspases to promote an immune silent environment. We propose that

inhibition of caspase activity leads to a decrease in DNMT1 levels, resulting in decreased methylation of ERVs. RNA Pol III then transcribes endogenous retrovirus loci forming dsRNAs that trigger innate immune pathways and induce type I and type III interferon production. Current studies are focused on determining whether these ERVs are bound to MDA5 upon pancaspase inhibition. These data further highlight the diverse regulatory events viruses must contend with in order to maintain host genome stability and avoid immune stimulation during infection.

Poster #16: John Tabor, postdoctoral researcher
(Dr. Lindsey James, Molecular Therapeutics)
Title: Discovery of a potent and selective targeted NSD2 degrader for reduction of H3K36me2
Abstract: Nuclear receptor-binding SET domain-containing 2 (NSD2) plays important roles in gene regulation, largely through its ability to dimethylate lysine 36 of histone 3 (H3K36me2). Despite aberrant activity of NSD2 reported in numerous cancers, efforts to selectively inhibit the catalytic activity of this protein with small molecules has been unsuccessful to date. Here we report the development of UNC8153, a first-in-class NSD2 targeted degrader that potently and selectively reduces the cellular levels of both NSD2 protein and the H3K36me2 chromatin mark. UNC8153 contains a simple primary alkylamine warhead that confers proteosome-dependent degradation of NSD2 through a novel mechanism. Importantly, UNC8153-mediated degradation of NSD2 results in the down-regulation of pathological phenotypes in multiple myeloma cells including mild anti-proliferative effects in MM1.S cells containing an activating point mutation and antiadhesive effects in KMS11 cells harboring the t(4;14)-translocation which up-regulates NSD2 expression. Subsequent efforts from our labs have identified a more potent degrader, UNC8732. Current work is aimed at studying the mechanism of action of UNC8732-mediated degradation.

Poster #18: Yijun Zhou, postdoctoral researcher
(Dr. Dirk Dittmer, Virology)
Title: Large-scale purification of extracellular vesicles by heparin chromatography identifies two EV subclasses.
Abstract: Most conventional extracellular vesicle (EV) purification methods are limited to a smaller scale. We use a tangential-flow filtration and Capto Core 700 (TFF-CaptoCore700) based method for large-scale EV purification and intentionally avoid strong physical force to preserve EV integrity. Separating EV into subclasses that carry unique cargos and markers is essential. This is the prerequisite for any accurate phenotypic tests and even more importantly for EV manufacturing. Here, we present on-going work to further separate TFF-CaptoCore700 purified EV into two populations by heparin chromatography. The first does not bind to the heparin column and can be collected in the flow-through, namely a non-heparin-binding (NHB) fraction. The second binds to the heparin column and can be eluted by higher salt, thus is a heparin-binding (HB) fraction. Proteomics and functional analysis show NHB carries most conventional EV markers while HB is enriched in extracellular matrix binding protein and histones. Heparin chromatography would be an effective way to isolate EV subclasses at larger scale.

Poster #20: Peter Buttery, graduate student
(Dr. Stephen Frye and Dr. Lindsey James, Molecular Therapeutics)
Title: Development and characterization of covalent chemical probes for chromodomain proteins MPP8 and CDYL2
Abstract: Histone post-translational modifications are interpreted by specific classes of epigenetic "reader" proteins to help regulate gene expression. Methyllysine (Kme) reader proteins recognize and bind to Kme marks to help convey methylation signals. The specific contexts in which Kme readers bind

