UPMC Hillman Cancer Center

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Poster No. 1
Cancer Biology Program

CITEgeist: Antibody-based spatial deconvolution of same slide multiomics reveals increased midkine signaling as a microenvironment regulator in ESR1 D538G tumors

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Spatial transcriptomics elucidates tissue architecture, yet its resolution hinges on deconvolving gene-expression signals against single-cell RNA-seq (scRNA-seq) atlases—an approach that is expensive, technically demanding, and often infeasible for the limited material obtained from core biopsies. We introduce CITEgeist, an open-source framework that replaces external scRNA-seq references with antibody-derived protein measurements captured on the same slide. By casting the joint inference of cell-type proportions and gene-expression profiles as a sparsity-constrained optimization problem, CITEgeist offers a biologically grounded, cost-effective, and interpretable alternative that scales to thousands of spots in minutes on a laptop.

Benchmarking against Tangram, Cell2location, Seurat, and other leading methods on simulated mosaics and public breast-cancer sections shows a >20 % reduction in cell-type assignment error at a fraction of the cost. When applied to paired pre- and post-therapy estrogen-receptor-positive (ER+) breast tumors from a Hillman-led clinical study, CITEgeist uncovered stromal-immune niches that earlier approaches missed. Downstream integration with interoperable signaling tools revealed heightened midkine (MDK) signaling within D538G ESR1-mutant tumors, pointing to an emergent microenvironmental vulnerability after endocrine resistance. Western blot and ELISA validation in D538G model cell lines confirmed the actionable accuracy of this prediction.

This work showcases the UPMC Hillman Cancer Center's complete translational pipeline: Hillman's clinically annotated tumor samples supplied by the Pitt Biospecimen Core; the Health Sciences Sequencing Core produced high-quality spatial and proteomic data; and Hillman's basic science program provided the infrastructure for rapid wet-lab validation. Collaborative algorithmic development with Carnegie Mellon University complemented Hillman's experimental strengths, enabling CITEgeist to eliminate the need for costly reference atlases while accelerating discovery in patient-derived specimens.

Poster No. 2

Cancer Epidemiology and Prevention Program

Clinical and sociodemographic determinants of sustained head and neck cancer survivorship clinic attendance

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Importance: Despite increasing recognition of the value of survivorship care for head and neck cancer (HNC) patients, little is known about the patterns and predictors of sustained survivorship clinic attendance.
Objective: Identify factors associated with sustained HNC survivorship clinic attendance
Methods: This single center retrospective cohort study included 625 HNC patients who presented to the UPMC HNC Survivorship Clinic at least once and had at least 42 months of follow-up. The overall cohort included a subset treated with radiotherapy (n=510) and a subset who did not receive radiotherapy (n=115). We defined sustained survivorship clinic attendance as 1) the total number of visits that occurred before completing treatment and within three years post-treatment, and 2) adherence scores, calculated as the number of recommended timeframes during which at least one visit occurred: pre-treatment completion (for the radiotherapy subset only) and one-, two-, and three-years post-treatment. We used Chi-square testing for descriptive statistics and Poisson regression to estimate prevalence ratios (PRs) for sustained attendance.
Results: Among 625 patients, 248 (39.7%) had only one visit to the Survivorship Clinic. In the radiotherapy subset, 41.4% of patients had an adherence score of 1 compared to 70.4% in the non-radiotherapy subset. Patients in the overall cohort who were treated with radiotherapy had more total visits than those who were not (PR: 1.41, 95% confidence interval [CI]: 1.19-1.68), adjusting for clinical and sociodemographic factors. Greater neighborhood socioeconomic deprivation (PR: 0.82, 95% CI: 0.70-0.97) and farther distance from the clinic (PR: 0.81, 95% CI: 0.69-0.95) were independently associated with fewer total visits.
Conclusion: Disparities in engagement with HNC survivorship care highlight the need for targeted interventions to improve compliance with follow-up recommendations.

Poster No. 3
Genome Stability Program

Investigating the mechanisms of the replication stress response to telomeric oxidative damage

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Oxidative damage to DNA arising from oxidative stress conditions plays a role in many human pathologies including cancer, aging-related diseases, and degenerative diseases. 8-oxoGuanine (8-oxoG) is among the most common mutagenic oxidative lesions, and telomeric sequences are preferred sites of 8-oxoG formation. We previously found that telomeric 8-oxoG induces premature senescence in non-diseased human cells by triggering replication stress, as evidenced by telomere fragility and activation of the DNA damage response (DDR). However, the mechanism of the replication stress response to telomeric 8-oxoG remains unknown. We hypothesize that replication fork reversal modulates the response to telomeric 8-oxoG-induced replication stress to prevent fork collapse and cellular senescence induction. To test this hypothesis, we utilized CRISPR-Cas9 genome editing to knock out the DNA translocases HLTF, SMARCAL1, and ZRANB3 and the fork restoration helicase RECQL1 in RPE-hTERT cells. To measure the impact of these fork reversal factor knockouts on the replication stress response to telomeric 8-oxoG, we selectively induced 8-oxoG at the telomeres using our chemoptogenetic tool, and are performing growth analyses to measure senescence, IF-FISH to measure DDR+ dysfunctional telomeres, and metaphase spreads to measure fragile telomeres. We found that RECQL1 knockout cells demonstrated an exacerbated growth reduction and increased DDR+ dysfunctional telomeres after the induction of telomeric 8-oxoG, suggesting that RECQL1 suppresses telomeric 8-oxoG-induced senescence. Further investigating the replication stress response to oxidative damage at the telomeres can expand our molecular understanding of disease pathogenesis and may allow us to identify therapeutic targets for diseases characterized by oxidative stress, including cancer.

Poster No. 4

Cancer Immunology and Immunotherapy Program

Phase 2 Trial of TIL Therapy for Metastatic Uveal Melanoma: Evaluating Cellular Potency and Tumor Transcriptomic Predictors of Response

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Background:
Uveal melanoma (UM) is a rare eye cancer that metastasizes to the liver and is resistant to current immunotherapies. We previously reported that adoptive transfer of autologous tumor-infiltrating lymphocytes (TIL) achieved a 35% objective response rate (ORR) in metastatic UM (NCT01814046). Here we present a follow-up phase 2 trial designed to validate these findings and prospectively identify predictors of response.

Methods:
In this single-center phase 2 trial (NCT03467516), metastatic UM patients underwent metastatectomy to generate early TIL cultures. Cultures demonstrating autologous anti-tumor reactivity and growth potential were expanded for infusion. Patients underwent non-myeloablative lymphodepletion followed by infusion of TIL and high-dose interleukin-2. The primary endpoint was ORR (RECISTv1.1). Exploratory analyses included pooled comparison (NCT03467516 and NCT01814046) of clinical response versus TIL potency and a recently defined in situ transcriptomic tumor biomarker (UMIS).

Results:
As of 6/2/2025, 31 UM patients received TIL therapy. Most had high disease burden (81% stage M1b/M1c, 77% elevated LDH, 71% both hepatic and extrahepatic metastases) and were heavily pretreated (median prior metastatic therapies=2, 61% checkpoint blockade, 32% tebentafusp). Liver metastases were the most common TIL source (52%). Infusion products contained a median of 5.24E10 TIL, were predominantly CD8+ (median=61%), and demonstrated autologous anti-tumor reactivity (62%). Among 30 evaluable patients, initial ORR was 33% (10/30), confirmed ORR (cORR) was 20% (6/30) with 10.8 month median duration of response (longest ongoing at 52 months). In the pooled cohort (n=46), infusion of tumor-reactive TIL yielded a cORR of 41% (11/27) versus 0% (0/19) with nonreactive products (P=0.001). Source metastasis UMIS predicted early TIL reactivity (n=50; P=3.65E-5) and clinical response (n=48; P=0.008).

Conclusions:
This study confirms TIL therapy has efficacy against advanced, pretreated metastatic UM. TIL autologous anti-tumor reactivity was the strongest predictor of response. Transcriptomic biomarkers, such as UMIS, may optimize patient selection and improve outcomes by identifying metastases harboring potent TIL.

Poster No. 5
Cancer Therapeutics Program

Lighting the Way: MEK Inhibitors Boost 5-ALA Photodynamic Therapy in Pediatric Brain Tumors

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Diffuse midline gliomas (DMGs) are highly aggressive pediatric brain tumors affecting central nervous system midline structures. These tumors commonly harbor H3K27M mutations and exhibit MAPK/MEK pathway dysregulation. Standard treatments are limited by poor brain tissue penetration and systemic toxicity, highlighting the need for more targeted local therapies. 5-aminolevulinic acid photodynamic therapy (5-ALA-PDT) is a light-based approach that selectively generates reactive oxygen species (ROS) in tumor cells. Clinical trials have demonstrated its safety and feasibility in high-grade gliomas. Importantly, 5-ALA-PDT may synergize with MEK-targeted therapies due to shared pathway vulnerabilities in DMG. We evaluated the therapeutic potential of combining MEK inhibition with 5-ALA-PDT in DMG models. In vitro studies using trametinib (TRAM) showed enhanced cell death and apoptosis, accompanied by reduced phospho-ERK levels. The combination also suppressed cell migration and invasion by elevating intracellular ROS. Immunofluorescence revealed decreased PDGFRA and HSP70, suggesting inhibition of tumor-promoting signals and stress responses. In vivo efficacy was tested using two DMG mouse models: an RCAS/tv-a-based genetically engineered model and a patient-derived xenograft (PDX). Treatment with 5-ALA-PDT and the MEK inhibitor mirdametinib (MIR) led to increased tumor cell death, enhanced neural stem cell differentiation and T-cell infiltration. These findings support that MEK inhibition augments the efficacy of 5-ALA-PDT in H3K27M-mutant DMGs, providing a proof of concept for a combinatorial therapy with potential for clinical translation.

Poster No. 6

Exploring the symptom experience of patients taking oral anticancer medications for multiple myeloma: A qualitative, descriptive study

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Purpose: Long-term oral anticancer medications (OAMs) are a mainstay of life-sustaining treatment for patients with multiple myeloma (MM) but can have dose-limiting symptoms and side effects.

Significance: The aim of this study is to explore the often overlooked and poorly understood symptom and side effect experience of patients taking OAMs for MM.

Methods: This qualitative, descriptive study was a secondary analysis of semi-structured interviews (n=17) conducted between 3/2022-3/2023 as part of a larger mixed methods study. Patients were recruited from cancer clinics in Western Pennsylvania. Sociodemographic and clinical characteristics were collected via self-report and medical record review. The Edmonton Symptom Assessment Scale (ESAS) characterized overall (sum score range: 0-100) and individual (range: 0-10) symptom severity. Two reviewers independently analyzed interviews, using content and thematic analyses, and identified qualitative codes.

Findings and Interpretations: Most patients were male (n=13, 76.5%), non-Hispanic white or Black (n=12, 70.6% and n=3, 17.6%) or Hispanic “other” race (n=2, 11.8%) and taking either lenalidomide (n=13, 76.5%) or pomalidomide (n=4, 23.5%). The median overall symptom severity score was 13 (IQR = 24), with the most severely rated individual symptoms being sleep, fatigue, and pain (median scores: 3, 1, and 1, respectively). Four main symptom experience themes emerged: ambiguous patient attribution of symptoms (unclear whether symptoms originated from cancer, OAM side effect, or other cause); burdensome symptoms that interfered with life (causing psychological distress and life role limitations); variable provider interventions for symptoms (patient education, pharmacological intervention, specialty referrals); and the need for self-management of symptoms (lifestyle modifications, coping strategies, acceptance).

Discussion: Findings highlight the burdensome symptom and side effect experiences of patients taking OAMs for MM. Symptom scores were low overall but varied. Future mixed methods research should prospectively examine the symptom experience of patients taking OAMs for MM to better understand and support patient OAM adherence.

Poster No. 7
Biobehavioral Cancer Control Program

Geographic Access to Hospices with High Quality Ratings

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Background: Hospice care has experienced significant growth in the U.S., with concerns raised about variability in quality and access. To date, there has been no examination of county-level variation in hospice access and quality.

Objectives: (1) Assess county-level access to hospice care providers in the U.S. and (2) examine sociodemographic differences between counties with and without access to high quality hospice providers.

Research Design: National, cross-sectional descriptive study.

Measures: Hospice quality was assessed using 2023 CMS CAHPS Hospice Survey Star Ratings (with 3 or more stars indicating high quality). County-level sociodemographic characteristics (age, gender, race, ethnicity, education, socioeconomic status, insurance status, and rurality) were obtained from the U.S. Census Bureau's American Community Survey (ACS) 2023 five-year sample.

Results: Of 7,024 hospice providers, only 29.2% had publicly available quality ratings, and of those rated, 55.3% were high quality. Out of 3,222 counties, 1,355 (41.4%) had access to at least one hospice provider, while only 762 (23.6%) had access to a provider with high-quality ratings. Counties with high-quality hospice providers tend to be more urban, larger ($p < 0.001$), higher income ($p < 0.001$), lower rates of poverty ($p < 0.001$), uninsurance ($p < 0.001$), and lower shares of adults without a high school degree ($p < 0.001$).

Conclusions: The majority of U.S. counties do not have any hospice providers, and access to high quality hospice care is markedly lower in rural and poor counties. The high rate of hospices without any star rating limits the utility of publicly available quality data for patient and family decision making.

Poster No. 8

Cancer Immunology and Immunotherapy Program

Targeting DPP4 to Enhance T Cell Infiltration and Improve Cancer Immunochemotherapy

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Cancer immunotherapy has transformed cancer treatment, yet its efficacy remains limited by a “cold” tumor immune microenvironment (TIME) characterized by poor T cell infiltration. Induction of immunogenic cell death (ICD) has been shown to improve T cell infiltration by promoting the release of T-cell-recruiting chemokines, such as CXCL10. However, the overall outcome may be limited by dipeptidyl peptidase IV (DPP4), a serine protease that degrades CXCL10 and related chemokines. We herein report that ICD-inducing chemotherapeutic agents transcriptionally upregulate DPP4 in cancer cells, potentially acting as a negative feedback mechanism that dampens the magnitude of immune response. To improve the delivery of both DPP4 inhibitor and ICD-inducing agent while minimizing the systemic off-target side effects, we developed a hyaluronic acid (HA)-dendrimer nanocarrier for tumor-targeted co-delivery of the two therapeutic agents. HA is a natural ligand for CD44 that is overexpressed on both tumor cells and tumor endothelial cells, enabling precise targeting. In preclinical animal tumor models, this strategy significantly enhanced T cell infiltration, anti-tumor immunity, and the overall therapeutic efficacy while minimizing systemic toxicity. Our work establishes a novel immunochemotherapy platform that synergizes chemotherapy and immunotherapy via targeted DPP4 inhibition, offering a promising strategy to improve clinical outcomes in immunotherapy-resistant cancers.

Poster No. 9

Inhibition of ACSS2 by CD44-targeting nanocarrier to overcome chemo-immune-resistance

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Multifaceted chemo-immune resistance remains a major obstacle in the effective treatment of cancer patients, often driven by metabolic adaptations that allow tumor cells to survive under stress. Acetyl-CoA synthetase 2 (ACSS2), an enzyme that converts acetate to acetyl-CoA, enables cancer cells to utilize acetate as an alternative nutrient source for growth. However, the impact of disrupting acetate metabolism on chemo-immune resistance remains unclear. Here, we demonstrate that chemotherapeutic agents upregulate ACSS2 transcription in cancer cells both in vitro and in vivo, potentially promoting survival and resistance. Additionally, these agents induce cyclooxygenase-2 (COX-2), further contributing to the immunosuppressive tumor microenvironment. Notably, co-treatment with an ACSS2 inhibitor (ACSS2i) sensitizes tumor cells to chemotherapy under metabolic stress. To advance these findings toward clinical application, we developed a hyaluronic acid (HA)-based nanocarrier conjugated with 5-aminosalicylic acid (5-ASA), a COX inhibitor, termed HASA. This platform enables the efficient co-delivery of 5-ASA, doxorubicin (DOX), and ACSS2i, with selective tumor accumulation via CD44 targeting, which is overexpressed in both tumor and tumor endothelial cells. Further in vivo efficacy studies are therefore warranted. Together, our data suggest that targeting ACSS2 may provide a promising strategy to overcome chemo-immune resistance in cancer therapy.

Poster No. 10

Biobehavioral Cancer Control Program

The TeleHealth Resistance Exercise Intervention to Preserve Dose Intensity and Vitality in Elder Breast Cancer Patients (THRIVE-65) Trial

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Background: Older women with breast cancer fare worse than younger patients. Age-related declines exacerbate treatment toxicity, contributing to poorer outcomes and lower received dose intensity (RDI). RDI reflects both chemotherapy dose and timing of administration. A RDI below 85% raises recurrence and mortality risk 1.5 to 3-fold. Exercise and higher protein diet interventions targeting physical function may enhance RDI and survival in this vulnerable population.
Methods: The TeleHealth Resistance Exercise Intervention to Preserve Dose Intensity and Vitality in Elder Breast Cancer Patients (THRIVE-65) trial is a randomized controlled trial evaluating a multicomponent resistance and aerobic exercise intervention with protein intake support versus Health Education and Support (HES) on RDI in 270 older breast cancer patients undergoing chemotherapy. Secondary outcomes include chemotoxicities; physical function, psychosocial status; quality of life, fatigue, sleep; healthcare utilization; physical activity; dietary intake; strength; body composition; and treatment alterations. Eligibility criteria are women age >65 with stage I-III invasive breast cancer, 18-50 kg/m2 BMI, starting at least 10 weeks of cytotoxic chemotherapy for curative intent, able to walk for 6 minutes, not currently exercising, no weight-loss medication, and English speaking. The HES group receives a tablet with supportive programming (e.g., meditation, yoga, non-tailored nutrition programs) and Fitbit. The virtually-delivered THRIVE-65 intervention includes twice-weekly supervised resistance exercise, 90 minutes/week of unsupervised aerobic exercise, and diet counseling aimed to achieve a protein intake of 1.2 g/kg/day, along with tablet and Fitbit. Measurements include anthropometrics, questionnaires, comprehensive geriatric assessment, body composition and objectively measured physical activity. The primary analysis assumes 65-75% of the HES group will achieve an RDI of 85% and hypothesizes that 82-89% of the THRIVE-65 intervention will achieve an RDI of 85% (80% power, two-sided 0.05 significance level). To date, 124 women have been randomized across 3 US medical centers. Support: U01CA271277-03, T32CA186873-11 Clinicaltrials.gov identifier: NCT05535192

Poster No. 11

Genomic profiling of circulating tumor DNA in metastatic invasive lobular (ILC) and no special type (NST) breast carcinoma

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Introduction
Liquid biopsy enables minimally invasive cancer monitoring through blood circulating tumor DNA (ctDNA) sequencing. In this study, we analyzed ctDNA sequencing data from 27 cases of metastatic invasive lobular breast cancer (ILC) and 72 metastatic cases of no special type/invasive ductal cancer (NST/IDC) from UPMC Hillman Cancer Center and Medical University of Graz, Austria.
Methods
For NST, we analyzed 62 samples from first-line treatment, and 31 longitudinal samples (2-6 per patient, median 3). For ILC, we examined 96 blood samples collected over time (2-7 per patient, median 3). DNA was extracted from plasma and sequenced using shallow whole genome sequencing targeting 0.1x coverage.
Results
The primary focus of our analysis is on ILC ctDNA characteristics and longitudinal dynamics. Patients with ILC had a median survival of 43 months (range 6-124 months) with a median of 5 treatment lines. IchorCNA analysis showed 85 samples (91.4%) had tumor fraction (TFx) >3% (mean TFx 12.5%).
Patient-level analysis demonstrated predominant copy number gains versus losses. MDM4 is the most frequently amplified breast cancer related gene (88% of cases), followed by ELF3 and IKBKE. Top copy number loss genes were ZFHX3, TP53, and MAP2K4.
Longitudinal analysis revealed the fluctuating TFx correlated with treatment, as well as higher average continuity index in the top 100 gained genes (0.699) versus top 100 lost genes (0.588), which confirms the effectiveness of liquid biopsy in tumor monitoring.
We also compared ILC cases to NST/IDC cases in genomic copy number variation. We discovered a chromosome 11q13.3 amplicon enriched in ILC cases. This region showed preferential gain in genes CCND1, FGF3, FGF4, and FGF19, suggesting a potential ILC oncogenesis driver locus. Additional ILC and NST/IDC samples are being prepared for sequencing to validate these findings.
This study demonstrates the dynamics of ctDNA in metastatic ILC, and discovers potential oncogenesis driver for ILC disease.

Poster No. 12

Standard chemotherapy in patients with pancreatic ductal adenocarcinoma modulates B cell education within and outside tertiary lymphoid structures

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Pancreatic ductal adenocarcinoma (PDAC) is the third leading cause of cancer related deaths in the world. The standard treatment for PDAC consists of surgical resection when possible and perioperative combination chemotherapy regimens, including Gemcitabine-Abraxane (Gem/Abraxane). Recent studies have shown increased survival probability associated with higher B cell infiltration. B cells are a heterogenous population and can promote antitumor immunity by mediating humoral immunity in response to antigen stimulation. Naïve B cells will differentiate into memory B cells (MBCs) or plasma cells through a germinal center (GC)-associated response and/or the extrafollicular (EF) response. The GC-associated response occurs within tertiary lymphoid structures (TLS), ectopic lymphoid organs that arise in sites of chronic inflammation. Therapeutics, such as chemotherapy, can alter prevalence and activity of TLS by increasing tumor antigen availability and activating an inflammatory signaling cascade leading to an influx of immune cells. We hypothesized that Gem/Abraxane would increase the production of antibody secreting cells (ASCs) through amplification of the GC-associated and EF response. We aimed to understand these responses in PDAC patients via multispectral imaging of patient tumors and in vitro modulation of B cells via patient derived organoids (PDOs). Our multispectral imaging analysis showed an increase in GC-associated responses and increased TLS activity in the Gem/Abraxane-treated patients compared to the treatment naïve patients. Our in vitro modulation using conditioned media (OCM) from PDAC treatment naïve or Gem/Abraxane PDOs showed the naïve B cells cultured with the Gem/Abraxane OCM compared to the treatment naïve had increased production of ASCs at an earlier time point. Together, our findings highlight the importance of understanding the effects of standard treatment on altering the humoral response which modulates MBC and ASC production and can lead to an improved antitumor response. These findings provide rationale for designing B cell targeted immunotherapies in PDAC patients in complement standard.

Poster No. 13
Cancer Biology Program

Title: Revealing targetable metabolic vulnerabilities in breast cancer liver metastasis

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Rationale: Liver is the second most common site for breast cancer metastasis; yet the molecular mechanisms that drive breast cancer liver metastasis (BCLM) are underexplored. Preliminary studies showed that BCLM acquire unique metabolic adaptations to survive in the metabolically active liver environment. We aimed to further interrogate metabolic adaptations in BCLM and hypothesized that BCLM upregulate normal liver metabolic pathways to progress.

Methods: We injected 66CL-4-lucZSgreen mouse mammary carcinoma cells into the portal veins or spleens of syngeneic mice. At endpoint, tissue samples were collected from liver metastases (LM, N=4), normal tissue adjacent to metastases (NAL, N=4), and age-matched healthy mice (normal liver or NL, N=5). Samples were assessed by liquid chromatography-mass spectrometry. Metabolic enzymes of interest were examined in publicly available RNA sequencing data (GEO) and using Kaplan-Meier plotter.

Results: When comparing LM to NL, 197 metabolites of 1855 detected were significantly altered. Arginine ($p=0.047$) and creatine ($p=0.0096$) were two of the most upregulated metabolites in LM versus NL. Analysis of three metastatic breast cancer datasets revealed increased expression of creatine biosynthesis enzymes GAMT (FC=3.1) and GATM (FC=10.9) in BCLM compared to breast tumor specimens. High expression of creatine metabolism enzymes GATM ($p=0.00016$) and CKMT1A ($p=0.02$) predicted decreased overall survival in TNBC but not in the other breast cancer subtypes.

Conclusions: These results indicate that BCLM rewire their metabolic profile to enhance creatine metabolism. Future studies will continue to assess the impact of creatine metabolism on BCLM metabolic reprogramming, including testing whether this pathway is a therapeutic vulnerability of BCLM.

Poster No. 14
Cancer Biology Program

HO-1-Derived Bilirubin Disrupts Macrophage Efferocytosis via NFkB Activation to Promote Breast Cancer Liver Metastasis

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Over 50% of metastatic breast cancer patients develop liver metastasis, but this metastatic site remains resistant to most treatment approaches and results in a poor prognosis of just 2-3 years. Heme oxygenase-1 (HO-1) is a heme-degrading enzyme that serves as a potential therapeutic target as it may alter the microenvironment through its metabolites to allow metastasis establishment. One such metabolite, bilirubin, impairs macrophage antigen presentation and phagocytosis. Our mRNA-sequencing of primary murine bone marrow derived macrophages revealed that bilirubin may limit expression of efferocytosis-related genes. The role of efferocytosis in tumor progression is debated, as some propose that impaired efferocytosis can drive angiogenesis and tumor growth while others argue that efferocytosis promotes metastasis. We chose to examine the impact of bilirubin on macrophage efferocytosis using the pH-sensitive dye pHrodo and flow cytometry. We found that 10μM bilirubin significantly suppressed both efferocytosis and expression of the efferocytosis receptor MerTK in murine RAW264.7 macrophages when compared to the vehicle. Similar results were seen in primary human liver resident macrophages, also known as Kupffer cells, suggesting that macrophages in the bilirubin rich microenvironment of the liver maintain sensitivity to bilirubin. To elucidate the mechanism by which bilirubin alters efferocytosis, we also examined the impact of bilirubin on NFkB signaling and showed that bilirubin induced phosphorylation of NFkB/p65 and IL-6 secretion in mouse and human macrophage models. These findings suggest that inhibition of HO-1 could prevent accumulation of bilirubin in the liver metastatic microenvironment, and in turn alter macrophage function to limit the establishment of liver metastases.

Poster No. 15

^{177}Lu-PSMA-617 Targeted Radionuclide Therapy Increases the Efficacy of B7-H3 Targeted CAR-T Cell Therapy in Prostate Cancer

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Metastatic castration-resistant prostate cancer (mCRPC) remains a devastating disease with a median overall survival (OS) of just 25.6 months, underscoring an urgent clinical need for improved treatment regimens. The introduction of ^{177}Lu-PSMA-617 targeted radiopharmaceutical therapy marked a significant advancement in the treatment of mCRPC. Approximately half of patients exhibit a significant prostate-specific antigen decline of 50% or greater, a prognostic biomarker for improved survival, with many achieving a state of minimal residual disease. However, long-term remissions remain elusive, and disease progression typically occurs mere months after treatment completion. The dramatic tumor burden reduction in mCRPC from ^{177}Lu-PSMA-617 may render these solid tumors more susceptible to chimeric antigen receptor (CAR) T-cell therapy. While CAR T-cells have induced unprecedented remission rates in patients with acute lymphoblastic leukemia, patients with solid tumors show limited efficacy due to impaired T cell trafficking into the tumor microenvironment (TME) and immunosuppressive mechanisms in the TME. Additionally, the eradication of large tumor burdens by CAR T cell therapy is not achieved without cytokine release syndrome (CRS). Herein, we demonstrate the synergy of ^{177}Lu-PSMA-617 TRT with B7-H3 targeted CAR T-cells in a pre-clinical in vivo model. The combination of ^{177}Lu-PSMA-617 (400 µCi) and B7-H3 CAR T-cells (2 million) significantly improved survival compared to either treatment alone, with hazard ratios of 4.98 (p = 0.02) versus ^{177}Lu-PSMA-617 alone and 4.46 (p = 0.03) versus B7-H3 CAR-T alone (log-rank test). Additionally, complete tumor responses increased in a dose dependent manner with regards to ^{177}Lu-PSMA-617, as 0%, 25%, 57%, and 83% of mice pre-treated with 0, 200, 400, and 800 µCi before B7-H3 Car T-cell therapy experienced complete responses, respectively. This dose-dependence highlights the importance of tumor de-bulking before CAR-T. Future studies will investigate CAR T cell activity and infiltration into bone metastases, a clinically relevant metastatic site in prostate cancer.

Poster No. 16
Cancer Virology Program

Oncogenic PARG Suppresses HBV Restriction by Destabilizing the HBx-DDB1 Axis

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Hepatitis B virus (HBV) X protein (HBx) promotes viral episomal covalently closed circular DNA (cccDNA) transcription by targeting the host restriction complex SMC5/6 for proteasomal degradation through recruitment of the DDB1-CUL4 E3 ligase. However, we observed that this HBx-mediated degradation is inefficient in hepatoma-derived HepG2 cells. In contrast, EBV BNRF1 and KSHV RTA—known SMC5/6-targeting viral proteins—remain effective in these cells via DDB1-independent mechanisms. Comparative analyses revealed that DDB1 protein levels are significantly reduced in hepatoma cells compared to primary human hepatocytes (PHHs) and non-hepatic cells, resulting in an imbalanced DDB1-CUL4-SMC5/6 stoichiometry in liver cancer cells.

Restoration of DDB1 expression in HepG2 cells re-enabled efficient HBx-mediated SMC5/6 degradation and facilitated cccDNA chromatin remodeling, as shown by ChIP assays indicating increased H3K27ac and HBx enrichment, along with decreased SMC6 association. Given previous reports that DDB1 can be degraded via poly(ADP-ribose) glycohydrolase (PARG)-mediated dePARylation in hepatocarcinogenesis, we further explored this regulatory pathway. PARG knockdown or pharmacological inhibition increased DDB1 PARylation, enhanced its stability, and promoted SMC5/6 degradation upon HBx expression or HBV infection. Mechanistically, DDB1 serves as a critical molecular bridge linking HBx and PARG. Using co-immunoprecipitation and proximity ligation assays (PLA), we identified the PARG catalytic domain as essential for its interaction with DDB1. A catalytic-dead PARG mutant (ΔCAT) disrupted DDB1 binding and failed to suppress HBV transcription. These findings reveal a novel role of oncogenic PARG in undermining host restriction of HBV by destabilizing DDB1, offering new insights into virus-host dynamics during HBV persistence and hepatocellular carcinogenesis.

Poster No. 17
Cancer Biology Program

ETV6 Rearrangements Impair T Cell Infiltration via Activating β-Catenin Signaling in Triple-Negative Breast Cancer

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Triple-negative breast cancer (TNBC) is the deadliest breast cancer subtype accounting for 10-20% of all breast cancer cases but a disproportionately large number of deaths. Chemotherapy remains the mainstay of intervention for TNBC due to the lack of well-defined targets. Recently, immune checkpoint blockade (ICB) emerged as an effective therapy for TNBC patients in both adjuvant and neoadjuvant setting. However, >85% TNBC patients do not derive benefits from combining ICB with chemotherapy. In our previous study, we discovered a novel TNBC-specific gene fusion involving the prototype cancer gene ETV6 and its immediate telomeric neighbor BCL2L14. In this study, our analyses of WGS data detected TNBC-specific ETV6 intragenic rearrangements (IGRs) leading to exon duplications or deletions as well as additional 3' ETV6 fusions with different 5' partners. Together, ETV6 rearrangements are detected in ~13% of TNBC, including ~35% of the mesenchymal (M) and ~12% of the basal-like 1 (BL1) subtypes. More interestingly, tumors characterized by ETV6 rearrangements exhibit a notable scarcity of immune cell infiltration and interferon-γ signature. Mechanistic studies suggest that ETV6 rearrangements may act as a dominant-negative of wild-type ETV6 and activate β-catenin and TGF-β1 pathways to induce EMT, impair T cell trafficking, and dictate a relative cold tumor immune microenvironment (TIME). Conversely, knockdown of the duplicated E2 exon in HCC1187 cells harboring ETV6 exon 2 duplication led to increased interferon responses and NF-κB pathway activation, suggesting restoration of immune responsiveness. Pharmacological inhibition of β-catenin signaling with PRI-724 significantly reduced oncogenic signaling and enhanced CD8+ T-cell recruitment. Collectively, these findings highlight ETV6 rearrangements as novel, actionable targets for therapeutic intervention in TNBC and suggest their potential utility as biomarkers for patient stratification in future therapeutic strategies targeting Wnt/β-catenin signaling.

Poster No. 18
Cancer Biology Program

AP-2β Defines a Mammary Epithelial Subpopulation with a Potential Role in Lobular Carcinoma Development

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Background:
Invasive Lobular Carcinoma (ILC) is the most common special subtype of breast cancer, accounting for ~15% of cases, and is defined by the loss of the cell adhesion molecule E-Cadherin (CDH1). Despite distinct clinical and molecular features, patients with ILC are treated similarly to those with breast cancer of no special type (NST), contributing to worse long-term outcomes. Improved understanding of the molecular drivers of ILC is urgently needed. Our lab identified the transcription factor TFAP2B (AP-2β) as one of the most hypomethylated genes in ILC. Previous studies show AP-2β has enhanced expression in ILC, showing a potential role in ILC tumor initiation

Methods:
We analyzed AP-2β expression in normal mammary tissue across species (human, mouse, rat) and in C57Bl/6-derived organoids using immunoblotting, immunohistochemistry (IHC), and immunofluorescence (IF). Publicly available single-cell RNA-seq datasets were also interrogated.

