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Poster No. 1

Cancer Immunology and Immunotherapy Program

Targeting Myeloid Cell in Head and Neck Squamous Cell Carcinoma: A Kinase Inhibitor Library Screening Approach

Mohamed Y. Zaky1, Jessy John1, Monika Vashisht1, Arpitha H Shivarudrappa1, Priya Singh1, Mohammad A.I. Al-Hatamleh, Karen Siddoway1, Zhangguo Chen1, Jing H. Wang1,2,*

Head and neck squamous cell carcinoma (HNSCC) creates a complex tumor microenvironment that promotes the proliferation of myeloid cells, contributing to tumor progression and immune evasion. In this study, we established a co-culture system to investigate the interaction between myeloid cells and HNSCC tumor cells. Using CellTrace™ Violet, we demonstrated that HNSCC cells significantly enhance the proliferation of myeloid cells. To identify potential therapeutic targets, we screened a custom library of 70 kinase inhibitors to assess their ability to suppress myeloid cell proliferation. Our results revealed several inhibitors that effectively reduced the population of CD11b+ F4/80+ tumor-associated macrophages (TAMs), monocytic myeloid-derived suppressor cells (M-MDSCs), and polymorphonuclear myeloid-derived suppressor cells (PMN-MDSCs). Additionally, some inhibitors increased the population of Ly6G-Ly6C- double-negative cells, indicating a potential shift in the immune landscape within the tumor microenvironment. Our findings highlight specific kinase inhibitors that can modulate myeloid cell proliferation in HNSCC, offering potential therapeutic avenues for enhancing anti-tumor immune responses and improving patient outcomes. Further investigation into the mechanisms of action and in vivo efficacy of these inhibitors is warranted to develop targeted therapies for HNSCC.

Automating the Detection of Treatment Progression in Lung Cancer Patients Using Large Language Models

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Background: Accurate assessment of treatment responses is fundamental to advancing patient care, particularly in lung cancer, where treatment modalities are diverse and complex. The extraction of detailed treatment response, particularly disease progression information, from Electronic Health Records (EHRs) is essential for several reasons: It facilitates generation of real-world evidence from real-world data, enables personalized treatment planning, and contributes to a broader understanding of cancer therapeutics. Traditional methods of extracting this information from unstructured EHRs are labor-intensive, error-prone, and inefficient, presenting significant barriers to timely and accurate real-world evidence studies. The advancement of Natural Language Processing (NLP) technologies, especially Large Language Models(LLMs), presents a transformative opportunity to automate the extraction of treatment responses.

Methods: Our study involved a cohort of 1953 lung cancer patients from the University of Pittsburgh Medical Center, analyzing over 113,000 clinical notes. We focused on identifying instances of treatment progression, following the RECIST guidelines which define progression as an increase in tumor size or cancer markers after therapy. Annotations were meticulously performed by a Hematology Oncologist to create a dataset for treatment progression extraction. We fine-tuned LLAMA-2(7B) model, which is a state-of-the-art open-source LLM, to create an information extraction pipeline. The process of fine-tuning involved adjusting the model's parameters specifically to improve its ability to recognize and classify instances of treatment progression from the unstructured text found in EHRs. This model was then evaluated against a traditional rule-based NLP system to establish a baseline for comparison.

Results: Our analysis demonstrated a significant enhancement in performance metrics with the LLM-based NLP model compared to the traditional rule-based approach. The LLM model exhibited a remarkable increase in sensitivity by approximately 37%, indicating its superior ability to accurately identify instances of treatment progression. Additionally, the model maintained high specificity and positive predictive value (PPV), achieving scores nearly comparable to the rule-based system but with a notable improvement in the F1-score by nearly 14%.

Table 1: Performance of the NLP algorithms

Model	Sensitivity	Specificity	Positive Predictive Value(PPV)	F1-score
Rule-based NLP	0.67	0.96	**0.92**	0.80
LLM-based NLP	**0.92**	**0.91**	0.89	**0.91**

Conclusions: Our research highlights the transformative potential of LLM-based NLP algorithms in automating the extraction of treatment responses from EHRs. This methodology not only provides a scalable and efficient mechanism for processing large volumes of clinical text but also significantly enhances the accuracy of lung cancer treatment response assessments. This expansion promises further advancements in clinical informatics, potentially revolutionizing patient treatment planning and outcome monitoring in lung cancer care.

Poster No. 3
Genome Stability Program

Stromal mediated DNA damage promotes high grade serous ovarian cancer initiation

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High grade serous ovarian cancer (HGSOC) is the most common and most lethal subtype of ovarian cancer accounting for 70-90% of diagnoses. Despite high mortality, the mechanisms of initiation are unclear. Most HGSOC originates in the fallopian tube epithelium (FTE) with support of underlying mesenchymal stem cells (MSCs). MSCs are stromal progenitor cells that support FTE cell growth and enrich tumor cell progression and metastasis. Despite their tumor supportive properties, the role of MSCs in initiation has been neglected. I recently uncovered an MSC type exhibiting a tumor-supportive epigenome and phenotype that is detectable at all stages of HGSOC transformation including precancerous stages. This suggests that this MSC niche, herein referred to as high risk MSCs (hrMSC), may play a role in HGSOC initiation. Shockingly, BRCA1 and p53 mutant FTE cells initiated following long-term co-culture and subsequent injection into NSG mice and demonstrated malignant features consistent with HGSOC (histopathology, structural variant mutational signature). Further investigation revealed that hrMSCs induce DNA double strand breaks (DSB) in FTE cells via lipid peroxidation. Restoring feedback inhibition of oxidative stress and lipid peroxidation ablated hrMSC-induced FTE DNA DSBs. Cumulatively, our data suggests that FTE transformation may rely on chronic exposure to hrMSC-induced aberrant lipid peroxidation.

Poster No. 4
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). The function of structural mutations in tumor immune evasion is ill-studied in cancer in general. Studying the role of ETV6 rearrangements in TNBC immune evasion could shed light on novel therapeutic strategies for combating more aggressive subsets of TNBC.

Poster No. 5

Cancer Immunology and Immunotherapy Program

The HSP-CD91 axis provides co-stimulation for adaptive immune responses to tumors

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Our lab has shown that CD91 is required for cancer immunosurveillance in mice. CD91 is a heat shock protein (HSP) receptor expressed on antigen-presenting cells (APCs) that allows for efficient HSP-tumor antigen endocytosis and cross-presentation. Binding of HSPs to CD91 also leads to phosphorylation of two tyrosine residues on the β chain of CD91 and initiation of an intracellular signaling pathway. While activation of NF-κB and STAT1 as well as increased cytokine production have been observed upon HSP binding, the proximal signaling pathway of CD91 is yet to be investigated. Here, we identify adaptor proteins and kinases that interact with CD91 upon HSP binding by crosslinking and coimmunoprecipitation of CD91 following stimulation with the immunogenic HSP gp96. Mass spectrometry identified the kinases Fgr and Axl to associate with CD91 upon HSP binding. Additionally, phosphorylation of downstream signaling mediators was observed upon time course stimulation of HSPs. Kinase inhibition prevents HSP-induced phosphorylation of this signaling cascade and cytokine production. Work is being continued to further elucidate the signaling network for other HSPs (calreticulin and hsp70). These results are going to be tested in vivo using APC-specific knockout of these kinases to investigate the impact on cancer immunosurveillance. This project will allow us to understand the CD91-mediated pathways implicated in priming immune responses to emerging tumors.

Poster No. 6

Cancer Immunology and Immunotherapy Program

Hyperglycemic culture conditions during therapeutic T cell expansion impair tumor immunity and repress T cell signaling linked to altered intracellular glycosylation states

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Adoptive cell therapy (ACT) is a cancer treatment in which T cells are isolated from a patient, enriching or engineering tumor specificity, and expanding the cells prior to transferring them back into a patient. While these therapies have shown remarkable success in some cancers, many cancers have no response and relapses are common. The cell culture and expansion steps use media formulations contain hyperphysiologic concentrations of many nutrients, most notably glucose. We hypothesize that hyperglycemic conditions used to expand T cells may favor expansion but perhaps at the cost of cellular function and longevity. We generated therapeutic T cells from mice and expanded them in glucose concentrations of commonly used culture media and assessed the cells functionally and metabolically on day 7 of culture. We also transferred tumor-specific T cells cultured in high and low glucose into tumor-bearing mice to assess tumor growth and survival. T cells cultured in hyperglycemic conditions have a paradoxical reduction in proliferation throughout expansion. Cells cultured in supraphysiologic levels of glucose also fail to produce cytokines as robustly following TCR restimulation, demonstrating a bell-shaped curve of T cell polyfunctionality as it relates to glucose concentration. T cells cultured in hyperglycemic media show dramatically reduced efficacy when used to treat tumor-bearing animals. Lastly, T cells cultured in hyperglycemic conditions have elevated proteome-wide glycosylation, most notably O-linked □-N-acetylglucosamine (O-GlcNAc). Our data suggest that T cells cultured in increasing glucose have poorer T cell polyfunctionality and anti-tumor immunity due in part to altered intracellular glycosylation. Our working hypothesis suggests that increased glucose in culture media may blunt T cell signaling. Future work will identify which proteins are differentially O-GlcNAcylated and how O-GlcNAcylation mechanistically alters T cell signaling and tumor clearance.

Poster No. 7

What are we saving this for? Mitochondrial citrate export drives excess carbon storage and dysfunction in exhausted T cells.

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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, the combination of chronic antigen stimulation and metabolic stress drives an altered differentiation state, termed exhaustion. Indeed, exhaustion remains a significant hurdle for immunotherapeutic success. We have shown that the tumor microenvironment represses mitochondrial metabolism, which directly impacts antitumor effect. These features evoke an image of starving T cells that are unable to sufficiently fuel their effector function. However, we and others have observed that exhausted T cells accumulate stored carbon outside the mitochondria in the form of lipid droplets and protein acetylation. It remains unclear whether this accumulation of stored carbon contributes to exhausted T cell dysfunction or represents an untapped source of fuel that may be the key to their reinvigoration. We evaluated the effect of deleting Slc25a1 on T cell exhaustion both in and in vitro model of T cell exhaustion and in an in vivo tumor model. We find that exhausted T cells, both in vitro and ex vivo, accumulate stored carbon due to heightened export of citrate from the mitochondria. Inhibition of mitochondrial citrate export via genetic deletion or pharmacologic inhibition of the citrate carrier, resulted in reduced lipid content and protein acetylation. Additionally, deletion of Slc25a1 reduces exhaustion and improves tumor control in adoptively transferred T cells in tumor-bearing hosts. Taken together, our results suggest that as exhausted T cells experience mitochondrial stress, they shuttle TCA-generated citrate to the cytosol where it becomes acetyl-CoA and fuels lipid accumulation and protein acetylation. This pathway may be targeted to delay exhaustion or reinvigorate exhausted T cells within tumors. These data provide new insight into the metabolic mechanisms of T cell exhaustion and may inform future immunotherapeutic development.

Poster No. 8
Genome Stability Program

The Fanconi anemia proteins prevent MLL-AF9-induced leukemogenesis through suppressing NHEJ-mediated genomic instability

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Fanconi anemia (FA) is a genetic disorder associated with bone marrow (BM) failure and progression to acute myeloid leukemia (AML). Previous studies have established a DNA damage repair-based FA pathway, majorly involved in DNA interstrand crosslink (ICL) repair. Mixed lineage leukemia (MLL) is an H2K4me-depositing protein active during early development. MLL rearranged (MLLr) leukemia are responsible for about 10% of all acute leukemia. Among these, the translocation t(9;11)(p22;q23) is mainly associated with AML and fuses AF9 to MLL (MLL-AF9; MA9). However, the interplay between these two major leukemic pathways remains to be elucidated. Here we analyzed FA patient samples and found that t(9;11) translocation was excluded from FA AML in human. Deletion of FA genes, such as Fanca or Fancd2 in MA9 transgenic mice was embryonic lethal. FA haploinsufficiency exacerbated leukemia development mediated by MA9 in vivo. Consistently, forced expression of MA9 in hematopoietic stem and progenitor cells (HSPCs) isolated from FA-deficient mice (Fancd2, Fancc, and Fanca), led to clonal expansion of leukemia stem cells (LSCs) in vitro and produced extremely aggressive leukemia in the recipient mice. Further analysis reveals that MA9 leukemic cells deficient for FA genes exhibited DNA damage accumulation and genome instability. Mechanistically, we observed a hyper-active error-prone non-homologous end joining (NHEJ) in the FA-deficient leukemia cells. And inhibition of NHEJ pathway limited ex vivo LSC clonal expansion and delayed leukemia development in vivo. Together, our findings uncover a previously unknown role of FA protein in MA9-mediated leukemia and identify FA pathway as a potential therapeutic target for MA9 leukemia treatment.

Poster No. 9

HES1 Is Required for Mouse Fetal Hematopoiesis

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Hematopoiesis in mammal is a complex and highly regulated process in which hematopoietic stem cells (HSCs) give rise to all types of differentiated blood cells. Previous studies have shown that hairy and enhancer of split (HES) repressors are essential regulators of adult HSC development downstream of Notch signaling. In this study, we investigated the role of HES1, a member of HES family, in fetal hematopoiesis using an embryonic hematopoietic specific Hes1 conditional knockout mouse model and found that loss of Hes1 in early embryonic stage leads to smaller embryos and fetal livers, decreases hematopoietic stem progenitor cell (HSPC) pool, results in defective multi-lineage differentiation. Functionally, fetal hematopoietic cells deficient for Hes1 exhibit reduced in vitro progenitor activity and compromised in vivo repopulation in the transplanted recipients. Further analysis shows that fetal hematopoiesis defects in Hes1fl/flFlt3Cre embryos are resulted from decreased proliferation and elevated apoptosis, associated with de-repressed HES1 targets, p27 and PTEN in Hes1-KO fetal HSPCs. Finally, pharmacological inhibition of p27 or PTEN improves fetal HSPCs function both in vitro and in vivo. Together, our findings reveal a novel role for HES1 in regulating fetal hematopoiesis and provide new insight into the differences between fetal and adult HSC maintenance.