to chromatin and the resulting biological outcomes are the subject of much study. One way in which we seek to elucidate the functions of these proteins is through the development of chemical probes that serve as antagonists for the reader domains. Two targets for which such chemical tools are lacking are the chromodomain-containing proteins MPP8 and CDYL2. MPP8 participates in the Human Silencing Hub (HUSH) complex to mediate heterochromatin formation through H3K9me3 recognition and recruitment of methyltransferase SETDB1. HUSH mediated silencing has been implicated in a number of cancers including AML, making MPP8 a promising therapeutic target. CDYL2 similarly acts to recruit methyltransferases G9a and EZH2 at H3K9me2 and H3K27me3 marks and has been implicated in triple negative and ER+/HER2- breast cancer. As such, we seek to create chemical probes for these proteins to both elucidate their biological functions and to further evaluate their therapeutic potential. Our lab has successfully developed chemical probes for a number of Kme readers, yet such endeavors are often hampered by potency and selectivity challenges due to shallow binding pockets and the high sequence homology within Kme reader families. To address these issues, we have designed targeted covalent antagonists that contain electrophilic warheads to engage a cysteine residue within the binding pocket of these proteins. Targeted covalent inhibitors afford a number of benefits over traditional non-covalent inhibitors, including prolonged duration of action, resilience to drug resistance mechanisms, and improved potency and selectivity. This strategy represents a novel approach for developing chemical probes for these proteins.

Poster #22: Dalia Fleifel, graduate student
(Dr. Jean Cook, Cancer Cell Biology)
Title: The role of the c-Myc oncoprotein in ensuring complete DNA replication
Abstract: Origin licensing is a tightly regulated process that occurs in G1 phase where MCM helicases are loaded onto DNA to "license" multiple sites for DNA replication in S phase. MCM loading requires the cooperative action of origin licensing factors: Cdc6, Cdt1, and the ORC1-6 complex, in addition to the MCM2-7 complex. Licensing must occur only in G1, whereas origin firing must occur only in S phase. Thus, G1 is the only window available to license enough origins before entering S phase. Insufficient licensing in G1 leads to incomplete replication and sensitizes cells to DNA damage in S phase, which results in genome instability. The origin licensing rate is defined as the speed of MCM loading throughout the nucleus in G1 phase. Notably, G1 lengths vary greatly among different cell types, and licensing rates together with G1 length determine the amount of loaded MCMs. Thus, the rate must be tightly coordinated with G1 length to ensure enough loaded MCMs before S phase begins i.e.: a short G1 needs to be counterbalanced by rapid licensing. Our goal is to identify factors that control licensing rates in proliferating cells while maintaining their genome stability. We and others have shown that the G1 in iPSCs is very short, yet they have rapid licensing and can still load as many MCMs as cells with long G1 phases. Notably, a cocktail of Oct4, Klf4, c-Myc, and Sox2 (OKMS) factors can reprogram differentiated cells that have long G1 phases and slow licensing into induced Pluripotent Stem Cells (iPSCs) that have very short G1 phases and fast licensing. We hypothesize that one or more of the four OKMS factors directly or indirectly accelerates licensing to ensure complete replication and maintain genome stability. We have used non-transformed epithelial cells to overproduce the OKMS factors individually and in combinations to define their role in regulating licensing rates. Using single cell quantitative flow cytometry, we recently discovered that c-Myc or OKMS overproduction in epithelial cells shortens G1 and accelerates licensing. We also found that c-Myc overproduction increases total Cdt1 at both the mRNA and protein expression levels. Interestingly, OKMS overexpression did not change Cdt1 protein levels but instead led to increased Orc4 chromatin binding in G1. Our findings indicate that distinct mechanisms underlie how c-Myc overproduction, alone or in combination with the other OKS factors, regulates licensing. We also discovered that L-Myc, another member of the oncoprotein family, elicits a significantly weaker effect on origin licensing speed and G1 length, compared to c-Myc. We are currently

investigating how c-Myc regulates origin licensing by overproducing c-Myc variants as well as examining structural and functional differences between c-Myc and L-Myc. Importantly, c-Myc plays a dual role enhancing iPSCs generation as well as in tumor transformation. Our results will help identify both overlapping as well as context-dependent roles of c-Myc. Moreover, identifying c-Myc downstream targets that accelerate origin licensing will allow us to manipulate licensing rates in Myc-driven cancers, which might sensitize them to DNA damaging agents.

