Session I, 9:15-11:15 AM

10:15-10:30 AM: Joseph Szulczewski (Hahn lab, Postdoctoral Fellow)
Title: Development of Novel Tools to Probe the Role of Durotaxis in Cancer Cell Metastasis
Abstract: Collagen alignment strongly affects the invasiveness and metastasis of breast cancer. Recent work has shown that aligned collagen fibers facilitate cancer cell invasion by polarizing cells along the axis of alignment and restricting off-axis protrusion formation. Currently, it remains unclear exactly how collagen organization affects cancer cell protrusion formation and dynamics. Recent studies have shown that aligned collagen fibers are stiffer along the axis of collagen alignment, and that cells respond to cell-scale stiffness gradients in the aligned collagen. Cells sense stiffness differences using components of cell-extracellular matrix contact points known as focal adhesions (FA) through a process known as durotaxis. Vinculin, known as the cells mechanical clutch, is a well characterized FA protein responsible for translating force from the cell to the extracellular matrix. We hypothesize that collagen alignment affects the forces felt by individual focal adhesions, which in turn locally impact production of nearby protrusions. In order to test this hypothesis, we have developed novel methods to optogenetically modulate vinculin activity through insertion of a LOV domain in the vinculin tail region. Additionally, we engineered a sensitive new biosensor approach involving the strategic insertion of known SsrA tag that co-localizes with SspB protein only upon the the stretch-induced exposure of binding sites in p130Cas and vinculin. Using these tools, we are able to study how local tension acts through focal adhesions to generate different protrusion behaviors depending on the localization of mechanical gradients in the extracellular matrix, thus shedding light on the mechanism of durotaxis in cancer cell metastasis.

10:30-10:45 AM: Jordan Koehn (Weeks lab, Postdoctoral Fellow)
Title: Transcriptome-wide discovery of RNA-ligand target sites
Abstract: Currently, only 0.05% of the human genome has been drugged with fewer than 700 proteins targeted. Similar to proteins, certain RNA molecules can fold back on themselves to form complex structures that can contain pockets or clefts with sufficient structural sophistication to allow specific and high-affinity binding by small molecule ligands. Small molecule binding to messenger RNAs can modulate protein gene products by upregulating or downregulating protein translation efficiency or by altering mRNA abundance or stability. Recent studies have revealed that diverse motifs within a transcriptome can be highly structured and may represent a substantial untapped opportunity for discovering therapeutically useful RNA-ligand interactions. Therefore, a robust method for discovering ligands and mapping RNA-ligand interactions across the transcriptome would have high therapeutic value, especially in cancer biology. Our goal is to develop a technology for transcriptome-wide identification of RNA-ligand binding sites and discovery of novel small molecule ligands that bind RNA with high-affinity and selectivity. Our strategy will employ an innovative fragment-based ligand discovery approach coupled to a concise and direct read out of RNA-ligand binding sites by high-throughput sequencing. Once validated, we hope our technology will enable rapid identification of therapeutically relevant RNA motifs with high-information-content structures able to bind function-modulating small molecule ligands.

Session II, 11:15 AM-12:00 PM

11:15-11:30 AM: Katherine Barnett (Ting lab, Postdoctoral Fellow)
Title: An epithelial-immune circuit amplifies IL-1β and IL-6 release in COVID-19
Abstract: As new SARS-CoV-2 variants emerge, the COVID-19 pandemic remains a global burden, causing economic disruption, morbidity, and mortality. Certain patient groups are at high risk for severe COVID-19, including patients with hematologic cancers or patients receiving immune checkpoint inhibitor therapy. Elevated IL-1β and IL-6 are hallmarks of severe COVID-19 and are thought to facilitate fatal cytokine storm. Here, we investigate how SARS-CoV-2 infection promotes secretion of these cytokines and if this process is enhanced in severe disease. Primary human airway epithelial cells (HAE) support SARS-CoV-2 replication and have multiple functional inflammasomes, but the inflammasome is inactive in infected HAE. In leukocytes, SARS-CoV-2 does not replicate yet upregulates inflammasome gene transcription, and IL-1β secretion in leukocytes requires a second signal provided by the infected epithelium. COVID-19 autopsy lungs show active inflammasomes in myeloid cells lacking SARS-CoV-2 antigens. Patient single cell RNA sequencing (scRNAseq) data reveal that priming of multiple inflammasomes is heightened in severe COVID-19 as compared to mild/moderate disease. Once IL-1β is produced, it stimulates robust IL-6 secretion from HAE, promoting a burst of pro-inflammatory cytokine production. 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 pro-inflammatory cytokine circuit that is exacerbated in severe COVID-19.

11:30-11:45 AM: Minguk Jo (Gupta lab, Postdoctoral Fellow)
Title: Mre11 dependent cGAS-STING cytosolic DNA sensing pathway suppresses breast tumorigenesis
Abstract: We conducted an in vivo CRISPR screen to identify DDR gene mutations that accelerate tumorigenesis in a murine model of triple-negative breast cancer driven by Myc oncogene overexpression and p53 deficiency. The most significantly enriched tumor suppressor gene identified was Mre11, disruption of which was associated with reduced latency of breast tumorigenesis and higher rates of tumor cell proliferation. Time-lapse imaging of primary Myc-overexpressing and p53-deficient mammary epithelial cells revealed a post-mitotic cell cycle arrest phenotype that was associated with cGAS positive micronuclei, which is deficient in Mre11 mutant cells. Unexpectedly, Mre11 is also required for cytoplasmic DNA sensing of cGAS in both mouse and human cells. Time-lapse imaging reveals initial recruitment of Mre11 to cytoplasmic DNA that is followed by cGAS foci formation, suggesting a direct role as a positive regulator of cGAS activation. And Single-cell analyses reveal a Mre11/cGAS transcriptional signature whose loss is associated with genome instability and poor prognosis in breast cancer patients. Thus, Mre11-mediated cGAS-STING pathway activation is a critical genome integrity checkpoint that is commonly bypassed in genomically unstable triple-negative breast cancers, with possible implications for immunotherapy susceptibility.