Poster No. 10

Cancer Immunology and Immunotherapy Program

Uncovering mechanisms which control the infiltration of regulatory T (Treg) cells into the tumor.

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The development of immune checkpoint inhibitor (ICI) therapy has revolutionized the field of cancer treatment, resulting in striking clinical responses in patients with certain cancer types. However, a significant number of patients fail to exhibit durable responses to currently used therapies. CD4+ regulatory T (Treg) cells, dependent on the transcription factor Foxp3, suppress effector T (Teff) cell responses and thus prevent autoimmunity but contribute to tumor immunosuppression. The recruitment of Treg cells and expression of immune checkpoint molecules on cancer cells contribute to the restriction of tumor clearance by Teff cells. Favorable prognosis and survival in the absence of immunotherapeutic treatment is generally associated with low Treg to Teff cell ratios in the tumor. However, there is limited understanding of the signaling mechanisms and non-Treg interaction partners that contribute to the localization of Treg cells in the tumor. The development of approaches which allow pairing of precise spatial information with gene expression profiles will allow us to identify Treg cell-specific mechanisms contributing to their localization within or outside of the tumor, as well as the identity of their cellular interaction partners and signals. We will be leveraging spatial sequencing approaches using the GeoMx digital spatial profiler (DSP) system to examine gene expression profiles of spatially located Treg cells within and outside of the tumor in both human and murine samples to identify candidate genes which may be contributing to the infiltration of tumors by Treg cells. We hope that using this approach will unveil new and previously underappreciated immunotherapeutic targets.

Poster No. 11
Cancer Immunology and Immunotherapy Program

Going the distance: Helicobacter hepaticus colonization drives immunotherapy response in melanoma

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While melanoma outlook has improved with the introduction of anti-PD1 immunotherapy, 50% of patients are treatment resistant with largely unknown barriers to response. Recent studies correlated the gut microbiome composition with
immunotherapy response in melanoma; however, the mechanism behind these correlations remains unclear. We previously demonstrated that Helicobacter hepaticus
(Hhep) colonization drives CD4+ T cell dependent anti-tumor immunity in a murine
colorectal cancer model, but whether Hhep supports anti-tumor immunity at distant tumor sites was unknown. We hypothesize that Hhep drives anti-tumor immunity at distant sites through bacteria-specific T cell polarization in the presence of anti-PD1.

In an anti-PD1 resistant B16 intradermal model of melanoma, Hhep colonization without
anti-PD1 therapy increased tumor burden. However, Hhep colonization combined with
anti-PD1 decreased tumor growth. Transferred Hhep-specific transgenic CD4+ T cells
were found intratumorally, regardless of anti-PD1 treatment. However, Hhep only colonized the tumors of anti-PD1 treated mice. This combination of Hhep and anti-PD1 shifted both Hhep-specific and endogenous intratumoral CD4+ T cells from TFH to TH1 phenotype. Additionally, tumor-specific CD8+ T cells were less exhausted. However, the effects seen by both Hhep alone and Hhep with anti-PD1 intradermally were lost when tumors were moved to subcutaneous layer, suggesting that the tissue location of the tumor is critical for bacteria-driven anti-tumor effects. Here, we show that addition of a single bacterial species confers anti-tumor immunity in a distant, refractory tumor model and illuminates the contributions of the tissue microenvironment in modulating this response. Ultimately, these findings provide novel insight into the mechanism by which microbiome modulation can boost immunotherapy response at distant tumor sites – a phenomenon currently recognized but previously mechanistically undefined.

Poster No. 12
Cancer Virology Program

Targeting Methyltransferase 3 (METTL3) Enhances Kaposi's Sarcoma-Associated Herpesvirus (KSHV) Reactivation from Latency and Lytic Transcriptional Program

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Infection by Kaposi's sarcoma-associated herpesvirus (KSHV) is causally linked to Kaposi's sarcoma, the most prevalent cancer among AIDS patients, and several other malignancies. The life cycle of KSHV comprises latent and lytic phases, both crucial for the development KSHV-induced cancers. Despite its importance, the regulation mechanisms governing KSHV latency and lytic replication are not fully understood. N6-methyladenosine (m6A), a prevalent post-transcriptional RNA modification, is abundant on KSHV transcripts and has been implicated in the regulation of the KSHV life cycle. However, the specific stages, components of the m6A machinery involved, and underlying mechanisms remain elusive. This study investigates the role of methyltransferase 3 (METTL3), a key protein in the m6A "writer" complex, in KSHV lytic replication. Utilizing a KSHV replication trackable system, iSLK-RGB-BAC16, we demonstrated that siRNA-mediated knockdown of METTL3 enhances KSHV lytic replication induced by sodium butyrate. This enhancement is evidenced by increased production of infectious virions and elevated expression of viral lytic genes, including immediate-early gene RTA (ORF50), early gene ORF-K8, and late gene ORF65, at both mRNA and protein levels. These findings were further corroborated by pharmacological inhibition of METTL3 using two specific inhibitors, STM2457 and UZH1. Our results suggest that METTL3 and m6A modifications inhibit KSHV lytic replication by regulating the expression of KSHV immediate-early lytic genes. It appears that KSHV may exploit METTL3 and m6A to finely tune its lytic replication and latency in response to various intracellular and extracellular signals. Understanding this regulatory mechanism opens new avenues for targeted therapies against KSHV-associated malignancies.

Poster No. 13
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 for selectivity against RB1-deficient cells, and identified potential candidates, some of which are FDA approved (e.g. Pyrvinium, HHT, Paclitaxel, Methylene Blue, Taxifolin, Mycophenolate Mofetil, PD0332991, and Rapamycin). The drug candidates will be further tested in pairwise combinations. For the best combinations, we will dissect the downstream mechanism responsible for increased selectivity. We aim to identify the most effective pair of FDA-approved drugs that selectively kills RB1-deficient cancers.

Poster No. 14

Roles of IFNγ Producers within the Tumor Microenvironment in Response to Immunotherapies

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Despite the clinical success of immune checkpoint blockades (ICBs) in treating patients of various cancer types, the majority of patients do not respond or develop resistance to ICB monotherapies. Thus, a deeper understanding of mechanisms in ICB response is needed. Recent studies have demonstrated a correlation between ICB efficacy with interferon gamma-(IFNγ) and its downstream gene expression in cancer patients. IFNγ is an essential proinflammatory cytokine induced by ICBs that enhances the ability of immune system to recognize and eliminate cancer cells. Conversely, it can promote tumor progression by inhibitory receptor upregulation and induction of angiogenesis. Therefore, it remains unclear about how IFNγ expression impacts immunotherapy response or resistance due to its pleiotropic nature. To further investigate the immunophenotypes of different *Ifng*$^{+}$ T cell populations in the TME, we generated mouse lines that have IFNγ expression selectively removed from each major IFNγ-producing cell population and a novel lineage tracing mouse model of *Ifng*$^{+}$ cell – *Ifng*$^{iCreERT2\text{-}Ametrine}$ *Rosa26*$^{LSL\text{-}EYFP}$. Using these models, we found mice with IFNγ-deficient CD8^{+} T cells lost response to anti-PD1 treatment while there was no significant change in therapy response when IFNγ expression was removed from NKs or T_{regs}. These findings confirmed an essential role of IFNγ produced by CD8s in anti-PD1 response. IFNγ production from T_{regs} or NKs is not required for a robust immunotherapy response by providing help to CD8^{+} T cells. We anticipated that IFNγ secreted by CD4^{+} T conventional cells is also needed for immunotherapy response. With the use of spectral flow cytometry technique and the lineage tracing model, we can gain a comprehensive molecular phenotypic profile of different IFNγ-expressing populations in the TME by readouts of reporter proteins, effector markers and inhibitory molecules.

Poster No. 15
Cancer Therapeutics Program

Leveraging ChatGPT for literature-based inference of drug-gene relationships in cancer

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Approximately 5,000 new articles are indexed by PubMed every day, many of which reveal crucial insights into novel gene-drug interactions. This vast amount of data poses a significant challenge for researchers seeking to identify specific pharmacogenomic links. Emerging large language models, such as GPT-4o developed by OpenAI for ChatGPT, offer promising methods to address this challenge. This study presents a retrieval-augmented GPT-4o model to infer drug-gene relationships within the context of cancer. We have developed an automated pipeline to augment GPT-4o by integrating relevant literature (sentences or abstracts) retrieved from PubMed. With prompt engineering techniques, the pipeline reliably summarizes relevant articles based on a given gene, drug, and disease. As a result, it generates an inference of the drug-gene relationship, a detailed explanation of the inference process, and a confidence level for the inference.

Notably, our pipeline successfully confirmed known pharmacogenomic relationships, such as between palbociclib and CDK4, and accurately identified the primary target of CX-5461 as TOP2B, correcting a previous mischaracterization of its mechanism. A systematic evaluation demonstrated that the pipeline achieved a 94% area under the ROC curve (AUROC), which is a considerable improvement over using GPT-4o alone without augmentation by literature. In our comparative analysis, we tested the performance of GPT-4o, Gemini of Google, and several open-source large language models. We found that GPT-4o performed slightly better than Gemini, both of which outperformed other models by large margins. To make our pipeline broadly accessible to cancer researchers, we implemented a user-friendly web tool called GeneRxGPT. Users can simply enter the gene, drug, and cancer type of interest, GeneRxGPT automatically searches PubMed, retrieves relevant literature, feeds the information to GPT-4o, and generates an accurate inference with references. Taken together, our pilot research lays the groundwork for further utilization of large language models in drug discovery and development.

Poster No. 16

Mesenchymal Stem Cells Regulate E-cadherin Level, Proliferation, and Immuno-Resistance on Breast Cancer Cells

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Breast cancer is the most common cancer in women worldwide. Triple-negative breast cancer (TNBC) is the most aggressive subtype of breast cancer, while estrogen receptor-positive breast cancer (ER+BC) exhibits the longest dormancy phenomenon, wherein breast cancers recur as distant metastases even decades after removal of the primary tumor. Therefore, this study will focus on both TNBC and ER+BC.

Mesenchymal stem cells (MSCs) were previously shown to be recruited to the primary breast cancer site and also to be present in breast cancer metastasis. Their wide distribution allows them to act on both primary and metastatic tumor cells. We are expanding this research area to determine how MSCs regulate the more epithelial-mesenchymal phenotypes including immuno-resistance, proliferation, and E-cadherin-mediated interactions with parenchymal cells. Our preliminary results demonstrate that MSCs inhibit the E-cadherin level of specific TNBC cell lines and enhance the proliferation of TNBC cells and ER+BC cells; thus driving a more progressive phenotype. Fas ligand (FasL)-induced apoptosis will be measured to represent the non-specific immune responses. After that, whether this signal is paracrine or juxtacrine will be determined by the results of direct co-culture and indirect co-culture experiments including Transwell co-culture and conditioned media (CM) experiments. Signaling molecule screening will be performed by siRNA library screening, multiplex immunoassay, or small RNA sequencing to look for possible therapeutic targets, with the specific screening method is dependent on the co-culture results. Finally, we will confirm our conclusions including the effect on epithelial-mesenchymal phenotypes of BCCs and screened signal molecules in animal models and ex vivo MPS models which mimics human liver microenvironments. By studying the mechanism behind this regulation, we hope to define a new therapeutic approach to suppress the metastatic outgrowth of dormant breast cancer.

Poster No. 17
Cancer Biology Program

STAN, a computational framework for inferring spatially informed transcription factor activity across cellular contexts

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Transcription factors (TFs) drive significant cellular changes in response to environmental cues and intercellular signaling. Neighboring cells influence TF activity and, consequently, cellular fate and function. Spatial transcriptomics (ST) captures mRNA expression patterns across tissue samples, enabling characterization of the local microenvironment. However, these datasets have not been fully leveraged to systematically estimate TF activity governing cell identity. Here, we present STAN (Spatially informed Transcription factor Activity Network), a linear mixed-effects computational method that predicts spot-specific, spatially informed TF activities by integrating curated TF-target gene priors, mRNA expression, spatial coordinates, and morphological features from corresponding imaging data. We tested STAN using lymph node, breast cancer, and glioblastoma ST datasets to demonstrate its applicability by identifying TFs associated with specific cell types, spatial domains, pathological regions, and ligand-receptor pairs. STAN augments the utility of ST to reveal the intricate interplay between TFs and spatial organization across a spectrum of cellular contexts.

Poster No. 18
Cancer Therapeutics Program

Design and Evaluation of (Z)-2-Arylcyclopropane Carboxamide as Androgen Receptor Antagonists

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We have developed a series of 3rd generation small molecule androgen receptor antagonists to improve the stability of our 2nd generation lead compound (–)-JJ-450 as potential therapeutic for castration-resistant prostate cancer (CRPC). We synthesized a series of analogs assessing different properties, including liver microsome stability, prostate-specific antigen inhibition in LN95 cells and inhibition of patient derived CRPC cell lines. These combined efforts resulted in a lead compound (+)-ST-76-931 that have favorable metabolic stability with potent on target and phenotypic effects. (+)-ST-76-931 was also effective in a rodent LN95 prostate tumor xenograft model, showing significantly improvement outcomes in both survival and tumor growth reduction in vivo.

Poster No. 19

Impact of the Affordable Care Act on Receipt of Guideline-concordant Care for Colon Cancer

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Background: Access to high-quality care, as determined by receipt of guideline-concordant treatment, is crucial for cancer outcomes. Insurance coverage is an important determinant of receipt of guideline-concordant cancer treatment, and lack of coverage contributes to disparities in health outcomes. This study investigated the effect of the Affordable Care Act (ACA), which expanded insurance coverage for non-elderly adults, on receipt of guideline-concordant colon cancer care, overall and for underserved groups.