Poster #24: Aditi Kothari, graduate student
(Dr. Natalia Isaeva and Dr. Wendell Yarbrough, Virology)
Title: Interaction between NF-κB and NRF2 signaling pathways leads to better survival in HPV-associated head and neck cancer
Abstract: The human papillomavirus (HPV) is a causative agent in a proportion of head and neck squamous cell carcinoma (HNSCC) displaying a significantly favorable prognosis and overall better survival as compared to HPV negative HNSCC. More control of the primary tumor site has attributed to this improved survival and reduced tumor recurrence. Incidence of HPV related HNSCC is on the rise due to an increase in the incidence of HPV infection, while the prevalence of HPV negative HNNSCC is on the decline due to a control in smoking. Treatment includes chemotherapy along with intense radiotherapy leading to severe side-effects like difficulty swallowing, changes to voice, hair loss and lymphoedema among others. Precision medicine would revolutionize the treatment of head and neck cancer by accurately identifying patients that are at low risk of recurrence, reduce treatment related side-effects and aid in overall improvement in quality of life of patients after treatment. This generates the need to develop biomarkers to identify patients with less aggressive tumors as candidates for treatment de-escalation. Using molecular characteristics of HPV+ HNSCC and based on the presence or absence of NF-κB activating mutations, two intrinsic subtypes of HPV+ HNSCCs were identified. The subtype harboring inactivating mutations of NF- κB inhibitors tumor necrosis factor receptor-associated factor 3 (TRAF3) or cylindromatosis lysine 63 deubiquitinase (CYLD) is associated with activated NF-κB, maintenance of episomal (non-integrated extra-genomic) HPV, and improved patient survival. We found that tumors with increased NF-κB activity had a reduced nuclear factor erythroid 2-related factor (NRF2) signaling. NRF2 activation has been associated with resistance to treatment and a reduction in NRF2 activity seen in NF-κB active tumors may be a possible explanation of improved patient survival. We are exploring the hypothesis that NF- κB -driven intrinsic tumor characteristics contribute to increased sensitivity of NF- κB active HPV+ head and neck tumors to radiation, providing patients survival benefits. Indeed, TRAF3 or CYLD deletion dramatically increased radiation sensitivity of HPV+ head and neck cancer cells. We also found that activation of NF-κB significantly correlated with marked downregulation of NRF2 activity in tumors from 3 independent cohorts, as well as in HPV+ HNSCC cells that harbor constitutively active NF-κB due to deletion of TRAF3 or CYLD. Interestingly, TRAF3 CRISPR KO cells had lower NRF2 protein levels that were restored by MG132 treatment, indicating an involvement of KEAP1/CUL3 mediated proteasomal degradation of NRF2. In summary, our data showcases an inverse correlation between NF-κB and NRF2 pathways in HPV+ HNSCC. NF-κB activity or mutations in NF-κB pathway would be an invaluable tool for clinicians to identify patients with HPV-associated HNSCC with favorable prognosis as candidates for de-escalated therapy, as well as identification of patients who need intensive therapy.

Poster #26: Colleen Steward, graduate student
(Dr. Yuliya Pylayeva-Gupta, Immunology)
Title: Unraveling B cell differentiation in pancreatic cancer using engineered neoantigens
Abstract: Pancreatic cancer is the third-leading cause of cancer-related mortality and a growing public health burden. Late-stage diagnosis and poor response to existing treatments, including immunotherapy, contribute to a bleak 5-year survival rate of 11%. There is, therefore, a need to develop

combination therapies and harness immune cell populations within the tumor microenvironment. B cells represent a prominent population of infiltrating immune cells in pancreatic cancer and have the capacity to elicit both anti- and pro-tumorigenic responses. Effector B cells facilitate anti-tumor immunity, whereas regulatory B cells support tumor growth. The context in which B cells and antibodies encounter antigen shapes the immune response, yet it is unclear how antigen localization (membrane-bound or intracellular) impacts B cell responses within the tumor microenvironment. To address this question, we engineered a system to compare antigens expressed on the cell surface to intracellular antigens. In the model, hen-egg lysozyme (HEL) is either secreted from or expressed on the surface of murine pancreatic cancer cells. Given that the isolation of B cell specific tumor antigens in vivo has yet to be streamlined, our system enables us to control antigen localization and observe the resulting B cell response. Understanding the mechanisms by which tumor antigens shape B cell responses in cancer will help inform the design of B cell-directed immunotherapies to enhance anti-tumor immunity.