11:45 AM-12:00 PM: Joseph Kearney (Yeh lab, Postdoctoral Fellow)
Title: Cancer-Associated Fibroblast Heterogeneity Influence on Tumor Cells
Abstract: Background: The complex interaction of pancreatic cancer (PDAC) cells and their interplay with the cancer-associated fibroblast (CAF) subpopulations in the stroma is poorly understood. Translational work and failed clinical trials show that bluntly targeting CAFs produces poor patient outcomes. In-vitro characterization of CAFs based on their phenotypic effects on PDAC cell lines may be a better metric for subsequent pharmacologic targets in

development. We sought to characterize the in-vitro baseline behavior of two CAF lines, one myCAF and another inflammatory CAF (iCAF) in the context of CAF signals using conditioned media (CM) assays. Method: CAF subtypes were determined using RNA Sequencing. CM was acquired from p70216 (myCAF) and p40227 (iCAF) CAFs and mixed with FBS for a final concentration of 5% FBS. A scratch assay was performed on PDAC line p30411 evaluating effects of the conditioned media on cell migration. Results: We evaluated 16 scratches over four days: Four control DMEM:F12, Six p70216CM, Six p40227CM. Scratches were measured and the cells that migrated into the original scratch area were counted daily. Scratch areas between the groups were not significantly different on ANOVA analysis ($p = 0.55$). A multiple linear regression was done to evaluate cell count migration rates between each CM group, showing decreased migration among PDAC cells when exposed to p70216CM ($p = 0.03$). Conclusions: Baseline characterization of each CAF line's effect on PDAC lines reveals different behavior between lines, possibly representative of distinct CAF subpopulations. Using PDAC-based phenotypes as a baseline metric for evaluating CAF behavior may lead to more rigorous translational findings.

Session III, 12:45-2:45 PM

1:45-2:00 PM: Maria White (Damania lab, Postdoctoral Fellow)
Title: Inhibition of NEK2 as a novel therapy for primary effusion lymphoma
Abstract: Kaposi's sarcoma-associated herpesvirus is an oncogenic human virus responsible for several cancers. One of these malignancies, primary effusion lymphoma (PEL), is a form of non-Hodgkin lymphoma (NHL) with a poor survival prognosis and no cure. Previous work in the Damania lab identified the cellular protein NIMA Related Kinase 2 (NEK2) as a kinase that was upregulated in PEL (as well as other NHL) relative to healthy primary lymphocytes. Studies in other cancers have shown that NEK2 overexpression is associated with chromosome instability, tumorigenesis, metastasis, and resistance to chemotherapy. Thus, I hypothesized that NEK2 overexpression in PEL contributes to disease severity and that NEK2 could represent a novel therapeutic target in PEL. My data show that knockdown of NEK2, as well as small molecule inhibition of NEK2, lead to PEL cell death via caspase-3-mediated apoptosis. Additionally, activation of the downstream NEK2 effector proteins β-catenin and MDR1 is decreased upon NEK2 inhibition and, similarly, expression of the pro-survival proteins Bcl-2 and Bcl-xL is suppressed post-NEK2 inhibition. Finally, treatment of PEL-bearing mice with a NEK2 inhibitor significantly decreased tumor burden and prolonged survival with no apparent toxicity. Overall, these data suggest that targeting NEK2 represents a promising therapeutic candidate in PEL and offers an additional therapeutic approach for this rare but aggressive cancer.

2:00-2:15 PM: Rahul Mirlekar (Pylayeva-Gupta lab, Postdoctoral Fellow)
Title: Reprogramming of naïve B cells in pancreatic cancer subverts humoral immunity
Abstract: B cells frequently infiltrate human tumors, and the intra-tumoral abundance of plasma cells can correlate with improved patient prognosis. However, many tumors are devoid of plasma B cells, and strategies to enhance anti-tumor B cell responses are needed. We report the existence of a negative regulatory signaling network that reprograms naïve B cells in pancreatic cancer to antagonize anti-tumor plasma B cells. This network is driven by IL-35-mediated STAT3 activation, which directly stimulates upregulation of the pioneer transcription factors Pax5 and Bcl6 in naïve B cells and impedes plasma cell differentiation while simultaneously activating regulatory B cell phenotypes. Significantly, inhibition of Bcl6 reversed this tumor-associated reprogramming of naïve B cells, enabling intra-tumoral accumulation of plasma cells, and reduced tumor growth. Our data provide evidence that B cell dysfunction in

cancer involves a potentially targetable suppression program that alters the differentiation potential of naïve B cells.

Session IV, 2:45-3:15 PM

2:45-3:00 PM: Travis Nelson (Hathaway lab, Postdoctoral Fellow)
Title: Modulating TP53 expression through small molecule epigenetic modifiers
Abstract: Epigenetic pathway malfunction is a central mechanism of pathogenesis in a broad spectrum of diseases, including a significant number of human cancers. Drugs that offer epigenetic regulation of oncogene suppression and tumor suppressor gene promotion are attractive targets for development. However, these drugs often act in a broad-target manner, leading to unintended off-target effects and limited on-target effectiveness. The high specificity of CRISPR/CAS9 makes it an attractive tool to target dysregulated genes but current art requires the addition of large, exogenous regulatory proteins to deliver their intended effects. Recent work in our lab has shown an improved, dose-dependent control system to control gene expression that recruits endogenous chromatin regulatory proteins instead, using a catalytically dead CAS9 (dCAS) fused to with the FK506 binding protein (FKBP) and small molecule chemical epigenetic modifiers (CEM) to direct the endogenous machinery to the target gene. With the limitations of prior art and the advantages of our dCAS-FKBP-CEM system in mind, we present preliminary work in attempting to apply it in the context of modulating expression of the cancer-controlling protein p53, which plays a key role in a complex signaling pathway that is associated with the maintenance of cellular homeostasis, response to cellular stresses, and tumor suppression. Utilizing the dCAS-FKBP-CEM system's site-specific targeting ability to recruit desired epigenetic enzyme machinery to the TP53 gene in a dose-dependent manner, we anticipate being able to therapeutically mitigate dysregulation at the root, opening new options for genetically directed treatments of cancer in phenotypes with unmutated-yet-silenced p53.