Results:
IHC revealed sparse AP-2β expression in normal tissue but robust, uniform expression in ILC tumors. IF confirmed co-expression with luminal markers including FOXA1, GATA3, CK8/18, estrogen receptor (ER), and androgen receptor (AR). Single-cell RNA-seq localized AP-2β to hormone-responsive (HR) luminal epithelial cells, with enriched AR signaling and ROS pathways. CDH1-deficient mouse-derived organoids exhibited apoptosis and growth arrest; ongoing studies are testing if AP-2β overexpression rescues cell survival.

Conclusion:
AP-2β marks a specific luminal epithelial cell population in the mammary gland with potential resistance to CDH1 loss. These findings support a model in which AP-2β contributes to early lobular tumorigenesis by promoting survival of CDH1-deficient cells and driving progression from normal epithelium to LCIS and ultimately ILC.

Poster No. 19
Genome Stability Program

TRAF3 deficiency promotes lymphomagenesis in B cells that experienced VH gene replacement (VHR)

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B cells are prone to genomic instability due to DNA double-stranded breaks (DSBs) arising from physiologically programmed DNA recombination processes, e.g., V(D)J recombination generating the variable region of antibody molecule (a.k.a. B cell receptor or BCR). VH gene replacement (VHR) is a secondary V(D)J recombination process detected in mouse and human bone marrow B cells and in human lymphoma cell lines and thought to contribute limitedly to primary B cell repertoire due to its low frequency. It remains unknown whether and under what conditions aberrant VHR-associated genomic instability can promote lymphomagenesis. Using the hen egg lysozyme (HEL) BCR model, we showed that VHR occurred frequently in peripheral B cells, accompanied by increased expression of VDJ recombinase RAG-1. Nevertheless, VHR-experienced B cells did not undergo clonal expansion or become malignant. TRAF3 deficiency significantly predisposed HEL-specific B cells to develop lymphoma with VHR footprint. Mechanistically, TRAF3-deficient B cells survive more effectively upon RAG-induced DSBs, in a NF-B2 dependent manner, thereby allowing B cells to accumulate genetic lesions, evidenced by chromosomal translocations in lymphomas. Whole-genome sequencing analysis revealed translocation breakpoints preferentially targeting genetic loci enriched with RAG-targeting cryptic recombination signal sequences. We conclude that synergistic effects of TRAF3 deficiency-enhanced B cell survival and VHR-associated genomic instability significantly promote lymphomagenesis, furthermore, signaling molecules like TRAF3 serve as non-classical checkpoints to maintain B cell genome stability. Our studies uncover fundamental insights into under what conditions B cells decide to revise their identity and what pathogenic mechanisms make such process go awry.

Poster No. 20
Genome Stability Program

PROX1 Regulates Bone Marrow Niche Plasticity and Hematopoiesis Through POU3f3-ALK-1 Signaling Pathway

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Regeneration of bone marrow (BM) niche following myeloablation entails dynamic transitions between mesenchymal stromal cells (MSCs) and adipocytes, involving both MSC-to-adipocyte differentiation and adipocyte de-differentiation back to MSC-like states. However, the molecular mechanisms governing this bidirectional plasticity remain poorly understood. In this study, we investigate the role of transcription factor PROX1 in maintaining BM niche integrity. Using a mesenchymal-specific Prox1 conditional knockout mouse model (Proxfl/flPrx1Cre), we demonstrate that Prox1-deficient mice exhibited heightened sensitive to 5-fluorouracil (5-FU)-induced myeloablation attributed to compromised hematopoietic support by MSCs. Mechanistically, PROX1 deficiency inhibited adipocyte de-differentiation, thereby restricting the capacity of niche-resident adipocytes to revert to functional MSC-like cells under stressed conditions. Cytokine profiling revealed that expression of hematopoietic-supportive cytokine ALK-1 is marked reduced in Prox1-deficeint BM stromal cells, implicating it as a downstream effector of PROX1. Consistently, systemic administration of recombinant ALK-1 Fc in wild-type (WT) mice phenocopied the hematopoietic defects observed in Prox1-cKO mice, whereas activation of ALK-1 signaling via BMP9 restored MSC function and hematopoiesis in the context of PROX1 deficiency. Integrated ChIP-seq and RNA-seq analyses identified POU3f3 as a direct transcriptional target of PROX1, which in turn regulates ALK-1 expression. Collectively, these findings delineate a previously unrecognized PROX1-POU3F3-ALK-1 signaling axis that modulates MSC plasticity and hematopoietic support, offering novel insights into BM niche regeneration and highlighting potential therapeutic avenues for enhancing stromal remodeling following myeloablative injury.

Poster No. 21

Cancer Immunology and Immunotherapy Program

Tumor antigen-specific T cells increase tertiary lymphoid structure response in murine melanoma

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Immunotherapies such as immune checkpoint blockade (ICB) harness the adaptive immune system to control tumors, but their efficacy varies widely across cancer types. Tertiary lymphoid structures (TLSs) – aggregates of T, B, and myeloid cells – are linked to better prognosis and ICB efficacy. They are thought to act as niches for antigen drainage and to provide a pro-inflammatory microenvironment to enhance anti-tumor T cell responses. Despite the widely recognized importance of TLSs in cancer, the factors that are thought to drive TLS formation have yet to be thoroughly interrogated. In particular, the importance of antigenic determinants of TLS formation is not well defined, and, whether T and B cells recognizing tumor antigens interact to form TLSs in tumors is unknown. We hypothesized that the interactions between tumor-antigen specific Tfh cells and tumor reactive B cells are critical for TLS formation, and these interactions lead better tumor control. To determine if/how the presence of tumor antigen-specific T cells influences the TLSs and tumor microenvironment, we performed intraperitoneal injections of B16 or B16-OVA cell lines into C57Bl/6 mice and then adoptively transferred naïve CD4 OTII T cells. After the endpoint, the tumors were removed, weighed and processed for scRNA-seq, spatial transcriptomics and flow cytometry. Presence of both Ag-specific T cells and the antigen on tumor led to increased TLS formation and Tfh cells, while decreasing tumor burden. Using Slide-seq, we were able to map tumor-infiltrating T, B, and myeloid cells, showing TLS-like structures where T-B interactions are present. We are currently testing whether expanded Tfh TCR clones from tumors are reactive to the tumor, and if they can be mapped in a spatial manner. The completion of these studies will lead to a greater understanding of the T-B interactions that fuel TLS formation, allowing the development of antigen-specific therapies that enhance tumor clearance.

Poster No. 23
Genome Stability Program

TRABECTEDIN AND LURBINECTEDIN DISRUPT TRANSCRIPTION-COUPLED NUCLEOTIDE EXCISION REPAIR, LEADING TO DNA BREAKS IN ACTIVELY TRANSCRIBED GENES

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Our previous studies demonstrated that trabectedin exerts its cytotoxicity through transcription-coupled nucleotide excision repair (TC-NER), where it induces persistent single-strand breaks (SSBs) due to blockage of the second incision reaction of XPG (1). By leveraging this mechanism, we developed TRABI-Seq (trabectedin-induced break sequencing) to map TC-NER and transcriptional activity at a genome-wide scale. Given the mechanistic similarity between trabectedin and lurbinectedin, we extend this approach to investigate lurbinectedin's activity in small cell lung cancer (SCLC), a transcription-driven malignancy for which lurbinectedin is an approved treatment.

Utilizing the methodologies established in our previous work, we observe that lurbinectedin induces DNA break patterns highly similar to trabectedin, both in frequency and genomic distribution, reinforcing their shared mechanism of action. This study aims to further elucidate lurbinectedin's mode of action and to enhance its potential for precision oncology by refining its application in transcriptionally driven malignancies. These findings will contribute to a deeper understanding of TC-NER dynamics and provide a foundation for improving the clinical utility of lurbinectedin in SCLC.

Poster No. 24

Cancer Immunology and Immunotherapy Program

DGAT: A Dual-Graph Attention Network for Inferring Spatial Protein Landscapes from Transcriptomics

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Spatial transcriptomics (ST) technologies provide genome-wide mRNA profiles in tissue context but lack direct protein-level measurements, which are critical for interpreting cellular function and microenvironmental organization. We present DGAT (Dual-Graph Attention Network), a deep learning framework that imputes spatial protein expression from transcriptomics-only ST data by learning RNA–protein relationships from spatial CITE-seq datasets. DGAT constructs heterogeneous graphs integrating transcriptomic, proteomic, and spatial information, encoded using graph attention networks. Task-specific decoders reconstruct mRNA and predict protein abundance from a shared latent representation. Benchmarking across public and in-house datasets—including tonsil, breast cancer, glioblastoma, and malignant mesothelioma—demonstrates that DGAT outperforms existing methods in protein imputation accuracy. Applied to ST datasets lacking protein measurements, DGAT reveals spatially distinct cell states, immune phenotypes, and tissue architectures not evident from transcriptomics alone. DGAT enables proteome-level insights from transcriptomics-only data, bridging a critical gap in spatial omics and enhancing functional interpretation in cancer, immunology, and precision medicine.

Poster No. 25
Cancer Biology Program

TGFBI, a potential therapeutic target functions through immune cell infiltration

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Ovarian Cancer (OvCa) is one of the deadliest cancers in women with 25-30% 5-year survival rate. Suggesting anti-tumor immunity can help control tumors, patients whose T cells (especially CD8+ cytotoxic T) can infiltrate tumor islets have a better prognosis. However, tumor immune exclusion (TIE) can develop and prevent immune benefits. We recently reported that TIE can be driven by cancer-associated mesenchymal stem cells (CA-MSCs). The exact mechanisms whereby CA-MSC drive TIE remain unclear. We found that CA-MSC secrete Transforming Growth Factor Beta-Induced (TGFBI) and TGFBI expression directly correlated with TIE and lack of response to immunotherapy. TGFBI is present in tumor stroma and has many domains to cellular binding. We hypothesize that TGFBI contributes to immune exclusion by binding to immune effector cells in the tumor stroma and preventing their infiltration into tumor islets.
To test this, we subcutaneously injected UPK-10 Ova-T low (immune hot) tumor cells into 10-12 weeks old C57BL/6 female mice (WT) and TGFBI knockout (KO) mice. Suggesting an immunosuppressive role for TGFBI, tumors in the KO mice showed significant reduced growth compared to the WT mice. Analyzing tumor immune compartment by multispectral flow cytometry revealed significant increase in CD3e+, CD8+ and alpha-SMA+ cells, and a significant decrease in Ly6G+ cells in the KO mice by day 18 compared to the WT mice. We validated these findings using multiplex immunofluorescence. To directly assess the role of CA-MSC produced TGFBI, we co-injected adipose-derived MSCs from WT or TGFBI KO mice in a 1:1 ratio with UPK-10 tumor cells. We found significantly increased infiltration of CD3e+, NK1.1+, NKT+, CD8+ TCR$\gamma\delta^{+}$ T cells in the tumors in KO-MSC co-injected mice compared to their counterpart. To assess the impact of TGFBI on reducing tumor infiltrating T cells, we performed adhesion and migration assays. Suggesting TGFBI is directly inhibiting effector immune cell infiltration, activated CD8+ T cells showed both increased adhesion to and migration through TGFBI KO-MSCs, compared to WT controls.
Our findings demonstrate that TGFBI plays a critical immunosuppressive role directly regulating effector immune cell tumor infiltration and increasing immunosuppressive Ly6G+ cells. This work supports TGFBI as an important immunologic target for cancer therapy.

Poster No. 26

Cancer Immunology and Immunotherapy Program

PTX3 as a Tumor-Intrinsic Modulator of Myeloid Cell Function and a Therapeutic Target in HNSCC Immunotherapy

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Head and neck squamous cell carcinoma (HNSCC) remains a major clinical challenge due to an immunosuppressive tumor microenvironment (TME) and frequent resistance to immune checkpoint inhibitors (ICIs). We identify the innate immune molecule Pentraxin 3 (PTX3) as a key factor promoting the expansion of immunosuppressive myeloid cells in the TME, especially M2-like tumor-associated macrophages (TAMs), which facilitate tumor progression and inhibit $CD8^+$ T cell–mediated immunity. Using a genetically engineered murine HNSCC model harboring p53 deletion and PIK3CA hyperactivation, we showed that PTX3 expression is markedly elevated in ICI-resistant tumors. Moreover, PTX expression correlates with worse prognosis in HNSCC TCGA dataset. Genetic ablation of PTX3 in tumor cells significantly slows tumor growth in vivo and shifts the TME toward a less suppressive state. PTX-3-deletion mediated tumor inhibition was dependent on CD8 T cells; however, PTX-3 KO tumors were not sensitive to ICI treatment. Mechanistically, we showed that PTX3 promotes M2-like TAM differentiation through interaction with CD44 which serves as a receptor for PTX-3 and activation of downstream PI3K/AKT signaling pathways in myeloid cells. These findings provide a rationale for targeting PTX3, alone or combined with chemotherapy or other immunomodulators, to improve therapeutic responses in HNSCC patients. Our study highlights PTX3 as a novel immunomodulatory target in HNSCC and supports development of PTX3-based combination therapies.

Poster No. 27
Cancer Biology Program

Targeting Secreted Anterior Gradient-2 to Reverse Immune Exclusion in Breast Cancer Liver Metastases

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Background: About 50% of metastatic breast cancer cases spread to the liver, a site that responds poorly to chemo- and immunotherapy, resulting in poor prognosis. Despite their prevalence, there is a relative lack of research on the immune microenvironment of breast cancer liver metastases (BCLM). Our preliminary studies identified two distinct BCLM phenotypes in human specimens based on immune cell abundance: immune cold and immune hot. Further analysis revealed significantly elevated levels of anterior gradient-2 (AGR2) in immune cold BCLM. We hypothesized that AGR2 supports an immune cold state in BCLM by suppressing T cell infiltration.
Methods: We performed Western blotting and immunohistochemistry to examine AGR2 expression in human patient-derived (HCl-002 and HCl-010) and mouse mammary carcinoma cell lines (4T1-luc-ZSgreen and 66Cl-4). Mouse mammary carcinoma-derived organoids were co-cultured with activated CD8+ splenocytes ± recombinant AGR2. We monitored the co-culture every 4 hours for 3 days with fluorescent dyes, including Caspase-3/7 Red to detect cell death.
Results: AGR2 was expressed in HCl-010 but not in HCl-002 cells. AGR2 was undetected in 4T1-luc-ZSgreen and 66Cl-4 cells. Thus, AGR2 was supplemented in the media for all co-culture assays. T cells were significantly more distant from organoids in the presence of AGR2 when compared to co-cultures treated with vehicle control.
Conclusion: These results suggest that AGR2 contributes to an immune cold phenotype in BCLM by repressing T cell infiltration, revealing its potential as an immunotherapy target in BCLM. Future directions include co-culturing human-derived T cells and organoids, using AGR2-overexpressing HCI-002 cells and, eventually, BCLM patient-derived organoids.

Poster No. 28

Deep Learning–Driven HCC Morphologic Index from H&E Predicts Survival and Reveals Distinct Clinical and Molecular Phenotypes

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Background: Hepatocellular carcinoma (HCC) is a leading cause of cancer-related mortality. Although multiple transcriptomics-based classification systems exist, none are clinically implemented. With increasing use of tissue biopsies in targeted therapy trials, there is an opportunity to develop integrated molecular and histologic approaches for HCC stratification.

Methods: Public spatial transcriptomics data with paired hematoxylin and eosin (H&E) images from 10 HCC slides were used to train deep learning models to predict Hoshida subtype (S1, S2, S3) signatures within spatial transcriptomics spots from the corresponding H&E tiles. Models were evaluated using an 80/20 training/test split, then applied to H&E whole-slide images from The Cancer Genome Atlas (TCGA; n=340). Tile-level predictions were averaged to generate patient-level histologic scores. These scores were then reduced via principal component analysis to two dimensions (HCC Morphologic Indexes [HMI1 and HMI2]), which captured 97% of score variability. Optimal thresholds for overall survival (OS) stratified patients into high-, intermediate-, and low-risk groups, which were assessed for clinical and molecular correlates.

Results: Models achieved holdout AUROCs of 0.93 (S1), 0.92 (S2), and 0.94 (S3). In TCGA, risk groups predicted OS across all comparisons (high vs low, $p=4.4\times10^{-11}$; high vs intermediate, $p=0.0034$; intermediate vs low, $p=1.9\times10^{-5}$) and disease-free survival (DFS) in most comparisons. Each group was associated with distinct etiologies (e.g., alcohol, MASLD, HBV) and molecular phenotypes identified through gene set enrichment analysis and mutational profiling: high-risk with proliferation (e.g., cell cycle, *MKI67* mutation), intermediate-risk with inflammation (e.g., complement cascade), and low-risk with metabolism (e.g., mitochondrial β-oxidation, *MTOR* mutation).

Conclusions: By predicting spatial subtype signatures from H&E alone, we developed a histology-based HMI with greater prognostic power than bulk RNA-based Hoshida subtypes. The associated clinical and molecular features suggest biologically distinct phenotypes, supporting the potential of HMI to guide patient stratification in clinical trials and inform personalized therapeutic strategies.

Poster No. 29
Cancer Virology Program

CASTOR1 Deletion Impairs Antiviral Immunity and Alters Immune Responses to Murine Gammaherpesvirus 68 Infection in Mice

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The mechanistic target of rapamycin complex 1 (mTORC1) regulates immune cell activation, differentiation, and antibody production in response to metabolic and nutrient cues. In B cells, mTORC1 activity is finely tuned to support processes such as germinal center formation, class-switch recombination, and affinity maturation. CASTOR1, a cytosolic arginine sensor, suppresses mTORC1 under nutrient-limited conditions, yet its role in antiviral immunity remains poorly understood. To address this, we infected CASTOR1-/- and wild-type (WT) C57BL/6 mice intranasally with murine gammaherpesvirus 68 (MHV68), a natural pathogen of mice, and evaluated viral replication, latency, and immune responses at defined post-infection time points. Preliminary results showed that CASTOR1-/- mice exhibited disrupted splenic architecture, with altered germinal center organization compared to WT controls. Additionally, CASTOR1-/- mice displayed differential antibody responses to MHV68, indicative of a dysregulated B cell maturation pathway. Histopathological analysis of the lungs revealed increased inflammatory infiltrates and tissue damage in CASTOR1-/- mice relative to WT. These findings suggest that CASTOR1, through modulation of mTORC1 activity, plays a critical role in humoral immunity and in resolving tissue injury following infection. Ongoing studies aim to elucidate the underlying mechanisms of these effects.

Poster No. 30

Mechanisms of LGALS1 blockade in sensitizing HNSCC to immune checkpoint inhibitor

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Head and neck squamous cell carcinoma (HNSCC) accounts for 90% of HN cancers. Immune checkpoint inhibitors (ICI) improved HNSCC treatment; however, the response rate remains low (10-20%). Thus, it is vital to identify new targets that can sensitize HNSCC to ICI therapy. Here, we showed that LGALS1 is upregulated in HNSCC in a subtype- and stage dependent manner. Contrary to prior findings, LGALS1 is predominantly expressed in SCC tumor cells, tumor-infiltrating myeloid cells and activated CD8 T cells intracellularly. A lower level of intracellular LGALS1 in myeloid cells and a higher serum LGALS1 level correlated with ICI unresponsiveness in mice and worse survival in ICI-treated HNSCC patients. Blocking LGALS1 sensitized tumors to anti-PD-L1 and prolonged recipient survival drastically. LGALS1 inhibitor does not affect LGALS1 expression level, instead, its efficacy depends on tumor-intrinsic and tumor-extrinsic mechanisms. Combination of LGALS1 blockade and anti-PD-L1 modulates differentiation of tumor-associated macrophage. LGALS1 blockade directly activated CD8 T cells in a STAT3-dependent manner. We conclude that LGALS1 exhibits prognostic significance in predicting ICI response in HNSCC and targeting molecules like LGALS1 may serve as ideal approaches to sensitize tumors to immunotherapy because its blockade affects tumor cells, myeloid and CD8 T cells simultaneously to promote anti-tumor immunity.

Poster No. 31
Cancer Biology Program

Fibroblasts modulate targeted therapy response dynamics in HER2+ breast cancer

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HER2-overexpressing (HER2+) breast cancer accounts for 15-20% of breast cancer diagnoses. Disease recurrence and resistance to HER2-targeted therapies such as lapatinib pose treatment challenges. Stromal fibroblasts in the tumor microenvironment are associated with poor survival outcomes and contribute to resistance. Cancer cells dynamically adapt to therapeutic stress through cell-intrinsic or cell-extrinsic mechanisms. However, existing studies investigating stroma-mediated therapy resistance have focused on endpoint response, thereby overlooking temporal changes to drug sensitivity. We investigated the drug response dynamics of HER2+ breast cancer cells cultured with fibroblasts and challenged with lapatinib then we applied mathematical models to these data to decouple intrinsic and microenvironment-mediated mechanisms of resistance.

A panel of 11 HER2+ breast cancer cell lines (AU565, BT474, EFM192, HCC1419, HCC1569, HCC1954, HCC202, MDA361, SUM225, UACC812, UACC893) were seeded in monoculture or coculture with AR22 mammary fibroblasts in a 96-well plate and treated with 0-3μM lapatinib for 96h. Tumor cells expressed H2B-GFP and were incubated with ethidium homodimer to distinguish live from dead cells. Cell count measurements were performed every four hours using time-lapse fluorescence microscopy. Four models of time-dependent death rates (constant, increasing, decreasing, or transient death rate) were fit separately to monoculture and coculture drug response data for each of 9 cell lines (18 conditions) and the best-fitting model was selected using the Akaike Information Criterion.

Lapatinib induced a cytotoxic response in some cell lines (BT474, EFM192) but inhibited the growth of others (HCC1419, HCC1954). Fibroblasts attenuated the effects of lapatinib in some cell lines. Modeling revealed heterogeneous death dynamics in monoculture in which some cell lines exhibited constant (MDA361), increasing (AU565), decreasing (UACC812), or transient (EFM192) death rates. Coculture with fibroblasts altered the death dynamics of some cell lines (AU565: increasing to transient).

Understanding tumor adaptation to therapy may inform timing for alternative or combination therapies to overcome resistance.

Poster No. 32
Cancer Biology Program

Ovarian Cancer–Driven Fibroblast Activation Facilitates Collagen Remodeling and Monocyte Infiltration in the Tumor Microenvironment

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Cancer-associated fibroblasts (CAFs) are critical components of the tumor microenvironment (TME), driving cancer progression through secretion of growth factors, cytokines, and chemokines, as well as remodeling the extracellular matrix (ECM). In ovarian cancer, the mechanisms by which tumor cells reprogram fibroblasts and influence immune cell infiltration remain poorly defined. This study investigates how ovarian cancer cell lines (UPK10, 2F8, ID8 p53 KO) reprogram murine dermal fibroblasts (NIH 3T3) via paracrine signaling, promote ECM remodeling, and modulate monocyte infiltration.

Fibroblasts were co-cultured with ovarian cancer cells using 0.4 μm transwells to allow soluble factor exchange without direct contact. Activation was assessed by immunofluorescence staining for αSMA, FAP, and S100A4, compared against TGF-β–treated positive controls. For ECM remodeling, activated fibroblasts were embedded in 3D collagen droplets (1–2 mg/mL) and monitored over 120 hours for gel contraction and collagen fiber organization using confocal microscopy and NHS Ester staining. Monocyte infiltration assays were conducted by seeding bone marrow–derived murine monocytes atop fibroblast-containing collagen gels and quantifying infiltration at 0, 24, and 48 hours.

Fibroblasts co-cultured with UPK10 exhibited significantly elevated αSMA expression compared to controls ($p < 0.0001$), indicating a strong CAF phenotype, whereas ID8 p53 KO induced minimal αSMA despite promoting fibroblast proliferation. Collagen remodeling correlated with activation status, with UPK10-induced CAFs displaying greater gel contraction and altered collagen organization. Monocyte infiltration mirrored activation patterns, with the highest recruitment observed in UPK10 conditions.

These results demonstrate that ovarian cancer cells differentially reprogram fibroblasts via soluble mediators, producing distinct CAF phenotypes that drive ECM remodeling and immune cell recruitment. Understanding these interactions may identify therapeutic strategies targeting fibroblast activation and tumor–stroma crosstalk in ovarian cancer.

Poster No. 33

Cancer Immunology and Immunotherapy Program

Bacterial translocation following PD1 blockade drives immunotherapy response through microbiome-specific T cell polarization

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Despite improved melanoma outlook following the introduction of anti-PD1 therapy, 50% of patients bear treatment resistant tumors with largely unidentified barriers to resistance. However, recent studies have correlated gut microbiome composition with response to anti-PD1 therapy in melanoma, although the mechanisms behind these correlations remain unclear. Previously, we demonstrated that *Helicobacter hepaticus* (*Hhep*) colonization drives $CD4^+$ T cell dependent anti-tumor immunity in a murine model of colorectal cancer. We have since learned that *Hhep* colonization, when combined anti-PD1, drives anti-tumor immunity in an otherwise resistant melanoma model. We hypothesized that *Hhep* translocation to the tumor fosters anti-tumor immunity via *Hhep*-specific T cell polarization in the presence of anti-PD1.

Interestingly, *Hhep* is only present in tumors of mice treated with both *Hhep* and anti-PD1 and overall intratumoral bacterial burden is increased upon PD1 blockade. Dual treatment results in a significant increase in *Hhep*-specific CD4+ T_{H1} cells intratumorally contrasting lower infiltration of *Hhep*-specific T_{FH} cells, demonstrating a shift not only in quantity but also function. Additionally, direct intratumoral injection of *Hhep* results in colonization within tumors of both *Hhep* and *Hhep* plus anti-PD1 mice, but only *Hhep* plus anti-PD1 mice experience decreased tumor burden, revealing the dependence on anti-PD1 not only for *Hhep* to translocate to and colonize the tumor, but also for successful anti-tumor immunity.

Overall, we have found that combined *Hhep* and PD1 treatment decreased tumor burden, altered the intratumoral *Hhep*-specific T cell population, and promoted translocation of bacteria to the tumor. This model will reveal how checkpoint inhibitors and microbial translocation synergize, which has the potential to drive microbe-targeted therapeutic intervention for treatment resistant tumors in the clinic.

Poster No. 34

S1PR1: Mediator of TCR/MHC Class I Independent Activation of CD8+ T Cell Against Cancer

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T cells play a critical role in the immune system's defense against cancer. CD8+ T cell function is traditionally understood through the lens of TCR recognition of antigens presented on MHC class I. However, there is a growing body of evidence to suggest that CD8+ T cells can function outside of TCR-peptide/MHC recognition. Our lab has found that unstimulated CD8+ T cells are strongly activated and cytotoxic towards chemotherapy-treated, MHC class I null tumor cells. When co-culturing naive CD8+ T cells with chemotherapy-treated, β2M-KO tumor cells, we find that CD8+ T cell activation markers are highly upregulated and correlated with T cell egress receptor, S1PR1. In addition, we find that inhibition of S1PR1 and one of its downstream mediators, PI3K, significantly reduces CD8+ T cell activation and cytotoxicity in our co-culture system. Finally, we verified our results by developing an E8i-Cre S1PR1 conditional knockout mouse model. We find that S1PR1 null CD8+ T cells have substantially reduced activation in co-culture with chemotherapy-treated, β2M-KO tumor cells. These experiments indicate that S1PR1 plays a critical role in the TCR-independent activation of CD8+ T cells against chemotherapy-treated tumor cells.

Poster No. 35
Cancer Virology Program

EBNA1 SUMOylation by PIAS1 Suppresses EBV Lytic Replication and Enhances Episome Maintenance

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Epstein-Barr virus nuclear antigen 1 (EBNA1) is essential for the replication and stable maintenance of the viral episome in infected cells. Here, we identify the SUMO E3 ligase PIAS1 as a key regulator of EBNA1 through site-specific SUMOylation. Our Chromatin-Immunoprecipitation Sequencing (ChIP-seq) analysis revealed that PIAS1 is enriched at the viral origin of plasmid replication (oriP), where it physically associates with EBNA1 and catalyzes its SUMOylation. Using mutational analysis, we identified three lysine residues on EBNA1—K17, K75, and K241—as major SUMOylation sites. Disruption of these sites compromises EBNA1's ability to restrict EBV lytic replication. In addition, both PIAS1 depletion and SUMOylation-deficient EBNA1 lead to reduced retention of EBNA1-OriP-based EBV mini-replicon, indicating the importance of EBNA1 SUMOylation in viral episome maintenance. Together, these results uncover a conserved post-translational mechanism by which PIAS1-mediated SUMOylation modulates EBNA1 function and EBV episome maintenance and suggests a broader role for SUMOylation in viral latency, lytic replication and persistence.

Poster No. 36
Cancer Virology Program

Proteomic screening identifies PHF19 as a caspase substrate and EBV restriction factor

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Epstein-Barr virus (EBV) infects 95% of the human population and contributes to approximately 2% of all human cancers. Our previous studies demonstrated site-specific cleavage of host restriction factors is a new way to promote EBV reactivation from latency. To identify novel EBV restriction factors that are cleaved by caspase, we utilized immunoprecipitation coupled with quantitative mass spectrometry to monitor protein cleavage in Akata (EBV+) cells upon lytic induction. Among over 1000 identified proteins that are cleaved during EBV replication, we focused on polycomb repressive complexes 2 (PRC2) proteins, specifically PHF19, for further functional analysis. PHF19 is responsible for recruiting PRC2 enzymes SUZ12/EZH2 to chromatin to facilitate H3K27 methylation, a marker for transcription repression. Here, using the CRISPR/Cas9 genomic editing method to disrupt PHF19, we found that PHF19 depletion significantly promotes EBV lytic replication. We demonstrated that PHF19 is efficiently cleaved upon lytic induction, and caspase-mediated cleavage separates Tudor and Extended Homology (EH) domains from C-terminal chromo-like domain (CD), providing a way to disable PRC2 and facilitate viral gene expression. We further demonstrated that wild-type PHF19 and a cleavage-resistant mutant suppress EBV replication upon B cell receptor activation. In addition, using co-immunoprecipitation assay, we found that PHF19 interacts with several key viral proteins, including ZTA, RTA, BGLF4, and BXLF1. Together our study identifies PHF19 as a novel caspase substrate and host restriction factor that represses EBV lytic replication. Ongoing study will determine the molecular mechanism by which PHF19 represses EBV replication through chromatin remodeling and/or regulation of viral proteins. We will also explore how PHF19 cleavage by caspase contributes to efficient EBV replication.

Poster No. 37

Sympathetic-Sensory Crosstalk Drives Oral Cancer Pain and Progression

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Solid tumors contain peripheral nerve innervation from both sympathetic and sensory nervous systems responsible for pain and autonomic responses in the tumor. We hypothesize that sympathetic and sensory nerves interact to drive tumor-associated symptoms and impact tumor progression.

We prospectively accrued OSCC patients (69.4±10, 65% male) for the assessment of patient-reported outcomes, circulating catecholamines, and tissue nerve innervation. Reverse translation into a syngeneic orthotopic tongue cancer mouse model (MOC2) was used to understand the underlying mechanism for sympathetic-sensory nerve coupling through behavioral assays, functional Ca2+ imaging and gene expression.

At diagnosis, the majority of OSCC patients reported severe pain associated with oral cancer. Function-evoked pain scores were reported to be higher (79 (5,183)) compared to spontaneous ongoing pain (41 (0,143)). The median circulating NE concentration was 105 (67,294) pg/ng with a significant increase in blood from OSCC patients compared to healthy controls. Spontaneous/ongoing pain positively correlated with NE concentration. Reverse translation using the MOC2 mouse model revealed a similar spontaneous pain and circulating NE phenotype. Evaluation of sympathetic neurons from MOC2 mice revealed transcriptomic plasticity in neurotransmission and signaling genes and increased acetylcholine-evoked Ca2+ responses. In sensory trigeminal neurons, tumor growth induced adrenergic receptor plasticity, marked by increased Adra1 expression and NE-induced excitation (91% vs. 9% responders in cancer vs. sham). Fiber type specific denervation differentially modulated the tumor; sympathetic ablation suppressed tumor growth and spontaneous pain while having no impact on acquired sensory adrenergic sensitivity, while sensory ablation reduced pain and prevented the acquired sympathetic plasticity to attenuate overall sympathetic tone.