Methods: Our retrospective study includes data from the Pennsylvania Cancer Registry for 3290 patients aged 26-64 diagnosed with Stage 3 colon cancer between 2010-2019. We used a review of the literature and NCCN guidelines to establish criteria for guideline-concordant care for stage 3 colon cancer, which consists of adjuvant chemotherapy and resection of affected regional lymph nodes. We conducted an interrupted time series analysis comparing receipt of guideline-concordant care among patients diagnosed with stage 3 colon cancer pre- and post-ACA implementation across several socioeconomic variables. Underserved groups were identified as those who live in higher ADI quartiles, who identify as non-Hispanic Black and Hispanic, or who live in rural areas.

Results: Across the study period, 82.8% of patients received guideline-concordant care. Receipt of guideline-concordant care increased post-ACA by 7.7% on average per year (p=0.0244) among patients in rural areas; by 3.5% on average per year (p=0.036) among patients from ADI Q4 (the most disadvantaged) neighborhoods; and by 7.8% on average per year (p=0.0135) among non-White patients.

Conclusions: There was a statistically significant increase in guideline-concordant care for colon cancer post-ACA among non-White patients and patients from rural areas and the most deprived neighborhoods in Pennsylvania. The ACA is associated with an increase in the quality of colon cancer care for underserved groups, indicating that availability of comprehensive insurance coverage is important for reducing disparities in cancer care and outcomes.

Poster No. 20
Cancer Immunology and Immunotherapy Program

Autophagy inhibition in pancreatic ductal adenocarcinoma cancer patients modulates tertiary lymphoid structure activity and B cell function

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Pancreatic ductal adenocarcinoma (PDAC) is the third leading cause of cancer related deaths in the world and only one-fifth of patients respond to immunotherapy (IO). Autophagy inhibition combined with IO is a promising strategy for PDAC treatment. The formation of tertiary lymphoid structures (TLS) in solid tumors improves patient outcomes and their responses to IO. TLS are ectopic lymphoid aggregates that contain T and B cells, high endothelial venules (HEVS), and follicular dendritic cells (fDCs). TLS state varies in solid tumors, with more developed TLS containing germinal centers (GCs), which are marked by fDCs with B and T cell zones. Further, GC-containing TLS have increased B cell activity. PDAC tumors have increased autophagy which creates an immunosuppressive microenvironment. **We hypothesized that autophagy inhibition and combination with anti-PDL1 would increase TLS activity, B cell proliferation and somatic hypermutation**. We analyzed TLS in primary resected PDAC patient tumors using multispectral imaging with our TLS activity panel. The patients were treated with either chemotherapy alone, a combination of chemotherapy and autophagy, or a combination treatment with chemotherapy adjuvant (anti-PDL1) autophagy inhibition. Spatial distribution of TLS did not change across the treatment groups. There was an increase in B cell proliferation, and somatic hypermutation within TLS after the combination of autophagy inhibition and anti-PDL1. This indicates a more active TLS, which was further solidified by increased fDC networks and GCs in the combination treatments. These findings highlight the benefits of autophagy inhibition in potentially altering the PDAC tumor microenvironment for improved TLS function and B cell activity, which could be directly related to remodeling of desmoplastic stromal populations in PDAC patients. Our studies will offer new insights on TLS modulation by PDAC therapeutics to identify new targets to amplify the IO response in patients.

Poster No. 21

Generative AI enhanced with NCCN clinical practice guidelines for clinical decision support: A case study on bone cancer.

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Background: Bone cancer is a complex and challenging disease to diagnose and treat in clinical practice. Recently, generative AI, especially large language models (LLMs), has demonstrated potential as a decision support tool for cancer. However, most implementations have overlooked the integration of available cancer guidelines, such as the NCCN Bone Cancer Guidelines, in fine-tuning the outputs of generative AI models. Incorporating these guidelines into LLMs presents an opportunity to harness the extensive clinical knowledge they contain and improve the decision-support capabilities of the model. Methods: In this study, the aim is to enhance the LLM with cancer clinical guidelines to enable accurate medical decisions and personalized treatment recommendations. Therefore, we introduce a novel method for incorporating the NCCN Bone Cancer Guidelines into LLMs using a Binary Decision Tree (BDT) approach. The approach involves constructing a BDT based on NCCN Bone Cancer Guidelines, where internal nodes represent decision points from the Guidelines, and leaf node signify final treatment suggestions. Then the LLM makes decision at each internal node, considering a given patient's characteristics, and guides toward a treatment recommendation in the leaf node. To assess the efficacy of Guideline-enhanced LLMs, an oncologist from our team created 11 hypothetical osteosarcoma patients' medical progress notes. Each note contains their demographics, medical history, current illness, physical exams, diagnostic tests. We tested three LLMs in the implementation (GPT-4, GPT-3.5, and PaLM 2) and compared the LLM-generated treatment recommendations with the gold standard treatment across four runs with different random seeds (random seeds is a setting to control the LLM outputs). The results are reported as the average of four runs. The original LLMs are used as baseline methods for comparison. Results: The table below provides a comparison between the performance of original LLMs and those augmented with cancer guidelines for osteosarcoma treatment recommendations. We can observe that the PaLM 2 model demonstrated superior performance compared to its counterparts, underscoring the effectiveness of integrating cancer guidelines into LLMs for decision support. Conclusions: The clinical decision support capabilities of the LLMs are promising when enhanced by NCCN Bone Cancer Guidelines using our approach. To fully exhibit the potential of our proposed method as a clinical decision support tool, further investigation into other subtypes of bone cancer should be conducted in the future study.

Poster No. 22

Cancer Immunology and Immunotherapy Program

Temporal evaluation of tertiary lymphoid structures reveals changes in activity and composition over time

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Tertiary lymphoid structures (TLS) are immune structures that form ectopically in cancer. TLS correlate with favorable clinical outcomes in patients with solid tumors, including lung adenocarcinoma (LUAD). Thus, TLS could be new promising therapeutic targets. However, mechanism of TLS development remains poorly understood due to lack of murine models with spontaneous TLS formation. We utilized a carcinogen (NNK) induced murine model of LUAD that reflects human disease to assess TLS over time. We learned that composition and activity of TLS change as tumor forms, hinting at presence of different TLS states. In the model, formation and activity of TLS is associated with increased intratumoral immunity whereas tumor size is influenced by TLS immune components. We mechanistically disrupted TLS by reducing B cells at varying timepoints. When disrupted at early timepoint before tumor and TLS form, TLS and germinal center were decreased in frequency and size with decreased B cells and increased T cells, leading to reduced humoral immunity and increased tumor size. On the other hand, reduction of B cells after TLS formed at later timepoint had similar phenotypic changes in TLS while humoral immunity and tumor were unaffected. Our findings of reduced impact on tumor and humoral immunity could be explained by reduced TLS activity at later timepoint. These data suggest that TLS function could be affected over time in tumor. In parallel with mouse studies, our lab evaluates the complexity of TLS in human LUAD using multispectral imaging and spatial transcriptomics to uncover pathways that could improve TLS formation and antitumor immunity. Thus, we have generated an oncolytic virus (OV) that can deliver the factors while generating immunogenic antigens and stromal space for TLS to thrive. These studies will increase mechanistic understanding of TLS formation for improved immunotherapies and will potentially provide new therapeutic interventions for cancer patients.

Poster No. 23

Exhausted CD8+ T Cell Subset Dynamics and Regulation by PD1

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Cell surface inhibitory receptors (IRs) are critical regulators of T cell signaling and function. While IRs help maintain immune self-control to prevent autoimmunity, they are also a major barrier for effective immunity to tumors due to their high expression on exhausted CD8+ T cells (TEX). In recent years, cancer immunotherapy has been a remarkable clinical advancement. One major category is the immune checkpoint blockade therapy, which aims to block the interaction between IRs and their ligands to restore T cell activity and reinvigorate TEX. Inhibitory receptors, programmed cell death protein 1 (PD1) and lymphocyte-activation gene-3 (LAG3), are the major therapeutic targets of immunotherapy. Although significant success has been achieved with PD1 blockade, a considerable number of patients do not respond to it. Some studies revealed the importance of understanding terminal TEX since they are the ones that remain unresponsive to checkpoint blockade therapy.

Three subsets of TEX are progenitor-like, intermediate, and terminal populations. Although PD1 is expressed across all subsets, pre-exhausted and terminally exhausted populations respond distinctly differently to PD1 blockade. Therefore, it is important to understand the unknown mechanisms of how PD1 regulates T cell exhaustion. PD1 signal is known to diminish the functional effector T cell response to tumor antigens and is also pivotal for maintaining a small population of memory precursor T cells. While the mechanisms that drive T cell exhaustion are becoming clearer, we have little understanding of what mechanisms shape the fate of TEX and to what extent do PD1 in TEX contribute to effector activity and memory development. Here, we aim to discover the role of PD1 in the maintenance of CD8+ T cell exhaustion and memory development or maintenance. Our central hypothesis is that PD1 is required to maintain CD8+ TEX and PD1 of TEX is necessary for developing long-lived memory CD8+ T cells.

Poster No. 24

A Digital Pathology Approach to Predict Spatial Subtype Signatures of Hepatocellular Carcinoma from Histologic Images

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Background: Hepatocellular carcinoma (HCC) is a leading cause of global cancer-related mortality. There are multiple classifications of HCC, including the Hoshida system, which stratifies HCC into 3 subtypes (S1, S2, and S3) with distinct pathomolecular features. Advancements in precision medicine for HCC have been delayed by a scarcity of tissue biopsies due to historical concerns of safety risks. However, with the advancement of clinical trials for targeted therapies, tissue biopsies are increasingly being utilized. Thus, there is an emerging opportunity to pioneer the use of digital pathology approaches to advance precision medicine for HCC.
Materials and Methods: We accessed published spatial transcriptomics data, paired with corresponding hematoxylin and eosin (H&E) images, from 7 HCC slides. The H&E images were divided into tiles corresponding to the spatial transcriptomic spots. Using a deep learning model specifically designed for digital pathology, we extracted an embedding vector from each tile. For each spatial transcriptomic spot, Hoshida subtype signatures were calculated and binarized using selected threshold values. Neural networks were trained to predict these signatures from the tile embeddings, utilizing 6 slides for training and validation and the remaining slide for testing.
Results: On the validation set, the precision-recall area under the curve (PR-AUC) values were 0.933, 0.914, and 0.940 for the S1, S2, and S3 signature prediction models, respectively. The F1 scores for the selected threshold values were 0.775, 0.794, and 0.849. On the test set, the PR-AUC values were 0.931, 0.665, and 0.774; the F1 scores for the selected threshold values were 0.864, 0.697, and 0.701.
Conclusions: Our preliminary results suggest that deep learning can effectively predict the spatial patterns of subtype gene expression signatures from H&E whole slide images. This highlights a promising role for digital pathology in HCC subtyping and precision medicine.

Poster No. 25
Cancer Virology Program

SWI/SNF Chromatin Remodeling Complex is Essential for Kaposi's Sarcoma-Associated Herpesvirus-Induced Cellular Transformation

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Kaposi's sarcoma-associated herpesvirus (KSHV) is one of the seven known oncogenic viruses. It is causally linked to the development of several human malignancies, including Kaposi sarcoma (KS), primary effusion lymphoma (PEL), a subset of multicentric Castleman's disease (MCD), and KSHV inflammatory cytokine syndrome (KICS). Notably, the incidence rate of KS is 20,000 times higher in patients with acquired immune deficiency syndrome (AIDS) than the general population. Nevertheless, the underlying mechanism of KSHV-induced oncogenesis largely remained elusive. Following infection, KSHV exploits the host replication machinery for its persistence and induction of cellular transformation. The switch/sucrose non-fermentable (SWI/SNF) complexes are groups of epigenetic modifiers that play crucial roles in chromatin remodeling and gene expression by controlling the accessibility of DNA by proteins involved in transcription, DNA replication and repair. In this study, we examine the role of the SWI/SNF complexes in KSHV-induced cellular transformation. We used ACBi1 - a proteolysis targeting chimera (PROTAC) to selectively degrades components of the SWI/SNF complexes. We found that proliferation of KSHV-transformed metanephric mesenchymal precursor (KMM) cells was significantly reduced with cell growth arrested at G0/G1 cell cycle phase following treatment with ACBi1. The inhibitor also abolished colony-formation of KMM cells in soft-agar in a dose-dependent manner. Mechanistically, KSHV latency-associated nuclear antigen 1 (LANA1) interacts with various components of SWI/SNF complexes. These results indicate that KSHV might hijack the SWI/SNF complexes to facilitate cellular transformation and the SWI/SNF complexes could be potential therapeutic targets for KSHV-induced malignancies.

Poster No. 26
Cancer Immunology and Immunotherapy Program

Investigating the interplay of IL-12 and IFN-gamma to induce Treg fragility within the tumor microenvironment

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Regulatory T cells (T_{regs}) are integral in promoting tumor growth, but remain necessary to maintain peripheral tolerance, highlighting the importance of investigating how to specifically target intratumoral T_{regs} therapeutically. We have previously shown that induction of T_{reg} fragility – characterized by expression of T_H1 effector molecules and maintenance of Foxp3 expression – by IFN-gamma was necessary for the anti-tumor response to anti-PD1 in mice. Additionally, it is known that IFN-gamma works with IL-12 to cause T_H1 differentiation in $CD4^+$ T cells, with IL-12 alone being abe to drive this differentiation with more potency compared to IFN-gamma alone. However, it remains unknown if IL-12 can induce T_{reg} fragility, and if this is necessary for proper anti-tumor responses. We have therefore developed a novel transgenic mouse with a T_{reg}- conditional deletion of IL-12Rβ2 (*Il12rb2.Thy1.1*$^{L/LhNGFR}$ *Foxp3*$^{Cre\text{-}YFP}$) to test the effect of IL-12 on T_{reg} fragility induction after immunotherapy, and the impacts on therapeutic efficacy.