3:00-3:15 PM: Xinjun Wu (Damania lab, Postdoctoral Fellow)
Title: Kaposi's sarcoma-associated herpesvirus (KSHV) Kinase Promotes Endothelial Cell Survival By Activating Akt Signaling
Abstract: Viruses have developed a variety of strategies to antagonize cell death and establish lifelong persistence in their host, a relationship that may contribute to the development of cancer. Understanding the mechanism by which viruses inhibit cell death is essential for understanding viral biology and pathogenesis. Kaposi's sarcoma-associated herpesvirus (KSHV) is associated with three different cancers in the human population including Kaposi's sarcoma (KS), primary effusion lymphoma (PEL), and multicentric Castleman's disease (MCD). Here, we report that a viral kinase (vPK) from KSHV protects cells from apoptosis mediated by caspase-3. Human umbilical vein endothelial cells (HUVEC) expressing vPK (HUVEC-vPK) have a survival advantage over control HUVEC under conditions of serum starvation and drug treatment. We found that KSHV vPK expressing endothelial cells exhibit increased phosphorylation and activation of cellular Akt kinase, a cell survival kinase, compared to control cells without vPK. In addition, we report that vPK directly binds the pleckstrin homology (PH) domain of Akt. Moreover, treatment of HUVEC-vPK cells with a pan-Akt inhibitor Miransertib (ARQ 092) reduced the overall phosphorylation of Akt, but vPK-expressing cells still exhibited higher phosphorylation of Akt compared to control cells at lower concentration of the inhibitor. At higher concentrations of Miransertib, the levels of phosphorylated Akt were similar in control and vPK expressing cells resulting in activation of caspase-3 and apoptosis. Collectively, these data

suggest that vPK augments cell survival through the activation of Akt signaling and further underscores targeting this pathway for the treatment of KSHV-associated cancers.

3:30-4:15 PM: Poster Session I, ITCMS GatherTown Poster Session Room

Poster #1: Tamara Vital (Davis lab, Graduate Student)
Title: Characterizing a small molecule inhibitor of chromatin accessibility in Ewing sarcoma
Abstract: Cancer genotyping projects have identified frequent mutations in epigenetic regulators, however, determining their functional consequences is often confounded by coincident mutations. Ewing sarcoma, a pediatric tumor, harbors a low mutational burden and is characterized by the critical translocation-associated fusion oncoprotein, EWS-FLI. EWS-FLI is re-targeted to genetic loci where it alters chromatin accessibility via unknown mechanisms. As such, Ewing sarcoma provides a model to interrogate mechanisms underlying chromatin dysregulation in tumorigenesis. We performed a high-throughput, chromatin-directed screen to discover small molecules capable of reversing EWS-FLI-mediated chromatin accessibility and identified UNC0621, a molecule with uncharacterized mechanism of action. Proteomic studies to identify the targets of UNC0621 indicated that UNC0621 associates with RNA binding and splicing proteins and chromatin. Surprisingly, interactions with chromatin and many RNA-binding proteins, including known interactors of the EWS-FLI fusion protein, were RNA-independent. In fact, UNC0621 enriches for EWS-FLI in an RNA-independent manner. Additionally, UNC0621 decreased cellular proliferation in Ewing sarcoma cell lines by inducing a G1/S arrest. Our findings suggest that UNC0621 reverses EWS-FLI-mediated chromatin accessibility by interacting with and altering the activity of RNA splicing machinery and chromatin modulating factors to ultimately inhibit Ewing sarcoma cell proliferation. This use of an oncogene-associated chromatin signature as a target represents a novel strategy to directly screen for modulators of epigenetic machinery and provides a framework for using chromatin-based assays in future drug discovery efforts.

Poster #2: Shelsa Marcel (Davis lab, Graduate Student)
Title: Genome wide cancer-specific chromatin accessibility patterns derived from archival processed xenograft tumors
Abstract: Chromatin accessibility states that influence gene expression and other nuclear processes can be altered in disease. The constellation of transcription factors and chromatin regulatory complexes in cells results in characteristic patterns of chromatin accessibility. The study of these patterns in tissues has been limited since existing chromatin accessibility assays are ineffective for archival formalin-fixed, paraffin embedded (FFPE) tissues. We have developed a method to efficiently extract intact chromatin from archival tissue via enhanced cavitation with a nanodroplet reagent consisting of a lipid shell with a liquid perfluorocarbon core. Inclusion of nanodroplets during the extraction of chromatin from FFPE tissues enhances the recovery of intact accessible and nucleosome-bound chromatin. We demonstrate that the addition of nanodroplets to the chromatin accessibility assay FAIRE (formaldehyde-assisted isolation of regulatory elements), does not affect the accessible chromatin signal. Applying the technique to FFPE human tumor xenografts, we identified tumor-relevant regions of accessible chromatin shared with those identified in primary tumors. Further, we deconvoluted non-tumor signal to identify cellular components of the tumor microenvironment. Incorporation of this method of enhanced cavitation into FAIRE offers the potential for extending chromatin accessibility to clinical diagnosis and personalized medicine, while also enabling the exploration of gene regulatory mechanisms.

Poster #3: Brigid Grabert (Gilkey lab, Postdoctoral Fellow)
Title: Improving HPV Immunization Practices: A process improvement project assessing a nursing education intervention to increase HPV vaccine uptake among active duty soldiers at Fort Bragg, NC
Abstract: Background: Human papillomavirus (HPV) is the most common sexually transmitted infection in the military. Vaccination against HPV is not required in the military, and rates of vaccination are low among active duty soldiers, with only 26% of women and 6% of men initiating the multi-dose series. Thus, we conducted a quality improvement project to recommend and administer HPV vaccine to soldiers at Fort Bragg, NC. Methods: In March 2021, we conducted a one-hour training about HPV vaccination for nursing staff (n=11) at the Medical One Stop, a medical processing facility for soldiers. Training included using presumptive recommendations to present HPV vaccine as the default choice in routine care. After the training, nursing staff integrated HPV vaccination into clinic procedures, and we tracked uptake among soldiers for 4 weeks. We used multivariable logistic regression to assess correlates of uptake of the HPV vaccine. Results: 611 soldiers presented for medical processing during the intervention period. Of these, 521 (85%) were eligible to receive and were offered HPV vaccine. Of the soldiers who were offered HPV vaccine, 203 (39%) received the vaccine. Soldiers <21 years accepted HPV vaccine more often than those ≥21 years (46% versus 33%, $p<0.01$). Conclusions: Our findings suggest training nursing staff to recommend and administer HPV vaccines to soldiers is feasible and may warrant wider-scale testing as a strategy to increase military readiness and protect soldiers from HPV-attributable cancers. Until guideline changes are implemented, use of education strategies across all provider levels is one path to increasing HPV vaccine coverage among soldiers to ensure protection from HPV-related diseases.