Together, these findings suggest that tumor growth induces plasticity in both sympathetic and sensory neurons, promoting bidirectional crosstalk within the tumor microenvironment that contributes to cancer pain and progression. Importantly, patient reported pain and stress may serve as clinical indicators to guide therapeutic strategies and improve outcomes.

Poster No. 38
Cancer Biology Program

Drug-Induced Whole Genome Duplication as a Driver of Drug Resistance in Breast Cancer

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Whole genome duplication (WGD) represents a key adaptive mechanism in breast cancer under therapeutic stress. Using cell cycle mapping, a single-cell proteomic imaging platform integrating multiplexed immunofluorescence with manifold learning, we found that DNA-damaging agents such as Talazoparib and Doxorubicin frequently induce WGD. Polyploid cells arise through mitotic slippage or cytokinesis failure, bypassing normal mitotic completion. These cells subsequently re-enter the cell cycle via distinct polyploid-specific routes—endoreplication or endomitosis—whose selection depends on cyclin/CDK accumulation patterns and genetic context, including impaired p21-mediated checkpoints. This re-entry sustains proliferative potential and contributes to resistance heterogeneity. Importantly, pharmacologic Wee1 inhibition compromises polyploid survival by triggering premature mitotic entry, leading to mitotic catastrophe and enhancing sensitivity to DNA-damaging therapies. These findings highlight WGD as a dynamic and reversible component of cell cycle plasticity, providing both a survival advantage under genotoxic stress and a potential therapeutic vulnerability.

Poster No. 39
Cancer Epidemiology and Prevention Program

HCC-CareAgent: A Pilot Multi-Agentic AI System for the Future of Cancer Care

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Artificial intelligence (AI) has been increasingly adopted in oncology for treatment decision support and cancer care. The recent emergence of generative AI and large language models (LLMs) has enabled the development of AI agents and agentic systems capable of autonomous adaptation, minimal human intervention, and seamless integration with specialized models. These systems can autonomously orchestrate complex, multi-step clinical workflows, a capability well suited to oncology, which inherently requires multidisciplinary expertise and collaboration. Despite this potential, agentic AI systems have yet to be explored in the context of oncology.

In this study, we present HCC-CareAgent, a pilot multi-agent AI system designed to simulate interdisciplinary expertise and collaboration as a prototype framework for future cancer care. The system handles complex, procedure-driven workflows, demonstrated here through a simulated cancer diagnosis and management scenario. HCC-CareAgent comprises: (1) an Orchestrator Agent that coordinates specialized agents for intake, radiology, pathology, surgery, oncology, and surveillance; (2) an Intake Agent that processes inputs from care providers and structured (e.g., demographics, past diagnosis and treatments) and unstructured data (e.g., oncology notes, progress reports, genetic reports) from electronic health records (EHRs); (3) a Radiologist Agent that takes the processed results from Intake Agent and recommends appropriate imaging (X-ray, CT, MRI) and interprets results to characterize tumor location, size, and spread; (4) a Pathologist Agent that determines the need for tissue sampling, confirms diagnosis through histologic review, and orders molecular testing when indicated; (5) a Surgical Agent that combines radiology and pathology data to assess resectability, recommend operative techniques, and advise on achieving optimal margins; (6) an Oncologist Agent that integrates all prior results to stage the disease and develop a guideline-based treatment plan, sequencing surgery, systemic therapy, and radiation; and (7) a Surveillance Agent that develops creates an individualized post-treatment monitoring schedule, detects recurrence early, and re-initiates the workflow when necessary. Each agent's logic is grounded in authoritative clinical practice guidelines (e.g., NCCN, CAP, ASTRO), ensuring alignment with current standards, traceability, and adaptability as guidelines evolve.

HCC-CareAgent was developed using the state-of-the-art Agent Development Kit (ADK) framework and the Agent2Agent (A2A) protocol, enabling secure and scalable inter-agent communication. Each agent is implemented using the open-source LLM LLaMA 3.2 model, deployed locally to ensure security and maintain data privacy. Preliminary evaluation of HCC-CareAgent on a small set of synthetic bone cancer cases demonstrated its potential for future applications in oncology care. Our future work includes expanding collaboration within HCC to incorporate broader expert input and conducting evaluations on real-world patient data across multiple cancer types.

Poster No. 40

Cancer Immunology and Immunotherapy Program

A small but mighty ROR(γt): RORγt supports peripherally induced regulatory T cell function and tumor growth

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Regulatory T cells (Tregs) are often correlated with poor prognosis in cancer. Recently developed Treg-targeted therapies show anti-tumor efficacy, but many patients experience immune-related adverse events due to the loss of systemic tolerance. RORγt+ peripherally-induced Tregs (pTregs) are a microbially-driven population of Tregs in the gut that have independent functions from thymic Tregs; however, mechanisms utilized by pTregs to promote tumor growth are unknown. We hypothesized that RORγt supports pTreg suppressive function and drives tumor growth in colon-
distant sites.

We have shown decreased tumor growth in male *Foxp3*^{CreERT2}*Rorc*$^{fl/fl}$ mice with no signs of detrimental systemic inflammation. We saw a decrease in overall Treg numbers and IL-10 production in the tumor, while CD4 and CD8 T cell infiltration was increased. We also saw stronger effector, tissue retention, and interferon stimulated gene signatures in tumor infiltrating T cells while having no negative effects in peripheral tissues. Interestingly, tumor growth did not differ in female mice, indicating a role for sex in our model. Strikingly, when microbiomes were normalized between males and females, female *Foxp3*^{CreERT2}*Rorc*$^{fl/fl}$ mice harboring a male microbiome showed reduced tumor growth, suggesting that the male *Foxp3*^{CreERT2}*Rorc*$^{fl/fl}$ microbiome is sufficient to induce attenuated tumor growth.

Here, we have shown that targeting pTregs can efficiently promote anti-tumor immunity without leading to systemic inflammation. Additionally, our findings suggest a link between sex, microbiome composition, and anti-tumor immunity at distant sites. Overall, these findings have the potential to identify novel pTreg targets that could enhance immunotherapy efficacy without detrimental toxicities for patients.

Poster No. 41

Loss of FOXD1 Suppresses Wnt Signaling and Enhances Anti-Tumor Immunity in mouse HNSCC model

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Forkhead box D1 (FOXD1) is a transcription factor whose functions have not been elucidated in head and neck squamous cell carcinoma (HNSCC). Based on analysis of TCGA datasets, FOXD1 is among the top 10 most differential survival genes in HNSCC. High FOXD1 expression is significantly correlated with poor survival of HNSCC patients. We propose that FOXD1 may serve as an independent prognostic marker for HNSCC. However, mechanistic basis for tumor-promoting and immune modulatory effects remains unknown. Here, we knocked out FOXD1 gene in murine HNSCC cell line MOC2 and then performed studies to elucidate its role in regulating functions of tumor and tumor immune microenvironment (TME). Bulk RNA sequencing and functional enrichment analysis, cytokine array and immunoblotting results revealed that FOXD1-KO cell lines have reduced expression of genes related to Wnt pathway in comparison to the wild-type (WT) tumor cell line. Prior studies suggest that there is an inverse relationship between tumor intrinsic Wnt signaling and anti-tumor immunity. To investigate the role of FOXD1 in anti-tumor immunity, we injected WT and FOXD1-KO cell lines in C57BL/6 mice and observed that FOXD1-KO tumor grew significantly slower than WT one. Further, mice bearing FOXD1-KO tumors exhibited significantly longer survival than WT tumors. FOXD1-KO tumors exhibited an immune profile consistent with enhanced anti-tumor immunity, including increased $CD4^+$ T-cells, elevated $IFN\gamma^+CD8^+$ and $IFN\gamma^+CD4^+$ T-cells and $IFN\gamma^+TNF\alpha^+$ dual-positive CD8+ and CD4+ T-cells. FOXD1-KO tumors contained reduced immunosuppressive populations such as $CD25^+CD4^+$ T-cells, $CD11b^+$ myeloid cells, and $CD86^-CD206^+$/$MHCII^-CD206^+$ TAMs. Our findings suggest that loss of tumor intrinsic FOXD1 results in not only downregulation of Wnt pathway-related genes but also reprograms the TME towards robust anti-tumor immunity. Thus, targeting FOXD1 may represent a promising therapeutic strategy to enhance anti-tumor immunity in HNSCC. Our future direction includes elucidating whether and how FOXD1 promotes Wnt-driven immune suppression in HNSCC.

Poster No. 42

Cancer Epidemiology and Prevention Program

Automated Extraction of Treatments and RECIST Responses From Lung Cancer Clinical Notes Using a Hybrid NLP System

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Background: Real-world oncology research often requires extracting treatment details and outcomes from unstructured clinical notes, a labor-intensive and error-prone process. We developed and evaluated a natural language processing (NLP) system to automatically identify cancer treatments and link them to RECIST-based response categories—complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD)—in non-small cell lung cancer (NSCLC) clinic notes.

Methods: A retrospective corpus of 250 NSCLC outpatient notes from UPMC Hillman Cancer Center (2017–2021) was annotated by oncologists for treatments and responses. The system combined (1) a rule-based entity extraction module to detect treatments (chemotherapy, immunotherapy, targeted therapy, radiotherapy, surgery) and responses, with negation and context handling, and (2) a machine-learning relation classifier using BioClinicalBERT embeddings and logistic regression to determine treatment–response links. Performance was assessed on a held-out test set (64 notes) and partially validated externally on a Mayo Clinic dataset.

Results: On the UPMC test set, entity extraction achieved macro-averaged precision 0.98, recall 0.81, and F1-score 0.87. Precision was highest for chemotherapy and immunotherapy (≥0.98), with lower recall for surgery (0.45). The relation classifier attained an AUC-ROC of 0.938 and F1-score of 0.92, effectively linking treatments to correct outcomes. External validation for chemotherapy–response relations yielded F1-scores of 0.51–0.64, reflecting annotation schema differences and institutional documentation variation.

Conclusion: This hybrid NLP approach accurately extracts structured treatment–response data from NSCLC clinic notes, enabling efficient real-world evidence generation and reducing manual abstraction burden. The system's high internal performance and moderate cross-institution results demonstrate feasibility and highlight the need for harmonized annotation standards. Integration into electronic health records could support oncologist decision-making and large-scale observational research.

Poster No. 43

Radiation-induced oral mucositis drives pain and sensory neuron plasticity

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Pain during and after radiation therapy (RT) for head and neck cancer (HNC) is a major clinical challenge due to its multifactorial etiology and variable management. Radiation-induced pain typically co-develops with oral mucositis (OM), an inflammatory condition defined as tissue damage to the oral mucosa resulting in highly symptomatic lesions. Preclinical research efforts are needed to develop and evaluate new therapeutic options; however, standardized preclinical RT-induced pain models are severely lacking and the impact of sensory neurons are not considered in OM pathology. Sensory neuron activation underlies the pain associated with OM and may contribute to disease pathology through neuropeptide release and subsequent neurogenic inflammation.

We developed a novel mouse model of RT-induced OM using a single fraction approach. Mucositis development and recovery as well as changes in body condition were clinically tracked in tandem with nociceptive (i.e. pain) behavior and sensory nerve plasticity using real time qPCR for gene expression and immunohistochemistry for protein validation.

Mice that received 15Gy single fraction RT localized to the anterior tongue region showed evidence of hypersalivation and tongue ulcerations as early as post-radiation day (PRD) 6. Peak weight loss (-26±3.4% loss from baseline) and ulceration occurred at PRD 12. Mice demonstrated a 66.1±25.0% increase in spontaneous pain behavior as measured by PainFace grimace assay and a 224.7±54.0% increase in function-induced pain behavior as measured by the Dolognawmeter assay. Lastly, sensory neurons in the mandibular trigeminal nerve branch had increased ATF3 and CGRP protein expression at PRD 12 compared to sham RT indicating increased nerve damage and neurogenic inflammation. While the nerve injury response persisted during OM resolution, nerves also increased Gap43 gene expression at PRD21 suggesting neuronal regeneration during healing. Highlighting the role of sensory neurons in RT-induced pain and OM lays the groundwork for novel prevention and treatment strategies.

Poster No. 44
Genome Stability Program

Investigating noncanonical roles for nucleotide excision repair proteins in repairing 8-oxoGuanine at telomeres

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Telomeres are regions of the genome that are especially sensitive to formation of the the oxidative lesion 8-oxoGuanine (8-oxoG), due to their G-rich sequence. Accumulation of these lesions at the telomeres triggers replicative stress and rapid premature senescence. Typically, 8-oxoG is repaired by base excision repair (BER), which employs polyADP-ribosylation (PARylation) to aid in recruiting repair factors. However, accumulating evidence indicates a role for nucleotide excision repair (NER) protein Xeroderma Pigmentosum C (XPC) in repairing 8-oxoG. Though NER is considered functionally distinct from BER, XPC stimulates the catalytic activity of BER glycosylase OGG1 and XPC deficiency has been linked to increased 8-oxoG lesions. We hypothesized that XPC repairs telomeric 8-oxoG in a PARylation-dependent manner. To investigate this, our lab has developed cell lines expressing a construct which, when exposed to a photosensitizer dye and 660-nm light, induces 8-oxoG exclusively at telomeres. We found that in XPC-deficient cells expressing this construct, the production of telomeric 8-oxoG induces premature senescence via p53 activation; their growth is significantly reduced compared to wild-type and OGG1-deficient cells. These data suggest that XPC stimulates OGG1 in a manner necessary for the timely repair of telomeric 8-oxoG. XPC-deficient cells exhibit an increased general PARylation response after induction, indicating a role in modulating the PAR response to telomeric 8-oxoG. We also asked whether other NER proteins modulate the response to telomeric 8-oxoG and have found that XPA-deficient cells are hyper-sensitive to these lesions. Our evidence indicates a noncanonical role for multiple nucleotide excision repair proteins in the cellular response to 8-oxoGuanine at the telomeres.

Poster No. 45
Cancer Virology Program

Arginine sensor CASTOR1 mediates colon epithelial homeostasis and repair in colitis by regulating interleukin IL-6/STAT3-mediated inflammation

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The mammalian target of rapamycin (mTOR) signaling pathway integrates signals of both environmental and growth factors to regulate cell growth and survival. However, how mTORC1 responds to acute inflammatory signals to regulate bowel homeostasis and repair is poorly studied. Cytosolic arginine sensor for mTORC1 subunit 1 (CASTOR1) is a newly discovered arginine sensor, which negatively regulates mTORC1 activity. In this study, we generated a CASTOR1 knockout mouse model and used it to investigate the role of CASTOR1-mTORC1 pathway in acute colitis. We found that mice with CASTOR1 knockout were resistant to body weight loss, maintained intact intestinal barriers, and showed increased cell proliferation and decreased epithelial apoptosis during dextran sulfate sodium (DSS)-induced intestinal epithelial injury and acute colitis. Mechanistically, CASTOR1 knockout promoted intestinal crypt proliferation and regeneration by inducing interleukin-6-associated reparative inflammation, STAT3 activation. Furthermore, treatment with berberine chloride induced mTORC1 activation and relieved intestinal impairment. Conversely, treatment with rapamycin enhanced the process of acute inflammation in wild type mice. In addition, supplying mouse IL-6 protein showed a superior immunosuppression activity or blocking IL-6/STAT3 signaling by stattic elevated inflammation in DSS indued colitis mice. In addition, we found CD4+ T cells were upregulated in DSS induced WT mice compared with DSS treated CASTOR1 KO mice. Conditional CASTOR1 was lineage specifically deleted with CD4 regulated Cre in T cells. CASTOR1 deficiency in T cells reduced colitis in mice. This was characterized by decreased inflammatory cell infiltration, increased colonic weight-to-length ratio. Together, these findings provided evidence that full body CASTOR1 deficiency and CD4 Cre conditional CASTOR1 KO could prevent and block intestinal inflammatory disorders. Our results indicate that CASTOR1 has protective effects on DSS-induced colitis, and facilitates colon epithelial regeneration by regulating mTORC1 and IL-6-STAT3 pathway, thus providing a new target for the treatment of colitis-related diseases.

Poster No. 46
Cancer Biology Program

Development of a Robust Breast Cancer Liver Metastasis Mouse Model

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Background: Breast cancer (BC) is one of the most common malignant cancers in women and it often metastasizes to the liver. However, breast cancer liver metastasis (BCLM) remains understudied. We previously showed that heme oxygenase-1 (HO-1) depletion in cancer cells limits metastatic outgrowth in a mouse model of BCLM. Since the field of BCLM research is hindered by a lack of robust animal models, we are developing a more reliable mouse model so we can further test the effects of HO-1 inhibition on liver metastasis. To do so, we injected parental mouse mammary carcinoma cells (66Cl-4-luc-ZSgreen) into the portal vein and spleen of mice. The resulting liver metastases were developed into cell lines (MW01 and MW03, respectively). We hypothesized that the MW01 and MW03 cell lines will exhibit increased growth rates, colony-forming abilities, and HO-1 expression than the parental cell line.

Methods: Cell viability and colony growth were assessed in 2D and 3D. HO-1 expression was determined with a Western Blot. The cell viability assay was repeated with Tin Mesoporphyrin (SnMP) and Cobalt Protoporphyrin (CoPP), a HO-1 inhibitor and activator respectively.

Results: MW03 cells were ~50% less viable than 66Cl-4-luc-ZSgreen and MW01 cells. When these cell lines were grown in 3D, the MW03 cells formed the fewest number of colonies, but their colonies size was the largest. Western blot analysis showed that MW03 cells expressed twice as much HO-1 compared to the parentals. Conversely, the MW01 cells had about half of the parental's HO-1 expression. Lastly, out of the three cell lines, the MW01 cells were the most sensitive to SnMP.

Conclusion: The 66Cl-4-ZSgreen and MW01 cell lines exhibited faster growth rates and stronger colony-forming abilities than the MW03 cell line. These results suggest that the MW01 cell line would be a better candidate for further BCLM model development.

Poster No. 47

AI-Driven Virtual Keratin Staining from H&E Slides for Enhanced Diagnostic Efficiency in Vulvar Carcinoma

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Introduction
Keratin immunohistochemistry (IHC) is valuable for identifying epithelial regions in vulvar carcinomas and supporting the development of computational pathology methods, but adds cost, time, and logistical complexity. Virtual staining using artificial intelligence (AI) can generate synthetic IHCs from routine Hematoxylin and Eosin (H&E) whole-slide images (WSIs), reducing the reliance on additional lab processing. This study proposes a deep learning framework for generating keratin-stained images from H&E WSIs, enabling accurate epithelial visualization without physical staining.

Methods
We utilized 35 pairs of WSIs stained with H&E and corresponding IHC keratin stains. Since the H&E and IHC WSIs are obtained from sequential tissue sections, we applied rigid and non-rigid co-registration using DeeperHistReg to align anatomical regions. The registered WSIs were tiled into 256 x 256 patches at 40X magnification and filtered to include ≥ 90% tissue. A Pix2Pix model with a U-Net generator and a PatchGAN discriminator was trained to translate H&E tiles to IHC tiles. The dataset was split into 30 training pairs and 5 test pairs. Performance was evaluated using Fréchet Inception Distance (FID), Structural Similarity Index Measure (SSIM), and Peak Signal-to-Noise Ratio (PSNR).

Results
The model was trained on approximately 13,145 H&E-IHC tile pairs from 30 WSI pairs and evaluated on 2,590 tiles from 5 WSI pairs. On the test set, the model achieved an average FID score of 167.34, SSIM of 0.362, PSNR of 12.01 dB. However, visual inspection revealed signs of mode collapse despite these qualitative results.

Conclusion
Our findings demonstrate the feasibility of using deep learning to generate virtual keratin-stained images directly from H&E slides in vulvar carcinoma. This approach offers a cost-effective alternative to physical IHC staining, preserving key epithelial-stromal structures and potentially streamlining histopathology workflows. Moving forward, we will incorporate additional images and explore alternative generative methods to address the mode collapse.

Poster No. 48
Genome Stability Program

Tyrosine kinase Fgr expression links both hematopoietic- acute radiation syndrome (H-ARS), and late effect radiation-induced pulmonary fibrosis (RIPF)

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Exposure to Ionizing radiation may lead to both to acute and late damages to the tissues. Acute damage to the hematopoietic system is known as the hematopoietic acute radiation syndrome (H-ARS), and the late effect of radiation cause damages to many tissues including radiation-induced pulmonary fibrosis (RIPF). There are FDA approved therapies for H-ARS, however none is known to be also effective for the treatment of RIPF. Hence there is an urgent need for novel medical countermeasures (MCMs) that would target the underlying mechanism shared by both H-ARS, and RIPF. We recently reported that tyrosine kinase Fgr is induced in irradiated mouse lungs in myeloid origin cells and Fgr inhibitor (TL02-59) treatment significantly reduces radiation-induced pulmonary fibrosis (Mukherjee et. al., Cell Death Discovery, 2021, 2023). Here we investigated whether Fgr expression in the bone marrow origin immune cells plays a key role in both acute, and late effects of radiation exposure. We observed that the deletion of Fgr (Fgr-/-) in mice improves survival following both total body irradiation (TBI) that causes H-ARS, and thoracic irradiation which leads to late effect RIPF. For the H-ARS arm of our study, we observed that Fgr knockout bone marrow cells are radioresistant relative to control C57BL/6 BM. In Fgr-/- mice following TBI, BM cellularity in the femur was preserved, pro-hematopoietic growth factors such as G-CSF, and GM-CSF were induced, and proinflammatory proteins such as CXCL5, and CCL2 were suppressed when compared with the BM of control C57BL/6 mice. For the RIPF arm of our study, we have transplanted control GFP positive BM into both control and Fgr-/- mice. Following thoracic irradiation there was significantly less GFP+ macrophage recruitment in the Fgr-/- lungs, and significant reduction in fibrotic, pro-fibrotic, and senescence genes were seen in mice that were transplanted with Fgr-/- bone marrow. Moreover, relative to control senescent lung fibroblasts, Fgr-/- lung fibroblasts released markedly less chemokines. Taken together, we have discovered a unifying mechanism where tyrosine kinase Fgr plays a key deleterious role in the pathogenesis of both H-ARS, and RIPF. Thus, targeting Fgr using small molecule inhibitor can be a valuable therapeutic option to treat both acute and late effects of radiation.

Poster No. 50

Pathway-informed deep learning enhances cancer dependency prediction using multi-source CRISPR screening data

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Precision cancer therapy relies on identifying tumor-specific genetic dependencies, which are genes critical for cancer cell survival. The Cancer Dependency Map (DepMap) datasets from the Broad and Sanger Institutes provide a comprehensive resource for systematically uncovering these dependencies through genome-wide CRISPR screens. These datasets have enabled the development of predictive models for cancer cell lines or tumors that cannot be directly screened. Recent machine and deep learning approaches have utilized gene expression data, with or without the integration of other omics profiles, to predict such dependencies. However, existing methods often fall short in capturing pathway-level interactions among genes. To address these challenges, we present a novel deep learning model that integrates pathway-level information and both DepMap datasets to predict the dependency of individual genes in cancer cell lines. In our approach, each cancer cell line is represented by activity scores across thousands of biological pathways, while each gene is modeled based on its involvement in these pathways. This design enables the model to comprehensively capture functional interactions and dependencies within the cellular context, rather than treating genes in isolation. To further enhance the representation of genes targeted for knockout in the model, we implemented a contrastive learning framework. This framework was pretrained on the Sanger CRISPR screens to classify whether two genes exhibit similar dependency profiles and was subsequently fine-tuned on the Broad CRISPR screens to predict the dependency score of each gene in a specific cell line. The final deep learning model demonstrated strong predictive performance and significantly outperformed baseline configurations that excluded pathway integration or contrastive learning. In summary, our novel deep learning approach improves cancer dependency prediction by leveraging pathway-level insights and integrating multiple DepMap datasets. Ongoing efforts are focused on systematic model interpretation to uncover the underlying mechanisms driving gene dependency.

Poster No. 51
Cancer Biology Program

HGF/MET/TWIST1 signaling promotes brain metastases and is a therapeutic target in lung adenocarcinoma brain metastases

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Lung adenocarcinoma (LUAD) has the highest incidence of brain metastases (BM), which has a poor prognosis and limited treatment options. We previously identified a significant enrichment of MET amplification in LUAD BM compared to primary LUAD. MET, a tyrosine kinase receptor, activated by hepatocyte growth factor (HGF), drives proliferation, epithelial-mesenchymal transition (EMT), and has been implicated in BM. The EMT transcription factor TWIST1 is overexpressed in BM and is required for MET tumorigenesis, leading us to hypothesize that the HGF/MET/TWIST1 axis promotes LUAD BM and is a therapeutic target. Using an ex vivo organotypic brain model, we demonstrated that exogenous HGF promoted brain colonization of MET wildtype (WT) LUAD lines, and a cell line with HGF overexpression (OE) similarly enhanced colonization. In patient-derived MET WT H2073 and paired MET amplified H1993 cells, the MET inhibitor capmatinib reduced colonization in H1993 but not H2073. TWIST1 inhibition with harmine also reduced brain colonization in MET amplified cells. EGFR TKI resistant, MET amplified HCC827R2 cells exhibited greater brain colonization than parental MET WT HCC827 cells and was inhibited by harmine. Furthermore, conditioned medium (CM) from H1993 cells increased astrocyte migration compared to H2073 CM in a MET-dependent manner: capmatinib reduced migration induced by H1993 CM ,while HGF induced migration in H2073 CM. In vivo, HGF OE mice developed BM more frequently and more rapidly than WT SCID mice. In syngeneic models, 40% of the mice injected with HGF OE cells developed BM, whereas none injected with WT cells did. Moreover, HGF OE cells grown in the brain showed a 2-fold faster growth rate compared to WT cells. Finally, capmatinib and harmine eliminated BM, with harmine producing a durable effect lasting over three months post-treatment. Harmine was also effective in MET TKI resistant models. These findings support the HGF/MET/TWIST1 axis as a driver of LUAD BM, identify TWIST1 inhibition as an effective approach in MET TKI-resistant disease, and establish this pathway as a promising therapeutic target in MET amplified LUAD BM.

Poster No. 53
Genome Stability Program

Chromatin compaction by N, N-dimethyldoxorubicin: implications for anthracycline induced toxicity.

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Anthracyclines have been a cornerstone of sarcoma treatment for over 50 years, with efficacy ascribed to DNA-damage via topoisomerase II poisoning. However, treatment responses remain modest, and accrued toxicity from DNA-damage limits lifetime dosage while making continued focus on genotoxic properties less than ideal. Recent studies have highlighted non-genotoxic anthracycline mechanisms, such as DNA minor groove intercalation leading to nucleosome disruption, as promising in sarcomas. These mechanisms have been observed in doxorubicin analogs aclarubicin and N, N-dimethyldoxorubicin, neither of which causes additional DNA-damage.

Doxorubicin itself was recently shown to induce chromatin compaction and subsequent transcriptional disruption independent of nucleosome disruption, a feature not observed in aclarubicin. This raises the question of whether N, N-dimethyldoxorubicin can cause chromatin compaction, suggesting previously unconsidered functional and mechanistic differences amongst anthracycline analogs.

To investigate this, we utilize epigenome profiling techniques, including ATAC-seq and CUT&Tag, to assess chromatin accessibility and targeted epigenetic changes. Additionally, we developed a custom imaging pipeline to quantify nuclear dark space and DAPI intensity as proxies for chromatin compaction. This work seeks to compare the relationship between anthracycline structure, chromatin state, transcriptional disruption, and toxicity with implications for safer sarcoma treatments.

Poster No. 54
Cancer Virology Program

KSHV MTA Modulates ALKBH5-Mediated m6A Dynamics to Promote Lytic Replication

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Kaposi's sarcoma-associated herpesvirus (KSHV) lytic replication is orchestrated by key viral genes, including the replication and transcription activator (RTA, ORF50), which is necessary and sufficient to initiate the lytic program by transactivating downstream viral genes, and the mRNA transcript accumulation protein (MTA, ORF57), which regulates viral transcript splicing, nuclear export, stability, and translation. N6-methyladenosine (m6A), the most prevalent mRNA modification, modulates transcript biogenesis, splicing, export, stability, and translation. Although m6A has been detected in viral and host transcripts of KSHV-infected cells and implicated in viral replication, the specific stages of KSHV replication and the roles of individual m6A regulatory components remain unclear. Here, we investigated the role of the m6A demethylase ALKBH5 in KSHV replication. We found that ALKBH5 expression is downregulated during lytic induction. Pharmacologic inhibition of ALKBH5 using two specific inhibitors, IN-3 and IN-5, enhanced KSHV gene expression and promoted lytic replication. These effects were confirmed by shRNA-mediated knockdown of ALKBH5, supporting its inhibitory role in KSHV lytic reactivation. Mechanistically, we identified an interaction between MTA and ALKBH5 and demonstrated that expression of MTA alone is sufficient to suppress ALKBH5 levels. Ongoing studies are aimed at elucidating how MTA modulates ALKBH5 to enhance the expression of specific viral genes and promote lytic replication. Defining the interplay between ALKBH5 and key lytic genes will refine mechanistic models of KSHV reactivation and may uncover pharmacologically tractable targets to modulate viral reactivation in KSHV-associated cancers.

Poster No. 55

Cancer Immunology and Immunotherapy Program

Targeting *NEBL* via a siRNA-loaded hybrid nanocarrier for advanced non-small cell lung cancer treatment

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Introduction: *NEBL*, a gene encoding protein LASP2, is critically involved in cell adhesion and actin filament architecture. It has recently been identified as an oncogene in non-small cell lung cancer (NSCLC), facilitating tumor metastasis, invasion, and progression. However, the role of *NEBL* in drug resistance and immune modulation remains poorly understood.

DNA hypermethylation significantly influences tumor progression by silencing tumor suppressor genes and immune-related genes. Azacitidine (Aza) is a DNA methyltransferase inhibitor (DNMTi) capable of reversing hypermethylation. However, as a monotherapy, Aza shows limited efficacy in solid tumors. Our RNA-seq analysis on Aza-treated 3LL tumors showed significant upregulation of Nebl following Aza treatment, suggesting that targeting *NEBL* could enhance Aza-based therapies in lung cancer.

Methods: MTT and wound healing assays evaluated the synergy between Aza and siNEBL (NEBL siRNA) in reducing tumor viability and invasion. RNA-seq and Western blot analyses explored mechanisms of siNEBL-induced cytotoxicity. We developed a novel Aza-based prodrug nanocarrier (PLL-PAza) to co-deliver Aza and siNEBL, and evaluated its efficacy in subcutaneous (S.C.) and orthotopic (O.T.) NSCLC mouse models. Immune profiling was conducted via flow cytometry.

Results: MTT and wound healing assays demonstrated effective synergy between Aza and siNEBL, reducing tumor cell viability and invasion. RNA-seq and Western analyses of siNEBL-treated human NSCLC cell line A549 revealed RIPK3/MLKL-dependent necroptosis and significant induction of CXCL10, a chemokine critically involved in T cell trafficking. Our biodistribution data showed that PLL-PAza effectively delivered siRNA to tumor tissues. Codelivery of Aza and siNEBL using PLL-PAza nanocarrier led to significantly enhanced antitumor activity and improved tumor immune microenvironment in both S.C. and O.T. murine cancer models.

Conclusion: Our study reveals *NEBL* upregulation as a new mechanism of resistance in Aza monotherapy of NSCLC. Targeting *NEBL*, combined with Aza, offers a promising new therapeutic strategy for NSCLC, enhancing both efficacy and immune response.

Poster No. 57
Cancer Virology Program

Oral Microbiome and Inferred Functions Predict Kaposi's Sarcoma Progression

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Kaposi's sarcoma (KS), the most common cancer in people with AIDS, is caused by Kaposi's sarcoma-associated herpesvirus (KSHV). Prior studies have linked alterations in the oral microbiome to KSHV infection and KS development, but its role in disease progression remains unclear. To investigate this, we performed 16S rRNA sequencing (V1/V2 and V3/V4 regions) on baseline saliva, peripheral blood, and tumor biopsies collected from 20 KS patients enrolled in the Antiretrovirals in Kaposi Sarcoma study (10 progressive, 10 stable). Clinical measurements, including HIV viral load, KSHV viral load, and CD4 counts, were not significantly different between patients with progressive and stable disease ($p>0.05$, t-test). Among these sample types submitted for 16S rRNA sequencing, the oral microbiome exhibited the highest diversity. Comparative analyses revealed significant microbiome differences in the oral cavity between progressive and stable KS ($p=0.04$, PERMANOVA), whereas tumor and blood samples showed no significant variations ($p=0.91$ and $p=0.86$, respectively). Seventeen oral microbial species were associated with disease progression, compared to only two in tumors and none in blood ($p<0.05$, ALDEx2). Notably, Mogibacterium diversum and Megasphaera micronuciformis, both short-chain fatty acid producers, were enriched in progressive KS (LFC=1.7 and 2.4). The identified differential microbes exhibited taxonomic relationships, notably within the families Prevotellaceae and Lachnospiraceae, where multiple genera and species in these two families were enriched in the oral cavity of patients with progressive KS. Functional pathway prediction using PICRUSt2 identified 39 differentially regulated pathways in the oral microbiome ($p<0.05$, t-test), including those involved in denitrification, ubiquinone metabolism, and arginine biosynthesis, whereas no significant pathway alterations were detected in tumor or blood. These findings suggest a strong association between the oral microbiome and KS progression, potentially mediated by microbial metabolic activity and inflammation. Further studies could elucidate its role in KSHV-induced cancers and identify novel biomarkers or therapeutic targets for KS management.