Using our model, we show that IL-12 inducing therapies increase intratumoral levels of IL- 12 and IFN-gamma compared to anti-PD1 treatment. However, when T_{regs} lost IL12Rβ2, tumor growth after treatment was similar to the control mice. Additionally, T_{regs} in these conditional knockout mice remain fragile, suggesting that IL-12 may be sufficient but not necessary for T_{reg} fragility induction. More specifically, these data indicate that the increase in IFN-gamma that is induced by IL-12-driven immunotherapy may circumvent the IL-12 signaling loss in our mouse model and induce T_{reg} fragility. We are currently investigating this hypothesis by using IFN-gamma receptor conditional knockout mice (*Ifngr1*$^{L/L}$ *Foxp3*$^{Cre\text{-}YFP}$) as well as a model of dual IL-12 and IFN-gamma receptor deletion in T_{regs} (*Ifngr1*$^{L/L}$ *Il12rb2.Thy1.1*$^{L/LhNGFR}$ *Foxp3*$^{Cre.YFP}$). While the mechanism of how IL-12 is effecting these changes are currently still unknown, this evidence suggests that IL-12 can indirectly induce fragility in T_{regs} and therefore augment the TME and the response to immunotherapy.

Poster No. 27

The Use of Remote Monitoring to Improve Patient-Reported Outcomes and Readmission Rates Following Radical Cystectomy

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Background and Significance: Following radical cystectomy for bladder cancer, almost a third of patients are readmitted. Enhanced monitoring of this vulnerable group represents an opportunity for improved quality of care. One approach is through the early identification and work-up of the signs and symptoms of complications via remote monitoring. In other fields of medicine, remote monitoring with wearable devices has prompted drastic improvements in clinical outcomes. Yet, the post-cystectomy period is defined by distinct characteristics and challenges that render patients uniquely vulnerable to complications including dehydration and infection, and thus in need of an intentionally designed remote monitoring intervention. As such, we aim to elicit and incorporate patient feedback into a remote monitoring program development. We also aim to establish the feasibility of real-time provider feedback in response to remote vital sign and patient-reported outcome changes after cystectomy.

Methods: Our group is conducting a randomized controlled trial evaluating the feasibility of incorporating real-time provider follow-up to changes in remotely collected patient-reported outcome and vital sign data. Our novel remote monitoring program incorporates consumer-grade wearable monitors, specifically Fitbit wrist watches, to collect vital signs. Changes in remote data trigger alerts to providers that facilitate early work-up and intervention of abnormal vital signs and severe symptoms, intended to promote outpatient and lowered intensity therapies, as well as reduce rates of readmission. Prior to this trial, we will conduct qualitative interviews with patients utilizing think-aloud and cognitive walkthrough protocols to obtain patient feedback and inform the remote monitoring program revision. Participant feedback will be organized by theme and frequency of discussion, and integrated into final program design.

Impact: This work builds on prior feasibility studies of remote monitoring following cystectomy, by including methods of user-centered design, incorporating data from wearable devices, and integrating real-time provider alerts to changes in physiologically significant remote data changes.

Poster No. 28

Cancer Immunology and Immunotherapy Program

DIFFERENCES BETWEEN ALPHA AND BETA-EMITTING RADIOPHARMACEUTICAL THERAPY ON THE TUMOR MICROENVIORNMENT USING SINGLE CELL ANALYSIS IN PRECLINICAL BRAIN TUMORS

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Aim: Our interest lies in using targeted radiotherapy (TRT) to enhance immunotherapy for brain tumors. This project compares the impact of α- vs β-emitting TRT (α/βTRT) on tumors and their microenvironment in a brain tumor model. We focused on antibodies targeting 1) EphA2, an antigen restricted to brain tumor cells, and 2) CD11b, a molecule on immunosuppressive myeloid cells. Single-cell (sc)RNAseq and scTCR sequencing quantified changes in cell phenotypes, frequencies, and T-cell clonal expansion, indicating anti-tumor immune responses following treatment of mice with syngeneic orthotopic gliomas.

Materials & Methods: We analyzed 130,514 cells using scRNAseq and scTCR sequencing (10X Genomics platform) from GL261 orthotopic gliomas in C57BL/6 mice, either 3- or 13-days post-RPT with 225Ac-DOTA-anti-CD11b, 177Lu-DOTA-anti-CD11b, 225Ac-DOTA-anti-EphA2, 177Lu-DOTA-anti-EphA2, or control antibodies at multiple doses. CellRanger, Seurat, and Loop-browser were used for QC, UMAP generation, reclustering, and T-cell receptor clonality analysis. SingleR identified cell clusters. Expert review and curated mouse gene sets from GSEA mSigDB quantified changes in cell subpopulations involving immune-activation, sensitization, suppression, apoptosis, and DNA damage pathways.

Results: Both 225Ac- and 177Lu-labeled TRT agents altered the tumor microenvironment and gene expression within tumor cells. Generally, 177Lu therapies robustly recruited immune cells into the tumor, while 225Ac therapies did so to a lesser extent. We identified 12 subpopulations of tumor-infiltrating myeloid cells with varying molecular phenotypes and differentiation states, showing different responses between treatments. TCR clonality from scRNA/TCRseq data revealed that T-cell infiltration was 4.7% in controls, 8.0% with 225Ac, and 11.9% with 177Lu. While 225Ac showed significant expansion of a few T-cell clonotypes, 177Lu showed broader expansion across T-cell clonotypes.

Conclusion: α- vs βTRT agents differ significantly in eliciting anti-tumor immunity. Further exploration of both α- and β-TRT's role in enhancing anti-tumor immunity is underway. This data supports expanding research into anti-tumor immunity responses with α- and βTRT.

Poster No. 29

Time to Head and Neck Cancer Survivorship Care

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Background: Although emerging evidence supports prehabilitation for improvements in quality of life (QOL) and function in head and neck cancer (HNC) survivors, survivorship programs often begin care years post-treatment. This analysis aims to evaluate the association of pre-treatment multidisciplinary survivorship care and patient-reported outcome measures (PROMs) of symptoms burden, QOL, and psychological distress.

Methods: Data was prospectively collected through a multidisciplinary HNC survivorship clinic from 2017-2022. Survivors with squamous cell carcinoma of the oral cavity, oropharynx, laryngopharynx, and other sites who underwent definitive or adjuvant radiotherapy were included. Those with recurrent disease, second primary, and/or distant metastasis were excluded. PROMs (University of Washington QOL, Neck Disability Index, Eating Assessment Tool-10, Insomnia Severity Index, Patient Health Questionnaire-8, and Generalized Anxiety Disorder-7) were obtained one-year post-treatment. Analysis was conducted using multivariable logistic and linear regression and propensity score matching.

Results: 312 survivors were included. Majority were male (240[76.9%]) and white (280[89.7%]), with a median age of 61.18 years (SD=9.66). Majority had oropharyngeal SCC (164[52.6%]), early T and N stages (162[51.9%] and 160[51.3%]), and adjuvant radiotherapy (166[53.2%]). Compared to survivors without a pre-treatment baseline visit (n=153), survivors with a pre-treatment baseline visit (n=159) had 6.28 points higher UWQOL physical (95% CI 2.38, 10.18, p=0.002), 5.41 points higher UWQOL social-emotional (95% CI 0.01, 10.81, p=0.050), 0.93 points lower PHQ-8 (95% CI –2.20, 0.34, p=0.152), 1.13 points lower GAD-7 (95% CI –2.17, -0.10, p=0.032), 4.00 points lower EAT-10 (95% CI –6.70, -1.30, p=0.004), 2.46 points lower ISI (95% CI –4.40, -0.51, p=0.014) and 2.12 points lower NDI (95% CI –4.46, -0.22, p=0.076) scores.

Conclusions: HNC survivors who received pre-treatment multidisciplinary survivorship care had a higher QOL and lower symptoms burden and psychological distress. Supporting the growing literature on prehabilitation, these findings highlight the positive impact of proactive multidisciplinary survivorship care on outcomes in HNC survivors.

Poster No. 30

Cancer Immunology and Immunotherapy Program

The Head and Neck Squamous Cell Carcinoma tumor microenvironment modulates circulating and intratumoral memory B cells.

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While immunotherapy has improved the prognosis of patients with head and neck squamous cell carcinoma (HNSCC), most patients fail to produce a durable response. B cells represent a possible target for immunotherapy as they are the second most abundant tumor infiltrating lymphocyte in many human tumors and correlate with increased patient survival. B cells can mediate the immune response to cancer by directing the local and peripheral antitumor immune response by producing tumor-specific antibodies, bolstering T-cell responses via antigen presentation, and generating antigen-specific memory B cells (MBCs). Together, these data highlight MBCs as a promising target for new immunotherapeutic options to complement T-cell centric therapies; however, there remains a need for a mechanistic understanding of MBC function in the tumor microenvironment. MBCs are a heterogenous population with functionally distinct subsets and may be generated either from germinal center (GC)-dependent reactions or GC-independent reactions. In HNSCC, we find accumulation of GC-independent MBCs in patient peripheral blood and tumors, correlating with advanced stage disease. Importantly, GC-independent populations are predominantly of IgG isotype, posing this MBC subset as an antigen specific population that can contribute to antitumor immunity through the production of tumor reactive antibodies. Here, we investigate the phenotype of intratumoral and circulating GC-independent MBC populations in HNSCC patients. We find that GC-independent MBC subsets express high levels of inhibitory receptors, such as PD-1 and FcRL5, suggesting this population is functionally impaired in the tumor microenvironment. Moreover, we find that GC-independent MBC populations upregulate adhesion molecules (CD18, CD11c) are hyporesponsive to antigenic stimulation, suggesting that altered expression of inhibitory receptors and adhesion molecules contribute to immunogenic tolerance in the tumor microenvironment.

Poster No. 31
Genome Stability Program

Meta-analytic framework for robust biomarker and cell subtype identification on single-cell and spatial transcriptomics data

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Single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics (ST) technologies have revolutionized scientific research. scRNA-seq highlights cellular diversity and distinct functional states within cell populations, emphasizing the characteristics of individual cells from a heterogeneous population, and allowing the identification of rare cell types. Meanwhile, ST maintains the spatial context of gene expression in tissues. The advanced capabilities of scRNA-seq and ST have led to a substantial accumulation of the data, and integrating datasets from multiple cohorts hold promise to detect more robust biomarkers. However, it presents significant challenges with batch effects that are caused by different pre-processing, library preparation, and sequencing platforms. Though, several tools are specifically designed to address batch effects, they often tend to overcorrect the data or lose valuable details.
To address these, meta-analysis provides an alternative approach to efficiently integrate data from multiple studies and avoid data distortion caused by excessive batch effect correction. In this project, we aim to establish a meta-analytic framework to integrate single-cell and spatial data from multiple cohorts, providing more robust and accurate detection of the biomarkers and cell subtypes. Our pipeline first detected biomarker and cell subtypes from individual cohort, followed by employing statistical meta-p-value integration to combine multiple studies. This innovative framework was applied to ST data on hepatocellular carcinoma samples for robust tumor marker detection, as well as scRNA-seq data on time-series acute kidney injury libraries to define fibroblast subpopulations. These applications have proved that our meta-analytic framework is able to identify robust biomarkers and subtypes compared with the existing batch effect correction methods. In addition, our pipeline will serve as a generalizable tool for wild application across diverse healthy and diseased samples. As future steps, novel biomarkers will be experimentally validated and the sophisticated meta-analysis framework will be optimized for application to more extensive and various datasets. This approach will help address batch effects challenges and enhance the quality and applicability of research findings.

Poster No. 32
Cancer Virology Program

Unraveling CASTOR1's Tumor Suppressive Role: Genetic Ablation of CASTOR1 Enhances Kras-Driven Mouse Model of Non-Small Cell Lung Cancer

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Cytosolic Arginine Sensor for mTORC1 Subunit 1 (CASTOR1) is a crucial regulator of mTORC1 activity and plays a significant tumor-suppressive role in various cancers. Its functions are modulated through several mechanisms, including inhibition by viral miRNAs in KSHV-associated cancers and degradation through AKT-mediated phosphorylation and RNF167-mediated ubiquitination in non-viral cancers. This study explores CASTOR1's role in non-small cell lung cancer (NSCLC) using a novel CASTOR1 knockout (KO) mouse model crossed with the Kras (LSL-KrasG12D/+) NSCLC model. The research investigates tumor incidence, growth, and oncogenic pathway activation in mice with Kras activation and homozygous CASTOR1 ablation. Results show that CASTOR1 deficiency significantly increases lung tumor incidence and size, accompanied by a higher proliferative index. Analysis revealed that high levels of phosphorylated CASTOR1 (pCASTOR1) in tumors correlated with advanced tumor stage and low total CASTOR1 levels, suggesting an active degradation process that promotes tumor progression. Increased mTORC1 signaling, indicated by elevated p4EBP and pS6 levels, was observed following CASTOR1 loss, suggesting that mTORC1 activation contributes to tumor development. Additionally, elevated pERK levels in CASTOR1 KO tumors indicate a feedback loop between CASTOR1/mTORC1 and the Kras/ERK pathway. Treatment with mTORC1 inhibitors enhanced sensitivity of Kras tumor organoids to KrasG12D inhibitors, while CASTOR1 overexpression increased sensitivity to KrasG12D inhibitors in NSCLC cells. These findings underscore CASTOR1's critical tumor-suppressive role in Kras-dependent LUAD and suggest that combined inhibition of Kras and mTORC1 may be an effective strategy for LUAD with hyperactivated Kras and reduced CASTOR1 levels.