Poster #4: Cole Edwards (Der lab, Graduate Student)
Title: YAP1-TAZ-TEAD transcriptional activity modulates KRASG12C-mutant cancer responses to KRASG12C inhibition
Abstract: Activated mutants of KRAS comprise the major oncogenic drivers in lung (LAC), colorectal (CRC), and pancreatic ductal adenocarcinoma (PDAC). Recent success in covalently targeting one KRAS mutant (KRAS G12C) has produced the first approved anti-KRAS therapy (sotorasib) for LAC. Whereas an overall objective response rate (ORR) of ~40% has been reported for LAC, CRC has shown less response and the response for PDAC remains to be established. In addition to de novo resistance, treatment-induced acquired resistance to G12Ci treatment has been observed in LAC and CRC. While ERK-MAPK reactivation is associated with a subset of acquired resistance mechanisms, the mechanisms of resistance in ~50% of patients remains to be determined. One candidate mechanism of resistance involves the transcriptional co-activator paralogs, YAP1 and TAZ. To address whether YAP1 activation can bypass sensitivity to G12Ci, we ectopically overexpressed doxycycline-inducible, constitutively active YAP1 (YAP1 S127A) in a panel of KRAS G12C-mutant LAC, CRC, and PDAC cell lines. In all cell lines tested, overexpression of YAP1 S127A substantially diminished G12Ci cytostatic and cytotoxic activity. In contrast, genetic suppression of YAP1/TAZ caused a 4-to-28-fold enhancement of G12Ci efficacy. Since potent and selective YAP1/TAZ inhibitors remain to be developed, we evaluated the activity of pharmacological inhibitors of the canonical YAP1/TAZ transcriptional partners, the TEAD family transcription factors (TEADi). We found that TEADi phenocopied YAP1/TAZ knockdown and sensitized cells to G12Ci, supporting the enhanced anti-tumor efficacy of TEADi in combination with G12Ci.

Poster #5: Alyssa Peace (Hayes lab, Postdoctoral Fellow)
Title: Establishing an Orthotopic Xenograft Murine Model for Desmoplastic Small Round Cell Tumor
Abstract: Introduction: Desmoplastic Small Round Cell Tumor (DSRCT) is a rare and aggressive pediatric sarcoma with a patient population that is 85-90% male. Patients develop sarcomatosis with notable tumor implants in proximity to the seminal vesicles. The purpose of this study was to establish an orthotopic xenograft model based on a hypothesis that the organ of origin of DSRCT is androgen-based. Methods: Four groups of male, NOD/SCID/gamma mice were injected targeting the seminal vesicles with luciferase-transfected JN-DSRCT-1 tumor cells. Groups 1 and 2 received bilateral intraperitoneal (IP) injections of $1x10^6$ cells and $2.5x10^6$ cells, respectively, with one mouse in each group receiving a single IP injection of $5x10^6$ cells. Groups 3 and 4 received single IP injection of $5x10^6$ cells. Tumor growth was measured via bioluminescence using the Ami HT Optical Imaging System (Spectral Instruments Imaging) weekly for 5-8 weeks and necropsies were performed to assess tumor burden. Results: After 5-8 weeks, the single mice from Groups 1 and 2 that received IP injections of $5x10^6$ cells demonstrated a significant increase in tumor growth compared to the mice that received bilateral injections. Group 3 mice that received single IP injection of $5x10^6$ cells had variable tumor uptake, whereas all the young 28-day old mice that underwent single IP injection of $5x10^6$ cells had consistent, exponential tumor growth when analyzed with bioluminescence imaging. Necropsies of the Group 4 mice revealed sarcomatosis consistent with what is seen in patients with DSRCT. Conclusion: Targeting the seminal vesicles of pediatric mice reliably produced sarcomatosis in an orthotopic xenograft model of DSRCT and can be used in future treatment studies.

Poster #6: Caroline Fraser (Davis lab, Graduate Student)
Title: Investigating the connection between EWS-FLI1 and PAX7 in transcription and oncogenesis in Ewing sarcoma
Abstract: Recent sequencing studies have shown mutations in chromatin regulating factors to be primary drivers of a wide array of human cancers, highlighting the role of chromatin dysregulation in oncogenesis. One such cancer is Ewing sarcoma, an aggressive pediatric cancer, characterized by a chromosomal translocation resulting in the fusion of the FLI1 and EWSR1 genes. The resulting chimeric oncoprotein, EWS-FLI1, maintains the transcription factor activities of the parental FLI1 protein but is retargeted to distinct loci consisting of GGAA microsatellite repeats and functions at these sites to alter the chromatin landscape, creating *de novo* enhancer elements. As EWS-FLI1 does not have intrinsic chromatin remodeling functions, EWS-FLI1 binding and subsequent oncogenesis requires the recruitment of other chromatin modulators. We utilized a proximity-labeling approach to identify proteins interacting with EWS-FLI1 in its native chromatin environment. A protein identified was the transcription factor PAX7. PAX7 localizes to GGAA microsatellite repeat elements and the PAX7 gene is itself an EWS-FLI1 target gene, resulting in uniquely high and ubiquitous PAX7 expression in Ewing sarcoma tumors and cell lines. Furthermore, knockdown of PAX7 expression results in a loss of proliferation of Ewing sarcoma cells, indicating PAX7 is required for proper cellular functions. Studies have shown that PAX7 is capable of inducing chromatin accessibility, suggesting PAX7 could be playing a similar role at GGAA microsatellite repeats in Ewing sarcoma. Characterizing the role of PAX7 in Ewing sarcoma will provide insight into the mechanisms by which EWS-FLI1 drives chromatin dysregulation and identify potential Ewing sarcoma therapeutic targets.