Poster No. 58

Differential Roles of Glut1 in Treg Cell Function Across Tissue Niches

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Regulatory T cells (Tregs) are essential for maintaining self-tolerance and immune homeostasis. In different tissue environments, Tregs differentiate into diverse subtypes, enabling them to acquire specialized properties and regulate a wide range of immune responses. We and others have reported that glucose metabolic conditions define Tregs with distinct phenotypes and functional properties. Glucose transporter 1 (Glut1) is primarily responsible for regulating glucose uptake in Tregs; however, its specific role in controlling Treg function across different tissue environments remains poorly understood.

Using a specific Glut1-receptor binding domain (Glut1-RBD), we assessed surface Glut1 levels in Tregs from various tissues. We found that surface Glut1 is highly expressed in Tregs from the skin and gut, but relatively low in lymphoid organs. To further investigate the role of Glut1 in Tregs across tissues, we employed Slc2a1flox/flox Foxp3GFP-Cre-ERT2 mice to selectively delete Glut1 in Treg cells following tamoxifen administration.

In B16-F10 and MC38 tumor models, Glut1 deletion in Tregs accelerated tumor growth, accompanied by increased expression of inhibitory molecules and enhanced suppressive activity within the tumor microenvironment. In the experimental autoimmune encephalomyelitis (EAE) model, Glut1-deficient Tregs conferred enhanced protection against CNS autoimmunity. In striking contrast, mice with Glut1-deficient Tregs exhibited increased susceptibility to imiquimod-induced skin inflammation and DSS-induced colitis, characterized by rapid disease onset, reduced Treg infiltration, and impaired suppressive function.
These differential phenotypes across tumor and inflammatory models suggest that Glut1, play context-dependent roles in modulating Treg responses according to their local metabolic environment.

Poster No. 59
Genome Stability Program

Doxorubicin Analogs Promote Histone Eviction Without DNA Damage and Reduce Tumor Growth

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Sarcomas are rare solid tumors derived from mesenchymal lineages. They fall into two categories: bone and soft tissue. Mainstay treatments include surgical resection and chemotherapy. However, with high rates of recurrence and metastasis, patient outcomes remain poor. Clinicians routinely use anthracyclines such as doxorubicin (doxo) in sarcoma therapy but due to toxicity effects such as dose-dependent cardiotoxicity, alternative treatments are needed. The prevailing hypothesis is that doxorubicin acts on the DNA damage pathway through topoisomerase II inhibition; emerging literature suggests DNA damage, via this mechanism, may not be doxorubicin's only major mode of action in sarcoma but rather chromatin disruption via histone eviction. In this study, we used various in vitro sarcoma models and mouse models to assess the efficacy of doxorubicin analog dimethyl-doxorubicin (dime-doxo) to target the chromatin environment without inducing DNA damage. Through immunoblots for γ-H2AX, we observed dimethyl-doxorubicin and aclarubicin induced significantly less DNA damage than doxorubicin without any significant changes in cytotoxicity. CUT&RUN assays revealed significant depletion of H3K27ac in sarcoma cells without significant changes in global H3K27ac levels. In the C57BL/6J and NSG mouse models, doxorubicin and dimethyl-doxorubicin significantly reduced tumor growth, but mice treated with dimethyl-doxorubicin exhibited significantly less weight loss compared to doxorubicin, suggesting dimethyl-doxorubicin may be less toxic. These results demonstrate that dimethyl-doxorubicin can effectively disrupt the chromatin environment without inducing DNA damage and slow tumor growth progression in mice with significantly reduced side effects.

Poster No. 60
Cancer Biology Program

Redox-Mediated HER2 Regulation Reveals a Novel Therapeutic Vulnerability in Anchorage-Independent Ovarian Cancer Cells

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Anchorage-independence (a-i) is mandatory for ovarian cancer (OC) metastasis. During this event, cells adapt to detachment from the primary tumor and disseminate into the peritoneal space. We previously demonstrated that a-i induces rapid upregulation of antioxidant defenses to detachment-related increases in superoxide anions ($O_2^{\cdot-}$), supporting survival following detachment. Superoxide dismutation leads to the production of hydrogen peroxide (H_2O_2), but the consequence of these redox changes in a-i on OC cells remain unknown. Evidence of H_2O_2 at sublethal levels has been shown to serve as a signaling molecule by modulation of cysteine thiol oxidation to aid in pro-metastatic signaling in cancer. Thus, we hypothesize that spatial-temporal regulation of H_2O_2 is important for priming OC cells for metastasis. Using cytosolic and mitochondrial-localized ratiometric, live cell redox sensor HyPer7 with structured illumination microscopy, we find a gradient distribution of H_2O_2 in a-i tumor spheroids, with highest H_2O_2 levels at the periphery and lowest levels at the core. To understand the transcriptomic effect of H_2O_2 in a-i, bulk RNA-sequencing utilizing compartment-targeted H_2O_2 scavengers, cytosolic catalase (CAT) and mitochondrial-targeted catalase (mCAT), revealed redox-dependent changes in *ERBB* signaling. Furthermore, we found that CAT/mCAT overexpression and pharmacological targeting of the $O_2^{\cdot-}$- H_2O_2 axis by mitoquinone (MQ) downregulates protein expression of HER2 in a-i. Consequently, CAT/mCAT overexpression and MQ both exhibit dampened pro-survival signaling via AKT and increased cellular death in a-i. With MQ's previous Phase I clinical trial approval, we found that when used in tandem with HER2-targeted therapy trastuzumab (TZM), further decreases in HER2 expression, signaling, and survival of a-i OC cells are observed. Altogether, our data highlight the importance of spatial redox regulation in a-i survival and how understanding these changes can be used to therapeutically exploit metastatic OC.

Poster No. 61

3D-printed plugs enhance cell usage efficiency for single-cell migration and neuron axon guidance assays

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Metastasis remains the leading cause of breast cancer mortality, yet drug discovery efforts have largely neglected tumor cell migration, a critical early step in dissemination. We developed a high-throughput single-cell migration screening platform that integrates microfluidics, robotic liquid handling, and automated image analysis, enabling the monitoring of hundreds of thousands of individual cells and quantification of migratory heterogeneity. While microfluidics generally reduces reagent and cell usage for high-throughput studies, significant sample loss inevitably occurs due to dead volume at the microfluidic interface, caused by the mismatch between macro-scale liquid handling and micro-scale microfluidic devices. To overcome this challenge, we developed a 3D-printed plug as a meso-scale interface solution that minimizes sample loss and enhances cell usage efficiency, seamlessly connecting microfluidic systems to conventional well plates. The plug concentrates cells near the region of interest for chemotaxis, reducing cell number requirements and featuring tapered structures for efficient manual or robotic liquid handling. Comprehensive testing showed that the plug increased cell usage efficiency in single-cell migration assays by eightfold, maintaining accuracy and sensitivity. We also extended our approach to neuron axon guidance assays, where limited cell availability is a constraint, and observed substantial improvements in assay outcomes. This integration of 3D printing with microfluidics establishes low-loss interfaces for precious samples, advancing the capabilities of microfluidic technology.

Poster No. 62

Molecular and Immunological Characteristics of Pharyngeal Tumors Stratified by Tumor-Immune Proximity and HPV Status

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Human-papillomavirus (HPV)-related head and neck squamous cell carcinoma (HNSCC) is a malignancy with high immunological heterogeneity and limited biologic prognostic biomarkers. The crosstalk, specifically the physical proximity between tumor cells and tumor infiltrating lymphocytes (TILs), is known to regulate all types of HNSCC progression and therapeutic response. HPV-related cancers specifically have high immunologic phenotypes and may have unique spatial relationships between tumor cells and TILs. There is a critical need to understand how HPV viral etiology affects the tumor immune microenvironment and spatial relationships between tumor-immune cells to drive survival, as well as the underlying molecular mechanisms of immune activation. Our group developed an automated, deep-learning-based system for comprehensive spatial analysis of hematoxylin-and-eosin (H&E) slides. We calculated the Tumor:Lymphoid G-cross (Gfx) score for 48 HPV+ and 84 HPV- HNSCC patients with pharyngeal tumors, stratifying these tumors into low, mid, and high-Gfx groups based on their Gfx scores. We then performed bulk and single-cell RNA sequencing to determine the changes in gene expression, pathway activation, and TILs composition between immune-inflamed (high-Gfx) and immune-excluded (low-Gfx) tumors, in both HPV+ and HPV- groups. Our data showed that HPV+ tumors with high-Gfx exhibited elevated expression of stromal remodeling genes (INHBA, AADAC) and increased activation of epithelial–mesenchymal transition (EMT) and tumor necrosis factor-α (TNF-α) signaling pathways. However, HPV- tumors with high-Gfx showed elevated expression of metabolic genes (FBP2, PPP1R1A) and increased activation of oxidative phosphorylation and myogenesis pathways. Moreover, CD8+ and CD4+ populations were higher in HPV+ and high-Gfx tumors, reflecting a canonical virally-inflamed state. In contrast, HPV- tumors with high-Gfx lacked effector lymphocyte enrichment and maintained myeloid dominance regardless of spatial patterns. These findings demonstrate that spatial organization drives distinct transcriptional and immunological states, with HPV status determining whether tumor-immune proximity results in productive immune engagement or persistent exclusion in HNSCC.

Poster No. 63

Cancer Immunology and Immunotherapy Program

Impact of SYK inhibition on the Ovarian Cancer Tumor Microenvironment

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Ovarian Cancer (OvCa) is an aggressive malignancy with poor survival outcomes, driven in part by an immunosuppressive tumor microenvironment (TME). Tumor-associated neutrophils (TANs) and macrophages (TAMs) promote immune tolerance and resistance to immunotherapy, limiting treatment efficacy. We identified tumor-secreted epidermal growth factor-like 6 (EGFL6) as a promoter of an immunosuppressive phenotype in infiltrating myeloid cells. By binding with B3 integrin, Egfl6 induces the activation of spleen tyrosine kinase (SYK) signaling. Spatial transcriptomic analyses of human OvCa tissues revealed a strong correlation between tumor EGFL6 and SYK expression in myeloid cells. Dual immunohistochemistry confirmed an increase in SYK+ macrophages in OvCa compared to normal ovary. In this study, we investigated whether SYK inhibition could reverse Egfl6-dependent reprogramming of myeloid cells. In vitro, the SYK inhibitor R788 (fostamatinib) reduced the proliferation of ovarian tumor cells, as well as myeloid cells, in a dose-dependent manner. In syngeneic OvCa mouse models, administration of R788 significantly improved the survival rate and reduced ascites volume in both control and Egfl6 overexpressing tumors. Flow cytometry and cytokine array analysis showed that R788 decreased the number of TANs and reduced VEGF, M-CSF, and CXCL5 levels in ascites. Moreover, single-cell RNA-sequencing revealed that R788 drastically alters the immune TME, with SYK inhibition associated with a) depletion of specific CCL8+ TAMs and CXCL2+ TANs; and b) reduced number of exhausted TIGIT+CTL4+ CD8 T cells. Next, we sought to investigate whether SYK inhibition could enhance chemotherapy response. Combination therapy of R788 and Paclitaxel moderately improved overall survival compared to R788 and Paclitaxel alone. Ongoing studies are evaluating whether this combination modulates innate immune response. Our findings establish the role of EGFL6 in OvCa progression and identify SYK inhibitor, R788, as a promising therapeutic agent to modulate the anti-tumor immune response in OvCa patients.

Poster No. 64
Cancer Virology Program

Merkel Cell Polyomavirus Small T Antigen Recruits HSP70 to Remodel Large T Helicase for Viral Replication

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Merkel cell polyomavirus (MCV) is the causative agent in ~80% of Merkel cell carcinomas, a highly aggressive skin cancer. The MCV early gene encodes large T antigen (LT), which initiates viral DNA replication by assembling into a head-to-head dodecameric helicase at the viral origin sequence. Another early gene isoform, small T antigen (ST), markedly enhances LT-driven replication. Both LT and ST contain DnaJ domains that interact with the Heat Shock Protein 70 (HSP70) chaperone, but the functional role of this interaction in replication has been unclear. Using single-molecule optical tweezer–fluorescence microscopy (C-Trap), we observed that HSP70 serves as a molecular bridge between ST and LT on origin DNA. This bridging facilitates LT remodeling into replication-competent dodecamers and increases its stability. Mutations in the DnaJ domains of LT (LT.D44N) or ST (ST.D44N) abolish ST-mediated replication enhancement. The ST-HSP70-LT complex is also disrupted by HSP70 inhibitors. Förster resonance energy transfer (FRET) experiments confirm the adjacent binding between labeled ST and LT. Our data suggest that ST recruits HSP70 to LT multimers at the viral origin, remodeling them into active dodecamers. This process diverges from the canonical eukaryotic HSP70 chaperone function, instead resembling the bacterial DnaK–DnaJ–GrpE system, which remodels replication proteins DnaB during bacteriophage λ replication. These findings reveal a role for HSP70-dependent remodeling in eukaryotic replication that may inform novel cellular replication mechanisms.

Poster No. 65

Intragenic Rearrangement Burden Predicts Immunotherapy Benefit in TMB-Low Tumors

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In tumor-mutation-burden–low (TMB-low) cancers, reliable predictors of immune-checkpoint blockade (ICB) benefit remain limited. Building on our prior link between intragenic rearrangement (IGR) burden and T-cell inflammation, we present a clinical-grade IGR model that improves clinical utility and portability. The model (i) applies a coverage-aware normalization that divides IGR counts by the weighted effective length of callable genes to yield a platform-agnostic score, and (ii) restricts estimation to non-recurrent (patient-unique) IGRs to enrich private neo-epitopes. Validated across five independent ICB datasets, the normalized IGR burden outperformed raw IGR counts and conventionally normalized TMB for predicting benefit, improving cross-cohort consistency. In esophageal adenocarcinoma treated with durvalumab, IGR burden computed from RNA-seq–unique structural variants stratified time-to-event outcomes alongside PD-L1. In high-grade serous ovarian cancer receiving neoadjuvant chemotherapy with or without pembrolizumab, IGR burden correlated with cytotoxic T-lymphocyte infiltration and associated with overall survival in the NeoPembrOv study following platinum-based chemotherapy plus pembrolizumab. In triple-negative breast cancer (TNBC), pre-treatment IGR burden predicted radiologic response to chemoimmunotherapy in a phase II metastatic cohort (n=15), whereas in a retrospective UPMC TNBC cohort (n=46; 83% chemotherapy) higher IGR burden tracked with poorer overall survival, supporting a predictive but not purely prognostic role. Collectively, these findings establish non-recurrent IGR burden as a previously under-recognized neoantigen source and demonstrate that weighted-coverage normalization yields a robust, single-assay metric capable of stratifying ICB benefit across diverse TMB-low, IGR-dominant tumors. We are now developing a targeted IGR "hotspot" panel to enable cost-effective assessment from pre-treatment biopsies and to extend precision immunotherapy to patient populations underserved by existing biomarkers.

Poster No. 67
Genome Stability Program

Investigating the mechanisms of immune evasion in ATRX-deficient sarcomas

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Sarcoma is a rare type of cancer that originates from the mesenchymal tissue with more than 100 distinct subtypes. Undifferentiated Pleomorphic Sarcoma (UPS) is an aggressive soft tissue sarcoma accounting for 5–10% of all adult soft tissue sarcoma cases. UPS is clinically heterogeneous, making it challenging to treat with standard chemotherapy. One of the most common genetic alterations in sarcoma is the loss of Alpha Thalassemia/Mental Retardation Syndrome X-linked (ATRX), a chromatin remodeler essential for heterochromatin maintenance. ATRX loss is, observed in ~30% of UPS and Leiomyosarcoma (LMS), associated with poor clinical outcomes in LMS. Preliminary data showed that ATRX-deficient tumors exhibit de-repression of transposable elements (TEs), a known trigger of innate immunity pathways; however, these tumors do not appear to activate innate immune pathways, suggesting a compensatory immune-evasion mechanism. ADAR1, an RNA-editing enzyme, prevents activation of double-stranded RNA (dsRNA) sensors that respond to TE expression. We hypothesized that ATRX-deficient tumors depend on ADAR1-mediated dsRNA editing for immune evasion. Using shRNA- directed ADAR1 knockdown, we show that ATRX- deficient cells are not dependent on ADAR1 activity, pointing to an alternative mechanism. Immunoblotting show that ATRX-deficient cells retain an intact upstream IFNα-JAK-STAT pathway, evidenced by robust phosphorylation of STAT1 upon stimulation with dsRNA mimic. However, these cells induce a limited downstream transcriptional immune response that may be attributed to silencing of key interferon-stimulated genes (ISGs), potentially through epigenetic mechanisms. Furthermore, TCGA-SARC dataset reveals that ATRX-loss tumors have downregulated expression of dsRNA-sensors, like MDA5 and DHX58. Through this ongoing study, we aim to understand this mechanism of immune-evasion in ATRX-deficient sarcomas and to explore novel therapeutic strategies. Future studies will investigate a synergistic therapy by increasing immunogenic dsRNA burden using ADAR1 inhibition combined with epigenetic drugs to reverse ISG silencing to re-sensitize ATRX-deficient sarcomas to innate immune activation.

Poster No. 68
Cancer Therapeutics Program

Context-aware pathway analysis and interpretation using large language models: a pan-disease evaluation and case study in acute myeloid leukemia

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Background: Pathway enrichment analysis is critical for interpreting high-throughput omics data by identifying biological pathways associated with specific conditions. However, interpretation is complicated by the inherent redundancy and interdependence in pathway annotations. Clustering enriched pathways can extract coherent biological themes, yet most existing approaches do not explicitly incorporate the biological context of the experiment, potentially missing context-specific patterns.
Framework development: We present a context-aware framework for clustering and interpreting pathway enrichment results using state-of-the-art large language models (LLMs). The workflow involves: 1) Data acquisition: We retrieved KEGG canonical pathways and their definitions, along with 42 disease contexts including acute myeloid leukemia (AML) and other cancer types. 2) Contextual pathway summarization: For each disease, original KEGG definitions were refined by LLMs to incorporate disease-relevant biological details and enhance contextual specificity. 3) Semantic embedding conversion: Context-enriched pathway summaries were transformed into high-dimensional vector embeddings capturing pathway-level semantics. 4) Similarity-based clustering: Pathways were clustered by embedding similarity to identify pathways with shared contextual relevance. 5) Evaluation: Biological relevance of clusters was assessed using ClusterR2, which quantifies the variance in pathways explained by between-cluster differences. Cross-disease clustering were further compared using Adjusted Rand Index (ARI) and Normalized Mutual Information (NMI).
Results: Across all disease contexts, Cluster R2 were consistently significant ($p<0.01$), indicating that context-enriched pathway definitions and embeddings captured the variance in pathway relevance. Cross-context analysis showed that clustering patterns were more similar among biologically related diseases (e.g. neurodegenerative disorders, hematopoietic cancers, ARI>0.70); unrelated diseases showed markedly lower similarity, reflecting distinct, context-specific pathway relationships. In AML, pathways linked to myeloid differentiation and leukemogenesis formed distinct, high-relevance clusters, revealing context-specific biology absent in baseline methods.
Conclusion: Compared to gene overlap-based pathway clustering and original KEGG descriptions without context, our approach generated clusters with higher internal contextual relevance and improved biological interpretability. These improvements were consistent across the entire dataset, underscoring the utility of integrating LLM-derived context into pathway clustering analyses.

Poster No. 69

Cancer Immunology and Immunotherapy Program

Interrogating the phenotype and function of mature regulatory DCs in solid tumors

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Despite the success of immune checkpoint blockade (ICB), a substantial proportion of patients with solid tumors fail to respond to therapy. Although immunotherapies typically target CD8+ T cells, many systemic and tumor microenvironment (TME) factors influence responsiveness. Recently, a mature regulatory population of dendritic cells (mregDCs) was discovered in both murine models and human non-small cell lung cancer (NSCLC) limiting anti-tumor immunity. This mregDC program represents a unique state adopted by DCs from exposure to TME-derived factors. mregDCs are characterized by a mature phenotype with co-expression of regulatory markers. Although mregDCs have been attributed to poor antitumor immunity, much remains unknown, including the cues driving their formation and whether they can therapeutically be targeted to promote antitumor immunity. To address these questions, we curated an atlas from publicly available single-cell RNA sequencing datasets from NSCLC, head and neck squamous cell carcinoma, and melanoma to evaluate the abundance of mregDCs and whether their transcriptional state is conserved across solid tumors. Across tumor types, we developed transcriptional signatures enabling identification of each DC subset in the TME. The conserved signature of mregDCs revealed previously unrecognized regulators of the mregDC state, including transcription factors that may drive or sustain their phenotype. To begin interrogating whether mregDCs can be therapeutically targeted to promote antitumor immunity, we have data demonstrating that aspects of the mregDC phenotype can be recapitulated in vitro using human monocyte-derived DCs. Overall, better understanding of mregDC phenotype and function across solid tumors will enable new therapeutic strategies to improve antitumor immunity.

Poster No. 70

Next-Generation VH-Fc Fusion Proteins for Mesothelin Targeted Theranostics in Solid Tumors

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Mesothelin (MSLN) surface biomarker is highly expressed in multiple cancers, making it an attractive target for molecular imaging and targeted radionuclide therapy. Full-length antibodies (~150 kDa) have been traditionally used for MSLN-targeted imaging and therapy, but they often limit tumor uptake and penetration. Anti-MSLN VH-Fc (~80 kDa) fusion proteins offer improved tumor uptake, penetration and have optimal pharmacokinetics compared to monoclonal antibodies. However, VH-Fc fusion proteins can bind to Fc receptors on immune cells leading to undesirable sequestration in organs like liver and spleen. We engineered Fc-mutant anti-MSLN VH-Fc fusion proteins incorporating either G236R/L328R (GRLR) or L234A/L235A/P329G (LALAPG) mutations to diminish FcγR binding of VH-Fc fusion proteins. Both mutants retained high purity (>95%) and strong MSLN binding affinity (KD 2.2–3.7 nM) while eliminating FcγR interaction in *ex vivo* and *in vivo* assays. Following radiolabeling with zirconium-89, PET imaging in multiple MSLN-expressing xenograft models revealed that [^{89}Zr]Zr-2A10-VH-Fc$_{LALAPG}$ achieved substantially higher tumor uptake and reduced Fc-organ accumulation compared to wild-type (WT). In HCT116 tumors, LALAPG reached 13.0 ± 0.1 %ID/g at 120 h post-injection versus 4.2 ± 0.6 %ID/g for WT, with liver uptake reduced from 19.8 ± 2.8 to 4.3 ± 0.6 %ID/g and spleen from 95.0 ± 39.3 to 9.3 ± 0.1 %ID/g. High tumor uptake was also observed in AsPC-1 (21.2 ± 8.7 %ID/g), A431-G9 (16.5 ± 4.5 %ID/g), and A431-H9 (11.3 ± 2.4 %ID/g) models. To evaluate therapeutic potential, in a pilot study using alpha-emitter actinium-225 labeled 2A10-VH-Fc$_{WT}$ in HCT116 xenografts we observed extended median survival to 53 days compared to 23 days for vector control (AB6-VH-Fc$_{WT}$). These findings demonstrate that VH-Fc fusion proteins and particularly Fc-engineered LALAPG mutant, achieved high tumor specificity, reduced off-target uptakes. In addition, observed pilot survival benefits highlights strong therapeutic potential supporting their advancement as next-generation agents for targeted theranostics.

Poster No. 71

Do health information seeking behaviors among patients taking oral anticancer medication for multiple myeloma differ by socioeconomic status? An exploratory, qualitative, descriptive study

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Purpose
Multiple myeloma (MM) treatment includes costly, life-long oral anticancer medication (OAM), requiring knowledge for disease and medication self-management. We explored health information seeking behavior (HISB) among these patients and whether HISB themes varied by socioeconomic status (SES).

Significance
HISB contributes to self-management among patients with MM. Lower SES has been associated with less engagement with HISB and trust in health professionals.

Methods
This qualitative, descriptive study is a secondary analysis of semi-structured interviews (n=17) conducted in a larger mixed-methods study. Patients were recruited from cancer centers in Pennsylvania. Participant characteristics were collected via self-report. SES was operationalized using Area Deprivation Index national percentiles, range: 1-100, least to most disadvantaged, dichotomized as low (ADI-L=1-50 [n=8]) and high (ADI-H=51-100 [n=9]). Two reviewers independently analyzed interviews to generate qualitative codes and themes, which were compared by ADI group.

Findings and Interpretations
Most participants in both groups were male (ADI-L n=5, 62.5%; ADI-H n=8, 88.8%) and non-Hispanic white (ADI-L n=7, 87.5%; ADI-H n=5, 55.5%), with median age around 60 (ADI-L range 35-73; ADI-H range 45-80). ADI-L had higher levels of education (n=8, 100% college or beyond) relative to ADI-H (n=5, 55.5% no diploma or no college degree; n=4, 44.4% associate's degree or beyond). Four themes emerged across both groups: desire for information (side effects, disease, funding, emerging treatments); trust in physician as primary source and clarifier of information; passive receival of information (from doctor, pharmacist, or nurse); and mixed confidence in online health information. Two additional themes emerged for the ADI-H group: desire for information from peers and distrust of the physician.

Discussion
Findings reveal HISB similarities between SES groups, while ADI-H included additional themes of desire for information from peers and distrust of the physician. Future larger, diverse, prospective studies are needed to understand HISB to support self-management.

Poster No. 72

Interrogating CD4+ regulatory T cell mediated immunosuppressive modulation of antigen presenting cells in the tumor microenvironment

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Immunotherapy is clinically efficacious, but not all patients benefit. New immunotherapeutic strategies are required to improve cancer patient outcomes. CD4+ regulatory T (Treg) cells in the tumor microenvironment (TME) suppress antitumor immunity via interactions with antigen presenting cells (APCs) and CD8+ T cells. While some Treg-derived mediators of immunosuppression are known (such as IL10 or IL35), a comprehensive analysis of Treg/APC interactions and their consequences is lacking. We hypothesize that an immunosuppressive feedback loop exists whereby Treg cells drive formation of immunosuppressive APCs in response to the inflammatory stimuli in the TME.

To address this hypothesis, we interrogated Treg/APC intercellular communication using three computational methods (CellTalker, CellChat, and NicheNet) in existing head and neck squamous cell carcinoma (HNSCC) and melanoma (MEL) single-cell RNA sequencing datasets. All methods of cell-cell communication inference revealed that Treg-derived colony stimulating factor 1 (CSF-1) was a source for CSF1R on APCs. In the TME, CSF-1-producing Treg cells had transcriptional signatures previously attributed to suppressive function including TNF-family receptors and IFN-signaling related genes. Analysis of spatial transcriptomics data from HNSCC revealed CSF-1-producing Treg cells in proximity with CSF1R-expressing APCs, strengthening the inference these two populations are communicating. Further, NicheNet revealed that CSF1-mediated signaling in APCs drives upregulation of IL-1B, which was identified as a potential regulator of CSF-1 expression in Treg cells by NicheNet. To mechanistically validate the immunosuppressive role of CSF1-producing Treg cells and their responsiveness to IL-1B signaling, we are performing in vitro suppression assays with Treg cells, APCs, and CD8+ T cells and are evaluating the phenotype of APCs and their ability to promote CD8+ T cell activation and proliferation.

Our study has revealed novel insights into immunosuppressive pathways in the TME driven by intercellular communication. Abrogating TME-specific Treg functionality offers the possibility of disrupting an immunosuppressive feedback loop to promote antitumor immunity.

Poster No. 73
Cancer Virology Program

Restoration of host polymerase alpha expression in primary mouse hepatocytes enables HBV cccDNA formation via the intracellular recycling pathway

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HBV fails to establish cccDNA in the livers of HBV transgenic mice or HBV-inoculated hNTCP transgenic mice, posing a major obstacle to developing a convenient mouse model for HBV infection. However, cccDNA formation appears achievable in a mouse hepatocyte-derived cell line AML12, indicating that cellular transformation or immortalization may breach an intrinsic intracellular barrier to cccDNA formation in mouse hepatocytes. In our search for additional mouse cell lines supporting cccDNA formation in the context of Adeno-HBV transduction, we discovered that the FL83B cell line supports a robust cccDNA formation. The successful cccDNA formation in FL83B cells was further confirmed by stable HBV transfection. However, FL83B cells stably expressing huNTCP failed to support de novo cccDNA formation from HBV infection, further highlighting the different mechanisms of cccDNA formation between de novo infection and intracellular recycling pathway. Next, comparative transcriptomic analysis of FL83B cells and primary mouse hepatocytes (PMHs) revealed that mouse polymerase alpha 1 (mPOLA1) expression is absent or extremely low in PMHs. We prioritized mPOLA1 for further study among other differentially expressed genes because its human homolog (POLA1) has been shown to play a key role in the intracellular recycling pathways of cccDNA foramtion in human hepatoma cells. Furthermore, RT-qPCR and western blot analyses confirmed the low levels of mPOLA1 expression in adult mouse hepatocytes and livers. Analysis of the NCBI RNA-seq database for various mouse tissues indicated that mPOLA1 is highly expressed during early embryonic stages but becomes downregulated in the liver during development. Lastly, reconstitution of mPOLA1 expression in Ad-HBV-transduced PMHs enabled cccDNA formation in vitro. Currently, we are developing HBV and mPOLA1 double transgenic mice to evaluate cccDNA formation in vivo.

Poster No. 74
Cancer Biology Program

Mitochontrol: Adaptive mtRNA filtering of single cell RNA sequencing data

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We propose Mitochontrol, a novel preprocessing method for single cell RNA-sequencing data, which performs adaptive quality control at the cell-type resolution to identify and remove compromised cells.
Single cell RNA-sequencing (scRNA-seq) quantitatively measures the gene expression profiles of individual cells by counting mRNA transcripts. This data is acutely vulnerable to noise resulting from artifact reads and unhealthy cells. Extensive preprocessing is performed to ensure data quality. One critical quality control step evaluates cell quality based on the fraction of mitochondrial transcripts (mtRNA). High mtRNA levels often indicate forms of cellular distress such as apoptosis, but mtRNA levels also fluctuate due to metabolic demands, which are closely tied to cell type and tissue of origin. The current standard for mtRNA filtering is arbitrarily chosen fixed thresholds, applied uniformly to the full sample, which disregards the biological diversity of mtRNA levels and results in the unavoidable inclusion of compromised cells and/or exclusion of intact cells. This presents an urgent need for robust, adaptive mtRNA filtering methods which ensure the observed expression profiles accurately reflect the sample's biological state without bias from compromised cells. Existing adaptive methods have failed to appropriately address these concerns in an unbiased and efficient manner.
Mitochontrol addresses these concerns by selecting adaptive thresholds for each independent cell type using data-driven confidence bounds; these bounds are derived by applying online expectation maximization to assign cells to components of a Gaussian Mixture Model. The removed fraction of cells is validated by comparative pathway enrichment analysis, to confirm the removed cells reflect a 'compromised' functional profile.
We demonstrate that Mitochontrol results in higher retention of intact vs compromised cells and significantly reduced bias in cell type retention rates to create a biologically informed mtRNA quality control protocol which removes compromised cells while respecting the biological diversity of mtRNA levels.

Poster No. 75

The Sean Karl Cohort: An International Collaborative Effort to Study the Landscape of Recurrent and Metastatic Ewing Sarcoma

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Ewing sarcoma (EwS) is a fusion oncoprotein-driven bone cancer that demonstrates vast intra- and inter-tumoral heterogeneity. EwS occurs in about 250 children and young people a year in the US, and patients who present with recurrent or metastatic disease have few treatment options and a poor prognosis. How the tumor microenvironment (TME) evolves between primary disease and recurrent or metastatic disease remains poorly understood. Since EwS is a rare cancer, paired patient samples to study TME evolution between primary and recurrent or metastatic disease are infrequent, necessitating collaborative efforts. A better understanding of tumor, stromal, and immune populations in the TME of recurrent or metastatic EwS could lead to identification of novel therapeutic strategies for treatment of this patient population. To address this problem, we have established an international collaborative effort (the Sean Karl cohort) to collect retrospective paired EwS patient tumors and conduct single-cell RNAseq (scRNAseq) analyses to study TME evolution between primary and recurrent or metastatic disease. Individual cells were isolated from FFPE scrolls or slides and prepared for scRNAseq using the GEM-X Flex Gene Expression protocol (10x Genomics). Alex’s Lemonade Stand Foundation Data Lab established a customized data processing and cell annotation workflow to create a harmonized dataset for downstream analysis. To date, we have generated data from 73 specimens, yielding over 340,000 sequenced cells. Four analytic teams will utilize this data to assess tumor and immune cell subpopulations, therapeutic vulnerabilities, and cellular interactions in the TME. To make progress toward improving outcomes for patients with aggressive EwS, a detailed understanding of heterogeneous tumor cell subpopulations and therapeutic vulnerabilities is needed. We have established a multi-institution and multi-analytic approach to facilitate discovery as a first step towards improving such outcomes. In doing so, we are generating the largest, openly shared, and annotated EwS scRNAseq dataset currently available.