Poster #28: Margarita Dzama, postdoctoral researcher
(Dr. Jesse Raab, Cancer Genetics)
Title: Identification of new potential targets in liver cancer using focused CRISPR-Cas9 screens
Abstract: Hepatocellular carcinoma (HCC) is a highly deadly disease with few available treatment options for patients with unresectable tumors and not eligible for chemotherapy. Unfortunately, these existing systemic therapies only moderately prolong the lifespan and often result in emerging resistance. It has been previously shown that mutations in the SWI/SNF chromatin remodeling complex can be collectively detected in 40% of HCC cases. Patients carrying these mutations showed worse overall survival. In this project, we focused on the identification of new therapeutic targets as well as their combination with FDA-approved systemic therapy and therapy in the final phase of clinical trials by performing focused CRISPR knockout screens using different HCC models. We constructed a CRISPR library of 6000 guide RNAs targeting 737 genes involved in chromatin-mediated gene regulation. We chose to perform this screen in the presence of the multikinase inhibitor sorafenib, as it remains the most commonly used drug for HCC treatment. We also chose the additional kinase inhibitor donafenib, a deuterated sorafenib derivative that shows lower side toxicity in late-stage clinical trials. The CRISPR screens performed in various hepatoblastoma (HepG2) and hepatocellular carcinoma (HLF, PLC/PRF/5, Huh7) cell lines revealed new targets for HCC, including some members of the menin-MLL1 complex such as MEN1 and ASH2L. Evaluation of potential resistance in hepatoblastoma cells revealed KEAP1 and members of the SWI/SNF family (SMARCC1, ARID1A, and ARID1B) as important modulators of sorafenib-specific resistance. We confirmed this result by generating a mutant HepG2 cell line with a mutated ARID1B gene using CRISPR-Cas9 and performing sensitivity assays. A CRISPR screen on HCC cell lines in the presence of donafenib revealed that a knockout of KEAP1 increases sensitivity to the drug, which is the opposite effect of sorafenib treatment in the hepatoblastoma cell line. We intend to investigate further the striking differences of KEAP1 knockout in HCC cell lines upon treatment with sorafenib and donafenib. Overall, these data suggest differences in drug response could be driven by cell model or genotype. We believe that understanding this is critical for rational drug choice. We anticipate that epigenetic regulators are novel targets and represent an appealing therapeutic strategy for HCC treatment. A better understanding of acquired resistance may also help in the development of new treatment strategies and improve therapy outcomes.

Poster #30: Jonathan DeLiberty, graduate student
(Dr. Kirsten Bryant and Dr. Channing Der, Molecular Therapeutics)
Title: PIKfyve Inhibition is a Fyve-Out-of-Fyve Strategy for Targeting Autophagy in Pancreatic Ductal Adenocarcinoma

Abstract: Pancreatic ductal adenocarcinoma (PDAC) is characterized by KRAS- and autophagy-dependent growth. Autophagy is a multi-step, lysosomal-mediated process whereby cells degrade and recycle macromolecules to sustain growth. We and others have demonstrated that inhibition of the RAS-RAF-MEK-ERK pathway resulted in upregulated autophagic flux, and that dual treatment with the autophagy inhibitor hydroxychloroquine (HCQ) and ERK-MAPK inhibitors synergistically blocked PDAC growth. HCQ is limited in terms of specificity and potency, and we sought to identify a more efficacious autophagy inhibition strategy. We performed a CRISPR/Cas-9 mediated genetic loss-of-function screen with a library targeting cancer signaling pathways and identified PIKfyve as an essential autophagy-related gene in PDAC cells. PIKfyve is a lipid kinase critical for the recycling dynamics of endosomes and lysosomes. We hypothesized that PIKfyve inhibition could be a more potent anti-autophagy therapy in PDAC. Accordingly, inhibition of PIKfyve with apilimod (PIKfyvei) resulted in an accumulation of the canonical autophagy markers p62 and LC3B-II, suggestive of autophagy inhibition. Additionally, we observed a build-up of large intracellular vacuoles staining positive for LAMP1, a marker of lysosomes and late endosomes. Since autophagy is a lysosomal-mediated process, we sought to determine if these enlarged lysosomes were still functional. Indeed, PIKfyve inhibitor treatment significantly reduced the acidity of lysosomes. When we inhibited MEK (with mirdametinib (MEKi)) and PIKfyve concomitantly, we observed a decrease in MEKi-induced autophagic flux. This suggested that combined PIKfyve and MEK inhibition could be an efficacious strategy for PDAC treatment. Accordingly, dual MEKi and PIKfyvei showed substantial synergy across a panel of PDAC cell lines, due in part to a significant induction of apoptosis following combination treatment. This synergistic relationship was maintained in patient derived PDAC organoids. Taken together, these findings suggest dual MEK and PIKfyve inhibition is a potentially efficacious therapeutic strategy and warrants further investigation in more advanced models of PDAC.