Poster #7: Kevin Field (Davis lab, Graduate Student)
Title: Developing a Screen for CAR-T 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, which typically have a low mutation burden and therefore intact antigen genes, 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 expression of disialoganglioside GD2, the target antigen of CAR-T treatment of neuroblastoma. Pharmacologic inhibition of EZH2, the catalytic component of the PRC2 chromatin modulating complex, induces GD2 expression in previously GD2-negative cell lines. However, it remains to be seen whether other epigenome-modifying small molecules can induce the same effect, or whether these small molecules can induce GD2 expression in cell lines non-responsive to EZH2 inhibition. This work establishes the conditions for a medium-throughput, flow cytometry-based screen for cell viability and GD2 expression. This method allows us to probe a panel of Ewing sarcoma and osteosarcoma cell lines using small molecule libraries directed at known epigenetic targets, such as the UNC EpiG Diamond set, with the aim of identifying novel epigenetic regulators of GD2 expression for rapid translation to the clinic.

Poster #8: Danielle File (Perou lab, Postdoctoral Fellow)
Title: Genomic features of metastatic breast cancers with intrinsic treatment resistance
Abstract: Background: Metastatic breast cancer (MBC) is incurable with currently available therapeutics however survival beyond metastatic diagnosis is highly variable, ranging from months to years. Clinical features provide some prognostic information however there remains a great need to further the understanding of genomic features of primary treatment-refractory tumors. Methods: Using an institutional database of 2,000 patients treated for MBC over the past ten years, we identified patients with de novo MBC who had an untreated breast tumor and synchronous untreated metastasis available and performed whole transcriptome sequencing. We then stratified patients based on survival beyond metastatic diagnosis and performed supervised analysis using Significance Analysis of Microarrays with genes and gene signatures on a) the primary breast tumors, b) metastatic tumors, and c) paired primary breast versus metastatic tumors from patients with short and longer survival. Rationale: Analysis of de novo MBC eliminates concurrent detection of therapeutic resistance genes that resulted from treatment in the (neo)adjuvant setting, providing a unique opportunity to detect intrinsic resistance genes and identify novel vulnerabilities within tumors that are refractory to current therapeutic agents. Conclusion: Despite advances in the understanding of therapeutic resistance mechanisms in MBC, little is known about tumors that demonstrate intrinsic resistance to multiple therapies from the time of initial metastatic diagnosis. By comparing samples from patients with treatment-refractory MBC and treatment-sensitive MBC, we aim to provide new insights into the biology underlying the most lethal breast cancers.

Poster #9: Leiah Carey (Campbell lab, Postdoctoral Fellow)
Title: Oncogenic RAS Q61 mutants as novel targets for drug discovery efforts
Abstract: The three RAS genes (HRAS, KRAS and NRAS) encode for GTPases that regulate cellular growth via cycling between inactive and active states. When this regulation is

compromised, aberrant RAS signaling manifests in cellular disease, specifically numerous types of cancer. RAS is the most frequently mutated oncogene in cancer (~30%), with 99% clustered at one of three mutational hotspots (G12, G13 and Q61). It is becoming increasing clear that all RAS mutations are not created equal and their distinctive differences may harbor attractive therapeutic targets. Inhibitors to other oncogenic RAS mutants are needed. Biochemical and biological studies conducted on KRAS and NRAS Q61 mutants have yielded various mutation-specific results, suggesting that biochemical and functional consequences of Q61 mutations are distinct from those of G12/G13 mutants, but intriguingly, also residue- and isoform-specific differences within the Q61 codon. Understanding these differences will have important biological and clinical implications. Molecular dynamic (MD) and NMR studies on oncogenic RAS Q61 mutants suggest a subset adopt distinct structural ensembles that promote accessibility of druggable pockets. We hypothesize that select oncogenic RAS Q61 mutants may adopt conformations that are vulnerable to structure-based drug discovery efforts, and will present preliminary biochemical, NMR, X-ray and MD results for a subset of RAS mutations (KRAS Q61R; NRAS Q61R) that support differences in structure and ligand binding pockets. Our novel combinatory experimental and computational structure-based analysis to identify unique druggable binding pockets of mutated RAS Q61 GTPases may pave new directions for treatment of RAS oncogenic-driven cancers.

Poster #10: Wolfgang Beck (Kim and Vincent labs, Graduate Student)
Title: Effects of liver metastasis on urothelial cancer response to immune checkpoint blockade
Abstract: Urothelial cancer (UC), the cancer with the sixth highest incidence in the US, has >70% 5-year survival but only ~5% survival in the metastatic setting. Liver metastasis predicts particularly poor prognosis and poor response to immune checkpoint blockade (ICB) in UC. Liver metastasis, when adjusted for the effects of other clinical variables and metastasis sites, independently predicts worse ICB response and survival in UC. In UC patients treated with ICB, liver metastases grow faster and respond worse than other metastases, suggesting the local liver tumor microenvironment might reduce ICB efficacy and promote tumor growth. The tumor immune microenvironments of patients with liver metastases differ from those of non-liver metastasis patients, suggesting that both global immunity plays a role in liver metastasis mediated ICB resistance. Future work will seek to characterize the local and global effect of liver metastasis on ICB response in a UC mouse model.

Poster #11: Kevin Chen (Gomez lab, Resident Research Fellow)
Title: Computer Vision Analysis of Specimen Mammography
Abstract: Introduction: In breast conserving surgery, obtaining negative margins is critical to reduce breast cancer recurrence. Intra-operative specimen mammography is one commonly used technique to identify positive margins, but it can be inaccurate. We sought to create an algorithm using machine learning to identify positive margins on specimen mammography. Methods: From 7/2017 to 6/2020, specimen mammograms were collected as part of a surgeon's quality assurance process. After IRB approval, these images were matched with pathology reports and classified as positive or negative margins based on NCCN guidelines. This dataset was split into a 80/20 ratio for training and validation. Transfer learning and data augmentation were used. Results: The dataset included a total of 450 images with 208 positive and 242 negative. 315 images were used for training and 135 were used for testing. The overall accuracy was 63%. For positive margins, the sensitivity was 69%, while the specificity was 66%. The positive predictive value was 60% and the negative predictive value was 66%. Conclusions: Our project developed a prototype algorithm which assesses the margin status of specimen mammograms with modest accuracy. Our accuracy metrics compare favorably with published

literature at 63% vs 53%. We plan to improve the model by creating a more robust training set, evaluating with an independent test set, and developing image segmentation. Optimized version of this algorithm could reduce the rate of positive margins in breast-conserving surgery.