Poster No. 76
Biobehavioral Cancer Control Program

Monitoring clinical, functional, and psychosocial changes in adults with hematologic malignancies undergoing CAR T-cell therapy

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CAR T-cell therapy is used to treat adults with relapsed or refractory hematologic malignancies. However, symptom burden is high and can have negative long-term consequences. There is limited evidence surrounding changes in functional capacity and psychosocial health in CAR T recipients. PURPOSE: To assess changes in clinical, functional, and psychosocial outcomes in adults diagnosed with Diffuse Large B-Cell Lymphoma (DLBCL) or Multiple Myeloma scheduled for CAR T-cell therapy. METHODS: Objective physical function and patient-reported quality of life and symptoms were evaluated pre-CAR T infusion, post-discharge, +30-days CAR T, and +90-days CAR T. Results are presented as mean difference ± standard error. RESULTS: Nineteen patients are enrolled- 79% are male, 68% diagnosed with DLBCL, and on average are 65 years old. For the Six-minute Walk Test, the mean distance increased by 32.96±36.53 meters (p= 1.00) from baseline to 90-days post CAR T. For the Timed Up and Go, compared to pre-CAR T, walk-time +90 days decreased by 1.67±0.92 seconds (p=0.47). For the Thirty Second Chair Stand, the number of stands significantly increased by 3.42±1.07 (p=0.02) from baseline to +90 days. Scores on the Short Physical Performance Battery did not significantly change from baseline to +90 days (1.56 ±0.64, p=0.12). We also found that patients walked an average of 130 meters less than age- and disease-matched older adults (438 ± 111 m), which is clinically significant. Although the increase in the Thirty Second Chair Stand was statistically significant, the CAR T population exhibited lower chair stand values when compared to age- and disease-matched patients. CONCLUSION: These results show that although function did show improvement over time, physical function performance remains lower than similar hematologic malignancy populations. More research is needed to determine the best way to ensure continued exercise and how to maintain patients' physical function throughout the CAR T care journey.

Poster No. 77
Genome Stability Program

Elucidating the role of UBL7 in ovarian cancer cell quiescence and the ubiquitin-proteasome system

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Ovarian cancer causes more deaths than any other cancer of the female reproductive system and has a high rate of recurrence after chemotherapy. One relatively unexplored factor underlying cancer recurrence is cellular quiescence. Quiescence is often triggered by reduced nutrient signaling and is a reversible state of cell cycle arrest in which cells maintain a low level of metabolic activity but do not proliferate. Quiescent ovarian cancer cells have recently been isolated from proliferating cells, and expression levels of multiple protein factors involved in the ubiquitin-proteasome system, which maintains cellular protein homeostasis, increase when cells are quiescent. One such gene, UBL7, is upregulated in quiescent cells and contains two domains, a ubiquitin-like (UBL) and a ubiquitin-association (UBA) domain. These domains are present in other proteins that shutte ubiquitinated proteins to the proteasome for degradation. In addition, related proteins have been shown to undergo phase separation, where proteins self-assemble into biomolecular condensates, which impacts their function. However, UBL7 function is largely uncharacterized. We hypothesize that UBL7 plays an important role in ovarian cancer cell quiescence through binding to the proteasome by way of its UBL domain and that phase separation is important for this activity. Our results show that UBL7 co-immunoprecipitates with ubiquitinated proteins, and further experiments are being conducted to identify specific substrates and assess proteasome binding. Additionally, we have created both UBL7 knockout ovarian cancer cell lines to assess how UBL7 affects the rate of cell proliferation in the presence of chemotherapeutics and a GFP tagged version of UBL7 to perform live cell imaging under a variety of stress conditions and quiescence to visualize condensate formation. Investigating the role of UBL7 in quiescence and the ubiquitin-proteasome system will enhance our understanding of how protein quality control pathways impact the cell cycle and advance knowledge surrounding ovarian cancer drug resistance.

Poster No. 78
Cancer Therapeutics Program

Targeting androgen receptor nuclear localization in prostate cancer

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Prostate cancer (PCa) is the most frequently diagnosed cancer and second leading cause of cancer death in American men. More effective therapies for PCa are urgently needed. The androgen receptor (AR) is a key therapeutic target for PCa. AR appears to be overexpressed, stabilized, and nuclear-localized in castration-resistant PCa (CRPC). AR nuclear localization is necessary for its function as a transcription factor. We have identified two closely related pyrroloimidazoles, CPPI and EPPI, which can inhibit AR nuclear localization in CRPC. These small molecules inhibited all tested AR-positive prostate cancer cells, including enzalutamide-resistant CRPC. Further studies suggested that these small molecules can directly bind to AR and enhance AR ubiquitination and degradation in the nucleus, acting as nuclear AR degraders (NARDs). Since CRPC is associated with increased AR level and stability, inhibition or even partial inhibition of AR level may slow down the progression to CRPC. To explore the therapeutic potential of NARD, we have synthesized and characterized novel analogues of CPPI with the goal to identify new lead NARD compounds with submicromolar potency and high specificity for AR-positive PCa cells. We are presenting the preliminary structure-activity relationship in this series. Ongoing studies are aimed at the development of a therapeutic lead compound that slows down the progression of prostate cancer to castration resistance and provides an alternative approach to PROTAC AR degraders.

Poster No. 79
Cancer Biology Program

Loss of Predicted Cell Adhesion Molecule MPZL3 Promotes EMT in Ovarian Cancer

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Myelin protein zero-like 3 (MPZL3) is an immunoglobulin-containing transmembrane protein with predicted cell adhesion molecule function. Loss of 11q23, in which the MPZL3 gene resides, is frequently observed in cancer. Yet the role and consequences of altered MPZL3 expression have not been explored in tumor development and progression. We addressed this in ovarian cancer, in which both MPZL3 amplification and deletions are observed in respective subsets of high-grade serous specimens. Whereas high and low MPZL3-expressing populations are similarly observed in primary ovarian tumors from an independent patient cohort, metastatic omental tumors largely display decreased MPZL3 expression, suggesting that MPZL3 loss is associated with metastatic progression. MPZL3 knockdown leads to an increase in EMT gene expression in OVCAR4 and OVCA433 cell lines, a transcript signature that is associated with poor patient outcomes. MPZL3 promotes homotypic cancer cell adhesion, and decreasing MPZL3 expression enhances invasion and clearance of mesothelial cell monolayers. Conversely, MPZL3 loss abrogates cell-cycle progression and proliferation, with cells adopting senescence features. This was associated with decreased sensitivity to cisplatin and reduced DNA damage and apoptosis in response to treatment in OVCAR4 cells. Our study suggests that decreased expression of the predicted adhesion molecule MPZL3 is associated with low proliferation but increased metastatic potential during ovarian cancer tumor progression.

Poster No. 80

Cancer Immunology and Immunotherapy Program

Chronic TCR signaling rewires mitochondrial metabolism to promote citrate export driving T cell exhaustion

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The efficacy of immunotherapy depends on the presence and persistence of functional immune cells within the tumor. While tumor-specific T cells can be activated and infiltrate the tumor microenvironment, they are progressively rendered dysfunctional by the combination of chronic antigen stimulation and metabolic stress, resulting in an altered differentiation state, termed exhaustion. Exhaustion remains a significant hurdle for immunotherapeutic success. We have shown that the tumor microenvironment represses mitochondrial metabolism and T cell metabolic fitness directly impacts effector function. However, how persistent immunologic signals directly cross talk with mitochondria remains unclear. We observed that exhausted T cells accumulate stored carbon outside the mitochondria in the form of lipid droplets and protein hyperacetylation. We hypothesize that excess carbon is exported from dysfunctional mitochondria to the cytosol, where it is a substrate for both de novo fatty acid synthesis and protein acetylation.

We evaluated the effect of blocking mitochondrial carbon export via the citrate carrier (CIC, encoded by Slc25a1) both in vitro and in vivo. Using CRISPR-Cas9, we deleted Slc25a1 in primary murine OT-I T cells and assessed their metabolic capacity. We also adoptively transferred these cells into ovalbumin-expressing B16OVA tumor bearing mice to evaluate the effect of this gene deletion on antigen-specific T cells in the tumor microenvironment. Interestingly, deletion of Slc25a1 increased both oxidative and glycolytic metabolism and also reduced accumulation stored carbon. Additionally, deletion of Slc25a1 reduced exhaustion and improved tumor control in adoptively transferred, tumor-specific T cells in tumor-bearing hosts. Our results support a model in which mitochondrial citrate export drives accumulation of stored carbon and exhaustion in T cells. Our study provides new insight into the metabolic mechanisms of T cell exhaustion and may inform future immunotherapeutic development, as these pathways may be leveraged to both delay exhaustion or alter the function of pre-existing exhausted T cells.

Poster No. 81
Cancer Virology Program

KSHV LANA Stabilizes METTL16 to Drive Cell Proliferation and Transformation

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Kaposi's sarcoma-associated herpesvirus (KSHV), also known as human herpesvirus 8, is an oncogenic virus linked to multiple human cancers. While KSHV-induced oncogenesis has been extensively studied, the underlying molecular mechanisms remain incompletely understood. METTL16, an RNA methyltransferase that catalyzes N6-methyladenosine (m6A) modifications, regulates RNA splicing, stability, and translation, and plays a key role in gene expression. However, its role in KSHV-mediated oncogenesis has not been explored. Here, we report that METTL16 protein, but not mRNA, is markedly upregulated during KSHV latent infection. Cycloheximide chase assays revealed enhanced METTL16 stability, and proteasome inhibition by MG132 indicated regulation via the ubiquitin-proteasome pathway. Mutational analysis showed that KSHV latency promotes METTL16 ubiquitination through K48- and K63-linked chains. Mechanistically, the KSHV latent protein Latency-Associated Nuclear Antigen (LANA) directly interacts with METTL16 and reduces its ubiquitination, thereby stabilizing METTL16. Functional studies demonstrated that siRNA-mediated METTL16 knockdown inhibited proliferation and cellular transformation, induced G2/M cell cycle arrest, and decreased the S phase population in KSHV-latently infected cells. Moreover, METTL16-specific inhibitors effectively suppressed proliferation and transformation of KSHV-transformed cells. These findings reveal that KSHV LANA hijacks METTL16 to promote cell proliferation and transformation, highlighting METTL16 as a potential therapeutic target and its inhibitors as promising agents for treating KSHV-associated cancers.

Poster No. 82
Cancer Virology Program

Inhibition of PKM2 suppresses hepatitis B virus replication by promoting the phosphorylation-dependent viral polymerase degradation

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Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related death worldwide, with chronic hepatitis B virus (HBV) infection being a major driver. HBV replicates its DNA genome via reverse transcription of an RNA pregenome (pgRNA) within nucleocapsid. Post-translational modifications, such as protein phosphorylation, play critical roles in regulating viral protein stability, localization, and replication. While serine/threonine phosphorylation has been extensively studied in HBV biology, the role of tyrosine phosphorylation remains largely uncharacterized. To address this gap, we screened a tyrosine phosphatase inhibitor library and identified shikonin as a potent inhibitor of HBV replication. Shikonin treatment suppressed HBV pgRNA encapsidation and DNA replication in HBV transiently or stably transfected HepG2 cells without reducing the level of pgRNA. The in vitro endogenous polymerase reaction (EPR) on phosphonoformic acid (PFA)-arrested HBV nucleocapsids further confirmed a defective synthesis of both single-stranded DNA (ssDNA) and relaxed circular DNA (rcDNA) under shikonin treatment, highlighting a suppression in the reverse transcription step of HBV replication cycle. We then assessed the role of pyruvate kinase M2 (PKM2), a known host target of shikonin, in HBV replication. Knockdown of PKM2, or treatment with a more potent and specific PKM2 inhibitor 3K, phenocopied the antiviral phenotype of shikonin. Moreover, knockdown of PKM2 promoted proteasomal degradation of HBV polymerase (Pol). Notably, PKM2 depletion led to increased phosphorylation of HBV Pol, as revealed by Phos-tag gel analysis. This post-translational modification likely serves as a signal for Pol turnover, reducing the functional Pol level and suppressing replication. Additionally, we demonstrated that pyruvate kinase L/R (PKL), a PKM2 homolog highly expressed in primary human hepatocytes (PHHs), plays a similar compensatory role. Silencing PKL in HepG2 cells mimicked the effects of PKM2 knockdown, resulting in reduced HBV Pol level and impaired viral replication. In HBV-infected PHHs, PKL knockdown significantly decreased extracellular HBV genome secretion, further supporting its role in the viral life cycle. Collectively, these findings define a novel PKM2/PKL–HBV Pol regulatory mechanism, demonstrating that tyrosine phosphorylation critically influences HBV Pol stability and replication efficiency with potential implications for treatment of HBV and HBV-related liver cancer.

Poster No. 83

Processed food intake as a factor of response to immune checkpoint inhibitor therapy in advanced melanoma

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Immune checkpoint inhibitors (ICIs) targeting PD-1 and CTLA-4 have improved response and survival rates in advanced melanoma. In melanoma, dual PD-1/CTLA-4 ICIs produce high objective response rates (55%), with significantly improved progression-free survival (34%) and overall survival (49%) at 6.5 years. However, predicting individual outcomes remains challenging.

Multiple predictive biomarkers to ICIs have been described including CD8+ TIL-infiltrate, PD-L1 expression, tumor mutation burden (TMB), HLA class I haplotype and circulating CD8+ T cell immunophenotype. Outside of tumor microenvironment (TME)-centric biomarkers, work by our group and others has demonstrated the role of gut microbiome composition in mediating ICI efficacy and immune-related adverse event (irAE) development in advanced melanoma. Recent data suggests that dietary elements including soluble fiber may mediate ICI outcomes, possibly via gut microbiome composition. We aimed to relate dietary ultra-processed food intake to ICI outcomes using DHQIII, a web-based dietary recall questionnaire.

Dietary data was collected using DHQIII on 114 patients receiving ICI therapy for advanced melanoma. Using the NOVA classification system, all food types were categorized by processing level, and the percentage of gram intake was used to determine each patient's level of ultra-processed food (UPF) intake.

We observed that high UPF intake (defined using in-cohort quartiles) was associated with lower overall response rate (ORR) ($p = 0.034$) and poorer progression-free survival (PFS) (45.36 (Q1) vs. 20.95 (Q4) months, log rank $p = 0.023$) in ICI-treated advanced melanoma patients.

This supports our hypothesis that a high UPF diet will lead to poorer outcomes to immune therapy. Inflammation, a known driver of response to ICI therapy, is well documented to be increased in diets containing large amounts of highly processed foods. Further exploration will include metagenomic analysis of the gut microbiome and proteomic analysis of patients classified by ultra processed food intake. This will allow us to define the biological differences in patients driving the differences in response.

Poster No. 84
Cancer Biology Program

RHOV is a Detachment-Responsive Rho GTPase Necessary for Early Metastatic Reprogramming in Ovarian Cancer

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Ovarian cancer spreads primarily via transcoelomic metastasis, where cells disseminate into the peritoneal fluid, resist anoikis, and form multicellular aggregates that invade the peritoneum. This tumor progression represents the main driver of morbidity and mortality for ovarian cancer patients. The earliest adaptations necessary for metastasizing ovarian cancer cells to survive matrix detachment and initiate transcoelomic metastasis remain poorly defined. In this study we identify a conserved detachment-sensitive gene signature activated shortly after matrix-detachment across multiple ascites-derived ovarian cancer cell lines that was subsequently confirmed in patient-derived samples. Within this signature, RHOV, an atypical and constitutively active Rho GTPase, emerged as a top transcript. Notably, RHOV expression was enriched in omental metastases compared to matched primary tumors. Loss of RHOV impairs anoikis resistance, multicellular aggregate integrity, migration and invasion, and completely abolishes metastasis in vivo. Mechanistically, RHOV exerts these effects through integrating c-Jun dependent cytoskeletal remodeling to support pro-metastatic signaling. Rescue experiments show that both GTP-binding and membrane localization are required for RHOV's pro-metastatic function. Together, these findings define RHOV as a unique detachment-sensitive Rho GTPase and for the first time establish RHOV as a critical and necessary coordinator of early adaptations that prime ovarian cancer cells for metastatic progression. This work provides key insights into the molecular vulnerabilities of disseminating tumor cells, establishes the targeting of early molecular adaptations following matrix detachment as a potential new therapeutic strategy for metastatic disease, and uncovers new functions for an understudied member of the Rho GTPase family.

Poster No. 85

Fatigue, sleep disturbance, and anxiety over the first year of breast cancer treatment

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Introduction:
Breast cancer (BC) is the leading cancer diagnosis in women with up to 90% of women still reporting any symptoms more than a year after diagnosis. BC patients identify cancer-related fatigue as the most difficult persistent symptom with up to 30% of patients still reporting severe fatigue one year after diagnosis. Understanding how fatigue, anxiety, and sleep disturbance are experienced can give insight into how to target interventions to prevent and mitigate persistent symptoms.

Methods:
This secondary analysis used longitudinal data collected in a prospective study that examined the genetic underpinnings of nausea and vomiting in early-stage BC patients. Participants provided informed consent before initial BC surgery. Participants completed the Patient-Reported Outcomes Measurement Information System (PROMIS®29) Questionnaire after hospital discharge, weekly during active treatment, and monthly otherwise up to 12 months. Group-based trajectory modeling (GBTM) identified distinct temporal patterns for fatigue, sleep disturbance, and anxiety during the first year of follow-up.

Results:
Participants (n=295) had a mean age of 60.2 (SD=11.82) years. Using GBTM, we identified three distinct fatigue trajectories: mild/linearly decreasing (28.5%), mild/flat (55.6%), and moderate/flat (15.8%). Three flat distinct sleep disturbance trajectories were revealed: low (12.2%), mild (44.8%), and moderate (43.0%). Anxiety also had three distinct trajectories: low/quadratically decreasing (28.2%), mild/quadratically decreasing (43.5%), and moderate/flat (28.3%). Of the 276 participants having grouping information across all three symptoms were classified, 73 (26.4%) were classified into the same group with 20.5%, 46.6%, and 32.9% belonging to groups 1, 2, and 3, respectively; 171 (62%) had partial overlap, sharing two group assignments.

Conclusions:
Participants in this study maintained or decreased in symptom severity over the first year of treatment. Notably, those with higher symptom severity were less likely to experience symptom relief over time. Patients should be screened early and often, and if symptoms are reported, begin interventions earlier.

Poster No. 86

Investigating Stromal Mitochondrial Changes in High-Grade Serous Ovarian Carcinoma

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Background: Ovarian cancer (OC) is recognized as the deadliest gynecologic cancer due to late diagnosis and early metastasis. High-grade serous ovarian carcinoma (HGSOC) is the most common OC subtype, with over 70% of patients presenting with metastatic disease at diagnosis. The stromal tumor microenvironment (TME) plays a vital role in OC progression. Our lab focuses on a specific stromal cell population within the TME: mesenchymal stem cells (MSCs). We have shown that cancer-associated MSCs (CA-MSCs) promote OC growth, metastasis, and resistance to therapy. Our lab also showed that CA-MSCs have more total mitochondria compared to normal MSCs (nMSCs) and this is important for their pro-tumorigenic function. This study explores the metabolic changes in CA-MSCs.

Method: (1) RNA-seq of 14 nMSCs and 17 CAMSCs with differential expression analysis (DE) and pathway gene set enrichment analysis (GSEA) using R-Studio's DESeq2 and clusterProfiler packages, as well as the Broad Institute's GSEA software, to identify significant genes involved in metabolic function.
(2) To validate the RNA-Seq data, reverse transcription quantitative PCR (RT-qPCR) and western blotting were used.

Results: RNA seq analysis identified 9 genes of interest: CYP24A1, PDK4, TSPO, MTFR2, DNA2, CRLS1, SQOR, SUGCT, and CD36. Multiple rounds of RT-qPCR on different cell lines verified the upregulation of PDK4, CYP24A1, and TSPO and downregulation of MTFR2 and DNA2 in CA-MSCs versus nMSCs. Interestingly, the validated CA-MSCs upregulated genes are associated with increased cellular energy production through metabolic shift from glucose utilization towards fatty acid oxidation. Further, Western blot validation showed that ATP5a (a subunit of the ATP synthase complex) is upregulated in CA-MSC vs nMSCs.

Conclusion: CA-MSCs use different energy production pathways to support cancer cell growth. These transcriptional changes lead to increased mitochondrial mass and enhanced oxidative phosphorylation (OXPHOS), enabling the cells to generate more ATP, which, in turn, promotes OC progression.

Poster No. 87

Cancer Immunology and Immunotherapy Program

FOLFIRONOX and Gemcitabine treatment alters formation and activity of tertiary lymphoid structures in pancreatic ductal adenocarcinoma patients

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Pancreatic ductal adenocarcinoma (PDAC) is the third-leading cause of cancer mortality in the United States, with a 5-year survival rate of 13%. For patients with locally advanced PDAC, neoadjuvant chemotherapy with FOLFIRONOX (FFX) or Gemcitabine is first-line treatment with the goal of increasing resectability. Recent studies have shown tertiary lymphoid structures (TLS), ectopic aggregations of lymphocytes within tumor and non-tumor tissue, are associated with improved clinical outcomes in PDAC. There is less understanding, however, of how the spatial and transcriptomic features of TLS correlate with neoadjuvant therapy response, particularly between two different first-line chemotherapies, FFX and Gemcitabine. Using QuPath, machine learning software for digital pathology image analysis, we analyzed multispectral stained tumor tissue samples from n=67 PDAC patients. Using markers for CD4, CD20, and CD21, we were able to assess the composition and state of TLS, with a particular focus on the presence of CD21+ follicular dendritic cells (fDC), which mark the formation of germinal centers (GCs). We assessed TLS activity by measuring Ki67 for proliferating lymphocytes and AID for B cells undergoing somatic hypermutation within the TLS. GC formation is often associated with more active TLS and improved survival but is not abundant in human tumors. If less active TLS are present, they can still contribute to B cell education through extrafollicular differentiation, an alternate route of B cell activation that doesn't require GC education as B cells will differentiate to antibody secreting cells (ASC) depending on the inflammatory cues within patient tumors.

Using AI training models and custom scripts, immune aggregates were identified, immunophenotyped at single-cell resolution, and classified as a lymphoid aggregate, TLS, or TLS with GC based on composition. Patient TLS profiles were analyzed by comparing structure count, proximity to tumor, and the density, activity, and proliferation of T and B cells. Clinical correlates included overall survival, change in tumor size and CA19-9 levels, and recurrence. Preliminary analysis reveals that FFX patients had a better response and harbored a greater number of total immune aggregates. Gemcitabine patients had a higher number of GCs but still demonstrated less AID activity than the FFX cohort. To investigate whether B cells in the extrafollicular pathway were ultimately associated with a better response in patients with low TLS activity, we isolated healthy donor naïve B cells and differentiated them in extrafollicular conditions in media collected from treatment naïve, FFX or Gemcitabine PDAC patient derived organoids. B cell differentiation to ASCs was assessed by spectral cytometry. Testing the regulatory dynamics of the extrafollicular response in vitro could uncover new immunotherapeutic targets that would augment TLS function in PDAC patients. Ultimately, this work will aid us in understanding how standard of care neoadjuvant chemotherapies modulate B cell education within and outside of TLS.

Poster No. 88
Cancer Virology Program

Extrachromosomal circular DNA (eccDNA)-producing gene MAD1L1 identified in HBV-HCC cells disrupts HBV pregenomic RNA encapsidation

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Hepatitis B virus (HBV) is a major etiologic agent for primary hepatocellular carcinoma (HCC) globally. Recent interest in extrachromosomal circular DNA (eccDNA) as oncogenic entities prompted us to evaluate the eccDNA landscape in healthy primary human hepatocytes (PHH), HBV-infected PHHs, and HBV-related HCC cell lines (Hep3B and PLC/PRF/5). Our analysis identified several eccDNA-producing genes, notably the mitotic arrest-deficient-1-like 1 (MAD1L1) gene. Quantitative PCR revealed a negative correlation between MAD1L1 eccDNA abundance and its mRNA expression in HBV-HCC lines, supporting fragile-site-driven eccDNA formation. Endogenous levels of MAD1L1 in transiently and stably transfected hepatoma cells were also decreased at the mRNA level, suggesting that HBV replication can downregulate MAD1L1 expression. Thus, we hypothesized that MAD1L1 regulates HBV replication and is likely involved in HCC development. Overexpression of host MAD1L1 leads to a consistent decrease in HBV capsid-associated (core) DNA in HBV transiently and stably transfected hepatoma cells. This reduction corresponded to decreased encapsidation of HBV pregenomic RNA (pgRNA), without affecting total HBV RNA levels or capsid protein abundance, indicating a targeted disruption at or beyond pgRNA encapsidation. The precise component(s) and step(s) of HBV pgRNA encapsidation targeted by MAD1L1 are currently under investigation. Additionally, RNA-seq data from 21 paired HBV-HCC tissues (GSE94660) revealed significant upregulation of MAD2L1, the primary downstream effector of MAD1L1 involved in spindle assembly checkpoint (SAC) regulation. Collectively, our findings establish a potential connection between eccDNA formation, the DNA damage stress response, and HBV-driven genomic instability in cancer cells. This work provides foundational insights for future research exploring the interplay between eccDNA, HBV pathogenesis, and the mechanisms underlying genomic instability in HCC.

Poster No. 89

Cancer Immunology and Immunotherapy Program

IFN-γ$^+$ Tregs Drive Trm-Mediated Toxicity and Resistance in Cancer Immunotherapy

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Background: Immune checkpoint inhibitors (ICIs) have revolutionized cancer therapy, yet treatment resistance and immune-related adverse events (irAEs) in responders remain significant clinical challenges, and preclinical models remain limited.
Methods: To uncover mechanistic links between irAEs and ICI failure, we developed a novel murine model—Lifestyle-Induced Susceptibility to irAEs (LISA), which integrates diet–driven metabolic and microbial alterations to simulate human susceptibility to irAEs upon anti–PD-1 therapy. This model faithfully recapitulates human disease in tissues impacted, susceptibility, severity, and treatment response.
Results: scRNA sequencing and flow cytometry analyses revealed tissue-specific expansion of inflammatory CD4$^+$ and CD8$^+$ tissue-resident memory T cells (Trms), marked by increased T-bet and Eomes expression, and a concurrent loss of reparative Trm subsets across impacted tissues, including the colon, liver, and skin. In contrast, tumors from the same mice exhibited a reciprocal immune profile, with reduction in cytotoxic Trms. These inflammatory signatures were strongly associated with an expansion of IFN-γ$^+$ regulatory T cells (Tregs), which drove both systemic irAEs and reduced ICI efficacy.
Dietary intervention restored Treg stability and prevented Trm reprogramming, thereby ameliorating both toxicity and therapeutic failure. Surprisingly, anti-LAG3 combination therapy enhanced both inflammatory and reparative Trms alongside IFN-γ$^+$ Tregs, leading to improved tumor control without further aggravation of irAE symptoms. Finally, targeted deletion of IFN-γ or IFN-γ signaling specifically in Tregs (via IfngL/LFoxp3cre-YFP or Ifngr1L/LFoxp3cre-YFP) restored reparative Trms, suppressed inflammatory subsets, and significantly improved ICI response while reducing irAE incidence.
Conclusions: Our study reveals a novel immunoregulatory axis wherein IFN-γ–producing Tregs modulate tissue-resident T cell fate, thereby linking immune toxicity and resistance in ICI-treated hosts. These findings highlight the need for therapeutic strategies that balance Treg function and tissue inflammation to optimize immunotherapy outcomes.

Poster No. 91

Cancer Immunology and Immunotherapy Program

Geospatial Analysis of Public Health Disparities in Immune Checkpoint Inhibitor treated Advanced Melanoma

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Immunotherapies including immune checkpoint inhibitors (ICIs) and chimeric antigen receptor T cell (CAR-T) produce durable response in a subset of patients with solid tumors and hematologic malignancies. However, not all patients respond, and many responses are not durable. Disparities due to socioeconomic status (SES) impact development of cancer and cancer treatment outcomes including of ICI therapy. Prior studies have linked poor ICI outcomes to SES, with mixed results at the county level, and there is a need to investigate social determinants of health (SDoH) at a more granular level. Our study aimed to ICI outcomes between SES groups at the census tract level.

We calculated Area Deprivation Index (ADI) derived from the American Community Survey (ACS) data, determined impact of ADI upon outcomes of ICI and CAR-T in advanced cancer patients, and geographically tied the outcomes of advanced cancer to SES factors of deprivation. Using the Area Deprivation Index (ADI), patients were classified as “Low ADI/High SES” or “High ADI/Low SES”. High ADI/Low SES patients had poorer overall survival in ICI-treated melanoma (log rank $p = 0.07$) and CAR-T treated leukemia/lymphoma (log rank $p = 0.094$). Metagenomic analyses demonstrated that gut microbial α-diversity was directly associated with High ADI/Low SES (Inverse Simpson Index $p = 0.02$). Utilizing GIS mapping from the US Census Bureau, we observed that poorer survival was linked with High ADI/Low SES census tracts (Moran’s $I = 0.739$, I test p value = 0.001)

Our findings link High ADI/Low SES with poorer outcomes to ICI and CAR-T therapy. This may be linked to ADI-mediated changes in gut microbiome composition. Further research aims to uncover the link between diet-driven, gut microbiome-mediated impacts of SES status upon immunotherapy outcomes in southwestern Pennsylvania.

Poster No. 93

Cancer Immunology and Immunotherapy Program

Inhaled Microplastics Impair Macrophage Metabolism and Function

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Rationale: Microplastics are ubiquitous pollutants which have been observed in human lung tissue, sputum, and bronchoalveolar lavage. Yet, the effects of microplastics on the lung, and the pulmonary immune system in particular, remain undefined. Studies have identified plastic laden macrophages within plaques removed after carotid endarterectomy which correlated with a higher risk for myocardial infarction, stroke, and all cause mortality. In human precision-cut lung slices plastics are most often associated with pulmonary macrophages, which are incapable of degrading and clearing these particles. We hypothesized that inhaled microplastics cause both long-lasting pulmonary macrophage dysfunction as well as extra-pulmonary deposition with systemic effects. Methods: Macrophage cell lines (Raw264.7) and human monocyte-derived macrophages (MDMs) were cultured in vitro with fluorescent polystyrene microplastics of sizes 0.02µm, 0.1µm, 1.0µm, 4.0µm, and 10µm at various ratios and phagolysosomal processing was determined by immunofluorescence microscopy. Mitochondrial mass was quantified using MitoTracker, mitochondrial membrane polarization assessed with JC-1 staining, and cellular metabolism was measured by Seahorse assay. Macrophage antigen processing and presentation was determined via OVA stimulation of DO11.10 T cells. Female and male FVB/N mice were intranasally exposed to 1.0µm microplastics and extrapulmonary dissemination of microplastics in tissues was detected by flow cytometry and immunohistochemistry. Results: Exposure to any size microplastic reduced macrophage phagocytosis at 24hrs. Larger microplastics (10µm) inhibited lysosomal processing of E.coli by 24hrs whereas smaller plastics (1µm) reducing processing at 3 days post-exposure. Phagocytosed microplastics were found to amass in the late (Rab7+) phagosome but were largely absent from either the early (Rab5a+) phagosome or the lysosome (LAMP-1+). Metabolic analysis showed a reduce mitochondrial mass and loss of mitochondrial membrane polarization and a shift from oxidative phosphorylation toward glycolysis in microplastic-exposed macrophages. Microplastic exposure of Raw264.7 cells cultured with OVA protein or peptide resulted in a concentration-dependent decrease in T cell stimulation, suggesting impaired antigen processing and presentation, respectively. Following installation in mice, microplastics were detected in the lung, brain, liver, kidney, heart, and colon for up to 7-days after respiratory exposure. Conclusions: These findings indicate that microplastics can directly disrupt the Macrophages metabolism and inhibit the ability of macrophages to clear particulate matter, process antigens, and stimulate T cells. Findings suggest that microplastic-induced pulmonary macrophage dysfunction may lead to increase susceptibility to infection, chronic tissue damage, and ultimately lung cancer.