<u>Poster #32</u>: Breanna Jeffcoat, graduate student
(Dr. Russell Broaddus, Cancer Genetics, and Dr. Andrew Gladden, Cancer Cell Biology)
Title: Patient Derived Organoids as a Model System to Study Endometrial Cancer Pathogenesis
Abstract: Endometrial cancer (EC) affects many women around the world and is a health disparity in the United States with a 21% increase in mortality among African American women. There is a strong need to study the molecular mechanisms surrounding the differences in EC subtypes and the pathogenesis of disease. To aid in our understanding and exploration of this disease, we have developed Patient Derived Organoids (PDO) as a model to study EC. This model system is beneficial to study EC because there are multiple molecular subtypes of EC that have different characteristics and genomic features. PDOs are small, unique, 3-dimensional multicellular tissue culture model systems utilized for studying cancer pathogenesis. PDOs specifically have the ability to capture the characteristics of different subtypes in vitro, can express mutations and characteristics of the tumor of study from the original patient, and display patient specific drug response. The use of organoids as a model system for cancer research is not widespread but is growing in occurrence. We have developed multiple PDO model systems derived from 9 patient-derived xenografts (PDX) from African American and Non-Hispanic Caucasian women with various stages and subtypes of endometrial cancer. After collecting the PDX tumors grown in nude mice, we developed a protocol to digest, isolate and plate droplets of organoids in extracellular matrix. Upon plating, the organoids are incubated in expansion media before performing experiments. This 3D model system has allowed us to observe differences in the basal properties of each sample in vitro. This heterogeneity of the organoids reflects the heterogeneity of the native EC. We have observed differences in morphology with some samples forming round cystic like structures that may be recapitulating glands that are seen in well differentiated EC. Other samples have formed large, solid compacted structures correlating with the samples that came from grades 2 and 3 EC cases, which are known to have decreased glandular structures indicative of altered differentiation, resulting in more

solid architecture microscopically. The different organoid samples reach confluency in culture at different time points, reflecting heterogeneity in cell proliferation. Using immunofluorescence (IF), we have seen inconsistent levels of expression of the hormone receptor progesterone receptor in the samples while the expression of estrogen receptor has been consistently high through multiple samples. In addition, we have also observed inter-organoid variation of IF expression of β-catenin, E-cadherin, and vimentin, proteins playing a pivotal role in cellular contact, signaling, and epithelial-mesenchymal transition (EMT), commonly observed in invasive cancers. The observed organoid phenotypes and genotypes are preserved when comparing to the native EC. The use of this organoid model system is advantageous for studying EC because growth characteristics are similar to an in vivo model, allowing us to observe cellular interactions. Developing this model will allow us to study the impact of external stimuli on molecular mechanisms. A future study of interest is to understand the molecular underpinnings obesity has on EC pathogenesis. Such studies can lead to improved outcomes in African American and all women diagnosed with EC.