Poster #12: Denis Okumu (Johnson and Perou labs, Postdoctoral Fellow)
Title: Perturbation of the MAPK Pathway in TNBC elicits a dynamic and heterogeneous kinome-wide transcriptional and proteomic response
Abstract: TNBC is characterized by frequent elevation of MAPK signaling or increased copy number of receptor tyrosine kinases (RTKs). Heterogeneity is common in the genomic landscape of TNBC, with no dominant driving oncogenic mutations, and has hindered the development of effective targeted therapies. Inhibitors targeting kinases in the EGFR-MAPK pathway inhibit tumor growth, but resistance rapidly develops due in part to extensive remodeling of the kinome transcriptional and protein landscapes allowing for adaptive bypass of targeted inhibition. We screened a panel of TNBC cell lines against the MEK1/2 inhibitor (trametinib), explored dynamics of EGFR-MAPK pathway activation, and profiled kinome-wide changes using Multiplexed Inhibitor Bead capture coupled with Mass Spectrometry (MIB/MS). MEK1/2 inhibition caused acute loss in ERK1/2 activity that resulted in rapid degradation of c-Myc and dramatic induction of transcriptional responses characterized by differential up-regulation of RTKs. All cell lines exhibited time-dependent reactivation of the EGFR-MAPK signaling pathway, which correlated with degradation of c-MYC and the up-regulation of an overlapping but distinct cohort of RTKs in each cell line suggesting that kinome plasticity is treatment-specific and cell line-specific. Heterogeneity in kinome reprogramming and EGFR-MAPK reactivation dynamics was consistent with differential sensitivity to trametinib observed in our proliferation studies. Thus, understanding kinome remodeling and how it contributes to the activation status of the EGFR-MAPK signaling pathway will help us better elucidate mechanisms of drug resistance and design treatment strategies that make targeted therapies in TNBC more effective and durable.

4:15-5:00 PM: Poster Session II, ITCMS GatherTown Poster Session Room

Poster #13: Evan Dewey (Sekelsky lab, Postdoctoral Fellow)
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 misregulation causes accumulation of “scars” in the form of detrimental mutations within the genome, eventually leading to 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, better understanding mitotic crossover mechanisms is critical to the prevention of cancer and other genetic disease. 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 that is only possible in *Drosophila,* it is possible to now analyze resulting HDR products from CRISPR/Cas9 using Sanger sequencing. I have begun to use this tool to define mitotic crossover mechanisms for the first time in a multicellular organism. Preliminary results point to resolution of unligated double Holliday junctions as the primary mechanism. 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 mitotic crossovers in genome editing.

Poster #14: Priya Hibshman (Der lab, Graduate Student)
Title: Defining the role of MYC in KRAS-driven pancreatic cancer
Abstract: KRAS mutations are found in ~95% of pancreatic ductal adenocarcinoma (PDAC). The recent approval of the KRAS inhibitor sotorasib for KRAS G12C mutant lung cancer supports the importance of targeting KRAS for PDAC treatment. However, since KRAS G12C comprises only 2% of KRAS mutations in PDAC, alternative strategies are needed for the majority of KRAS-mutant PDAC. One promising approach involves inhibition of the key KRAS effector pathway, the RAF-MEK-ERK protein kinase cascade. However, clinical evaluation of ERK inhibitors have been limited by toxicity. One strategy to overcome this limitation involves inhibition of signaling downstream of ERK. I hypothesize that the MYC transcription factor and oncoprotein, both a substrate and gene target of ERK, is the key to effective therapeutic targeting of ERK. To address this possibility, I have taken two approaches. First, I determined that genetic suppression of MYC phenocopies KRAS-ERK suppression. Acute KRAS or MYC suppression caused significant cell enlargement, enhanced actin stress fiber organization, and expression of proteins associated with a mesenchymal to epithelial transition. Additionally, reverse phase protein array (RPPA) pathway activation mapping determined that KRAS or MYC suppression caused significant changes in both shared and distinct signaling networks. Second, RNA-Seq analysis of the KRAS/ERK- versus MYC-dependent transcriptome found significant overlap in metabolic processes including glycolysis, nucleic acid synthesis, and autophagy, but also key differences in steroid biosynthesis and cell membrane/structure dynamics. In summary, my studies support a key role for MYC in facilitating diverse KRAS-driven cellular activities.

Poster #15: Carolyn Turcotte (Sekelsky lab, Graduate Student)
Title: Mechanisms and regulation of meiotic recombination
Abstract: During meiosis, correct placement of crossovers prevents aneuploidy in offspring by ensuring proper segregation of homologous chromosomes. Crossovers are formed via homologous recombination, a process that repairs double-strand breaks (DSBs) using a homologous template. Many more DSBs than crossovers are formed, and crossover position is tightly regulated. Several pathways exist within meiotic recombination that repair DSBs as either crossover or non-crossover products. The pathway used to repair a DSB can be traced via SNPs and indels in heteroduplex DNA, DNA in which each strand is derived from a different parent molecule (e.g. homologous chromosomes). Normally, heteroduplex DNA is eliminated via mismatch repair corrections of SNPs between strands. Our lab has successfully eliminated the mismatch repair systems that mask heteroduplex DNA in *Drosophila melanogaster*, the only metazoan in which this has been achievable thus far, and found heteroduplex structures that conflict with the classical model of recombination, leading to a revised model in which most crossovers and many non-crossovers are derived from a different intermediate. Although we have analyzed heteroduplex DNA at a test locus, the number of recombinants at this locus is low. To continue to test and redefine the model of meiotic recombination, I am developing hetSeq, whole-genome sequencing of heteroduplex DNA. I am also testing relationships between meiotic proteins and crossover number and distribution using mathematical modeling. Together, these developments will further our understanding of crossover regulation and its relationship to meiotic recombination mechanism.