Poster No. 33
Cancer Therapeutics Program

Systematic Evaluation of Large Language Models for Gene Function Representation

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Quantitative representation of gene functions is critical for the statistical analysis of high-throughput experiments, such as the Gene Set Enrichment Analysis. Recently, large language models (LLMs) have demonstrated unprecedented capabilities in analyzing textual data. One of the key strengths of LLMs lies in the ability to generate rich "semantic embeddings," which map complex textual data, such as a paragraph describing functions of a gene, into a low-dimensional latent space. This process captures deep semantic information and brings semantically similar data points (e.g., functionally relevant genes) closer together in this space. However, the extent to which these embeddings accurately capture and represent gene functions has not been thoroughly evaluated. To address this gap, this study systematically assesses the ability of embeddings generated by LLMs to represent the functions and interactions of genes. We developed a statistical inference framework to evaluate gene-function associations captured by the embeddings. Specifically, we converted gene descriptions extracted from the NCBI Gene database and the narratives of Gene Ontology (GO) terms into embedding vectors. We then statistically tested the correlation between the embedding of a GO term and the embeddings of genes within the term, in comparison to other genes. Our evaluation included embeddings generated by seven state-of-the-art LLMs, including proprietary models such as text-embedding-3-large (OpenAI) and text-embedding-004 (Google), as well as five open-source models such as PubMedBERT (Microsoft) and Llama 3 (Meta). The results demonstrated that embeddings generated by text-embedding-3-large achieved the best performance, with 97.1% of GO terms showing a significant average correlation between the gene embeddings and the corresponding GO term embedding. Text-embedding-3-large was followed by text-embedding-004 (97.01%) and other methods (ranging from 93.06% to 50.01%). Overall, our framework provides a statistical assessment of embedding methods in capturing gene-function associations. It may offer new insights for future research on gene function analysis using LLMs.

Poster No. 34
Cancer Immunology and Immunotherapy Program

Exercise-induced microbial folate metabolite enhances antitumor CD8 T cell immunity and promotes immunotherapy response in ICI-resistant preclinical melanoma

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Exercise is associated with enhanced antitumor immunity and improved immunotherapy efficacy in multiple cancers. However, the mechanisms through which exercise mediates antitumor effects remain obscure. Here, we show for the first time that exercise-induced changes to the microbiota are a key mechanism by which exercise promotes antitumor immunity.

We first show that exercise-mediated tumor suppression relies upon CD8 T cells and an intact specific-pathogen-free microbiota in our model. Strikingly, we show that the compositionally distinct exercised microbiota is sufficient to confer tumor suppression, but must be metabolically active to do so. Accordingly, we find that exercise-microbiota produced metabolites are sufficient to restrain tumor growth in vivo and act directly on CD8 T cells to promote antitumor effector function in vitro. Through targeted and untargeted metabolomics approaches, we identify that exercise changes metabolic output of the microbiota by increasing bacterial folate (vitamin B9) metabolism. Accordingly, formate, a short-chain fatty acid and known intermediate of folate metabolism, is enriched in intestinal contents and serum with exercise. Excitingly, we identify that the bacterial enzyme pyruvate formate lyase, required for bacterial formate synthesis, is significantly enriched in feces of immunotherapy responder patients across eight different study cohorts. In our model, we find that oral administration of formate alone is sufficient to restrain tumor growth, promote CD8 T cell effector function, and enhance immunotherapy efficacy in in a manner dependent on Nuclear Factor Erythroid 2-related factor 2 (Nrf2) signaling. Lastly, we demonstrate that Nrf2 signaling is required for the exercise-mediated antitumor effect in vivo.

Overall, we have unveiled a novel mechanism in which exercise, by modulating production of a microbial metabolite, improves antitumor immunity in melanoma. Our study will provide a rational mechanistic basis to design novel exercise, precision dietary, and microbial metabolite combinatorial therapeutic strategies to alter microbial Nrf2 agonists in immunotherapy-resistant cancer patients.

Poster No. 35

Cancer Immunology and Immunotherapy Program

Pathogenicity of human CD91 SNPs

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CD91, expressed by antigen presenting cells, is a necessary receptor for cancer immunosurveillance. It binds multiple ligands including immunogenic heat shock proteins (HSP) and functions as both an endocytic receptor for antigen capture and a signaling receptor to upregulate cytokines and costimulatory molecules. Due to its size, there are more than 3500 identified single nucleotide polymorphisms (SNPs) for human CD91, however the functional consequences for these SNPs have not been addressed. Thus, we sought to investigate if certain SNPs result in altered function of CD91 and if these SNPs are linked with higher cancer incidence. Using a cohort of sarcoma patients, we identified 11 SNPs that resulted in missense mutations of CD91. Correlations between these SNPs and the immunophenotype of the respective tumors has identified several SNPs which correlate with poor immune infiltration and increased tumor antigenicity. Alternatively, a small number of SNPs correlate with above average immune infiltration and reduced tumor antigenicity, potentially enhancing CD91 function. In addition to our patient data, predictive folding algorithms were used to assess the potential effects of individual SNPs on protein folding. In order to directly assess the function of these SNPs, we have generated an in vitro model system using THP1 cells. Cells carrying single CD91 SNPs will be assessed for protein expression levels, HSP binding and internalization as well as costimulatory function.

Poster No. 36

Serum Arginase-1 is a Prognostic and Predictive Biomarker for Head and Neck Squamous Cell Cancer and Melanoma Patients

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Background: Recent advances in immuno-oncology have revolutionized cancer treatment, yet only a subset of patients achieves deep clinical responses. Biomarker-guided patient selection could improve the management of various malignancies by identifying those most likely to benefit from immunotherapy. One potential predictive biomarker is serum arginase-1 (sArg1). Novel data suggests that sArg1 is detected in a cohort of head and neck squamous cell carcinoma (HNSCC) and melanoma patients, but not healthy donors (HD). This study aims to identify the predictive potential and cellular source of sArg1.

Methods: Sera isolated from HNSCC and melanoma trial patients receiving chemoradiotherapy with pembrolizumab (CRT+pembro) or dendritic cell (DC) vaccines, respectively, were analyzed for sArg1 concentrations using Luminex, which were correlated to clinical parameters. Absolute cell counts of various leukocyte populations were correlated with sArg1 concentrations. Peripheral blood mononuclear cells (PBMC) from HD and non-trial melanoma patients were treated with TLR7-agonist imiquimod to identify potential cellular sources of sArg1 via flow cytometry.

Results: In HNSCC patients receiving sequential CRT+pembro, as well as melanoma patients receiving DC vaccines, elevated serum baseline sArg1 levels correlated with better overall survival. In contrast, elevated serum baseline sArg1 concentrations in HNSCC patients receiving concurrent CRT+pembro were associated with worse clinical outcome. sArg1 concentrations directly correlated with the absolute counts of circulating CD14lowCD3-CD19- myeloid cells in trial melanoma patients. In vitro treatments of PBMC with imiquimod-induced arginase-1 production in CD14low DC, as well as intermediate and classical monocytes, but not in CD14low non-classical monocytes and M-MDSC, from both healthy donors and melanoma patients.

Conclusion: Increased levels of serum sArg1 may serve as a regimen-dependent predictive biomarker for clinical outcomes in HNSCC and melanoma patients, with potential regimen specificity, and may serve as a surrogate biomarker for activated CD14low DC. Further validation is needed to link these observations to the clinical findings.

Poster No. 37
Genome Stability Program

Investigating the Role of UBL7 in the Ubiquitin-Proteasome Pathway in Ovarian Cancer Cells

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Ovarian cancer is one of the deadliest cancers affecting women worldwide, is highly resistant to common chemotherapeutics, and has a high rate of recurrence. One largely uninvestigated aspect of chemotherapy resistance and ovarian cancer recurrence is quiescence, whereby cells distal from a nutrient source can reversibly exit the cell cycle, remain in a non-proliferative state, but carry out a low level of metabolic activity. Single cell sequencing of quiescent cells showed a number of genes that are differentially expressed between quiescent and proliferating cells, including numerous genes associated with the ubiquitin-proteasome pathway. One such gene, UBL7, is more highly expressed in quiescent cells and—based on the presence of a ubiquitin-association domain (UBA) and a ubiquitin-binding domain (UBL)—is predicted to help recruit ubiquitinated proteins to the proteasome for degradation. Therefore, our goal is to identify UBL7 substrate(s) to understand its role in ovarian cancer quiescence. Initial results show that siRNA mediated knockdown of UBL7 does not increase overall poly-ubiquitination in HEK293 cells and ovarian cancer cells, indicating that UBL7 does not target all ubiquitinated proteins and may have more specific substrates. Additionally, there is no significant change in LC3B, a marker of autophagy, in these cells. Interestingly, a BLAST search revealed significant homology between three regions in UBL7 and a ubiquilin, which plays important roles in protein quality control, also possesses a UBA and UBL, and binds molecular chaperones. Further experiments will include immunoprecipitation and mass spectrometry to identify these and other UBL7 binding partners. In addition, design of a CRISPR UBL7 knockout ovarian cancer cell line, as well as future experiments examining sensitivity of these cells to common cancer therapeutics, will provide new insights into the role of UBL7 in chemotherapy resistance and ovarian cancer quiescence.

Poster No. 38
Cancer Immunology and Immunotherapy Program

Suppression of Anti-Tumor Immune Responses by Regulatory CD8+ T cells

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KIR+CD8+ regulatory T cells have been increasingly recognized for their critical role in modulating autoimmune responses in humans by suppressing pathogenic CD4+ T cells via their cytolytic activity. Given that a large fraction of tumor antigens originates from self-molecules, we hypothesized that these regulatory CD8+ T cells also contribute to the regulation of anti-tumor immune responses.

Our initial findings demonstrate an increased frequency and enhanced cytotoxicity of Ly49+CD8+ T cells, the mouse equivalent of KIR+CD8+ T cells, within the tumor microenvironment (TME) of MC38-bearing mice. Depletion of Ly49+CD8+ T cells in Klra6creDTA mice led to slower tumor growth and could further enhance the anti-tumor efficacy of PD-1 blockade in the MC38 model.

To elucidate the underlying mechanisms how Ly49+CD8+ T cells regulate anti-tumor immunity, we set up an in vitro co-culture of CD8+ T cells and irradiated E.G7-OVA tumor cells. While the irradiated tumor cells elicited a robust anti-tumor CD8+ T cell responses, we observed an even faster proliferation of Ly49+CD8+ T cells, indicating that these cells are induced upon anti-tumor immune responses. These in vitro expanded Ly49+CD8+ T cells co-expressed CD44 and CD122 and upregulated Granzyme B and Helios, consistent with the phenotypes of Ly49+CD8+ T cells observed in vivo. Moreover, addition of these Ly49+CD8+ T cells to cultures of Ly49-CD8+ T cells and E.G7 cells resulted in reduced proliferation of Ly49-CD8+ T cells. Additionally, an increase in OVA-specific CD8+ T cells was detected in the co-culture of E.G7 and CD8+ T cells from Klra6creDTA mice, further supporting their role in suppressing tumor-specific CD8+ T cells.

Taken together, our findings suggest that regulatory CD8+ T cells are induced within the TME to promote cancer immune evasion by suppressing anti-tumor CD8+ T cells, supporting them as a promising target for cancer immunotherapy.

Poster No. 39
Cancer Virology Program

Comparative profiling of extrachromosomal circular DNAs (eccDNAs) in HBV-infected primary human hepatocytes and HBV-related hepatocellular carcinoma cell lines

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Hepatitis B virus (HBV) is one of seven known oncogenic viruses. HBV-induced hepatocellular carcinoma (HCC) has been linked to the persistence of the HBV covalently closed circular DNA (cccDNA) genome and its transactivation by the viral X protein (HBx). Both cccDNA and host genes are subject to regulation by HBx, which can disrupt cellular pathways that contribute to hepatocarcinogenesis. In search of novel host targets of HBx, we explored the human extrachromosomal DNA (eccDNA) landscape of HBV-associated cancer cell lines and primary human hepatocytes (PHH) infected with wild-type and HBx-null mutant HBV. We hypothesize that oncogenic eccDNAs may be engaged by HBx in a manner similar to its regulation of HBV cccDNA. Bioinformatic analyses revealed the identities of genomic loci producing eccDNAs. Candidate eccDNAs were ranked according to their circularity confidence scores based on strictly defined structural variations. Quantitative real-time PCR (qPCR) was performed on total RNA lysates from cell lines and PHHs. Our preliminary study revealed differential expression of eccDNA producing oncogenes in HBV-related HCC cell lines Hep3B and PLC/PRF/5 compared to nonviral HCC cell line HepG2. In-depth annotation of the sequences housed in eccDNAs using Ensembl show that regulatory regions like enhancers, promoters and insulators are highly represented in these small host DNA episomes. Open chromatin regions are also highly represented in eccDNAs, prompting us to evaluate the posttranslational epigenetic modifications on eccDNA-producing loci using chromatin immunoprecipitation followed by PCR. We found differential gene expression and epigenetic modifications of eccDNA-producing genes, as well as altered protein expression of the candidate eccDNAs in HBV-associated cells compared to non-HBV immortalized or infected PHHs. This finding strongly suggests a link between eccDNA formation and expression of genes associated with HBV-induced hepatocarcinogenesis.

Poster No. 40
Cancer Immunology and Immunotherapy Program

A small but mighty ROR(γt): a population of RORγt+ peripherally induced regulatory T-cells promote growth of non-colonic tumors.

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The presence of regulatory T-cells (Tregs) in tumors is often correlated with poor prognosis. Recently, Treg targeting therapeutics have enabled anti-tumor responses but come with the risk of autoimmune-like adverse events, highlighting a need for further therapeutic optimization. A recent target of interest is RORγt+ peripherally induced regulatory T-cells (pTregs), a microbially driven population of T-cells in the gut that have increased suppressive capacity compared to thymic Tregs (tTregs). pTregs are well studied in autoimmune diseases, but their effect in cancer is relatively unexplored. Our objective is to elucidate the role of RORγt in the suppressive capacity of pTregs with the hope that targeting these cells will allow for anti-tumor immunity while sparing systemic tolerance. We hypothesize that RORγt+ Foxp3+ pTregs travel to distant tumor sites and promote sex-dependent tumor growth via cell-intrinsic and -extrinsic mechanisms. We have shown that conditionally knocking out RORγt in Foxp3+ CD4 T-cells can attenuate subcutaneous MC38 tumor growth in male mice, but not female mice. Utilizing flow cytometry, we showed a decrease in Foxp3 expression and IL-10 production in the tumors of knockout (KO) male mice compared to wild-type (WT). This indicates that the KO pTregs have a less suppressive phenotype in the tumor, and thus these mice have the potential for a stronger anti-tumor response. Additionally, we see an increase in intratumoral RORγt+ Foxp3- CD4 T-cells, also known as Th17 cells, however we see no differences in cytokine production of IL-17, IFNγ or TNFα at this early timepoint. At present, we have shown that RORγt expression in Foxp3+ T-cells is important for the maintenance of the pTregs suppressive capacity, however more extensive research is necessary to understand the mechanisms of anti-tumor immunity. Ultimately the knowledge gained can be used to improve targeted immunotherapies while minimizing autoimmune-like adverse events.