Poster No. 95

Cancer Immunology and Immunotherapy Program

Identification of a spatially resolved cellular neighborhood in HNSCC that limits response to immunotherapy

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Immunotherapy (IO) has transformed treatment for Head and Neck Squamous Cell Carcinoma (HNSCC), yet many patients do not benefit. While single-cell RNA sequencing (scRNAseq) has advanced understanding of tumor, stromal, and immune cell states, the spatial organization of the tumor microenvironment (TME) remains poorly defined. We hypothesize that a distinct cellular neighborhood at the tumor margin contributes to immune exclusion and IO resistance in HNSCC. To investigate this, we performed spatial transcriptomics (ST) on a tumor microarray (TMA) of 20 treatment-naïve HNSCC patients using Bruker's CosMx platform. We integrated a previously generated scRNAseq atlas with the ST data to enable robust cell identification. Cell neighborhoods were identified by performing k-means clustering on a matrix containing the proportion of cell types within 300 micrometers of a given cell. We identified six spatially organized cellular neighborhoods. Neighborhood 5 (N5) represented the tumor core and contained exhausted CD8+ T cells. Notably, Neighborhood 3 (N3), located at the tumor-stromal interface, displayed immunosuppressive signatures (e.g., TGFβ signaling) and extracellular matrix remodeling (e.g., integrin, collagen pathways), suggesting this neighborhood plays a role in immune exclusion. Cox regression analysis showed that a high enrichment of a signature derived from N3 correlated with worse outcomes. Interestingly, exhausted CD8+ T cells were highly enriched in N5, suggesting that adjacent N3 may restrict access to tumor antigens and prevent response to IO. To further study N3, we developed 3D spheroids with HNSCC tumor cells, fibroblasts, endothelial cells, and donor immune cells to model immune infiltration. This in vitro platform enables exploration of mechanisms driving immune exclusion and testing strategies to enhance immune cell infiltration. In conclusion, ST revealed a spatially distinct neighborhood (N3) that impedes immune infiltration and IO efficacy. Modeling N3 in vitro provides a foundation for interventions to improve immunotherapy responsiveness in HNSCC and other immune-excluded tumors.

Poster No. 96

Depletion of exhausted TIL and anti-tumor responses

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Exhausted $CD8^+$ T cells (Tex) accumulate in the tumor microenvironment (TME) after persistent antigen exposure and immunosuppressive conditions such as hypoxia drive effector T cell differentiation. Tex have limited effector functions, including reduced cytokine production, diminished cytotoxicity, and impaired proliferative capacity. Gene set enrichment analyses show that tumor-infiltrating Tex share transcriptional features with regulatory $CD4^+$ T cells (Tregs), including expression of CD39. Tex suppress antitumor responses through CD39-mediated adenosine production, in cooperation with CD73 on other cell types, a mechanism also utilized by Tregs. Depletion of Tregs in tumors enhances antitumor immunity and reduces tumor burden. We propose that Tex also play a suppressive role in the TME and that their removal will lead to enhanced immune activity. T cell exhaustion progresses from a $PD\text{-}1^+Tim\text{-}3^-$ progenitor state to a $PD\text{-}1^+Tim\text{-}3^+$ terminally exhausted state. Progenitor Tex retain responsiveness to PD-1 blockade, while terminally exhausted cells do not. We expect that depletion of the terminally exhausted subset will also enhance the response of the progenitor subset to PD-1 blockade in the absence of Tex suppressive activity.

Poster No. 97
Genome Stability Program

Evaluating a Chemokine-Modulatory Regimen Targeting Type-I Interferon Signaling, Toll-like Receptor 3, and the Prostanoid System as an Immunotherapy Adjuvant for Head and Neck Squamous Cell Carcinoma

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Head and neck squamous cell carcinomas (HNSCC) often exhibit a 'cold' tumor microenvironment (TME) with limited effector immune cell infiltration and poor responses to immune checkpoint inhibitors. To promote desirable immune cell migration, we are utilizing a chemokine-modulatory (CKM) regimen consisting of rintatolimod (Toll-like receptor 3 agonist), interferon-alpha 2b, and celecoxib (selective COX-2 inhibitor). CKM has shown promise in triple negative breast cancer clinical trials by inducing chemokines that bind CXCR3 and CCR5 – receptors found on cytotoxic T lymphocytes (CTLs), Th1 and NK cells – leading to enhanced effector immune cell trafficking into tumors.

Our current work focuses on evaluating mRNA expression of chemokines in HPV-negative and HPV-positive human and murine HNSCC cell lines treated for 24 hours with rintatolimod (125 ug/ml), interferon-alpha 2b (1000 U/ml), and/or celecoxib (10 uM) as single agents or in combination. Preliminary data in HPV-negative MOC1 cells show that the three-drug combination significantly increases expression of CXCL10 (>85-fold vs. vehicle, $p<0.01$) and CXCL9 (>50-fold vs. vehicle, $p<0.01$). CCL5 expression was also significantly increased by rintatolimod plus celecoxib (>25-fold vs. vehicle, $p<0.01$) or triple therapy (>30-fold vs. vehicle, $p<0.01$). In addition to expanding these studies to other cell lines, we are optimizing tumor explant models to evaluate effects of the CKM regimen, given that other cells within the tumor microenvironment contribute to chemokine production. Next steps will also include immune cell migration assays utilizing supernatant from tumor cells and explants treated with the CKM regimen and in vivo studies combining the CKM regimen with anti-PD-1 therapy in immunotherapy-resistant mouse models of HNSCC.

Modulating the chemokine landscape in HNSCC represents a promising strategy to promote influx of effector immune cells into the tumor microenvironment. By enhancing favorable effector chemokines, our goal is to amplify anti-tumor immunity and improve responses to immunotherapy in both HPV-positive and HPV-negative HNSCC.

Poster No. 98

Exploring the Impact of Lymph Node Environment on Exhausted T cells

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T cell exhaustion is a major barrier in cancer immunotherapies. In cancer, exhaustion occurs after T cells enter the tumor microenvironment (TME), are exposed to prolonged antigen stimulation, hypoxia, and a multitude of immunosuppressive factors known to contribute to T cell dysfunction. These cells can no longer mount an effective immune response, as they exhibit reduced cytokine production, increased expression of inhibitory markers, and diminished trafficking. With the loss of proper trafficking, exhausted T cells (Tex) become confined within the TME, where they continue to face immunosuppressive factors. In contrast, during acute infections, after a T cell has fought off its cognate antigen, it traffics back to the lymph node, which may help promote survival, renewal, and reinvigoration. The lymph node serves as a critical site for T cell homeostasis and function. It provides a supportive environment that aids in maintaining T cell survival and function through cytokines such as IL-7. Moreover, the lymph node lacks chronic antigen exposure, thereby preventing further exhaustion of T cells. This unique environment is essential for maintaining T cell efficacy. Our studies will address gaps in our understanding of T cell exhaustion and the impact of the lymph node's environment on Tex's phenotype. The results of this project have the potential to significantly influence the future of immunotherapy by providing new insights into the mechanisms that drive effective immune responses. Ultimately, the knowledge gained through these studies may guide the design of future immunotherapies, leading to improved outcomes for cancer patients.

Poster No. 99

Biobehavioral Cancer Control Program

Pre-radiotherapy multidisciplinary survivorship care and patient-reported outcomes in head and neck cancer survivors

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Purpose: This study aims to evaluate the association between receiving pre-radiotherapy multidisciplinary survivorship care and patient-reported outcomes measures (PROMs) pertaining to quality of life (QOL), symptom burden, and psychological distress at one-year post-radiotherapy among head and neck cancer (HNC) survivors.

Methods: Survivors who underwent radiotherapy from 2017–2022 and completed PROMs during their one-year post-radiotherapy visit at a multidisciplinary HNC survivorship clinic were included. Survivors with recurrent disease, second primary tumor, and/or distant metastasis were excluded. Differences in PROMs between propensity score-matched survivors who did and did not have a pre-radiotherapy visit were analyzed using multivariable regression models controlling for covariates.

Results: 310 survivors were included (mean [SD] age, 61.09 [9.58] years; 238 [76.8%] male; 159 [51.3%] pre-radiotherapy visit; 163 [52.6%] oropharyngeal; 161 [54.9%] early T stage; 159 [51.5%] early N stage). Compared to survivors without a pre-radiotherapy visit, survivors with a pre-radiotherapy visit had higher physical (+ 7.26 points, 95% CI [3.35, 11.18], $p < 0.001$) and social-emotional (+ 5.93 points, 95% CI [1.58, 10.29], $p = 0.008$) QOL scores and lower depression (-1.31 points, 95% CI [-2.61, -0.01], $p = 0.048$), anxiety (-1.18 points, 95% CI [-2.23, -0.13], $p = 0.027$), dysphagia (-3.77 points, 95% CI [-6.36, -1.19], $p = 0.004$), insomnia (-2.76 points, 95% CI [-4.61, -0.92], $p = 0.004$), and neck disability (-2.28 points, 95% CI [-4.41, -0.16], $p = 0.035$) scores one-year post-radiotherapy.

Conclusions: Receiving pre-radiotherapy multidisciplinary survivorship care was associated with higher QOL and lower symptom burden and psychological distress among HNC survivors. These findings support implementing proactive survivorship care in clinical practice to improve health outcomes in HNC.

Poster No. 100

Large language model embeddings enable quantitative gene function representation and hypothesis testing

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Accurate representation of gene function is essential for interpreting high-throughput genomic data. Large language models (LLMs) offer a scalable and adaptable alternative to traditional gene set-based functional annotation methods, with potential to uncover novel biological insights. However, their capability to quantitatively capture functional relationships remains largely unexplored. In this study, we systematically evaluate embeddings of genes and biological functions derived from seven state-of-the-art LLM models to assess their biological relevance, specificity, and interpretability. We benchmark these models on their ability to capture gene-gene functional similarities and gene-function relationships across diverse biological contexts. Beyond evaluation, we demonstrate applications in two real-world scenarios: (1) hypothesis testing where predefined gene sets are lacking, including newly characterized biological pathways and drug mechanisms; and (2) statistical assessment of novel hypotheses derived from LLM-inferred gene functions. By establishing a rigorous evaluation framework, this study highlights the potential of LLM-derived embeddings for scalable, quantitative gene function discovery.

Poster No. 101
Cancer Biology Program

Targeting Osteosarcoma with Disulfiram and Enzalutamide: Functional Outcomes and Drug Synergy Evaluation

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Introduction: Osteosarcoma is the most common primary bone tumor. Its aggressive nature and limited treatment options, particularly for metastatic disease, has contributed to decades of poor outcomes for patients. This study aimed to investigate the effects of enzalutamide, an androgen receptor antagonist, and disulfiram, a clinically approved anti-alcoholism drug with anticancer properties, on the primary osteosarcoma cell line SaOS-2 and its metastatic subclone LM-2 cells.

Methods: We performed a series of in vitro assays including cytotoxicity (WST-1), scratch migration, clonogenic survival, and IncuCyte-based growth curve analysis to evaluate drug efficacy and cellular responses. Cells were treated with disulfiram and enzalutamide either alone or in combination with one another, testing a series of dilutions.

Results: Significantly reduced viability was achieved in both cell lines with disulfiram and combination treatment compared to vehicle controls. Both cell lines showed a significant decrease in colony-forming ability when treated with disulfiram or combination therapies. Furthermore, the reduction in clonogenic survival was significant for both combination and disulfiram monotherapy groups but were not significantly different from one another. Growth curve analyses further supported these findings. Both disulfiram-treated and combination-treated cells displayed significantly reduced confluence over time. In contrast, enzalutamide-treated cells demonstrated growth patterns similar to vehicle controls in all assays tested. Scratch migration assays demonstrated no significant differences in migration rates across any treatment groups compared to vehicle controls.

Conclusions: Overall, our results suggest that disulfiram exhibits potent anticancer activity against osteosarcoma cells, both as monotherapy and in combination with enzalutamide. However, while additive effects were observed when both drugs were used together, disulfiram emerged as the primary driver of these responses, as there was no significant difference between disulfiram monotherapy and the combination treatment. These findings support further investigation of disulfiram as a potential therapeutic agent in osteosarcoma treatment strategies.

Poster No. 102

Surgical drain fluid as a novel liquid biopsy source for post-operative staging in PDAC

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Introduction: Pancreatic ductal adenocarcinoma (PDAC) is the most common type of pancreatic cancer, with poor outcomes partly due to late diagnosis and early early recurrence post-operatively. Blood-based liquid biopsy can predict progression, recurrence, and survival, but sensitivity in early-stage PDAC remains low (34–56%). To address the low sensitivity and specificity, we proposed utilizing surgical drain fluid as a novel liquid biopsy source, as its proximity to the resected tumor and tumor microenvironment may yield higher concentrations of tumor-derived nucleic acids and immune markers. We hypothesize that it could provide a more accurate and sensitive method for patient staging than blood-based assays.

Methods: In a prospective cohort of PDAC patients who underwent curative-intent surgical resection with pancreatectomy, paired surgical drain fluid and plasma samples were collected on post-operative day 1. Total cell-free DNA (cfDNA) concentration were measured via Qubit, fragment size distribution was measured via TapeStation, and KRAS G12D MAF was measured via ddPCR.

Results: Drain fluid contained an average of ~200-fold higher cfDNA concentrations than plasma, with ctDNA detectable in all drain fluid samples but only in 50% of matched plasma samples. Both plasma and drain fluid nucleic acid fragments were predominantly short (96–162 bp), consistent with cfDNA. The patient with the earliest recurrence exhibited the highest cfDNA concentration, with detectable KRAS G12D ctDNA in drain fluid but had no detectable ctDNA in plasma.

Conclusion: Surgical drain fluid is a rich source of tumor-derived nucleic acids, with greater DNA yield and higher ctDNA detection compared to plasma. These preliminary results suggest drain fluid liquid biopsy could substantially improve sensitivity for post-operative PDAC staging and recurrence prediction, meriting validation in larger cohorts. Future directions include increasing sample size, and additional characterization of immune-cell populations and determining if neutrophil-extracellular traps contribute to high cfDNA concentrations.

Poster No. 103
Cancer Therapeutics Program

Characterizing Tumor–Stroma Interfaces in Chemotherapy-Treated Ovarian Cancer via Spatial Transcriptomics

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The tumor–stroma interface (TSI) is a critical microenvironmental niche where cancer cells interact with surrounding non-malignant cells, influencing therapeutic resistance and disease progression. However, few studies fully characterize this region at the transcriptomic level, its interactions with tumor cells, and how these interactions contribute to differential treatment outcomes. This gap limits our ability to link microenvironmental features to patient outcomes in a reproducible and scalable manner.
We developed an integrative framework to identify and characterize TSI regions in high-grade serous ovarian cancer (HGSOC) using spatial transcriptomics (10x Visium). In this framework, tumor spots are detected at single-spot resolution with CancerFinder, followed by spatial graph analysis to define TSI regions. We first applied this framework to a post-chemotherapy dataset generated by Elena Denisenko et al. (n = 8; 3 good responders, 2 partial responders, and 3 poor responders) and identified four major clusters present in both good and poor responder groups. Pathway-level profiling revealed that one fibroblast-rich cluster formed a distinct extracellular matrix (ECM) barrier between tumor and normal regions, visible as a clear boundary on H&E staining and enriched for collagen organization and focal adhesion pathways. Another cluster, characterized by a high proportion of ribosomal genes, suggested a stress-response phenotype potentially associated with treatment resistance; this cluster was more prevalent among poor responders (>85%).
These findings highlight the functional heterogeneity of tumor–stroma interfaces and their potential role in modulating treatment response. By combining spot-level tumor detection, spatial graph-based interface mapping, and pathway-level characterization, our approach provides a scalable strategy to bridge histopathology and molecular profiling. This framework may enable the discovery of spatially informed biomarkers for predicting therapeutic outcomes in ovarian cancer and beyond. Our ongoing work extends to additional cohorts and therapeutic modalities, including immunotherapies, to yield broader translational insights.

Poster No. 104
Cancer Virology Program

Transferring Phenotype Labels from Histology to Spatial Transcriptomics via Virtual Staining and Deep Learning

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Background
Spatial transcriptomics offers molecular resolution with spatial context but lacks morphological detail, whereas hematoxylin and eosin (H&E) images capture tissue architecture without molecular information. We present ST2HE, a method to generate virtual H&E images directly from spatial transcriptomic data, enabling phenotype annotation using existing H&E-based resources without requiring unstained tissue images.

Methods
Spatial transcriptomic spots were overlaid on DAPI-stained images to create 512x512 patches with consistent gene sets and no cross-patch transcript sharing. A pix2pix model was trained on paired Xenium spatial transcriptomic and H&E data to synthesize virtual H&E images, followed by phenotype labeling with a pre-trained H&E classifier.

Results
In breast cancer subtype transfer (Xenium), virtual H&E images achieved an AUC of 81.3% compared to labels from real H&E images. In lung cancer subtype transfer (NanoString NSCLC), the Xenium-trained model virtually stained NanoString data, achieving an AUC of 96.2% for transferring pathologist annotations between stacks, despite differing gene panels and platforms. In Kaposi Sarcoma generalization, an unconditioned ST2HE model trained without skin tissue generated biologically meaningful H&E images for an in-house Xenium KS dataset. Virtual images reflected stage-associated spindle cell patterns and tumor architecture despite the model never encountering skin morphology during training. A stage classifier trained on real H&E achieved moderate tile-level AUCs (0.68–0.84) due to domain shift and morphological heterogeneity, but core-level pooling improved performance to 0.91–0.97 across stages.

Conclusions
ST2HE enables accurate phenotype transfer from histology to spatial transcriptomics in a platform-agnostic manner and generalizes to unseen tissue types. By integrating molecular and morphological features, virtual H&E images enhance interpretability, enable cross-platform and cross-tissue annotation, and broaden the applicability of spatial transcriptomics in precision oncology.

Poster No. 105

Protein Detection through Covalent Fluorophore Transfer via Aptamers

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Aptamers are single stranded oligonucleotides with target specificity that rival antibodies. Their low cost, chemical synthesis, lack of immunogenicity, easy modification, and high batch-to-batch consistency make them attractive alternatives to antibodies for applications such as cancer diagnostics and drug delivery. Despite these strengths, the field has been constrained by inherent limitations such as rapid in vivo clearance, susceptibility to nuclease degradation, short target engagement time, and potential off-target interactions. We have addressed these limitations by applying ligand-directed chemistry which allows for covalent modifications of a protein via electrophilic warhead installation on aptamers. Our method utilizes an aptamer to bring a cleavable electrophile bearing a selected payload into the proximity of nucleophilic amino acid residues on a specific target protein, resulting in the covalent transfer of the payload to a target with high specificity.

Previously, we applied this technology to label thrombin, a protein important for blood clotting, and showed fast and selective covalent label transfer. We then extended this novel approach to cell surface proteins such as PTK7, a cancer biomarker, to track expression and localization, and to deliver protein and small molecule cargos into cells.

Despite the emergence of covalent aptamers as a promising chemical tool, limitations persist in terms of limit of detection when compared to antibodies, in this study we evaluate the transfer of fluorophores for the detection of native proteins with high sensitivity toward the goal of replacing antibodies in research and diagnostic applications.

Poster No. 106

Synthesis and Characterization of Antibody Adaptors for Universal Chimeric Antigen Receptors

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Chimeric antigen receptor (CAR) T cells and antibody-drug conjugates (ADCs) are two powerful therapeutic modalities; however, the current antibody conjugates and antibody adaptors are heterogeneous mixtures with varying drug loads. Traditional antibody conjugation methods include the formation of covalent bonds to either lysine or cysteine residues, which are difficult to control with regard to site-specificity and stoichiometry. Advancements in site-specific conjugation techniques have allowed us to develop homogenous adaptors for a universal CAR system based on the SNAPtag self-labeling enzyme and benzylguanine (BG)-conjugated antibodies. Here, we are developing enzyme-mediated conjugation techniques to site-specifically conjugate a BG molecule to an antibody, resulting in defined and reproducible modification. We chose to use microbial transglutaminase (mTGase) as the enzyme in the antibody conjugation reactions, where we can site-specifically install two BG molecules to an antibody and then analyze the modification using protein mass spectrometry and reactivity toward SNAPtag. Site-specific antibody conjugates for trastuzumab were tested for in vitro activity and showed antigen-dependent and dose-dependent T cell activation for each antibody-antigen pair. We also generated an ADC adaptor by conjugating BG to the FDA-approved ADC trastuzumab emtansine (Kadcyla), targeting the HER2 antigen that is overexpressed on breast cancer tumor cells. The drug to antibody ratio (DAR) for both the DM1 and BG drug loads were determined by protein mass spectrometry. SNAP-CAR T cells co-incubated with the ADC-BG adaptors showed dose-responsive T cell activation and enhanced target cell lysis. In a human ovarian tumor xenograft mouse model, the ADC-BG adaptors showed enhanced anti-tumor activity. The implementation and advancement of site-specific antibody conjugation will improve SNAP-CAR T cell activation in response to antigen recognition.

Poster No. 107
Genome Stability Program

De-repression of immune pathway stimulating transposable elements with epigenetic drugs to enhance antitumor immunity in ATRX-deficient sarcomas

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ATRX, a member of the SWI/SNF chromatin remodeling complexes, plays a major role in epigenetically silencing genes. Undifferentiated Pleiomorphic Sarcoma (UPS) and Leiomyosarcoma (LMS) are two of the most frequent ATRX deficient sarcoma subtypes (20-30%). Clinical trials with immune checkpoint inhibitors (ICI) involving these two sarcoma subtypes have elicited mixed or poor response rates (i.e., ~25% in UPS), thus better therapies are needed. One such intervention is the idea of “epigenetic priming” with epigenome altering drugs where we anticipate the synergy with ATRX deficiency will drive immune response targeted cancer killing.
A growing interest in immunotherapy of cancer is the stimulation of immune response pathways via de-repressed transposable elements (TEs). These aberrant transcripts and possible encoded proteins could serve as predictive biomarkers and potential drug targets. When we knocked out ATRX in mouse and UPS cell lines, TEs were de-repressed including at ATRX-mediated H3K9me3 silenced regions. We treated UPS with multiple drugs and found that the DNA methyltransferase inhibitor, 5-azacitidine (already known to increase TE expression in cancers), and a HDAC inhibitor, chidamide, both induced expression of TEs. In addition, the drugs increased expression of downstream dsRNA sensing immune activation genes, such as MHC-1. RNA sequencing highlighted both an increase and decrease in TE expression after treatment; however it appears to be independent of the ATRX status. Additionally, pathway analysis of enriched differential genes involved with immune response, such as IFNα stimulated responses, also appear to be altered independent of ATRX. In conclusion, TE expression can be further altered in ATRX deficient sarcoma through treatment with epigenetic targeting molecules. More work remains to characterize these findings in ATRX deficient LMS, characterize changes in immune stimulating pathways, and identify additional epigenetic alterations in treated/non-treated ATRX deficient sarcomas that could additionally be responsible for antitumor immunity.

Poster No. 108

ADAM17 Inhibition as a Novel Strategy to Improve the Delivery of Anticancer Nanoparticles to CD44+ Cancer Cells

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Targeted Nanoparticles (NPs) are widely used in cancer therapy, enabling drug delivery to tumors and limiting off-target effects. CD44, an overexpressed receptor in many cancers, is a promising target for tumor-specific NPs; however, their clinical application has faced significant challenges. Continuous cleavage of CD44 by the metalloproteinase ADAM17, limits the delivery efficiency of targeted NPs. This study investigates the effect of ADAM17 inhibition on the tumor-specific delivery of CD44-targeted Hyaluronic acid (HA)-coated NPs, aiming to enhance their tumor specificity and therapeutic potential.

Our data show that pharmacological and genetic inhibition of ADAM17 significantly reduced CD44 cleavage in multiple cancer cell lines. Next, CD44-targeted HA-coated liposomes were optimized by adjusting HA/PEG ratios for improved targeting. Confocal imaging and flow cytometry showed that ADAM17 inhibition increased the uptake of HA-liposomes into CD44 wild-type (WT) cells, with no effect in CD44 knockout (KO) cells, confirming that the increased uptake was attributed to decreased cleavage of CD44. In 3D tumor spheroids, ADAM17 inhibition improved nanoparticle penetration into the spheroid core. In vivo, mice pretreated with ADAM17 inhibitor showed higher tumor accumulation and deeper intratumoral distribution of liposomes. Confocal and flow cytometry data further indicated enhanced nanoparticle migration away from blood vessels following ADAM17 inhibition.

While receptor-targeted nanoparticles have been widely explored in cancer therapy, little is known regarding the impact of receptor cleavage on the efficiency of delivery. This study addressed how ADAM17 inhibition decreases CD44 cleavage, preserves surface CD44 and thereby enhances the delivery efficiency of CD44 targeted nanoparticles. These findings define a practical strategy to enhance tumor-specific drug delivery. More broadly, they establish a modular approach to improve the efficacy of nanoparticle cancer therapies by increasing on-target accumulation and reducing off-target toxicity.

Poster No. 109
Cancer Virology Program

Spatial Single-Cell Transcriptomics Uncovers KSHV-Driven Remodeling of the Tumor Microenvironment in Kaposi's Sarcoma

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Kaposi's sarcoma (KS), a tumor caused by Kaposi's sarcoma-associated herpesvirus (KSHV), is marked by chronic inflammation, aberrant angiogenesis, and a complex, heterogeneous tumor microenvironment (TME). To investigate the cellular and spatial dynamics of KS progression, we employed spatial single-cell transcriptomics on 256 tissue samples from 43 KS patients—representing the full clinical spectrum (patch, plaque, nodular lesions)—alongside three normal controls.Our analysis revealed a dynamic interplay between KSHV-infected and uninfected cells in the TME, with significant shifts in cell type composition, gene expression, and intercellular communication as the disease progresses. High levels of lytic viral gene expression were detected, including viral interleukin-6 (vIL6), a key driver of inflammation and angiogenesis. CD34+ progenitor lymphatic endothelial cells (LECs) were identified as the primary targets of KSHV infection, and their clonal expansion dorve tumor growth. KSHV infection induced extensive cellular reprogramming across multiple cell types—including LECs, vascular endothelial cells, fibroblasts, and macrophages—leading to hybrid phenotypes that support tumor progression. In particular, KSHV+ macrophages were shown to promote inflammation, neoangiogenesis, and immune modulation, thereby facilitating tumor growth and immune evasion. Additionally, KSHV infection enhanced endothelial plasticity, neoangiogenesis, and immune modulation, reinforcing a pro-inflammatory, pro-angiogenic TME. Spatial analysis revealed distinct tumor-associated niches that evolve with disease stage, forming a core-to-periphery gradient linked to viral load, immune modulation, and cellular remodeling. These spatial patterns underscore the importance of microenvironmental context in KS pathogenesis. By leveraging spatially resolved single-cell data, we identified molecular programs predictive of disease progression and potential therapeutic targets. Our findings provide new insights into the cellular mechanisms of KSHV-driven tumorigenesis and support novel therapeutic strategies aimed at reprogramming the TME to restore immune function and limit KS progression.

Poster No. 110

Leveraging the Musculoskeletal Oncology Tumor Registry and Tissue Bank (MOTOR) to Drive Innovation in Sarcoma Research

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Introduction: Sarcomas are rare cancers with significant unmet clinical need. Their histological diversity and geographic dispersion make specimen acquisition difficult, limiting research opportunities and resulting in an underrepresentation in biobanks. To address this, the Musculoskeletal Oncology Tumor Registry and Tissue Bank (MOTOR) was established to collect and annotate sarcoma samples for collaborative research.

Methods: MOTOR is a tissue bank collecting fresh tumor tissue, blood, and clinical data from patients with musculoskeletal system malignancies such as sarcomas or bone metastasis. Specimens are processed into multiple formats, including cryopreserved, flash frozen, and OCT samples of tumor and normal tissue, as well as plasma and buffy coat that are processed and stored long term. The registry includes 43 distinct sarcoma subtypes. All samples are annotated with demographic and clinical information and continuously updated as new clinical information emerges.

Results: Currently, MOTOR houses 10,039 tumor, 1,253 buffy coat, and 4,121 plasma samples from 907 patients. The registry includes a diverse collection of sarcoma samples, with the highest representation from Sarcomas 'Not Otherwise Specified' (1,932 samples), demonstrating their histological diversity. Liposarcoma (1,554 samples) is the second most common subtype, followed by myxofibrosarcoma (1,545 samples), osteosarcoma (1,445 samples), chondrosarcoma (1,406 samples), leiomyosarcoma (1,105 samples), and Soft Tissue Sarcoma, Not Otherwise Specified (730 samples). Less common subsets include undifferentiated pleomorphic sarcoma (543 samples), rhabdomyosarcoma (430 samples), and Ewing sarcoma (376 samples), demonstrating their rarity and the importance of having such scarce samples available to collaborators. In addition, serial collections from 192 patients enable disease progression studies.

Conclusions: MOTOR serves as a critical resource for musculoskeletal oncology research by addressing the shortage of high-quality, annotated rare cancer specimens. MOTOR has enabled numerous domestic and international collaborations, advancing sarcoma research through publications, deeper insights into disease biology, and contributions to drug development and repurposing.

Poster No. 111

Cancer Immunology and Immunotherapy Program

Tributyrin Enhances PD-1 Blockade via Immune Modulation: A Preclinical Strategy to Improve Cancer Immunotherapy

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Immunotherapy has revolutionized cancer treatment, offering significant promise for durable responses in various malignancies. However, the therapeutic efficacy of immune checkpoint inhibitors (ICIs), such as PD-1 inhibitors, remains limited to a subset of patients, with response rates varying widely across different cancer types. Additionally, significant toxicity is often associated with combination therapies, leading to challenges in optimizing treatment regimens and limiting their broader clinical applicability. These limitations underscore the need for novel strategies that can enhance the effectiveness of ICIs while minimizing adverse effects. To address these challenges, we propose a novel strategy to enhance ICI efficacy while minimizing toxicity through the use of tributyrin, a prodrug of butyrate. Tributyrin delivers butyrate systemically, overcoming its limited bioavailability and modulating the gut microbiome to boost immune responses. Butyrate has been shown to increase the activity of CD8+ T cells, crucial effectors of antitumor immunity, and to favorably modulate the tumor microenvironment (TME) by promoting immune activation. Additionally, emerging evidence links the gut microbiome, particularly butyrate-producing bacteria, to improved responses to immune checkpoint inhibitors (ICIs). Specific gut microbiota, such as butyrate producers, can boost anti-tumor immunity by modulating immune cells, activating T cells, and shaping the tumor microenvironment. This connection suggests that the gut microbiome plays a critical role in shaping the efficacy of ICIs, offering new opportunities for therapeutic strategies that leverage microbiome modulation to optimize cancer immunotherapy. Our preliminary data show that a tributyrin-enriched diet enhances $CD8^+$ T cell activity and favorably modulates the tumor microenvironment, while reshaping the gut microbiome to increase beneficial taxa such as Lachnospiraceae, thereby improving the anti-tumor efficacy of PD-1 blockade in preclinical models. These findings suggest that dietary tributyrin supplementation may synergize with immune checkpoint inhibition to overcome resistance and improve therapeutic outcomes.

Poster No. 112

Cancer Immunology and Immunotherapy Program

Functional characterization of regulatory Ly49+CD8+T cells in the tumor microenvironment

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$CD8^+$ T cells expressing inhibitory killer cell immunoglobulin-like receptors (KIRs) have been recently recognized as a regulatory subset in humans that restrains autoimmunity and promotes maternal–fetal tolerance. Whether these $CD8^+$ regulatory T cells also suppress non-self, tumor-specific immune responses in the tumor microenvironment remains unknown.

In this study, we use murine tumor models to investigate the role of $Ly49^+CD8^+$ T cells, the mouse equivalent of human KIR^+CD8^+ T cells, in regulating anti-tumor immunity. We first observed a positive correlation between $Ly49^+CD8^+$ T cell frequency and tumor immunogenicity. Within tumors, $Ly49^+CD8^+$ T cells transition from a central memory phenotype in lymphoid tissues to an effector-like state and express cytotoxic molecules. Unlike conventional $CD8^+$ T cells that become exhausted under chronic tumor antigen stimulation, most tumor-infiltrating $Ly49^+CD8^+$ T cells do not recognize tumor antigens and retain a non-exhausted profile. Selective depletion of $Ly49^+CD8^+$ T cells increases the frequency of effector and tumor-specific $CD8^+$ T cells and elevates their IFN-γ and TNF-α production, without affecting $CD4^+$ T cell or myeloid populations. Depletion of $Ly49^+CD8^+$ T cells also sensitizes poorly immunogenic tumors, such as B16F10, to immune checkpoint blockade (ICB) therapy.