Poster #16: Kristina Drizyte-Miller (Der lab, Postdoctoral Fellow)
Title: Targeting mitochondrial function as a therapeutic strategy for KRAS-mutant pancreatic cancer
Abstract: The KRAS oncogene is activated in ~95% of pancreatic ductal adenocarcinoma (PDAC) patients and reprograms cell metabolism to support the bioenergetic demands of cancer cells. Emerging evidence suggests that PDAC cancer cells rely on altered mitochondrial function for their survival and that inhibiting mitochondrial activity will be a viable approach for PDAC treatment. However, the mechanisms by which aberrant mitochondrial function support PDAC growth are poorly understood. I propose two complementary studies to assess targeting mitochondrial function for PDAC treatment. First, it is established that the major KRAS effector signaling pathway, the RAF-MEK-ERK MAPK cascade, activates DRP1 to drive mitochondrial fission. To address the role of mitochondrial morphology and metabolism in PDAC, we suppressed DRP1, which increased mitochondrial fusion, impaired cell growth, and caused an increase in autophagy. Future studies include RPPA profiling to understand the signaling changes and examining the effects on cell metabolism conferred by changes in mitochondrial dynamics. As a second approach, we evaluated ONC201, a clinical candidate agonist of the mitochondrial protease ClpP, that causes proteolytic degradation of mitochondrial proteins. We found that ONC201 inhibited PDAC cell growth, impaired oxidative phosphorylation and caused a compensatory increase in glycolysis. Our ongoing studies are evaluating the consequences of ONC201 on PDAC cell metabolism, defining the ClpP substrates critical for ONC201 anti-tumor activity and identifying combination strategies that will enhance the long-term efficacy of ONC201. In summary, our results support a therapeutic value in targeting mitochondrial function for PDAC treatment.

Poster #17: Nila Pazhayam (Sekelsky lab, Graduate Student)
Title: Meiotic Crossover Patterning: The Centromere Effect
Abstract: Crossing over is a critical part of meiosis that prevents aneuploidy by ensuring proper segregation of homologous chromosomes. Crossovers are not randomly distributed along the chromosome, but instead show complex patterning; they do not occur close to one another (crossover interference) and are inhibited in centromeric and telomeric regions (crossover suppression). Erroneous crossover patterning can cause homolog missegregation, leading to chromosomal disorders such as Down syndrome, and even miscarriage. My goal is to investigate the poorly understood mechanisms behind crossover suppression around the centromere, also known as the centromere effect. We hypothesize that crossover exclusion in centromeric regions could be due to an absence of double-strand breaks in the pericentric satellite heterochromatin. To test this, I will study differences that arise in the number and positioning of DSBs in flies mutant for heterochromatin generation. Recent work from our lab has also shown that the centromere effect in adjacent euchromatin is dependent on distance to the centromere. I will further build on this result by looking at the effects of changing the distance of phenotypic markers on the X chromosome to the centromere. Based on studies in triploid flies, we also hypothesize that the strength of the centromere effect is dependent on the total number of centromeres. To test this, I will measure crossover rates in flies with a reduced number of centromeres, using compound chromosomes. Through these and other methods, my overarching goal is to separate the role of the centromere from that of pericentric heterochromatin in manifesting this effect, which I hope will further our understanding of the mechanisms behind it.

Poster #18: Jane Lee (Der and Cox labs, Graduate Student)
Title: Targeting valosin-containing protein (VCP), a regulator of the DNA damage response, for pancreatic cancer treatment
Abstract: Despite comprehensive knowledge of the genetic drivers of pancreatic ductal adenocarcinoma (PDAC), there are no clinically effective targeted therapies. One promising approach is inhibition of the RAS-RAF-MEK-ERK mitogen-activated protein kinase (MAPK) cascade. However, inhibitors of the ERK MAPK cascade have been ineffective in PDAC, due to normal cell toxicity and acquired resistance. To identify combination strategies to overcome these limitations, we applied a CRISPR-Cas9 loss-of-function (LOF) screen of the druggable genome, which identified DNA damage response (DDR) mechanisms as sensitizers to ERK inhibition. To further investigate the DDR, I performed a DDR-focused CRISPR-Cas9 LOF screen and identified valosin-containing protein (VCP) as a gene that modulates PDAC sensitivity to ERK inhibition. VCP is an ATPase with pleiotropic functions including regulation of endoplasmic reticulum (ER)-associated protein degradation, autophagy, and the cell cycle. We determined that inhibition of VCP resulted in growth inhibition and induction of apoptosis in KRAS-mutant PDAC cell lines. We then addressed the basis of VCP-dependent PDAC growth. First, we found that genetic and pharmacologic inhibition of VCP led to accumulation of DNA double strand breaks. Second, we addressed its role in ER function and found that VCP inhibition activated ER stress. Finally, we addressed its role in autophagy. Although VCP is a crucial component of autophagy initiation and autophagosome maturation, we unexpectedly found that loss of VCP increased autophagy. Furthermore, we found that VCP inhibition synergized with the autophagy inhibitor chloroquine. We show that targeting VCP may enhance the efficacy of autophagy inhibitors in treating PDAC.

Poster #19: Siyuan Su (Liu lab, Postdoctoral Fellow)
Title: Targeting ATR/SPOP signaling in Treating Relapsed Chemo-Resistant Ewing Sarcoma
Abstract: Ewing sarcoma is a pediatric cancer that predominantly occur in patients in their childhood and early adulthood. The fusion of two genes, EWSR1 and FLI1, into EWS-FLI1, present in 85% of all occurrences, is the major driving force underlying all types of Ewing sarcoma. However, there is no effective cures directly targeting EWS-FLI1 for inhibition as Ewing sarcoma therapy. Currently, chemotherapy is the standard first-line therapy for Ewing sarcoma, although responsive at the beginning, recurrence occurs in 30-40% of patients. The lack of biochemical understanding of chemotherapy resistance hinders development of new cures. To this end, we found chemotherapeutic agents increased EWS-FLI1 protein abundance as well as its transcriptional activity. In support of this observation, the protein level of SPOP, originally has been identified by us to be a bona fide E3-ligase for EWS-FLI1, was reduced by DNA damaging agents. Importantly, the above observation has been validated in mouse xenograft model. Further analysis showed chemotherapy promoted phosphorylation of SPOP, likely mediated by ATR kinase. Inspired by these observations, we validated our hypothesis that both ATR inhibitors, VE-822 and AZD6732, showed synergistic effect with chemotherapeutic agents in eliminating Ewing sarcoma cells in vitro. In addition to EWS-FLI1 transcriptional targets, chemotherapeutic agents also promoted the expression of a battery of “stemness genes” in A673 cells. Further studies will be required to test whether ATR inhibition was able to reverse the effect of chemo-drugs on Ewing sarcoma cells, as well as mouse xenograft tumors, in terms of EWS-FLI1 transcriptional activity and stemness gene expression.