Poster No. 41
Cancer Immunology and Immunotherapy Program

Investigating the mechanisms of *Helicobacter hepaticus* mediated lymphangiogenesis and tertiary lymphoid structure formation

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Recent studies have shown that the composition of a patient's gut microbiome and the presence of tertiary lymphoid structures (TLS), ectopic lymphoid structures that form in peripheral tissues during inflammation, are associated with improved immune checkpoint blockade response in a variety of cancers. However, we lack data indicating that TLS are causative for better cancer outcomes and are not just a byproduct of a robust anti-cancer immune response. Our group has shown that *Helicobacter hepaticus* (*Hhep*) colonization in mice reduces colorectal tumor burden, supports TLS formation and maturation through lymphangiogenesis and immune cell recruitment; however, the initial signals that drive this process remain unknown. Additionally, we have found that *Hhep* colonization increases genes associated with lymphangiogenesis such as *Lyve1*, *Pdpn*, and *Vegfc*, and increases lymphatic vessels surrounding mature TLS. Therefore, we hypothesize that lymphangiogenesis is required for TLS initiation and maturation.

Our previous work showed that *Hhep* drives mature TLS by day 14 post-colonization, however it was unknown what occurs prior. We used *Hhep* colonization, without tumor, as a model to investigate the spatial and temporal dynamics of TLS formation. We found that TLS are induced as early as day 2 post-*Hhep* colonization. Strikingly, TLS with germinal centers, the most mature TLS stage, were only found in *Hhep* colonized colons. Here we show that the *Hhep* colonization provides us the unique opportunity to elucidate the mechanisms promoting lymphangiogenesis and the formation and maturation of TLS in health and disease. Understanding the mechanisms governing these processes holds wide therapeutic potential including improving responses to immunotherapy

Poster No. 42
Cancer Virology Program

Arginine Sensor CASTOR1 Modulates Cellular Senescence and Oncogenesis

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Cytosolic Arginine Sensor for mTORC1 Subunit 1 (CASTOR1) is a recently identified regulator that negatively influences mTORC1 activity. Prior research demonstrated CASTOR1's role as a tumor suppressor in KSHV-induced transformation and breast cancer through its impact on mTORC1 activity and cell proliferation. However, its involvement in cellular senescence and oncogenesis had not been explored until now. Ras proteins, crucial GTPases, orchestrate signaling networks that determine cell fate, yet the interplay between Ras activity, cellular senescence, and proliferation remains undercharacterized. In this study, we examined CASTOR1's role in senescence and tumorigenesis using mouse embryonic fibroblasts (MEFs) from a novel CASTOR1 knockout (KO) mouse model crossed with the KrasG12D mutant transgenic model. Our findings reveal that CASTOR1 KO impairs cell proliferation and induces senescence through altered mTORC1 activation and increased reactive oxygen species (ROS) levels. Notably, CASTOR1 KO also exacerbates senescence induced by the KrasG12D mutant. Foci formation assays showed that CASTOR1 KO or KrasG12D mutant cells formed numerous large foci upon prolonged culture, unlike wild-type (WT) cells. Furthermore, CASTOR1 KO and KrasG12D mutant cells developed large colonies in soft agar assays. Conversely, KrasG12D mutant cells exhibited fewer and smaller colonies compared to CASTOR1 KO and KrasG12D mutant cells. These results suggest that CASTOR1 knockout and KrasG12D mutation enable cells to bypass senescence, sustain proliferation, and drive malignant transformation. Thus, CASTOR1 emerges as a critical link between oncogenic signaling and the regulation of cell fate and proliferation.

Poster No. 43

Identifying Antigenic Drivers of Tertiary Lymphoid Structure Formation in Solid Cancers

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Tertiary Lymphoid Structures (TLSs) are ectopic lymphoid structures that can be formed in the presence of inflammation or tumors and are niches for antigen drainage that allow for cellular traffic and interactions. The structure of TLSs provides nearby compartments to support cognate interactions of T follicular helper (Tfh) and B cells, which facilitates antigen-specific, anti-tumor responses. The presence of TLSs and infiltrating immune cells in cancers is associated with favorable prognosis and increased patient survival. Despite the widely recognized importance of TLSs in cancer, we do not know how they are formed, but there are currently three hypotheses: stromal remodeling, antigen-driven, and intrinsic properties of the tumor itself. We believe that this process is antigen driven, but in absence of the knowledge of the antigens, it is difficult to understand their contribution to TLS formation. Knowing what antigens are recognized by Tfh cells in TLSs will lead us to a greater understanding of the processes that lead to TLS formation and influence therapeutic outcome. By implementing a murine melanoma model of B16-OVA, we are able to control whether or not TLSs are formed based on injection site. Using this model, we were able to determine the presence of tumor-specific Tfh cells found in the tumor. We also performed scRNA-seq on the tumors that formed TLSs to identify Tfh-specific antigens via SABRs. To fully investigate the mechanism of TLS formation in cancer, novel engineered receptors (BCR-SABRs) will be used to initiate TLS formation in mice whose tumors do not from TLSs.

Poster No. 44
Cancer Virology Program

Unveiling the Vital Role of FAM50A in KSHV-Mediated Cellular Transformation Through RNA Splicing Reprogramming

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Kaposi's sarcoma-associated herpesvirus (KSHV) is a known culprit in various human cancers, notably Kaposi's sarcoma (KS), which remains a significant threat, particularly in AIDS patients. Our previous CRISPR-Cas9 screening of matched primary rat mesenchymal stem cells (MM) and KSHV-transformed MM cells (KMM) identified FAM50A, a spliceosome component, as indispensable for KSHV-induced cellular transformation. To unravel how KSHV manipulates FAM50A to reprogram cellular splicing, we sequenced MM and KMM cells with and without FAM50A knockout. We delineated 335 differentially expressed alternative splicing events between KMM and MM cells, with notable enrichment in Skipping Exon (SE) and Alternative 3' splice-site (A3) events. Motif enrichment analysis uncovered potential FAM50A-regulated motifs, including AGAGGAGGGG and AGAGGAAGGG. Gene ontology analysis underscored enrichment of MAPK pathways, particularly the ERK1/2 cascades implicated in KSHV-induced cellular transformation. Remarkably, FAM50A knockout altered SHP2 splicing, elevating a transcript isoform with enhanced enzymatic activity, resulting in reduced STAT3 Y705 phosphorylation in KMM cells. Additionally, we unveiled the interaction between the KSHV latent protein LANA and FAM50A, enhancing its expression to drive KMM cell proliferation and transformation. Both LANA and FAM50A directly interacted with histone variant H2AZ, suggesting LANA's role in recruiting splicing regulatory proteins to histones. These findings shed light on how KSHV harnesses FAM50A to reshape global transcript splicing, fueling cellular transformation. FAM50A-mediated SHP2 splicing activation underscores its pivotal role in KSHV-induced oncogenesis.

Poster No. 45
Cancer Immunology and Immunotherapy Program

Microbe-rich TLS drive TLS activity and immunotherapy response in melanoma patients

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Background: Immunotherapy results in durable long-term survival in some melanoma patients; however, predicting which patients will respond remains a challenge. Current predictive biomarkers for immunotherapy response include tumor mutational burden, PD-L1 expression, and the gut microbiome, all of which don't consistently predict patient clinical outcomes. Tertiary lymphoid structures (TLS), immune cell clusters that form at sites of chronic inflammation and antigen exposure are associated with superior immunotherapeutic response; however, what factors support TLS formation and activity and how this impacts immunotherapy response remains unclear. Maximal TLS activity is currently hallmarked by the formation of germinal centers (GCs) with increased B/T cell proliferation and B cell somatic hypermutation. We have recently found that some immunogenic bacteria, like *Helicobacter hepaticus,* reside within TLS and directly support their activity in mouse models. Therefore, we sought to determine the link between the microbiome, TLS activity, and overall prognosis in melanoma patients.

Methods: Tumor biopsies were collected before treatment with Pembrolizumab and high dose interfeon (Baseline) and after surgery (Post), and tumor sections were screened for TLS using CD20 staining. Sections were stained using Fluorescence *in situ* hybridization (FISH) to assess bacteria localized near or within tumor-associated TLS. Microbe-rich/poor TLS were analyzed for TLS activity using multispectral imaging (Akoya MOTIF).

Results: Patients who had an increase in TLS number and activity from baseline to post surgery showed an increased pathological response to Pembrolizumab and high dose interferon. Interestingly, TLS organization and activity (increased GC formation, B/T cell proliferation and B cell somatic hypermutation) was correlated with the presence of bacteria within the TLS. Patients with microbiome-rich TLS had the best overall response to immunotherapy, whereas those with microbiome deficient TLS had poor response with stable disease in most cases.

Conclusions: For the first time, we have shown a direct correlation between microbe-rich tumor-associated TLS and positive immunotherapy responses in melanoma patients. This not only demonstrates that microbiome-rich TLS may be a beneficial biomarker for immunotherapy in melanoma patients, but also suggests that administration of TLS-supporting bacteria may represent a new treatment modality to boost immunotherapy responses in melanoma.

Poster No. 46

Cancer associated psychological distress and pain in head and neck cancer patients.

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Background: Head and Neck Squamous Cell Carcinoma (HNSCC) is associated with worse pain, stress, and quality of life, than other cancers. The objective of our study is to explore the relationship between HNSCC patient-reported pain and psychological symptom burden prior to surgery.

Methods: This prospective, cohort study included patients diagnosed with squamous cell carcinoma of the oral cavity and oropharynx who underwent surgery between July 2020-December 2023. Baseline general head and neck (HN) and oral pain (Brief Pain Inventory, University of California San Francisco Oral Cancer Pain Questionnaire, respectively), depression [Patient Health Questionnaire (PHQ-8)], and anxiety [Generalized Anxiety Disorder-7 (GAD-7)] were included in the analysis. Multivariable regression was used to investigate the association between pain scores and anxiety/depression while controlling for sex, age and tumor staging.

Results: A total of 70 participants were analyzed, of which a majority had oral cavity cancer (mean [standard deviation]: 65 [91.5%]), with a tumor stage of T1-2 (45 [64.3%]) and nodal stage of N0 (45 [64.3%]). After controlling for age, sex, and stage, patients with higher levels of oral pain (p=0.004, beta=0.804, 95% CI [0.254, 1.355]) or general head and neck pain at its worst in the last 24 hour (p=0.034, beta=0.498, 95% CI [0.038, 0.957]) were more likely to experience higher levels of depression. Higher levels of oral pain (p=0.100, beta=0.437, 95% CI [-0.083, 0.958]) or general head and neck pain at its worst in the last 24 hour (p=0.244, beta=0.250, 95% CI [-0.170, 0.670]) were not associated with higher levels of anxiety.

Conclusion: Higher levels of depression were strongly associated with higher levels of HNSCC-related oral and general head and neck pain.

Poster No. 47
Genome Stability Program

ATRX LOSS IN UTERINE LEIOMYOSARCOMA IS ASSOCIATED WITH WORSE OVERALL SURVIVAL AND A DISTINCT PATTERN OF GENE EXPRESSION ACROSS SUBTYPES

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ATRX, a-thalassemia/mental retardation syndrome X-linked, is a chromatin remodeling protein that is frequently deficient in sarcomas. Somatic ATRX loss typically occurs in 10-30% of patients with leiomyosarcoma (LMS) and undifferentiated pleomorphic sarcoma (UPS). We sought to evaluate the molecular and clinical impact of ATRX alterations in sarcoma subtypes by leveraging survival, RNA-seq, and genome-wide DNA methylation data in a cohort from The Cancer Genome Atlas (TCGA). Of the 234 patients, 21% of patients (n=50) had ATRX loss of function alterations. ATRX loss most frequently occurred in UPS (34%) followed by uterine LMS (31%), DDLPS (24%), MFS (20%), and non-uterine LMS (7%). Patients with ATRX loss had a trend towards shorter median overall survival compared to those with ATRX (54 months vs. 76 months, p=0.25), and this difference increased and was significant when we excluded DDLPS (35 months vs. 81 months, p=0.0059). When we excluded DDLPS, ATRX loss was associated with an increased risk of mortality (HR=1.86 [95% CI: 0.90, 3.84], p=0.093), especially among those with uterine LMS (HR=5.18 [95% CI: 1.06, 25.30], p=0.042), adjusting for tumor size. Gene expression analysis identified 32 differentially expressed genes associated with ATRX loss (FDR <0.05). We identified over 500 differentially methyled CpG sites associated with ATRX loss (FDR < 0.05). Previous work suggested that ATRX loss in UPS caused de-repression of transposable elements (TEs) resulting from changes in epigenetic regulation. We identified 134 differentially expressed TEs (p < 0.05), and ATRX loss was associated with increased expression for almost all these sites. Therefore, ATRX loss in sarcoma subtypes is a potential prognostic biomarker particularly among patients with uterine LMS. ATRX loss is associated with genome-wide DNA methylation and tumor-relevant gene expression. Ongoing analyses of gene expression and immune infiltrates will improve our understanding of the impact of ATRX loss on sarcoma biology and prognosis.

Poster No. 48
Cancer Virology Program

Heterogeneity of Oncogenic and Survival Signaling Pathways in Primary Effusion Lymphoma: Implications for Precision Targeting and Combination Therapy

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Cancer's inherent heterogeneity presents a major challenge in developing effective therapies. Primary Effusion Lymphoma (PEL), a rare and aggressive lymphoproliferative disorder driven by Kaposi's sarcoma-associated herpesvirus (KSHV) and frequently co-infected with Epstein-Barr virus (EBV), typically manifests as lymphomatous effusions in body cavities. This malignancy primarily affects immunocompromised individuals, such as those with HIV/AIDS, and is associated with poor prognosis and limited therapeutic options, with an average survival rate of less than 6 months. The role of pathway heterogeneity in PEL's poor prognosis and the consequent therapeutic implications remain inadequately explored.