In summary, our findings suggest that $Ly49^+$ regulatory $CD8^+$ T cells promotes tumor immune evasion by suppressing anti-tumor $CD8^+$ T cell responses. Targeting this population may enhance the efficacy of immunotherapy, particularly in ICB-resistant tumors.

Poster No. 113

EnSEMBLE: An Integrated Framework for Enhancer-Set Enrichment Analysis and Biological Interpretation

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Introduction: Enhancers are critical cis-regulatory elements that dictate cell type-specific gene expression programs, yet their collective activities and functional implications remain challenging to interpret. The accessibility of enhancers, measured by techniques like ATAC-seq, and the transcription of enhancer RNAs (eRNAs) are hallmarks of their activity. To systematically analyze these complex regulatory landscapes, we developed EnSEMBLE (Enhancer-Set Enrichment Method with Biological ExpLanation), a comprehensive computational framework designed to decipher the functional roles of enhancers from genomic data.

Methods: EnSEMBLE integrates three core analytical modules. First, it performs Enhancer Set Enrichment Analysis (ESEA) to identify differentially active enhancer sets between sample groups, providing a powerful alternative to single-enhancer analysis and complementing traditional Gene Set Enrichment Analysis (GSEA). Second, it implements single-sample ESEA (ssESEA), which calculates enrichment scores for enhancer sets within individual samples to characterize each sample's unique enhancer landscape. Third, it features a Variant-to-Enhancer Mapping (VEM) module that functionally annotates non-coding genetic variants by assessing the enrichment of disease-associated SNPs within curated, disease-relevant enhancer sets.

Results: We compiled a comprehensive library of 489 enhancer sets from multiple databases. Applying EnSEMBLE to TCGA data demonstrated that our enrichment approach is more powerful than single-enhancer analysis, particularly for detecting signals from sparse eRNA data. ESEA on SNAI1 knockdown cancer cell lines revealed a shift in the enhancer landscape toward a more stem-like, EMT-associated state, helping to prioritize key pathways from GSEA results. Furthermore, ssESEA scores calculated for 448 CCLE cell lines successfully stratified them based on drug response, with specific enhancer sets showing predictive power for particular compounds. Finally, our VEM analysis of Type 2 Diabetes (T2D) GWAS variants revealed a significant enrichment in fetal islet and ductal enhancers, suggesting a developmental endocrine mechanism for T2D risk.

Conclusion: EnSEMBLE is a powerful, multi-faceted tool that enables robust analysis of enhancer activity from high-throughput genomic data. By linking enhancer landscapes to cellular states, drug responses, and disease-associated genetic variants, it provides novel biological insights into gene regulation. To facilitate user-friendly interpretation of these complex results, we also provide a specifically designed AI agent as a complementary tool for explaining the analysis outputs.

Poster No. 114

The Incidence of CNS Metastases in HER-2 Negative Metastatic Breast Cancer: Possible Protective Effects of CDK4/6 Inhibition

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Purpose

Central nervous system (CNS) metastases in metastatic breast cancer (MBC) are challenging to manage and are often a harbinger of poor survival outcomes. Evolving MBC treatment options such as CDK4/6 inhibition may prevent rapidly fatal systemic disease, but due to the blood brain barrier, do not offer the same CNS protections. The purpose of this study was to: 1) describe the incidence of brain metastases in patients with HER2- metastatic breast cancer (MBC), regardless of hormone receptor status, over the course of MBC disease, and 2) compare incidence in the context of available treatment options.

Methods

The Metastatic Breast Cancer REDCap database is a single-institution 25-year annotation of clinical, demographic, and treatment factors among patients with MBC. Data were dichotomized to pre-2015 and post-2015 (first CDK4/6i was FDA approved) and exported to SPSS for descriptive and comparative statistics.

Results

Aim 1 Total cohort N = 1,118 (HER2- MBC); n = 262 cases developed CNS mets; 150 pre-2015; 112 post-2015. The incidence rate ratio (IRR) = 1.49, 95% CI [1.17, 1.91], $p < 0.001$ suggests a 49% increase in incidence of CNS metastases from 2015 to the present compared to pre-2015. The distribution between groups was statistically significant: $X2$ (1, N = 1,118) = 13.10, $p < 0.001$. Aim 2 The median time to develop CNS mets increased from pre-2015 to post-2015 (16 vs 21.5 months), $z = -2.19$, $p = .029$.

Discussion

This descriptive study shows an increasing incidence of CNS metastases in the HER2- MBC population, likely secondary to improved systemic treatment options in the post-2015 period. Furthermore, in the post-2015 period the median time to develop CNS metastases increased, suggesting a possible protective effect by treatments introduced during that time.

Poster No. 115
Genome Stability Program

Uracil-Modified DNA Evades cGAS–STING–Mediated Immune Activation

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Extrachromosomal double-stranded DNA (dsDNA) activates cGAS-dependent innate immune responses, including interferon-β (IFN-β) production, essential for controlling infection and tumor growth. The noncanonical base 2'-deoxyuridine (dU) is prevalent in the cancer genome due to its incorporation during antimetabolite chemotherapy and activating mutations in cytidine deaminase; however, its impact on immune activation remains unexplored.

Here, we find that synthetic dsDNA fragments containing dU (dU-DNA) fail to activate type I interferon (IFN) signaling. Mechanistically, dU-DNA does not bind to key DNA sensors, cGAS or IFI16, and blocks STING-dependent signaling. Instead, dU in DNA is excised by SMUG1, generating abasic sites that are recognized by the DHX36 helicase, which unwinds and destabilizes the dsDNA. Disruption of DHX36 restores activation of cGAS-STING signaling by dU-DNA.

To explore the physiological relevance of this finding, we generated B16F10 melanoma cells deficient in uracil-DNA glycosylase (UNG), the key enzyme responsible for removing uracil from DNA. We previously showed that ATR inhibitor (ATRi) promotes dU incorporation into genomic DNA. Here, we show that ATRi significantly reduces type-I IFN signaling in UNG-deficient cells compared to wild-type cells.

Together, these findings reveal that aberrant dU in dsDNA suppresses the cGAS-STING-dependent signaling. More importantly, we discovered DHX36 as a suppressor of DNA sensing, and it could be a potential target to enhance immunotherapy, particularly in the context of dU-inducing chemotherapeutics.

Poster No. 116
Cancer Virology Program

Agentic AI for Cancer Biomarker Discovery in the Era of Spatial Omics

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Spatial transcriptomics, proteomics, and multiplex imaging provide unparalleled insight into tumor microenvironment organization and molecular states, enabling discovery of spatially resolved biomarkers. Yet, their complexity and scale demand advanced coding skills and domain expertise, limiting adoption in wet labs and clinics. The absence of interactive analysis tools and slow communication between bioinformaticians and clinicians further delays discovery.

To address these limitations, we introduce spatiAlytica, an agentic artificial intelligence (AI) platform designed to facilitate spatial omics-driven cancer biomarker discovery without writing any code. spatiAlytica enables researchers to perform end-to-end, multi-modal spatial analyses through natural language interaction, eliminating the need for extensive programming expertise. This empowers domain experts, including biologists and oncologists, to directly engage with spatial omics datasets for high-resolution cancer biomarker identification.

spatiAlytica uses a modular multi-agent architecture to enable fully autonomous spatial omics analysis from natural language input. Planning agents deconstruct user queries into ordered workflow steps, memory agents retain analytical context for iterative refinement and reproducibility, and action agents execute domain-specific tasks including normalization, spatial graph construction, clustering, marker identification, enrichment analysis, and niche annotation. The platform integrates Scanpy, Squidpy, Scikit-learn, and Pandas for single-cell and spatial transcriptomics.

A key innovation is ReSep, a vision-language model trained to extract structured insights from scientific figures and text, allowing real-time cross-referencing of spatial results with published literature. Users interact via natural language through GUI or CLI, supporting dynamic, multi-turn exploration of tumor microenvironments, hypothesis refinement, and cohort comparisons.

We demonstrate spatiAlytica utility across multiple spatial omics datasets derived from diverse cancer types, including Kaposi's Sarcoma and NSCLC. In each case, spatiAlytica autonomously identified cell-type-specific expression patterns, localized immune infiltration zones, and prioritized spatially distinct biomarkers. Results were consistent with previously published findings and required no user-authored code.

Poster No. 117

Cancer Immunology and Immunotherapy Program

Dynamics of Regulatory CD8+ T cells During Cancer Immunotherapy

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KIR+CD8+ T cells have been identified as an important immunosuppressive subset in humans. They are elevated in patients with autoimmune diseases and function to suppress pathogenic CD4+ T cells. As many tumor antigens are derived from self-molecules, we hypothesize that KIR+CD8+ cells are also activated during anti-tumor immune responses, potentially contributing to tumor immune escape.

Since immunotherapy is widely used to enhance anti-tumor immunity in cancer patients, we sought to assess the changes in KIR+CD8+ T cells in the peripheral blood of head and neck squamous cell carcinoma (HNSCC) patients following immunotherapy. In HNSCC patients enrolled in a phase II clinical trial (UPCI 18-139) receiving anti-PD-1 (nivolumab) alone or in combination with anti-LAG3 (relatlimab) or anti-CTLA-4 (ipilimumab), we observed an increased frequency of KIR+CD8+ T cells and significantly increased GZMB+ and proliferation among this cell population post treatment. Similarly, both the abundance and proliferative activity of KIR+CD8+ T cells increased in HNSCC patients (UPCI 19-082) following HPV-16 vaccine (ISA101b) plus pembrolizumab (anti-PD-1) therapy. Furthermore, 10x Genomics single cell RNA and TCR sequencing analysis on the sorted KIR+CD8+ T cells revealed an increased proportion and clonal expansion of a cytotoxic subset post-treatment.

Taken together, these findings suggest that regulatory CD8+ T cells are induced upon activation of anti-tumor immune responses triggered by immunotherapy, possibly acting as a negative feedback mechanism that limits therapeutic efficacy.

Poster No. 118

Synthesis and Characterization of Small Molecule Adaptors for Universal Chimeric Antigen Receptors

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Chimeric antigen receptor (CAR) therapy has been used to treat cancers by modifying a patient's own T cells to express a receptor that targets a tumor-associated antigen (TAA). Upon recognition and binding of the TAA, an immune response is activated, and the tumor cell is killed. This methodology has seen clinical success, in particular when treating B-cell malignancies, however, there remain limitations. Systemic overaction and "on-target, off-tumor" effects can lead to a variety of adverse reactions to this treatment, the most prominent being cytokine release syndrome. To address this, we have developed a series of small molecule adaptors that are able to direct T cell recruitment to specific cancer biomarkers. We have chosen to use the universal CAR system, SNAP-CAR, for this work. This CAR system contains the self-labelling enzyme SNAP-tag, which irreversibly binds to its substrate benzylguanine (BG). Our compounds contain three main components: a SNAP-tag binding domain, a PEG linker, and an antigen binding domain. Upon treatment with these adaptors, they will bind to their antigen target and then recruit SNAP-CAR T cells, allowing for specific immune activation only in the areas in which ternary complex formation as occurred. We have developed several of these adaptors, targeting specific cancer biomarkers such as carbonic anhydrase IX (CAIX), folate receptor alpha (FRα), fibroblast activation protein (FAP), and prostate-specific membrane protein (PSMA). These adaptors demonstrated varying degrees of immune activation, measured by the upregulation of immune activation markers, as well as specific lysis of tumor cells. Furthermore, adaptors with different linker lengths demonstrate a structure-activity relationship, revealing that a longer length generally leads to slightly higher immune activation and cell killing. The implementation and further study of these small molecule adaptors will improve the field of CAR T cell therapy and mitigate some of the adverse effects seen with traditional treatment.

Poster No. 119
Cancer Biology Program

Drp1 alternative splicing as a regulator of fission dynamics and ovarian cancer biology

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Integral to proper cell function and health, mitochondria must be tightly regulated. Key to mitochondrial maintenance is the regulation of mitochondrial shape. This is controlled by processes called fission and fusion. While the latter is known to enhance mitochondrial respiration, fission has been frequently linked to mitophagy, apoptosis, cell proliferation, and redox regulation. The balance between these two processes is integral to cell biology and has been extensively implicated in survival and behavior of cancer cells, including ovarian cancer. We have previously demonstrated that Drp1, the key driver of mitochondrial fission, is differentially spliced in ovarian cancer cells when compared to healthy fallopian tube, the site of initiation for ovarian cancer. Further, distinct expression profiles of Drp1 variable domain variants, notably higher expression of variant Drp1 (-/17) and lower expression of Drp1 (16/17), are linked to poorer ovarian cancer prognosis. We have shown that Drp1 (-/17), a Drp1 variant with reduced mitochondrial localization and fission induction capabilities, is beneficial to ovarian cancer cells as it maximizes mitochondrial respiration, enhances proliferation, and reduces chemosensitivity. While our original studies demonstrate a clear role for regulation of Drp1 variant expression in ovarian cancer cell biology, our original studies relied on overexpression of the Drp1 variants well above basal expression levels. We have now been able to establish OV90 cell lines which express single Drp1 variable domain variants at biologically relevant levels. Using these lines, we have been able to begin enhancing our understanding of the relevance of these variants in ovarian cancer cell health. Understanding how these variable domain variants impact cancer cells may provide an exciting new avenue of therapeutic target for ovarian cancer treatment.

Poster No. 120

Cancer Immunology and Immunotherapy Program

Therapeutic Induction of Tertiary Lymphoid Structures via Vaccinia virus in Lung Cancer

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Tertiary lymphoid structures (TLS) are ectopic immune structures that arise in sites of chronic inflammation including cancer. TLS are associated with improved survival and enhanced response to immunotherapy (IO) in patients with solid tumors, including lung adenocarcinoma (LUAD), making them a promising target for therapy. To understand the factors involved in TLS formation, our lab evaluated the complexity of TLS in human LUAD using multispectral imaging and spatial transcriptomics. We have found that TLS in patients can show incomplete expression of factors such as LIGHT/LTβ, CXCL13, CD40 ligand (CD40L), and IL-21. We have developed an oncolytic virus (OV) capable of delivering these factors while simultaneously generating immunogenic antigens and creating stromal space to support TLS formation and survival. Preliminary analysis of the OV utilized an in vivo subcutaneous murine model of syngeneic tumor cell line derived from carcinogen (NNK) induced LUAD murine model. OV treatment with TLS-inducing factors was shown to enhance the formation and maintenance of TLS compared to the empty vector. At the cellular level, tumors treated with the OV with all four TLS-inducing factors showed a higher tumor infiltration of CD4+ T effector cells and B cells, along with a reduced influx of Foxp3+ regulatory T cells. We are attempting to establish two additional murine lung cancer models to further test the efficacy of OV in TLS modulation: (1) KPAR, created by CRISPR/Cas9-mediated gene deletion, and (2) Lewis lung carcinoma (LLC), from a spontaneously occurring lung carcinoma model. These findings will deepen our understanding of TLS development and contribute to the advancement of immunotherapies for NSCLC patients.

Poster No. 121
Cancer Virology Program

Robust vessel detection from single-cell spatially resolved transcriptomics data

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Angiogenesis is crucial for tumor growth, and it is one of the hallmarks of cancer. Thus, accurate quantitative analysis of microvessels can serve as a potential prognostic marker. Newly grown vessels are often numerous and immature, making manual identification of microvessels an arduous task for pathologists.

We propose VESPA, an unsupervised vessel identification approach using scSRT data. We first detect vessel-associated cell types, such as vascular endothelial cells as it enables stronger transcriptomic validation beyond morphology. Based on cells' spatial coordinates, we build a neighborhood graph. The edge weights of the graph are designed to adapt seamlessly to vessels of varying sizes. Finally, a spectral graph clustering algorithm is used to delineate vessel regions and a concave hull is applied to outline each vessel.

VESPA was evaluated on KS skin tumors (n = 54), achieving a panoptic coverage of 0.513 and a panoptic quality of 0.63 (0.6 IoU). In addition to quantitative evaluation on KS tumors, we assesed VESPA on breast IDC and lung NSCLC (n=1) samples which indicates that VESPA generalizes well across tumor types and captures relevant vascular features. On the KS dataset, we compare microvascular features across tumor stages and show that VESPA effectively quantifies vascular characteristics. As the tumor progresses from control through patch, plaque, and nodular stages, both vessel density ($F = 15.41$, $p < 0.0001$) and microvascular area ($F = 18.9$, $p < 1e\text{-}6$) increase significantly. Meanwhile, the proportion of mature vessels—assessed by α-SMA coverage—steadily declines ($F = 5.97$, $p = 0.0006$), pointing to overall vascular remodeling and inflammation. These results demonstrate that VESPA enables stage-specific quantification of tumor-associated vascular changes, thus highlighting its potential as a valuable tool for studying tumor progression and microenvironmental remodeling in KS and related pathologies.

Poster No. 122

Non-linear IV pharmacokinetics of the ATR inhibitor berzosertib (M6620) in mice

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Introduction: The ataxia-telangiectasia and Rad3-related (ATR) protein serves as an apical initiator of DNA damage response pathways, promoting cell cycle arrest and facilitating DNA repair. Small molecule ATR inhibitors (ATRi) show promising antitumor activity when combined with DNA damaging agents and several ATRi are in clinical development including berzosertib (M6620, VX-970), which is currently being investigated in 11 clinical trials. Although clinical studies have examined plasma pharmacokinetics (PK) in humans, little is known regarding dose-exposure relationships and tissue distribution, which influence its therapeutic window. To support optimal deployment and clinical development of ATRi, several berzosertib PK studies were performed.

Methods: Dose proportionality was assessed using single IV doses (2, 6, 20, and 60 mg/kg) of berzosertib administered to BALB/c mice. Non-compartmental analysis (NCA) was performed to determine relevant PK parameters. Dose linearity was assessed by statistical comparison of dose-normalized plasma Cmax and tissue AUC across dose levels. Berzosertib plasma protein binding was assessed in vitro. An extensive PK study was also conducted in tumor-bearing mice to comprehensively determine exposure in plasma, tumor, and other tissues.

Results: Berzosertib displayed biphasic plasma concentration-time profiles. Increased doses were associated with less than proportional increases in early plasma concentrations and greater than proportional increases in tissue exposure. The fraction of unbound berzosertib increased at concentrations above 10,000 ng/mL, which is exceeded at the two highest doses and where non-linearity is observed. The following tissues had a tissue-to-plasma partition coefficient >1: bone marrow, kidney, liver, spleen, lung, small intestine, tumor, lymph nodes, thymus, heart, esophagus, muscle, and fat.

Conclusion: Berzosertib displayed non-linear PK. Increased doses were associated with non-proportional changes to plasma and tissue exposure, likely due to saturation of plasma protein binding. Our results will help to better understand preclinical pharmacodynamic data, and will help to inform optimal dosing and clinical deployment of berzosertib.

Poster No. 123
Cancer Biology Program

Investigating the role of Sox2 in promoting anchorage-independent survival of ovarian cancer cells

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Ovarian cancer (OVCA) is the most lethal gynecologic malignancy, often detected at an advanced stage. Malignant cells in the ascites adapt to survive under Anchorage-Independent (A-I) conditions by altering the expression of genes escalating anoikis resistance and metastasis. Our lab has shown that OVCA cells manipulate their transcriptomic profile to promote the expression of anoikis resistance genes in A-I. Expression profiling of genes upregulated in A-I revealed the transcription factor, SOX2, to be highly upregulated in response to OVCA cell detachment. While we have previously demonstrated that SOX2 is necessary for the A-I survival of OVCA cells, the specific mechanism underlying SOX2-dependent A-I survival remains unknown. RNA-sequencing studies performed in Ovcar3 cells revealed that the Leucine-rich repeat-containing G-protein coupled receptor 5, LGR5, is the most significantly upregulated gene in A-I compared to attached conditions. Importantly, amongst those genes that are upregulated in A-I, LGR5 is significantly downregulated following SOX2 knockdown in A-I. LGR5 modulates canonical Wnt signaling upon interacting with its ligand R-spondin1. Although SOX2 and LGR5 are established markers of cancer stemness in various tumor types, their direct relationship in promoting tumor progression and regulation of OVCA A-I cell survival remains unknown. Time-course experiments revealed a striking increase in LGR5 expression that peaked after 24 hours in A-I. Our studies confirmed the downregulation of LGR5 transcript and protein expression after SOX2 knockdown in established HGSOC cells and patient derived ascites cells, suggesting that LGR5 is a potential SOX2 target under A-I conditions. Furthermore, live/dead imaging showed that LGR5 expression is critical for A-I survival of ovarian cancer spheroids. Transcription factor enrichment analysis revealed WT1 as the most enriched transcription factor after LGR5 knockdown. Our current studies are focused on understanding how WT1 promotes the phenotypes driven by LGR5 and the role of LGR5 in driving OVCA A-I cell survival.

Poster No. 124

Cancer Epidemiology and Prevention Program

Effects From the p53 Signature, a Precursor of HGSOC, Could Drive Stromal Microenvironment Changes

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Ovarian cancer remains the fifth leading cause of cancer-related death among women. High-grade serous ovarian carcinoma (HGSOC) is the most prevalent and aggressive subtype. Early molecular events in HGSOC involve the gradual accumulation of genetic and epigenetic alterations, with the earliest being the “p53 signature,” characterized by 12 or more consecutive fallopian tube epithelium cells (FTEC) exhibiting aberrant expression of p53 and DNA damage markers, γ-H2AX and CDKN1A, despite maintaining normal morphology and Ki-67 indices.

We hypothesized that, despite their apparent dormancy, p53-mutant cells - particularly those with a wild-type allele reflecting the genetic context of early p53 signature lesions - could secrete factors capable of influencing the stromal microenvironment. This was investigated using an in vitro model of initial tumor transformation. Normal FTEC were transduced with common p53 hotspot mutations, and their transcriptome and secretome were analyzed. Results revealed upregulation and secretion of senescence-associated secretory phenotype (SASP) associated cytokines, suggesting an inflammatory, pro-tumorigenic microenvironment that may promote epithelial transformation, immune recruitment, and tumor initiation.

Further experiments assessed the impact of conditioned media from p53-mutant and p53 KD cells versus wild-type FTEC on fallopian tube-derived fibroblasts and macrophages. The secretome from mutant FTEC activated fibroblasts, evidenced by increased expression of mesenchymal markers and cytokines such as CCL2, CXCL1, CXCL2, IL6, IL8, and GMCSF. Conversely, normal FTEC appeared to suppress fibroblast activation and sustain an anti-inflammatory milieu. Additionally, mutant FTEC secretome skewed macrophage polarization toward an M2 phenotype, characterized by elevated CD163 and CD206 and reduced M1 markers (CD86, CD32). Validation via spatial proteomic profiling with Bruker CosMx confirmed that p53 mutations disturb the stromal immune landscape, fostering a tumor-supportive environment.

Collectively, these findings highlight that early p53 mutations can initiate stromal changes conducive to ovarian tumor development, emphasizing the importance of early detection and targeted interventions.

Poster No. 125
Cancer Virology Program

SWI/SNF Chromatin Remodeling Complexes Mediate KSHV-Induced Metabolic Reprogramming during Cellular Transformation

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Kaposi's sarcoma-associated herpesvirus (KSHV) is a human oncogenic virus implicated in multiple malignancies, including Kaposi's sarcoma and primary effusion lymphoma. The mechanisms by which KSHV drives cellular transformation remain incompletely understood. SWI/SNF chromatin remodeling complexes, including canonical BAF (cBAF), polybromo-associated BAF (pBAF), and non-canonical BAF (ncBAF), regulate gene expression and are frequently dysregulated in cancer. To elucidate their role in KSHV-mediated transformation, we treated primary metanephric mesenchymal (MM) cells and KSHV-transformed MM (KMM) cells with ACBi1, VZ185, or dBRD9, selective degraders of cBAF, pBAF, and ncBAF, respectively. ACBi1 markedly reduced KMM cell proliferation, induced G0/G1 cell cycle arrest, inhibited soft agar colony formation in a dose-dependent manner, and suppressed KSHV-driven tumorigenesis in nude mice, whereas VZ185 and dBRD9 showed no effect, highlighting a dominant role for cBAF. Similarly, BRM014, a SMARCA2/4 inhibitor, reduced KMM proliferation and colony formation. Mechanistically, KSHV latency-associated nuclear antigen (LANA) interacts with SWI/SNF components, particularly cBAF, and ACBi1 treatment disrupted multiple metabolic pathways in KMM cells, indicating cBAF's essential role in KSHV-induced metabolic reprogramming. These findings reveal a novel mechanism by which KSHV hijacks SWI/SNF via LANA to drive oncogenesis and identify SWI/SNF as a potential therapeutic target in KSHV-associated malignancies.

Poster No. 126

Cancer Immunology and Immunotherapy Program

Acute fasting enhances regulatory T cell suppressive phenotype

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Systemic nutrient availability fluctuates on short timescales, yet how acute changes in host metabolic state shape regulatory T cell (T reg) phenotype and function remains poorly defined. Previous work from our lab shows that short-term fasting impairs $CD8^+$ T cell metabolic capacity and anti-viral/anti-tumor function. Here, we examined how a 12-hour fast alters T reg phenotype, mitochondrial mass, and suppressive capacity. Mice subjected to a 12-hour fast were compared with ad libitum fed controls. We measured canonical T reg markers (CD4, CD25, Foxp3), activation/memory-associated markers, suppressive markers, and assessed mitochondrial mass by flow cytometry. In addition, we probed nutrient sensing signaling by measuring phosphorylation of Akt, 4E-BP1, and S6. In independent experiments, we performed microsuppression assays to quantify conventional T cell proliferation in co-culture with T regs. Compared with fed controls, T regs from fasted hosts showed increased expression of activation/memory and suppression-associated markers (including CD44 and CD73). In contrast, mitochondrial mass was higher across T cell populations from fed mice. Notably, T regs isolated from fasted mice were more suppressive in vitro than T regs from fed mice, demonstrating enhanced function despite reduced mitochondrial mass. Together, these data indicate that short-term caloric restriction promotes a functionally more suppressive T reg state while reprogramming T cell metabolic phenotypes. These findings have implications for experimental design, vaccination timing, and the manufacture/timing of cellular therapies, where host metabolic state could shift the regulatory vs effector T cell balance. Future studies will define molecular pathways linking nutrient sensing to T reg suppressive programs and test whether fasting-induced T regs alter immune outcomes in infection, autoimmunity, and tumor models. Effects of specific diets on T cells, such as Western, high fat, and high fiber, will also be explored.

Poster No. 127

Cancer Epidemiology and Prevention Program

Exploring Depression and Anxiety as Predictors of Radiation Non-Adherence in Head and Neck Cancer

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Background: Clinical guidelines recommend that head and neck (HNC) patients undergoing radiation therapy (RT) complete their treatment within seven weeks. Although HNC patients frequently report experiencing depression and anxiety, the relationship between pre-treatment depression or anxiety and adherence to the RT completion time remains unclear.

Objective: To evaluate the relationship between depression or anxiety and radiation completion time adherence.

Methods: This retrospective cohort study consisted of 363 HNC patients who had pre-radiation PHQ-8 and GAD-7 evaluations and were treated at UPMC between 2017 and 2024. The primary outcome of interest was RT completion time adherence, with patients who completed RT within 49 days classified as adherent. We generated descriptive statistics stratified by RT adherence, using Chi-square or Fisher's exact test to assess statistical significance for categorical variables and the Mann-Whitney U test for ADI. Pairwise Poisson regression, adjusting for clinical and sociodemographic factors, was used to estimate prevalence ratios (PR) for non-adherence for each comparison of depression or anxiety categories.

Results: Among 363 patients (90.9% White; 74.4% male), 283 (78.0%) adhered to RT while 80 (24.0%) did not. Among 187 patients who reported minimal levels of pre-treatment depression, 73.8% were adherent compared to 59.3% of those who reported moderately severe to severe levels of depression. Patients with minimal (PR: 1.96, 95% confidence interval (CI): 1.12-3.45) or moderately severe to severe (PR: 2.67, 95% CI: 1.35-5.26) levels of depression were more likely to be non-adherent compared to those with mild depression. The prevalence of RT non-adherence did not differ significantly by anxiety levels.

Conclusions: High levels of pre-treatment depression are significantly associated with non-adherent RT completion times in HNC patients. Providing mental health support alongside cancer treatment could enhance treatment adherence, potentially improving clinical and survival outcomes.

Poster No. 128
Cancer Biology Program

Investigating Stromal Mitochondrial Dysfunction and Metabolism in High-Grade Serous Ovarian Cancer

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High-grade serous ovarian cancer (HGSOC) is the deadliest gynecological malignancy due to its late-stage presentation, early and prolific peritoneal metastasis, and recurrence of increasingly therapeutic-resistant disease. Crucial to HGSOC progression is the complex crosstalk between the surrounding non-malignant microenvironment and tumor cells; prior work by our group has identified carcinoma-associated mesenchymal stem/stromal cells (CA-MSCs) as critical components of the tumor-supportive microenvironment. Previously we have demonstrated CA-MSCs provide metabolic support to HGSOC cells during metastasis by donating mitochondria to vulnerable tumor cells, allowing them to successfully metastasize and driving cellular heterogeneity at metastatic sites. However, how CA-MSC metabolism differs from normal MSCs, and how these differences influence HGSOC progression is poorly understood. Using primary patient-derived MSC/CA-MSCs, we demonstrate CA-MSCs significantly differ in metabolism and mitochondrial function. Seahorse analysis shows CA-MSCs have higher rates of both OXPHOS and aerobic glycolysis, indicating higher energy demand, as well as decreased efficiency in the coupling between OXPHOS and electron-transport chain components, indicative of mitochondrial dysfunction. Confocal and TEM imaging of CA-MSC mitochondrial networks reveals subtle but significant alterations in network organization and propagation of punctate/donut/spheroid morphologies consistent with oxidative stress. Flow cytometric analysis indicates increased mitochondrial ROS and decreased membrane potential in CA-MSCs compared to MSCs, further indicative of mitochondrial dysfunction. Using the fluorescent ROS-reporter MitoTimer, we find that CA-MSCs accumulate ROS-stressed mitochondria at a greater rate than MSCs. We further observe using MitoTimer that CA-MSCs preferentially donate ROS-stressed mitochondria to HGSOC cells. Finally, transcriptomic sequencing and subsequent validation shows significant upregulation of PDK4, an enzyme involved in promoting fatty-acid oxidation (FAO), as well as genes associated with FAO in CA-MSCs. Summarily, our work demonstrates enrichment of dysfunctional mitochondria and altered metabolism in HGSOC-stroma, and contributes to understanding how stromal-tumor metabolic interactions may influence HGSOC progression.

Poster No. 129
Genome Stability Program

Role of DDB2 in colorectal cancer tumor resistant to oxaliplatin

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Colorectal cancer (CRC) is the second most common cause of cancer death in the United States. Oxaliplatin is used as part of combination therapy and is considered standard of care for the treatment of curable CRC as adjuvant therapy and in the management of the majority of patients with metastatic CRC. Unfortunately, a significant percentage of patients treated with adjuvant FOLFOX (5-FU/leucovorin/oxaliplatin) inevitably recur and eventually become resistant to standard chemotherapy. Furthermore, oxaliplatin treatment has toxic side effects. Thus, it would be helpful to determine patients that would get the most benefit from this drug, to have reliable biomarkers for drug response, and to identify new drug targets. A recent study shows that oxaliplatin resistance in tumor cells is dependent upon the levels of DDB2, a first responder DNA repair protein involved in damage recognition in chromatin during global genome nucleotide excision repair (GG-NER). Therefore, developing a better understanding of how CRC tumors respond to oxaliplatin by investigating the mechanisms of how DDB2 acts to remove these DNA lesions fills an urgent gap in our knowledge. Our TCGA data base analysis and in vitro studies suggested that low DDB2 gene expression levels correlate with higher survival on CRC patients and lesser oxaliplatin IC50s, respectively. We have developed an IHC assay for DDB2 antibody and tested on colon cancer patient tissue, which depicts the differential expression of DDB2 protein. Our single molecule analysis reveals that DDB2 binds with 2 fold longer dwell time to oxaliplatin than cisplatin on lambda DNA lesions but binds equally to GXG site specific lesions. Furthermore, we demonstrated that oxaliplatin treatment dramatically facilitated DDB2 accumulation in nucleolus with mutual colocalization of NPM1 (a marker for nucleolar stress). These studies once completed will provide a new biomarker and therapeutic target in CRC.