Poster #20: Kanishk Jain (Strahl lab, Postdoctoral Fellow)
Title: Acetylation-mediated histone tail accessibility governs the read-write mechanism of H3K4
Abstract: Histone N-terminal 'tails' exist in a dynamic equilibrium between free/accessible and collapsed/DNA-bound states in nucleosomes, affecting their accessibility to the epigenetic machinery that utilizes them. Notably, acetylation of the H3 tail has been linked to increased recognition of H3K4me3 by the BPTF PHD finger; yet, whether this mechanism extends to other readers and writers of H3K4 is unknown. Here, we show that *cis* H3 tail acetylation provides general accessibility to a range of H3K4 methyl-dependent and independent readers – a finding that we also show extends to writers, notably MLL1, an H3K4 methyltransferase. *In vivo*, we show that rapid H3 acetylation correlates with increased levels of H3K4me in *cis*. Together, these observations reveal an acetylation 'chromatin switch' on H3 that modulates the accessibility and function of H3K4 methylation in chromatin. They also provide an answer to the long-standing question of how H3K4me3 levels are physically linked in *cis* with H3 acetylation throughout eukaryotes.

Poster #21: Sophie Roush (Fedoriw lab, Graduate Student)
Title: Transcriptomic Characterization of HHV8-associated Multicentric Castleman Disease
Abstract: Background: Human Herpesvirus 8-associated Multicentric Castleman Disease (MCD) is a polyclonal B-cell lymphoproliferative disorder occurring nearly exclusively in people living with HIV (PLWH). With the number of PLWH increasing, improvements in MCD diagnosis and risk stratification are necessary. Enhancing our understanding of MCD biology could help predict development and progression of MCD, and lead to novel therapeutic options. Methods: RNA from n=7 MCD, n=2 MCD with concurrent Kaposi Sarcoma and n=4 HIV+ lymphadenitis lymph nodes was sequenced using the TruSeq RNA Prep Kit v2 (Illumina). Differential Expression Analysis was performed with the DESeq2 pipeline and overlap with Hallmark Gene Sets was calculated with Gene Set Enrichment Analysis (MSigDB). Viral counts within the sequenced lymph node were determined using VirDetect. Results: Principal Component Analysis reveals two clusters of MCD samples. "MCD1" contains 5 samples, while "MCD2" contains 2 samples. Comparing MCD1 to MCD2, n=779 transcripts are up-regulated and n=1134 are down-regulated. Many transcripts related to immunoglobulins are up-regulated in MCD2. Additionally, the MCD2 samples have 8-fold higher EBV viral counts compared to the MCD1 samples (median 534 vs. 66). Gene sets related to cell cycle and metabolism share significant overlap with the transcripts up-regulated in MCD2. Discussion: We identified two subgroups of MCD that may represent transcription-level variation that has previously not been described. The MCD2 group over-expresses genes related to immunoglobulins, metabolism and cell cycle, possibly indicating immunologic collapse. Further investigation could lead to biomarkers that predict risk of developing MCD and risk of progression.

Poster #22: Jonathan DeLiberty (Bryant lab, Graduate Student)
Title: Identification and Characterization of Novel Targets for Autophagy Inhibition 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 recently demonstrated that inhibition of the RAF-MEK-ERK pathway resulted in upregulated autophagic flux, and that dual treatment with autophagy inhibitors such as hydroxychloroquine (HCQ)/chloroquine (CQ) and ERK and MEK inhibitors synergistically blocked PDAC growth. Currently, HCQ/CQ are the only clinically approved autophagy inhibitors; however, they are

limited by low potency. To identify genes whose loss enhance the anti-proliferative effect of HCQ treatment, we performed a CRISPR/Cas9-mediated genetic loss-of-function screen in the presence of CQ. Interestingly, we identified multiple genes that encode proteins that function in all key stages in the autophagic pathway. Preliminary analyses determined that treatment of PDAC cells with anti-autophagy combinations targeting multiple nodes in the pathway resulted in further reduction of autophagic flux relative to inhibition of any single node and also synergistically reduced PDAC cell proliferation. Furthermore, combining ERK inhibition with vertical inhibition of autophagy (dual inhibition of distinct nodes of a linear pathway) synergistically reduced cell growth in a panel of PDAC cell lines. Ongoing studies are aimed at delineating the mechanism underlying the synergy observed with anti-autophagy inhibitor combinations and further validation with additional preclinical models of PDAC. These studies will enhance our understanding of autophagy and aid in the development of novel combination therapies to target autophagy for PDAC treatment.

Poster #23: Philip Lange (Damania lab, Postdoctoral Fellow)

Title: Adenosinergic Signaling as a Novel Target for Viral Lymphomas

Abstract: Epstein-Barr virus (EBV) is a among the most prevalent human pathogens, causing lifelong infections in >90% of the adult population. Importantly, EBV is an oncogenic virus that is associated with numerous B cell lymphomas, lymphoproliferative disorders, and other malignancies. While current therapies have acceptable outcomes for some EBV+ lymphomas, others exhibit extremely poor prognosis despite aggressive chemotherapy. Thus, novel therapeutic targets are needed for the treatment of EBV-positive lymphomas. The adenosine signaling axis represents one of the most promising and targetable immunomodulatory pathways. Extracellular ATP and adenosine drive inflammatory and immunosuppressive signaling, respectively. For instance, ATP signaling via P2 receptors on lymphocytes and myeloid cells is critical for robust activation and inflammatory responses. On the other hand, adenosinergic signaling counteracts inflammatory stimuli, promotes tolerance, and induces immunosuppressive pathways. Ectonucleotidases regulate the extracellular purine concentrations by sequentially converting ATP to adenosine, thereby driving immunosuppressive adenosinergic signaling and opposing immune cell activation. Here I demonstrate robust ectonucleotidase expression and activity in EBV transformed primary B cells. Further, we observed similarly high ectonucleotidase expression in EBV infected tissues using a humanized mouse model of aggressive EBV lymphomagenesis. Notably, inhibiting ectonucleotidase activity in this model resulted in increased inflammatory cytokine expression, decreased viral burden and gene expression, and prolonged survival. *Thus, adenosinergic signaling represents a potential target for the treatment of EBV+ malignancies*.