Poster #34: Obed Ofori Nyarko, graduate student
(Integrated Physiology Program, University of Colorado School of Medicine Anschutz Medical Campus)
Title: Prevalence of Hypertension in Ghana: Analysis of an Awareness and Screening Campaign in 2019
Abstract: INTRODUCTION: Hypertension is an important public health menace globally and in sub-Saharan Africa. The prevalence of hypertension is on the rise in low- and lower-middle-income countries (LMIC) such as Ghana. This rise led to the adoption of the May Measurement Month (MMM) initiative, a global blood pressure screening campaign. We aimed to create awareness and present the findings of the 2019 MMM screening campaign in the Ashanti region of Ghana. METHODS: Ghana was 1 of 92 countries that participated in this global community-based cross-sectional study in May 2019. Participants (⩾18 years) were recruited by opportunistic sampling. The blood pressures of participants were measured 3 times and the mean of the last 2 was used for the analysis. Summary statistics were used to describe the data. Simple and multiple logistic regression models were used to determine the predictors of hypertension. RESULTS: We screened 3080 participants with a mean age of 39.8 ± 16.8 years. The prevalence of hypertension was 27.3% among participants. Two-thirds of the hypertensives were unaware of their condition and only 49.5% of participants with a history of hypertension on medication were controlled. Predictors of hypertension in a multiple logistic regression were increasing age (OR = 1.05 (CI 1.04-1.06), P < .001) and high body mass index (OR = 1.06 (1.02-1.10), P = .005). CONCLUSION: The MMM initiative is highly commendable and of huge public health importance in LMICs like Ghana. Population-based health programs such as the MMM initiative is encouraged to shape appropriate public health policies to reduce the prevalence of hypertension.

Poster #36: Ryan Robb, graduate student
(Dr. Kirsten Bryant, Molecular Therapeutics)
Title: Interplay and compensation between autophagy and macropinocytosis in ERK MAPK inhibited pancreatic cancer
Abstract: Alteration of essential metabolic pathways is a major mechanism by which oncogenic KRAS promotes tumor development and growth in pancreatic ductal adenocarcinoma (PDAC). KRAS-driven PDAC is dependent on nutrient scavenging pathways, including macropinocytosis and autophagy to fuel the high metabolic demand of rapid proliferation. Thus, these metabolic processes are attractive targets for the development of treatments for PDAC. KRAS loss results in downregulation of macropinocytosis in PDAC. Additionally, our lab demonstrated that KRAS loss or inhibition of ERK MAPK signaling decreased glucose consumption and glycolysis but increased autophagy, thereby enhancing dependency on autophagy for survival and growth. Accordingly, dual ERK MAPK and autophagy inhibition (via chloroquine) synergistically enhanced anti-tumor efficacy in PDAC. Early clinical data has demonstrated

that resistance to this treatment arises over time through unknown mechanisms. My preliminary data indicates that following ERK MAPK inhibition both autophagy induction and macropinocytosis downregulation is transient—with autophagic and macropinocytic activity returning to/surpassing basal levels after prolonged treatment. The underlying mechanistic and signaling crosstalk between autophagy and macropinocytosis remains poorly understood. We hypothesize that there is compensatory regulation between autophagy and macropinocytosis signaling following ERK MAPK inhibition. We propose that prolonged activation of autophagy upregulates macropinocytosis over time, consequently abrogating dependency on autophagy and therefore reducing sensitivity to autophagy inhibition. We demonstrate an inverse temporal relationship between autophagic flux and macropinocytosis following genetic loss of KRAS or pharmacological inhibition of the KRAS-ERK MAPK pathway. Furthermore, we show that chloroquine, which is commonly thought of as a lysosomotropic drug, does not prevent degradation of proteins engulfed via macropinocytosis. Together our data suggest that upregulation of macropinocytosis may mediate resistance to the combination of ERK MAPK inhibition and chloroquine. A better understanding of the signaling underlying these metabolic resistance pathways will inform future ERK MAPK inhibitor combinations.