Poster No. 130

Trajectories of Severe Pain and Fatigue in Patients Receiving Treatment for Head and Neck Cancer and Associated Risk Factors

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Purpose
This secondary analysis aimed to identify groups of patients with similar pain and fatigue severities during radiation for head and neck cancer (HNC) using group-based trajectory modeling (GBTM) and to identify risk factors.
Significance
Pain and fatigue commonly co-occur during HNC treatment and are associated with poorer survival and reduced quality of life (QOL). Few studies have explored symptom severity trajectories during treatment or the patient and clinical characteristics linked to more severe experiences.
Methods
In this prospective study, patients with squamous cell HNC (N=219) were enrolled from a survivorship clinic and followed from pre-radiation through 3 months post-treatment. Weekly symptom scores (weeks 0–7) from the MDASI-HN were analyzed using GBTM. Univariable screening ($p<.20$) identified candidate predictors for inclusion in multivariable models. Backward stepwise selection was used to retain variables that were statistically significant or worsened model fit based on the Bayesian Information Criterion.
Results
Most patients were white (87.1%), male (73.1%), with oropharyngeal (50.2%) or advanced-stage (52.1%) tumors. Three-group models emerged for both pain and fatigue, each with different increasing slopes. More severe pain was associated with higher baseline anxiety, while advanced-stage disease and better social-emotional QOL were linked to lower odds of severe pain. Severe fatigue was associated with worse neck disability and PEG use. Better social-emotional QOL and microvascular reconstruction were associated with lower odds of severe fatigue. Advanced-stage disease was associated with reduced odds of moderate fatigue.
Discussion
These findings support more personalized symptom management strategies during HNC treatment, and identified clinical and patient specific risk factors that were associated with more severe symptoms. Baseline assessments of functional impairments and psychosocial traits may prove to be important in anticipating and proactively treating those patients who may be at higher risk for more severe symptom trajectories during radiation for HNC.

Poster No. 131
Cancer Biology Program

The zinc finger of DNA Ligase 3α binds to nucleosomes via an arginine anchor

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Ligation of DNA single strand breaks is critical for maintaining genome integrity during DNA replication and repair. DNA Ligase III (LIG3α) forms an important complex with X-ray cross complementing protein 1 (XRCC1) during single strand break and base excision repair. We utilized a real time single molecule approach to quantify DNA binding kinetics of Halo-tagged LIG3α and XRCC1-YFP from nuclear extracts on long DNA substrates containing nicks, nucleosomes or nicks embedded in nucleosomes. LIG3α displayed higher affinity for nicks than XRCC1 with the LIG3α catalytic core and N-terminal zinc finger (ZnF) competing for nick engagement. Surprisingly, compared to single strand breaks in naked DNA, LIG3α bound even more avidly to an undamaged nucleosome reconstituted on the 601-sequence, with binding dependent on two arginine residues in the N-terminal ZnF. These studies reveal insights into nick detection and identify the role of a novel arginine anchor in LIG3α for engaging nucleosomes.

Poster No. 132

Cancer Epidemiology and Prevention Program

Chemical Exposomics Reveals Environmental Contributors to Lung Cancer Risk: A Nested Case-Control Study in the Pittsburgh Lung Screening Study

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While 80% of lung cancers (LCs) occur in ever smokers, only 15% of ever smokers develop LC, indicating that genetics, radon, and other lifestyle and environmental factors influence LC development. Prior studies have found that environmental factors, such as air pollution, diesel exhaust, and pesticides, were associated with increased LC risk, however many of these studies relied on geospatial or self-reported data. The chemical exposome (CE), the combination of endogenous and exogenous compound exposures encountered by an individual, can allow us to comprehensively evaluate the impact of individual-level chemical and metabolic exposures on LC risk. Among participants in the Pittsburgh Lung Screening Study Extension I (PLuSS-X), a cohort of heavy former and current smokers at high risk to develop LC, we conducted an "exposome-wide" analysis to identify associations between compounds detected in the CE and LC risk. High-resolution mass spectrometry was used to generate untargeted CE profiles in serum samples collected ~7 years prior to LC diagnosis from 122 case-control pairs in PLuSS-X that were matched on age, sex, smoking status, and time of sample collection. CE data was processed and putatively annotated using *TidyMass* in R. We evaluated the association between each compound (quantifying peak areas as undetectable, below median, above median) and LC risk using multivariable conditional logistic regression models. Among 2378 compounds included in our analysis, 63 and 41 compounds were associated with increased and decreased LC risk, respectively ($p < 0.05$). Notably, *N-nitrosomorpholine* (OR=2.04 [95% CI: 1.00,4.15]), is an IARC Group 2B carcinogens, where carcinogenicity has only been evaluated experimentally. *N-acetylneuraminic acid* (OR= 2.70 [95% CI: 1.02,7.13]), has been reported as a potential biomarker of lung cancer in previous study. These findings demonstrated the feasibility of using the CE to identify known and novel carcinogenic compounds in a population-based study of LC.

Poster No. 133
Cancer Biology Program

Investigating PDGFR signaling dependency in ZFTA-RELA fusion supratentorial ependymoma

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Ependymomas are rare, treatment-resistant pediatric brain tumors that remain poorly understood and lack effective therapies. In supratentorial ependymomas (ST-EPN), a common oncogenic driver is the ZFTA-RELA fusion, which promotes tumorigenesis through aberrant transcriptional regulation and signaling. Using patient-derived and syngeneic mouse models, we found that PDGFRA and PDGFRB are differentially expressed in ST-EPN compared to other ependymoma subgroups and normal neural stem cells. Isogenic neural stem cell models harboring ZFTA-RELA fusion confirmed that PDGFRA and PDGFRB expression is fusion-dependent. Functionally, PDGF-BB — the activating ligand for both PDGFRA and PDGFRB — markedly increased ST-EPN proliferation and stemness, whereas pharmacologic inhibition of these receptors with the selective small molecule CP-673451 profoundly reduced proliferation in ST-EPN, but not in controls. These findings suggest that ZFTA-RELA–driven ependymomas depend on PDGFR signaling for oncogenic growth, and that targeting this pathway with brain-penetrant small molecules holds strong therapeutic potential. Leveraging these insights, we are systematically mapping the downstream PDGFR signaling network using genetic perturbation and phospho-proteomics in disease models of ST-EPN. In parallel, we are assessing whether PDGFR inhibitors can extend survival in preclinical models, paving the way for rational, mechanism-based therapeutic strategies for children with these otherwise treatment-resistant tumors.

Poster No. 134
Cancer Biology Program

Integrated metabolic and epigenetic regulation in lethal pediatric ependymoma

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Posterior fossa group A ependymoma (PFA-EPN) is a lethal pediatric brain tumor characterized by distinctive epigenetic dysregulation and metabolic reprogramming. Our previous work demonstrated that the hypoxic tumor microenvironment is essential for both the initiation and sustained growth of PFA-EPN. Under hypoxia, expression of the EZH inhibitory protein (EZHIP) is markedly upregulated, leading to a global loss of H3K27me3 and predisposing tumor cells to acquire H3K27ac—an epigenetic mark associated with active transcription. While EZHIP's role in regulating H3K27me3 is well established, the mechanism underlying H3K27ac gain in PFA-EPN remains unclear.

Our transcriptomic and proteomic analyses identified Transketolase-Like 1 (TKTL1) as selectively upregulated in PFA-EPN, with expression strongly correlated to EZHIP levels. Functional studies revealed that TKTL1 knockdown in PFA cells leads to a marked depletion of H3K27ac, while enforced TKTL1 expression in isogenic neural stem cells increases H3K27ac. We therefore hypothesize that TKTL1 and EZHIP cooperate to drive both epigenetic regulation and tumor growth in PFA-EPN.

Consistent with this, independent or combined overexpression of TKTL1 and EZHIP in mouse neural stem cells significantly enhanced proliferation and stemness. TKTL1, a metabolic enzyme in the nonoxidative pentose phosphate pathway, is known for its role in glucose metabolism, but its involvement in epigenetic regulation remains poorly understood. Given the dynamic interplay between metabolism and epigenetics, we are investigating whether TKTL1 promotes epigenetic changes by generating metabolic cofactors for histone acetylation or by directly participating in histone modification. Ultimately, we aim to define how TKTL1 and EZHIP cooperate to sustain PFA-EPN growth and to identify downstream targets that could be exploited for therapeutic intervention.

Poster No. 135

HaloCAR – a Universal Chimeric Antigen Receptor (CAR) Programmable by Chloroalkane-Conjugated Adaptors

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Chimeric Antigen Receptor (CAR) T cell therapy is an adoptive cell therapy in which cells are genetically modified to express a receptor that binds and kills tumor cells via a specific target antigen. This approach has demonstrated clinical success against hematologic B cell cancers; however, to date, it has failed against solid tumors due to several issues, such as tumor antigen heterogeneity leading to incomplete tumor cell clearance and toxicities. To address these limitations, we are developing universal CAR T cells that target antigens on tumors via antibody and small molecule adaptors. Instead of directly binding to the antigen of interest, our most recent universal CAR, Halo-CAR, contains a mutated haloalkane dehalogenase, Halotag. Halo-CAR T cells are administered with one or more heterobifunctional antibody or small molecule adaptors containing chloroalkane (CA) motifs, forming a covalent bond with the Halotag receptor. We generated several antibody adaptors targeting several antigens (CD20, HER2, EGFR, and CAIX). We also synthesized several small molecule adaptors targeting CAIX, as well as folate-alpha (FOLR1) and folate-beta (FOLR2) receptors. In vitro, we observed potent specific lysis of tumor cells and activation of Halo-CAR T cells in a dose-dependent for both single and dual antigen targeting experiments. In vivo, we tested the ability of antibody adaptors to conjugate to Halo-CAR T cells and detected robust conjugation of the antibody adaptor to Halo-CAR T cells that remained on the cell surface for 1-2 days. Targeting of T cells using universal Halo-CARs and adaptors shows promise for developing therapies with programmable specificity.

Poster No. 136

Neural Network-Driven Radiomic Signatures for Predicting NF1, NF2, and TERT Mutations in Glioblastoma

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Purpose
To present a novel neural network–based radiomic approach for identifying key radiomic signatures predictive of NF1, NF2, and TERT mutations detected via Next-Generation Sequencing (NGS) in glioblastoma (GB) patients.

Methods
This retrospective study included 375 pathologically confirmed GB patients. Post-contrast T1-weighted, and FLAIR MRI sequences were segmented in 3D-Slicer. Regions of interest (ROIs) encompassed tumor enhancement, necrosis, edema, and contralateral white matter (cWM). After skull stripping, first-order features (e.g., skewness, kurtosis) and second-order features from the Gray-Level Co-occurrence Matrix (GLCM) at multiple angles were extracted. Image quantization at various gray levels yielded 195 second-order features per ROI. Features were normalized using the patient-specific cWM, and volume-independent features were derived by dividing the 195 original features by each ROI volume. Each ROI per sequence yielded 390 second order and 10 first-order features.

Random Forest was used for dimensionality reduction and feature selection. Neural network classifiers, with dense layers and sigmoid outputs, modeled correlations between features and mutations. The dataset was split into 70:30 for training and independent validation cohorts, with 3-fold cross-validation applied to the training cohort. Dropout and early stopping mitigated overfitting.

Results
Random Forest–based feature selection substantially reduced the feature space without compromising predictive performance. For NF1 mutation prediction, the neural network achieved an accuracy of 73.21%, sensitivity of 72.63%, specificity of 76.47%, and an AUC of 75.54% using 10 selected features. The NF2 model, based on 5 features, reached 80.36% accuracy, 72.72% sensitivity, 81.18% specificity, and an AUC of 73.9%. For TERT, 8 features yielded 72.32% accuracy, 73.08% sensitivity, 72.09% specificity, and an AUC of 73.75%.

Conclusion
Neural networks, combined with targeted feature selection, offer an effective strategy for predicting mutation status in GB patients. This approach shows promise for clinical gene mutation risk assessment, supporting personalized treatment planning and improved outcomes

Poster No. 137

Prognostic Value of Pre-Surgery Allostatic Load in Head and Neck Cancer

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Background
Allostasis is the bi-directional regulatory feedback pathway in response to chronic stress. Dysregulation of this process, termed allostatic load (AL), is linked to socioenvironmental stressors, psychiatric disorders, chronic disease, and worse survival. This study aims to investigate the association between AL and survival outcomes in head and neck cancer (HNC).
Methods
We analyzed 1,078 patients with HNC who underwent surgical resection at our institution. AL was assessed within one month prior to surgery using nine biomarkers: systolic and diastolic blood pressure, pulse, albumin, alkaline phosphatase, body mass index, creatinine, blood urea nitrogen, and white blood cell count. Points were assigned for values ≥75th percentile for all expect albumin (≤25th percentile was used). Cox proportional hazards regression estimated hazard ratios (HRs) for the association between AL and survival. Logistic regression estimated odds ratios (ORs) for the association between sociodemographic and clinical variables and AL, dichotomized into above vs below the median.
Results
Among 1078 HNC patients (78.11% male, 93.96% White, mean age: 61.26 years), the median AL was 2 (IQR:2). On multivariable logistic regression, age >61.3 years (OR = 1.86, 95% confidence interval [CI]: 1.44–2.40) was associated with higher AL, while female gender (OR = 0.53, 95% CI: 0.38–0.74) and alcohol use (OR = 0.84, 95% CI: 0.72–0.98) were associated with lower AL. On multivariable Cox regression, every one-unit increase in AL (HR = 1.10, 95% CI: 1.01–1.21) and tumor stage (HR = 1.14, 95% CI: 1.06–1.17) were associated with higher hazards of death.
Conclusion
Higher pre-treatment AL is independently associated with worse survival in HNC cancer patients. AL measurement may be a prognostic marker by capturing the cumulative impact of life stressors on physiologic function. Interventions such as pharmacologic therapy, cognitive behavioral therapy, and improved psychosocial support may help reduce AL and improve outcomes.

Poster No. 138

Cancer Immunology and Immunotherapy Program

Engineered antigen-presenting cells for enhancing anti-tumor T cell responses

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Despite their promise, the efficacy of immunotherapies for cancers has been limited. Several factors, including T cell exhaustion, inefficient T cell infiltration, heterogeneity of tumor antigens, and dysfunction of antigen-specific T cells, reduce efficacy. Although other immune cells in the tumor microenvironment (TME), including macrophages and dendritic cells, are known to exert anti-tumor activity through T cell priming and releasing proinflammatory cytokines, these responses are not necessarily antigen-specific in nature. Here, we propose a novel approach to enhance anti-tumor T cell responses by engineering antigen-presenting cells (APCs). Our lab has previously developed chimeric receptors called Signaling and Antigen-presenting Bifunctional receptors (SABRs), which can present a given antigen to T cells and then generate a functional response that can alter the activity of that T cell. We previously demonstrated the application of the SABR platform for discovering antigens recognized by CD4+ and CD8+ T cells. The initial iteration of SABRs (SABR-I and SABR-II) were constructed based on T cell costimulatory molecules, CD28 and CD3z. Here, we engineered SABRs with APC-specific signaling domains to drive signaling in professional APCs. We constructed SABRs presenting Ovalbumin (OVA) peptide in mouse class II MHC encoding for FcgR1-CD3z, ASGR1, DCIR, Dectin1, DNGR1 and DEC205 cytoplasmic signaling domains. Using dye dilution assays, we showed that SABR-expressing APCs enhanced T cell proliferation in vitro, compared to un-modified APCs or APCs expressing pMHC (peptide MHC) without signaling domains. We are currently performing experiments to test whether SABR-APCs can enhance tumor clearance by OVA-specific T cells in a B16-OVA melanoma model in vivo. We are testing whether SABR-APCs collectively induce strong anti-tumor T cell responses, characterized by increased proliferation and the release of pro-inflammatory cytokines, which in turn can modulate the tumor microenvironment. Our study will pave the way for novel approaches to improve the efficacy of antigen-specific immunotherapies for cancer.

Poster No. 139
Cancer Biology Program

Combined targeting of synthetic lethal partners in RB1-deficient cells

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RB1 is a tumor suppressor gene that is frequently mutated in various tumors, including retinoblastomas, small cell lung cancers, triple-negative breast cancers, prostate cancers, and osteosarcomas. RB1 is one of the most prevalent tumor suppressor genes driving metastasis. One therapeutic strategy for treating cancers with inactivated RB1 involves synthetic lethality (SL). A pair of genes can be defined as synthetically lethal when perturbation of either gene alone is not lethal but simultaneous perturbation of both becomes lethal. We performed a genetic screen for SL partners of Rb in the Drosophila eye and confirmed the validity of identified targets (splicing machinery, RAN, eIF4A3, and others) in human cancer cell lines and patient tumor samples. Furthermore, these SL interactions are preserved in the presence of additional oncogenic alterations (activation of Ras and loss of Pten). It is unlikely that monotherapy will be effective for eradication of RB1-mutated tumors, thus a combined targeting of two SL partners from different pathways is proposed for a synergistic effect. We created or obtained five pairs of isogenic cancer cell lines (prostate, lung, breast cancer), where RB1 is knocked out or downregulated. We further identified a library of 125 drugs against either SL partners or associated pathways. We screened drugs independently and in pairwise combinations and identified a drug combination of SN38 and MLN243, which showed selectivity against RB1-deficient cells. We will further dissect downstream mechanism of whether this increased selectivity is attributed to the increased apoptosis, senescence, necroptosis, or decreased proliferation of RB1-deficient cells.

Poster No. 141

Cancer Immunology and Immunotherapy Program

RUNX1 Rearrangements in Triple-Negative Breast Cancer: Insights into Immune Evasion and Resistance

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Abstract:
Triple-negative breast cancer (TNBC) is the deadliest breast cancer subtype, accounting for 10–20% of breast cancer morbidity. Chemotherapy remains the primary intervention for TNBC due to the lack of well-defined genetic targets, but it is often ineffective due to intrinsic and acquired resistance. Recent studies suggest a paucity of TNBC-specific mutations but identify novel genetic aberrations driving immune evasion. Our research reveals that intragenic rearrangements (IGRs) in RUNX1, a key transcription factor regulating hematopoiesis and immune response, contribute to immune suppression in TNBC. RUNX1 IGRs, detected in ~7% of TNBC cases, result in in-frame rearranged proteins that disrupt the RHD domain essential for DNA binding and interaction with CBFβ.
Our preliminary data show that RUNX1 IGRs repress NFκB target genes, increasing immunosuppressive cytokines like CCL5 and reducing pro-inflammatory cytokines like CXCL10. This creates a cold immune microenvironment, characterized by reduced CD8+ and CD4+ T-cell infiltration, larger tumors, and geographic necrosis. These tumors lack interferon γ signatures, contributing to worse clinical outcomes. This study is the first to identify and investigate the role of RUNX1 IGRs in solid tumors, highlighting their potential as both biomarkers and therapeutic targets.
Significance:
Intragenic rearrangements represent a largely unexplored aspect of TNBC genetics. The discovery of RUNX1 IGRs provides critical insights into immune evasion mechanisms, paving the way for novel immunotherapeutic strategies and precision oncology approaches for TNBC patients.

Poster No. 142

Cancer Immunology and Immunotherapy Program

Dark Antigen Burden as a Context-Dependent Biomarker of Checkpoint Blockade Benefit in Urothelial Carcinoma

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Existing biomarkers for immune checkpoint inhibitor (ICI) therapy—such as PD-L1 expression and tumor mutational burden (TMB)—only partially capture the determinants of clinical benefit. Dark Antigen Burden (DMA), reflecting non-canonical antigenic repertoire beyond classical tumor-associated antigens, emerges as an independent and complementary predictor of ICI response in urothelial carcinoma. Across cohorts, responders exhibited significantly higher DMA, with the most pronounced signal in PD-L1–low tumors containing non-exhausted $CD8^+$ T cells. In this “cold-but-competent” immune milieu, DMA outperformed TMB in identifying responders and stratifying survival outcomes. High baseline DMA was associated with evidence of pre-existing, effective immune surveillance and with improved disease-free and overall survival. The predictive value of DMA diminished in PD-L1–high or CD8-exhausted tumors, highlighting its dependence on immune context. Post-treatment analyses revealed marked declines in DMA, consistent with rapid immune-mediated antigen clearance. These findings support DMA as a promising biomarker to refine patient selection for ICI therapy, particularly in antigen-cold yet immune-competent urothelial cancers.

Poster No. 143
Cancer Virology Program

GPT-Powered Cell Typing for Highly Multiplexed Imaging

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Multiplex imaging enables accurate spatial analysis of single cells in tissue. However, data collected from multiplex imaging platforms often requires extensive preprocessing. Cell typing is particularly difficult and requires manual validation of the assigned cell types. Furthermore, current automated cell typing approaches like CellSighter and MAPS rely on traditional machine learning which can require thousands of hand-labeled examples for training. We leverage ChatGPT's multi-modal analytical power and the advanced prompt optimization technique TextGrad to automate cell typing in a more time and data efficient manner.

Our approach can operate without hand-labeled cells and can overtake existing approaches with as few as 20 hand-labeled examples per cell type. Input requirements are limited to a single-cell table with protein intensities, segmentation masks, and a mapping of cell types to their marker proteins. We compare against three existing automated cell typing approaches: CellSighter, CELESTA, and MAPS. Additionally, three published benchmark imaging datasets are leveraged facilitate comparison to the existing cell typing methods. We achieve an accuracy of over 90% for major cell types in each benchmark dataset. Reasoning for each cell type prediction is included in the output to ensure that GPT is leveraging sound logic to assign each predicted type. By combining the interpretability of marker-based rules with the flexibility of large language models, our framework offers a scalable solution for cell classification in multiplex imaging.

Poster No. 144

Distinct Proliferative Trajectories Enable Escape from MAPK Pathway Inhibition in Pancreatic Cancer

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Pancreatic ductal adenocarcinoma (PDAC) is an aggressive cancer frequently driven by KRAS mutations, which activate the MAPK pathway to promote proliferation. Although MAPK pathway inhibition is a promising therapeutic strategy, it fails to fully arrest all cells, revealing a spectrum of resistance across different cell lines. This phenomenon prompted us to investigate the alternative resistance trajectories that allow a subpopulation of cells to continue to proliferate.

We performed high-dimensional cell cycle mapping using a ~100-protein panel of cell cycle, signaling, and oncogenic markers to capture molecular trajectories of proliferation under drug treatment. This approach revealed two distinct resistance trajectories.

The predominant "main" trajectory, representing the majority proliferating cells, was defined by elevated global protein translation compared to arrested cells. Inhibition of translation significantly reduced proliferation, supporting a model where translation upregulation enables escape from drug-induced arrest. A second "alternative" trajectory, present as a small, pre-existing population in resistant cell lines, was characterized by low expression of the retinoblastoma (RB) protein, a key G1/S checkpoint regulator. Time-lapse imaging and treatment with the proteasome inhibitor MG132 suggested this reduction is not due to active degradation, hinting at transcriptional regulation. We observed time-dependent enrichment of this RB-low population from ~10% to ~25% after prolonged MAPK inhibition, indicating a selection and expansion of this population.

Together, these findings demonstrate that PDAC cells can evade MAPK pathway inhibition through at least two distinct proliferative strategies: sustaining high translation to maintain growth or bypassing the G1/S checkpoint via RB loss. By combining advanced experimental techniques with computational cell state analysis, we provide a detailed map of cell cycle adaptation under targeted therapy. These mechanisms highlight distinct therapeutic vulnerabilities that may be exploited to overcome resistance in PDAC.

Poster No. 145

Tumor-specific stem-like progenitor exhausted CD8+ T cell subsets diverge into terminally exhausted cells and long-lived memory T cells

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Chronic T cell stimulation in tumors and chronic viral infections leads to T cell exhaustion, a state of dysfunction. As the inhibitory receptor LAG3 is expressed by all exhausted T (T_{EX}) cells, we generated a *Lag3* lineage tracing mouse model (*Lag3*$i^{CreERT2}$*Rosa26*$^{LSL\text{-}tdTomato}$) to fate-map and characterize tumor-reactive $LAG3^{+}CD8+$ T_{EX} cells. In tumor-bearing mice, two distinct $tdTomato^{+}$ $CD8^{+}$ T cell subsets stratified by LAG3 surface expression ($LAG3^{+}tdT^{+}$ and $LAG3^{-}tdT^{+}$) exhibited contrasting anatomical distributions, functionality and transcriptional profiles, yet shared a common origin and T_{EX} epigenetic state. While $LAG3^{+}tdT^{+}CD8^{+}$ T_{EX} cells were terminally exhausted and restricted to the tumor microenvironment, $LAG3^{-}tdT^{+}CD8^{+}$ T_{EX} cells were stem-like progenitors that persisted in vivo and formed the T cell memory pool. This study highlights T_{EX} cell functional heterogeneity and plasticity, and characterizes a unique $LAG3^{-}tdT^{+}$ stem-like progenitor T_{EX} subset that drives an anti-tumor memory response, supporting antibody-based therapeutic targeting of $LAG3^{+}$ T_{EX} cells to unleash anti-tumor immunity and promote durability.

Poster No. 146

Cancer Immunology and Immunotherapy Program

Determining the Role of TOX in the Maintenance of CD8+ T cell Exhaustion and Memory Development

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Thymocyte selection-associated high mobility group box protein (TOX) is the master transcription factor of the T cell exhaustion lineage program, having been shown to be necessary for the formation of TEX cells. While TOX is highly expressed in TEX cells, little is known of the role of TOX after a T cell has entered the exhausted state. The primary objective of this proposal is to define the role of TOX in CD8+ TEX cells. Recent reports indicate that TOX has a critical role in determining the formation of memory CD8+ T cells; however, when in the TEX lineage program that TOX influences memory cell fates is currently unclear. To assess the impacts of the loss of TOX expression in TEX cells specifically, I have generated conditional knock-out mice to remove TOX in progenitor or terminally exhausted LAG3+ T cells, that either express a polyclonal TCR repertoire or a single TCR transgene, pMEL, that is specific for the gp100 B16 melanoma antigen. These CD8+ pMEL T cells will be utilized in adoptive transfer experiments to investigate the impact of TOX on cell survival, functionality, and the anti-tumor immune response. Collectively, these data will define the role of TOX in CD8+ TEX cells and enhance our understanding of how TOX influences memory T cell development. This proposed work will have broad implications for how T cells are maintained in an exhausted state and inform the prospect of targeting TOX as a potential cancer immunotherapy.

Poster No. 147

Interferon-gamma derived from exhausted T cells contributes to maintaining exhausted T cell population and host memory response in melanoma.

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Background: Exhausted T cells (TEXs) have traditionally been considered dysfunctional, exhibiting impaired production of cytotoxic cytokines. However, recent studies have provided evidence that these cells may not be fully dysfunctional and retain the ability to produce interferon-gamma (IFNg).
Method: To investigate how IFNg derived from those TEXs modulates the tumor microenvironment (TME), here we established a tamoxifen-inducible murine model that allows for assessing impacts of a temporal genetic deletion of Ifng from TEXs (Lag3iCreERT2-IfngL/L-Rosa26LSLtdTomato).
Results: Assessing the transcriptional profile of IFNg shows that IFNg+ CD8+ T cells are restricted to the TME, marked by increased inhibitory receptor expression. Our Lag3iCreERT2-IfngL/L-Rosa26LSLtdTomato model illustrates that IFNg ablation in TEX cells inhibits intratumoral TEX expansion and impairs host memory response in vivo upon tumor rechallenge.
Conclusion: These findings suggest IFNg produced from TEXs contributes to tumor memory response and the homeostasis of the TEX population. By utilizing novel murine models, this study highlights the functional capacity of IFNg+ TEX cells in the tumor immunity and their potential to be reinvigorated as therapeutic targets for cancer.

Poster No. 148

Cancer Immunology and Immunotherapy Program

Combination Therapy with anti-LAG3 and anti-PD1 Modulates Immunosuppressive Intratumoral CD4+ Regulatory T Cells in Solid Tumors

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Regulatory T cells (Tregs) maintain immune tolerance but, within the tumor microenvironment (TME), suppress anti-tumor immunity and limit the efficacy of immune checkpoint blockade (ICB). While most studies of ICB focus on effector T cells, the role of Tregs in shaping response versus resistance remains underexplored. To address this, we analyzed single-cell transcriptomic and TCR datasets from melanoma and head and neck squamous cell carcinoma (HNSCC) patients treated with combination anti-PD1 (aPD1) and anti-LAG3 (aLAG3) therapy. We identified seven transcriptionally distinct tumor-infiltrating Treg (TIL-Treg) subsets, most of which were conserved across tumor types. Among these, a subset expressing high levels of tumor necrosis factor receptor

(TNFR) genes—TNFR(hi) Tregs—was enriched in non-responders (NR) post-treatment and expressed elevated levels of suppressive molecules, including OX40, 4-1BB, and GITR, suggesting a role in adaptive resistance. Lineage tracing using TNFR(hi) Treg–restricted TCRs from untreated tumors revealed their distribution in post-treatment samples. In NR, these TCRs largely remained within the TNFR(hi) cluster, consistent with preserved suppressive function. In responders (R), the same TCRs redistributed across alternative Treg subsets, indicating a shift away from the TNFR(hi) suppressive state. Gene regulatory network analysis demonstrated that combination therapy induced distinct circuits in TNFR(hi) Tregs, with FOSL2 emerging as a key regulator. FOSL2 activity was enriched in R but reduced in NR, with pathway analysis implicating its role in inflammatory signaling, T cell activation, and differentiation. Together, these findings indicate that aPD1+aLAG3 therapy modulates Treg phenotype and function via FOSL2, where reduced FOSL2 activity maintains a suppressive TNFR(hi) Treg state, potentially contributing to therapeutic resistance.

Poster No. 149

Cancer Immunology and Immunotherapy Program

Impact of inhibitory receptors on T cell motility and migratin

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CD8+ T cells are a key component of the immune response against cancer and there has been intense research into improving CD8+ T cell effector functions and infiltration into the tumor microenvironment (TME). LAG3 is an inhibitory receptor that restrains T cell activation and preliminary work from our lab suggests that LAG3 also restricts T cell movement and migration. Here we investigated the impact of LAG3 on T cell motility using time-lapse microscopy and transwell migration assays to track the movement of activated CD8+ T cells in vitro. We found that LAG3 deficient CD8+ T cells move significantly faster and cover a greater distance compared to LAG3 sufficient counterparts. In addition, a greater number of LAG3 deficient cells migrated across the transwell membranes when compared to LAG3 sufficient cells. To assess whether LAG3 expression alters overall T cell migration and tumor infiltration we adoptively co-transferred pre-activated WT and LAG3 deficient PMEL cells into B16-GP100 tumor bearing mice. LAG3 deficient cells were present in both tumors and draining lymph nodes at significantly higher frequencies and numbers than their WT counterparts. Ongoing studies are aimed at continuing to investigate the impact of LAG3 on CD8+ T cell tumor infiltration.

Poster No. 150
Genome Stability Program

Undifferentiated Pleomorphic Sarcoma is Susceptible to Epigenetic-Targeting Drugs Independent of ATRX-status.

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Sarcomas are rare mesenchymal cancers in urgent need of better treatment. Many sarcomas feature pathologic chromatin dysregulation, including loss-of-function alterations to ATRX (alpha-thalassemia/mental retardation X-linked protein), an epigenetic regulator with roles including maintenance of heterochromatin domains and repression of the alternative lengthening of telomeres. ATRX loss in sarcoma is common, including in 20-30% of undifferentiated pleomorphic sarcomas (UPS), and is associated with reduction in disease-specific survival. Currently, no treatments exist for sarcoma tailored to ATRX status.

In this study, we investigated the treatment of ATRX-deficient sarcoma subtypes by targeting epigenetic dysregulation. Treatment of sarcomas featuring disruptions in epigenetic regulation with therapies targeting epigenetic mechanisms could specifically perturb or kill sarcoma cells while preserving the epigenetically-stable native tissues, as native tissues maintain crucial epigenetic regulatory and rewriting mechanisms which sarcoma may lack. We hypothesize ATRX-deficient sarcomas are preferentially susceptible to drugs which disrupt epigenetic mechanisms.

To test this, we treated UPS cell lines (ATRX-WT, ATRX-KO clone 1, ATRX-KO clone 2) with 8 epigenetic-dysregulating drugs to evaluate their cytotoxicity. In 2-3 biological replicates, we seeded 96-well plates with 2000 cells/well on day 0, and treated cells on days 1, 3, and 7, with 12 doses of each drug, from 10nM to 10uM. On day 8, cell viability was measured using CellTiter-Glo Viability Assay. Dose response curves were plotted and IC50 was determined via nonlinear regression. The epigenetic-targeting drugs used in our study were Tucidinostat, Belinostat, Vorinostat, AZD5153, A485, Decitabine, 5-Azacytidine, and Tazemetostat.

We found that all drugs in our study are cytotoxic against UPS, each with an IC50 of 2.2mM or lower, except for Tazemetostat, which was not cytotoxic at the doses tested. No drug showed a significant difference in cytotoxicity against the ATRX-KO UPS lines compared to ATRX-WT. Further research is necessary to evaluate combinations of epigenetic-targeting drugs against ATRX-deficient UPS.