To identify potential therapeutic targets for PEL, we analyzed three PEL cell lines including BC3, BCP1, and BCBL1, alongside a control B-cell line, BJAB, and KSHV-infected BJAB cells. Using Western blotting, we assessed the activation patterns of key signaling pathways involved in cell growth, proliferation, and survival, including mTORC1, NF-κB, PI3K/AKT, and FOXOs. We then evaluated the effect of pathway-specific inhibitors on these pathways to determine their roles in PEL cell proliferation and survival.

Our analysis revealed significant heterogeneity in pathway activation among the PEL cell lines. AKT was consistently activated across all PEL lines, while mTORC1, NF-κB, and FOXOs were strongly activated in BC3 and BCBL1 cells but were minimally active in BCP1 cells. BC3 and BCBL1 cells were highly responsive to inhibitors targeting their activated pathways. In contrast, BCP1 cells exhibited resistance to the mTORC1 inhibitor rapamycin; however, this resistance was mitigated by using dual-target inhibitors that address both PI3K/AKT and mTOR.

The findings highlight significant variability in oncogenic and survival signaling pathways among PEL cell lines, with distinct responses to pathway-specific inhibitors. This heterogeneity suggests that precision medicine approaches tailored to individual PEL patients may be essential for effective treatment. Furthermore, combination therapies targeting multiple pathways offer a promising strategy to improve therapeutic outcomes for PEL.

Poster No. 49
Genome Stability Program

NCOR1 IS AMPLIFIED IN LEIOMYOSARCOMA AND OSTEOSARCOMA WHERE ITS EXPRESSION LEVELS ARE ASSOCIATED WITH DISTINCT TRANSCRIPTIONAL STATES

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Osteosarcoma (OS) and leiomyosarcoma (LMS) are rare tumors derived from mesenchymal lineages with high rates of metastasis and recurrence. Typical treatment for OS includes surgery and chemotherapy with surgical resection being the mainstay in localized LMS treatment. Despite mild improvements in survival, results remain poor, leading investigators to seek more therapeutic options. Over several years, a diverse array of small molecules has emerged, targeting the cancer genome at the epigenetic level. Targeted sequencing identified NCOR1 amplification as the most common copy number variation among OS (21%) and LMS (19%) patients. NCOR1 encodes a nuclear co-repressor that complexes with HDAC3—a deacetylase involved in gene repression. We sought to understand the impact of NCOR1 amplification in OS and LMS clinical samples by determining gene amplification, protein expression levels, and changes in transcription. We first obtained forty-two soft-tissue and bone sarcoma samples from the University of Pittsburgh Musculoskeletal Oncology Tumor Registry and Tissue Bank. Fluorescence in-situ hybridization (FISH) analysis revealed 17% of samples exhibiting NCOR1 amplification based on a 1.75 ratio of NCOR1signals to centromere signals on chromosome 17. At the protein level, 50% of our samples had high NCOR1 expression by immunohistochemistry (IHC). RNA-seq of our samples, stratified by high versus low NCOR1 expression, revealed a significantly higher number of differentially expressed genes in samples with high NCOR1 expression. We also performed a retrospective chart review and clinical analysis of these samples and found no difference in overall, disease-free, and metastasis-free survival between NCOR1 groups. Following this analysis, we screened ten OS and LMS cell lines for NCOR1 gene amplification via FISH and protein expression via immunoblotting. While we did not observe any gene amplification as we did with the clinical samples, we did see variable protein expression, motivating future work to explore transcriptional and epigenomic changes in these cell lines.

Poster No. 50

Hypermetabolic expansion conditions imprint lasting dysfunction on adoptive cell therapies.

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Generating required cell numbers is a limiting factor in adoptive cell therapies like chimeric antigen receptor (CAR) T cell therapy, leading to the development of bioreactors designed for high scale proliferation. Culture conditions used for T cell expansion are extremely hypermetabolic, often 2 to 10 times richer in fuel sources like glucose compared to physiological levels. This may result in dysfunctional cells unable to persist and function in fuel deficient in vivo environments. Thus, we hypothesize that commonly used hypermetabolic expansion conditions contain high amounts of nutrients like glucose and may push T cells towards terminal differentiation, resulting in poor anti-tumor response and impaired memory formation in vivo. To compare the efficacy of commonly used T cell expansion conditions, Peripheral blood mononuclear (PBMCs) cells were used to generate anti-CD19 CAR-T cells in RPMI with increasing amounts of glucose (5mM, 1mM, 55mM) in gas-permeable Rapid expansion (G-Rex)R bioreactor or traditional flasks and analyzed for metabolic/functional parameters. T cells expanded in hypermetabolic conditions showed decreased mitochondrial capacity indicative of metabolic insufficiency and poor in vivo persistence. Additionally, CAR T cells expanded in physiological 5mM glucose activated better and were more functional in vitro and in vivo compared to cells expanded in hyperglycemic media in a NALM6 leukemia model. Our data suggests that commonly employed hypermetabolic culture conditions may imprint an unappreciated form of dysfunction. This suggests that modifying or engineering cell culture systems to more adequately mimic physiologic metabolic conditions may better prepare T cells to eradicate cancer in patients.

Poster No. 51

Poor Glycemic Control is Linked to Pathological Upstaging in Renal Cell Carcinoma

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Background: Diabetes has been associated with upstaging of localized clinical grade T1–T2 (cT1–T2) renal cell carcinoma (RCC) to the pathological T3 grade (pT3; with extrarenal tumor extension) [1]. However, the relation of diabetic severity—as measured by glycemic control—to upstaging risk remains unclear.

Hypothesis: Elevated hemoglobin A1c (HbA1c), a quantitative marker for glycemic control [2], in patients with cT1–T2 RCC is associated with increased odds of upstaging to pT3 and above (pT3+).

Methods: A single-center retrospective cohort study was performed including adult patients treated with nephrectomy between January 2019 to December 2022 who had HbA1c resulted within ±90 days of preoperative imaging and before surgery. Non-RCC and non-cT1–T2 RCC were excluded. Patients were categorized as “normal,” “pre-diabetes,” “well-controlled diabetes,” or “poorly-controlled diabetes.” HbA1c category, preoperative tumor size, days between preoperative imaging and surgery, BMI nearest to the imaging date, age at the imaging date, and gender were factored into a logistic regression model for predicting cT1–T2 to pT3+ upstaging.

Results: Two hundred ninety-four patients with cT1–T2 RCC were included. The mean age was 64.85 ± 10.60, and mean BMI was 32.81 ± 7.31. Most patients were male (n = 182; 62%). Forty-two (14%) and 70 (24%) patients had diabetic or poorly-controlled diabetic range HbA1c, respectively. Sixty-six (22%) patients exhibited pT3+ upstaging. The significant predictors of upstaging were poorly-controlled diabetic range HbA1c (OR = 2.39; 95% CI [1.02, 5.59]; p = 0.04), preoperative tumor size (OR = 2.75; 95% CI [1.73, 4.37]; p <0.001), and marginally, age at imaging (OR = 1.05; 95% CI [1.01, 1.08]; p <0.01).

Conclusions: Poorly-controlled diabetic range HbA1c is associated with increased odds of cT1–T2 to pT3+ upstaging. Clinicians should consider patient glycemic control during preoperative workup and counseling for localized RCC.

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Poster No. 52
Cancer Epidemiology and Prevention Program

INTRALESIONAL TREATMENT ASSOCIATED WITH SIMILAR POST-OPERATIVE OUTCOMES TO WIDE RESECTION FOR RENAL CELL CARCINOMA BONE METASTASES

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Objective:

In the management of renal cell carcinoma (RCC) long bone metastases, intralesional procedures (IL) generally enable reduced surgical morbidity and faster recovery but may pose an increased risk for disease recurrence/progression and the need for revision surgery compared to wide resection and megaprosthetic reconstruction (R&R). This is especially germane to RCC metastases, which demonstrate variable sensitivity to adjuvant radiation. We aimed to synthesize data from all long bone RCC metastases treated at our institution since 2005 to compare post-operative complications among patients who initially underwent IL versus R&R procedures, specifically the need for additional surgery.

Methods:

Patients who underwent surgical treatment of RCC metastases to long bones at our institution's orthopedic oncology practice between 2005-2024 were included (n=126). Medical charts were reviewed to collect data on surgical details, mortality, radiation history, and systemic therapy history. Two-sample proportion tests were used to compare mortality and need for additional surgery between patients who initially underwent IL versus R&R procedures.

Results:

Of 156 unique sites of long bone metastases in 126 included patients, 114 (73%) were initially treated with IL procedures and 42 (27%) with R&R. There was no difference in the proportion of metastases that required additional surgery between the IL (26/114, 23%) and R&R (9/42, 21%) groups (p=0.85). Mortality rate was similar among 89 patients whose first operation was an IL procedure (60/89, 67%) and 37 patients whose first operation was an R&R procedures (25/37, 68%) (p=0.99). Mean survival after first operation was not significantly different between the two groups (514 days vs. 718 days, p=0.09).

Conclusions:

We observed no difference in mortality and need for additional surgery following intralesional versus wide resection and reconstruction procedures. These results suggest that there may be no advantage to treating RCC long bone metastases with initial wide resection and reconstruction.

Poster No. 53

Tiny Intruders: Microplastics Inhibit Phagolysosomal Function in Macrophages

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Microplastics are near ubiquitous pollutants which have been observed in human lung tissue, sputum, and bronchoalveolar lavage. Yet, the effect of microplastics in the pulmonary environment, and the immune system of the lung in particular, is much unexplored. Macrophages serve at the interface with the environment in the lung, providing essential protective functions by engulfing and eliminating pathogens and debris. We hypothesize exposure to aerosolized microplastics may inhibit pulmonary macrophage function and over time, increase susceptibility to lung cancer.

To examine the direct effect of microplastics, Raw264.7 macrophages were cultured with fluorescent polystyrene microplastics of sizes 0.02um, 0.1um, 1.0um, and 10um. Using fluorescence microscopy, we determined particulate uptake per size and evaluated microplastics degradation through phagolysosomal vesicles in macrophages. Phagocytosis and lysosomal processing in microplastic exposed macrophages was evaluated via fluorescence detection following incubation with FITC- and pHrodo-labeled zymosan particles and E. coli.

Our findings illustrate that macrophages readily phagocytose microplastics which subsequently inhibit their ability to process pathogens. Microplastics of 0.01um induced the greatest inhibition of macrophage function and image analysis suggests that this deficit affects lysosomal processing but not phagocytosis. Microplastics result in sustained inhibition of macrophage function, which is present out to 3-days post-administration. Notably, addition of the AMP kinase activator AICAR (Acadesine) was able to restore macrophage function following microplastic exposure. Ongoing studies are evaluating the distribution of microplastics of various sizes across organs (lung, brain, liver, kidney, heart, colon) following intranasal administration in mice. This will allow us to assess intra- and extrapulmonary dissemination of the microplastics over time. Collectively, these findings indicate that microplastics can directly inhibit the ability of macrophages to clear particulate matter which may lead to increased susceptibility to infection, chronic tissue damage, and ultimately lung cancer.

Poster No. 54
Cancer Biology Program

Magnetic Hyperthermia Therapy Enhances the Chemoradiosensitivity of Glioblastoma

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Glioblastoma (GBM) is the most common and aggressive primary human brain tumor associated with very poor prognosis and survival.
Current standard-of-care treatment consists of maximal, safe tumor resection with adjuvant radiation therapy (RT) and concurrent and adjuvant temozolomide (TMZ) chemotherapy, collectively known as chemoradiation (CRT). However, GBM remains fatal due to the inability to completely resect tumor that has invaded eloquent regions of the brain, challenges with drug delivery across the blood-brain barrier (BBB), tumor heterogeneity, and the many ways in which GBM adapts to resist modern therapies.

Magnetic hyperthermia therapy (MHT) relies on magnetic iron oxide nanoparticles (MIONPs) that are activated by an alternating magnetic field (AMF) to generate local hyperthermia. Heating tumor tissue between 40-45 ^{O}C decreases cancer cell viability, radiosensitizes tumor cells, promotes anti-tumor immune signaling, and increases intratumoral blood flow for improved drug delivery and enhanced CRT.

Recent evidence from our laboratory provided proof of the therapeutic potential for GBM using a combination strategy based on MHT in combination with CRT treatment in multiple GBM models. MIONPs were characterized regarding heating efficacy and delivered intracranially via convection- enhanced delivery. Our in vitro experiments showed a significant reduced cell viability and increased apoptosis upon hyperthermia (HT) + CRT treatment compared to monotherapies across multiple GBM cell lines. *In vivo*, MHT+CRT decreased tumor burden and increased the overall survival compared to CRT alone in both PDX and syngeneic models. Furthermore, immunofluorescence analysis revealed an increased tumoral expression of γ-H2AX (DNA double-strand breaks), HSP90 (heat shock response), CD4 (T-cell recruitment), and IBA-1 (microglial activation) in MHT+CRT mice compared to those treated with CRT alone. In conclusion, adjuvant MHT enhances CRT efficacy in GBM models by inducing tumor chemo- and radio-sensitization, stimulating anti-tumor immune responses, and improving survival outcomes. These findings warrant further investigation of MHT+CRT combination therapy for GBM treatment.

Poster No. 55
Biobehavioral Cancer Control Program

Creating Community: Cancer Crushers Exercise Program

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Introduction: People living with and beyond cancer do not meet the recommended amount of exercise to mitigate treatment and disease-related adverse effects. Resources for safe exercise can be difficult to find and starting an exercise program can be intimidating for many people.

Objective: The objective of the Cancer Crushers exercise program was to provide people living with and beyond cancer with a free community-based exercise program while facilitating transition into independent exercise. This study also aimed to prepare participants for the Rush to Crush cycling fundraiser.