Poster #38: Karthik Adapa, graduate student
(Dr. Lukasz Mazur, Radiation Oncology)
Title: Developing an evidence-based implementation framework for implementing quality assurance checklists in radiation oncology
Abstract: BACKGROUND: Digital health technologies such as quality assurance (QA) checklists could improve patient safety in radiation oncology. However, there is limited evidence and guidelines for the successful implementation of QA checklists in radiation oncology clinical settings. To the best of our knowledge, no previous study has examined the determinants, and strategies for implementing QA checklists in radiation oncology. OBJECTIVE: The study aims to investigate the barriers and facilitators for implementing QA checklist, examine the strategies for implementing QA checklists and propose an implementation framework for implementing QA checklists in radiation oncology clinic METHODS: This study was conducted from January 2022 to August 2022 during pre-implementation, implementation, and post implementation of a dosimetry QA checklist (DQC) in a radiation oncology clinic of a large US academic cancer center. Following an abductive research approach with both inductive and deductive analysis, we used the Consolidated Framework for Implementation Research and the Expert Recommendations for Implementing Change to analyze the transcripts of 12 semi-structured interviews with dosimetrists (n=5) and physicists (n=5) and descriptive questionnaire data. The results of the qualitative analysis and the findings from the daily safety huddles, team meetings, field observations, and informal meetings, provided the basis for the development of the proposed implementation framework. RESULTS: The inductive analysis of the interview transcripts revealed two broad categories – the implementation process of DQC and the suggestions from participants for improving enhanced DQC's implementation in the clinic. Three themes emerged regarding the implementation process of enhanced DQC–QA checklist features, IT infrastructure and user training, and engaging users. The suggestions provided by interviewees were organized into two themes- involvement of users, user training, and support. The deductive coding revealed 4 CFIR constructs and 12 sub-constructs as barriers and 5 CFIR constructs, and 19 sub-constructs as facilitators. The suggestions for improving enhanced DQC's implementation were mapped on 7 clusters and 19 ERIC strategies. The proposed implementation framework includes 14 evidence-based strategies belonging to 4 clusters from the CFIR-ERIC matching tool and organized temporally in an implementation lifecycle. CONCLUSIONS: Implementing digital health technologies such as QA checklists in radiation oncology clinical settings should involve a comprehensive pre-implementation assessment and a continuous evaluation of the implementation conditions. Our proposed framework may guide healthcare system leaders, human factors engineers

and implementation science researchers with concrete, evidence-based, and step-by-step recommendations for implementing QA checklists in radiation oncology clinical settings.

Poster #40: Jiona Mills, graduate student
(Dr. Shakira Grant, Clinical Research)
Title: "If [multiple myeloma] is just for Black people, they don't care to study it, maybe that's why it is no cure": Dyadic perspectives on the legacy of Tuskegee and trust in medical care for multiple myeloma.
Abstract: BACKGROUND: Older Black adults are disproportionately burdened by multiple myeloma (MM) yet continue to face significant challenges in accessing high-quality cancer care, including opportunities to engage in research. Knowledge of experiments such as the Tuskegee Study, where Black persons experienced deliberate harm from research, has created mistrust of the healthcare system, especially in the Black community. We sought to examine racial differences in dyadic (patient-informal caregiver) knowledge of the Tuskegee study and understand their perceptions of care received for MM. METHODS: We conducted 21 in-depth semi-structured interviews with racially concordant patient-informal caregiver dyads living in North Carolina. Dyads were asked open-ended questions about the Tuskegee Study, mistrust, and their healthcare experiences. We used the Sort and Sift, Think and Shift approach for qualitative data analysis. RESULTS: Between November 2021 and April 2022, we enrolled 44 participants [(mean age, patients: 70 years (range=57-90), caregivers: 68 years (range=37-88)], and interviewed 42 (11 Black and 10 White dyads). Fourteen (67%) dyads (6 White, 8 Black) reported knowledge of the Tuskegee Study. We identified Black-White differences in how this knowledge influenced perceptions about the care received for MM, including provider and healthcare system interactions, where Black dyads reported mistrust because of this knowledge ("if [MM] is just for Black people, they don't care to study it, maybe that's why it is no cure"). Conversely, most White dyads reported no impact of this knowledge on their current level of trust in the healthcare system and expressed their discomfort with discussing the Tuskegee study and other events that led to the deliberate harm of Black persons. Black dyads emphasized the persistent nature of racial injustice in the healthcare system, creating a shared consciousness within the Black community that "Black patients don't get the attention... the care, that [their] counterpart does." Black dyads stressed the need for self-advocacy when interacting with providers and proactively sought to gain knowledge about their disease. Black and White dyads highlighted the importance of having a caregiver as an advocate, but Black dyads perceived caregiver presence as a potential mitigator of discrimination ("seeing a husband [and] wife, together. I think that makes a difference"). CONCLUSION: Black dyads often expressed knowledge of the Tuskegee Study, the related legacy of mistrust in the healthcare system, the need for self-advocacy, and knowledge of the disease when interacting with providers. These factors, including transparent communication with providers and acknowledgment of drivers of mistrust, are critical for enhancing the care experiences of older dyads affected by MM.

Poster #42: Bethany Ogbenna, graduate student
(Dr. Cher Dallal, Epidemiology and Biostatistics, University of Maryland School of Public Health)
Title: Association between a healthy lifestyle index and breast cancer risk among post-menopausal women in the Multiethnic Cohort (MEC) Study
Abstract: Consistent evidence supports maintaining a healthy weight, engaging in physical activity, and limiting alcohol consumption as potential strategies for reducing breast cancer risk. Sleep duration and sedentary behavior are also emerging breast cancer risk factors. While these cardiometabolic factors have been associated with an increased risk of breast cancer, prior studies have primarily investigated these factors separately. The association between a healthy lifestyle index (HLI) and breast cancer risk, particularly across racially and ethnically diverse postmenopausal women, has not been well studied. Within the Multiethnic Cohort study, ongoing analyses are examining associations between an HLI and

risk of invasive breast cancer among 65,561 eligible postmenopausal women (African American [18.7 %], Native Hawaiian [6.7 %], Japanese American [28.2%], Latina [21.1%], and White [25.3%]). During a median follow-up of 23 years, 4,555 incident invasive breast cancer cases were diagnosed between baseline (1993-1996) and the end of follow-up (2017). Self-reported baseline information on seven lifestyle factors was used to create an HLI based on the sum of scores (max HLI score=23): Healthy Eating Index 2010 (HEI 2010) score divided into quartiles (scored 1-4), metabolic equivalents (METs) of moderate and vigorous physical activity (1-3), time spent sedentary (1-3), smoking status and duration (1-5), alcohol consumption (1-3), body mass index (BMI) (1-3) and sleep duration (1-2). Preliminary analyses included descriptive statistics as well as age- and race and ethnicity-adjusted Cox proportional hazards models of invasive breast cancer to estimate hazard ratios (HR) and 95% confidence intervals (CIs) for the association with HLI score (tertiles, (T)) using age as the timescale. The mean HLI score in the overall population was 15.3 [Standard Deviation (SD): 2.1] with 51.4% of the analytic population in an HLI score tertile of ≥18 to 23 (T3; most healthy) and 13.2% in the lowest HLI score tertile ≥7 to <15 (T1; least healthy). The mean HLI scores by race/ethnicity were the following: African American 16.5 [SD: 2.5], Native Hawaiian 16.7 [SD: 2.6], Japanese American 18.2 [SD: 2.2], Latina 17.3 [SD: 2.2] and White 17.2 [SD: 2.6] women. Preliminary findings suggest that overall, women in HLI score T3 had on average, a BMI of 23.6 [3.7] (mean [SD] kg/m2), dietary intake HEI 2010 scores between 60-78.9, exerted > 6 METs of moderate and strenuous activity, spent < 6 hours a day sedentary, consumed less than 4.9 grams of ethanol per day, slept between 7 to 8 hours and were never smokers. In multivariable adjusted analyses, postmenopausal women with an HLI score in T2 (HR: 0.85 [95% CI:0.78-0.93]) and in T3 (HR: 0.75 [95% CI: 0.69–0.82]) (p-trend <0.001) were at reduced risk of breast cancer compared to women in T1. Ongoing analyses include race and ethnicity stratified multivariable Cox proportional hazards regression.