Methods: Participants were recruited from the UPMC Moving Through Cancer program and by word of mouth. The exercise program began in February 2024 for 12 weeks leading up to Rush to Crush in May 2024. Exercise sessions were scheduled for at least twice a week at 30-60 minutes with a combination of resistance, aerobic, and flexibility training. The intensity and type of exercise were based on functional capability, exercise goals, and their selected Rush to Crush event. Qualitative interviews were completed with participants to gauge program success and future directions.

Results: Thirteen different cancer types were represented by program participants, with 60% of participants on active treatment during the exercise program. Calculated exercise adherence was excellent, averaging 72.6%. Community engagement was high with features by the KDKA Talk Pittsburgh show and the UPMC social network.

Conclusion & Future Directions: The diversity of diagnoses that participated in Cancer Crushers demonstrates that exercise is feasible for many cancer types and treatment status is not prohibitive to exercise. The Cancer Crusher program was highly praised by participants and helped fulfill the need for an expert-led exercise program that provided guidance, goal setting, and peer support. As Cancer Crushers expands, clinical collaborations and referrals will be crucial in the program's continuation and success.

Poster No. 56
Cancer Virology Program

PIAS1 Targets Episome Maintenance Proteins of EBV and HPV16 to Regulate Viral DNA Replication

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Epstein-Barr virus (EBV) is a prevalent human pathogen that establishes latency in B cells and periodically reactivates in response to external stimuli. Our prior research revealed that Protein Inhibitor of Activated STAT1 (PIAS1) functions as an EBV restriction factor by binding to ZTA and RTA promoters. To determine PIAS1 binding profile across the entire EBV genome, we conducted PIAS1 Chromatin Immunoprecipitation-Sequencing (ChIP-Seq) experiments using EBV+ Burkitt lymphoma cells. Intriguingly, we found that PIAS1 is highly enriched within the origin of plasmid replication (OriP), a region supporting the replication and stable maintenance of EBV episomes in human cells. However, the role of PIAS1 in EBV episome maintenance remains unclear. Because EBV EBNA1 binds to OriP region and tethers EBV episome to human chromosome, we hypothesize that PIAS1 interacts with EBNA1 to regulate EBV episome replication. Through co-immunoprecipitation experiments in transfected cells, we found that PIAS1 and EBNA1 are in the same complex. PIAS1 is an E3 SUMO ligase for protein SUMOylation. In vitro SUMOylation analysis revealed that PIAS1 enhances EBNA1 SUMOylation. To determine whether PIAS1 and EBNA1 regulate EBV lytic replication, we overexpressed PIAS1 and EBNA1 together with ZTA to trigger EBV reactivation in HEK-293 EBV+ cells. We found that PIAS1 synergizes with EBNA1 to restrict EBV lytic reactivation. Interestingly, we also demonstrated that PIAS1 binds to other episome maintenance proteins, namely Kaposi's sarcoma-associated herpesvirus (KSHV) LANA and human papillomavirus 16 (HPV16) E2, and a non-direct episome maintenance protein, HBx, in Hepatitis B virus (HBV), highlighting the functional conservation of PIAS1-viral protein interactions across different human tumor viruses. We further demonstrated that PIAS1 overexpression enhanced the replication efficiency of HPV16 replicons. Ongoing study will determine the role of PIAS1 in EBV, KSHV and HPV16 genome maintenance and the molecular mechanisms underlying this regulation.

Poster No. 57

Patient concerns and self-management strategies among older adults with advanced cancer: A preliminary report

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Background: Older adults receiving advanced cancer treatment experience steep declines in quality of life. Understanding the salient concerns of this population can help to inform palliative oncology delivery models. Consequently, we are conducting a secondary analysis of data from a palliative oncology intervention trial to characterize the concerns and self-management strategies of older patients with advanced cancer.

Methods: We conducted a cluster-randomized controlled trial of an oncology nurse-led primary palliative care intervention for patients with advanced solid tumors receiving care at 17 community oncology clinics in Western Pennsylvania from 2016-2020. All visits were led by oncology nurses trained to address palliative care needs. Of 672 patients enrolled, 336 were randomized to the intervention arm, of this group, 179 were age 60 or older and the focus of this analysis. We used audio recordings from initial study visits (N=179) that were transcribed using HIPAA-compliant TranscribeMe, Inc. We are conducting qualitative descriptive content analysis using mixed inductive and deductive methods. All transcripts are reviewed by two coders with disagreements resolved by consensus.

Results: Of the 74 visits analyzed thus far, the most frequent patient concerns were physical in nature with fatigue being the most common (76%, 56/74) followed by loss of appetite (50%, 37/74), and pain (49%, 36/74). Outside of physical symptoms, the most common patient concerns were feelings of depression (38%, 28/74) and anxiety (35%, 26/74), functional concerns (32%, 24/74), and changes in self-concept (23%, 17/74). Only one patient had no concerns. In terms of self-management strategies, patients most frequently used supportive medications (76%, 56/74) followed by relationship with family (53%, 39/74) and using a nutrition plan (47%, 35/74).

Next Steps: We continue to refine our codebook and analyze transcripts. Ultimately, we expect that the findings from this analysis will help to inform areas of future innovation in palliative care delivery.

Poster No. 58
Cancer Biology Program

Revealing how CCNE1 oncogene reprograms nuclear metabolism in cancer.

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Cancers adopt metabolic adaptations for survival and excessive cell division, including differential metabolic compartmentalization allowing cells to maximize certain pathways. However, how oncogenic stress affects nuclear metabolic compartmentalization is unclear. Focusing on a key oncogenic driver, cyclin E1 (CCNE1), which drives aberrant S phase progression, DNA replication, and DNA damage, our data reveal that metabolic reprogramming in CCNE1-driven cells is partly specific to the nuclear compartment. Proteomics on isolated nuclei revealed ~30 metabolic enzymes enriched in the nucleus of CCNE1-driven cells compared to controls, suggesting translocation from the cytoplasm to the nucleus under oncogenic stress. Interestingly, many of these enzymes are related to nucleotide and specialized fatty acid metabolism, typically annotated to the cytoplasm and mitochondria/peroxisome, respectively. In silico analysis identified two candidates with a predicted nuclear localization signal (NLS), namely GMP synthase (GMPS) and Acyl-CoA Synthase Long Chain Family 4 (ACSL4). GMPS is a crucial enzyme in de novo purine biosynthesis, whereas ACSL4 is involved in polyunsaturated fatty acid metabolism. These data suggest that oncogenic stress induces translocation of metabolic enzymes, which may directly influence cancer phenotypes. Inhibition of nuclear import/export confirms compartmentalization of these enzymes between cytoplasm and nucleus and suggests its importance in chemoresistance. Future studies will determine the role of these enzymes in nuclear metabolic reprogramming of cancer cells using activity assays and metabolomics. Furthermore, we will determine whether NLS affects their nuclear translocation, then test if preventing nuclear localization counteracts chemoresistance. Together, these studies will establish that oncogenic stress drives differential compartmentalization of metabolic enzymes, suggesting development of therapies to target nuclear enzymes or their translocation.

Poster No. 59
Cancer Virology Program

Role of CBP/p300 histone acetyltransferase in Merkel Cell polyomavirus replication and Merkel Cell carcinogenesis

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Merkel cell polyomavirus (MCV/MCPyV) persistently infects unknown skin cells and causes human Merkel cell carcinoma (MCC) through persistent infection. ~ 80% of MCC harbor clonally integrated MCV in the tumor genome and constitutively express viral large T (LT) and small T (ST) oncoproteins from the T antigen gene. MCV-positive(+) MCC are fully dependent on the expression of viral oncoproteins to grow. Thus, viral oncogene expression is a rational target to control MCC tumorigenicity. Others and our work found that CBP/p300 histone acetyltransferase (HAT) is essential for MCV gene expression, affecting both virus replication and MCC growth.
We identified that individual treatment of MCV+MCC cells with two separate CBP/p300 inhibitors, A-485 and dCBP-1, ablated their proliferation and induced quiescence with increased p27kip1 expression. Furthermore, the inhibitors also induced neurite formation, a marker of neuronal differentiation, in a co-culture context for some MCC cell lines. ChIP experiments identified direct binding of CBP and p300 to the T antigen promoter with H3K18ac, an active histone mark acetylated by CBP/p300. The cellular Sox2 promoter, which is activated by MCV LT, is also bound by CBP and p300. Sox2 is essential for MCC growth and controls expression of the Merkel cell lineage factor Atoh1, a critical factor for Merkel cell development. We found that CBP/p300 inhibitor suppressed expression of MCC marker genes, including ATOH1, KRT20 and INSM1.
While we are currently searching for the direct CBP/p300 target genes in MCC by unbiased sequencing approach, our results suggest that CBP/p300 directly controls viral T antigen and its downstream cellular Sox2 oncoprotein, a driver that promotes the Merkel cell phenotype and tumor cell growth. Thus, targeting the CBP/p300 HAT activity might be a rational target for differentiation therapy in virus-positive MCC and further uncover the expression cascade downstream of LT that is responsible for controlling the Merkel Cell phenotype of MCC.

Poster No. 60
Cancer Immunology and Immunotherapy Program

Unraveling Host-Gene-Microbiome Interactions in Ovarian Cancer

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Tumor-associated macrophages (TAMs) are critical negative regulators of anti-tumor immunity. Understanding factors which regulate these cells could result in the identification of new approaches to enhance anti-tumor immunotherapy.

The epidermal growth factor-like protein 6 (Egfl6) is a factor secreted by tumor cells known to promote angiogenesis and tumor progression. Egfl6 expression is also upregulated in human obesity. Our recent data revealed that Egfl6 induces an immunosuppressive state of macrophages enhancing their expression of IL-10, and TLRs factors. The gut microbiota exerts potent effects on the immune system and recent work has linked microbiota-immune interactions . We found upregulation of Egfl6 expression in ovarian tissue of germ-free compared to conventional raised mice. Additionally, we found differential expression of genes associated with an M2-like macrophage signature, such as Trem2, Stat3 and Mapk1. These findings suggest a potential link between microbiota, Egfl6 expression, and macrophage polarization in the ovary. Moreover, we found that transgenic mice overexpressing Egfl6 developed spontaneous tumors, including ovarian cancer (OvCa), and their body weight was higher compared to C57BL/6J control mice. As body weight affects gut microbiota, we collected serial stool samples and used 16S marker gene sequencing to determine community composition. Compared to C57BL/6J mice, Egfl6 mice showed a significant loss of the beneficial commensal Akkermansia muciniphila and Turicibacter sanguinis. Conversely, we found an increased Helicobater hepaticus abundance, inflammatory bacteria associated with increased cancer risk in various preclinical models and in humans. Using a syngeneic mouse model of OvCa, we observed reduced abundance of A. muciniphila in Egfl6+ tumor bearing mice compared to control tumor bearing mice. In conclusion, this study elucidates the critical interplay between Egfl6, gut microbiota, and TAMs in OvCa, revealing how Egfl6-induced dysbiosis impacts tumor progression.

Poster No. 61
Genome Stability Program

Inhibition of tyrosine kinase Fgr mitigates hematopoietic acute radiation syndrome (H-ARS)

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Ionizing radiation (IR) causes acute damage to hematopoietic and gastrointestinal systems and late effect fibrosis. The hematopoietic damage is known as the hematopoietic acute radiation syndrome (H-ARS). A few FDA approved therapies exist to treat H-ARS which are effective after 24h and none is known inhibit radiation late effects to the lungs. Therefore, there is an urgent need for novel medical countermeasures (MCMs) targeting master regulators of inflammation, proliferation, and tissue regeneration in response to Total body irradiation (TBI) injury in both acute and late settings. We have recently reported that tyrosine kinase Fgr is induced in irradiated mouse lungs, and treatment with an Fgr inhibitor (TL02-59) significantly reduces radiation-induced pulmonary fibrosis (Mukherjee et. al., Cell Death Discovery, 2021, 2023). Here we investigated whether Fgr is a master regulator of inflammation in the bone marrow (BM) after TBI and whether Fgr inhibition could mitigate H-ARS. We observed significant increase in Fgr expression in mouse BM cells after TBI in mice and in non-human primates (NHP). Fgr knockout (Fgr-/-), mice showed significant increase in survival relative to control C57BL/6 mice after TBI treatment. We also observed significant improvement in survival of control mice that received the Fgr inhibitor TL02-59 24h after TBI. Interestingly, we have documented induced expression of endogenous proteins that mitigate ARS including G-CSF, GM-CSF, and TGF-beta following TBI in Fgr-/- BM relative to control mice and reduced upregulation of inflammatory proteins in the BM of irradiated Fgr-/- mice. Furthermore Fgr-/- mouse BM cells were radioresistant compared to control BM in the CFU-GEMM colony assay. By histological analysis we found that relative to control mice, Fgr-/- mice retained higher BM cellularity following TBI. Taken together we found that Fgr is a master regulator of inflammation in the hematopoietic compartment and Fgr inhibitor TL02-59 is a potent mitigator of H-ARS.

Poster No. 62
Cancer Biology Program

Genomic Landscape of Intragenic Rearrangements Unveils the Hidden Pathobiological Drivers in Triple-Negative Breast Cancer

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Breast cancer exhibits significant molecular heterogeneity, complicating treatment and prognosis and necessitating a deeper understanding of its genetic underpinnings. Intragenic rearrangements (IGRs) are crucial pathological events and druggable targets, which have been largely overlooked by previous cancer genomics studies. Herein, we conducted a comprehensive analysis of IGRs in breast cancer using whole-genome sequencing data from the Pan-Cancer Analysis of Whole Genomes, systematically identifying 8,046 IGRs and 14,831 gene fusions. IGRs are associated with Pathways in cancer and show significant differences in enrichment across different molecular subtypes, especially in triple-negative breast cancer (TNBC) and menopausal status. A high IGR burden significantly reduces overall survival, and selected candidate genes (RAD51B, RUNX1) not only correlate with survival but also serve as independent prognostic factors in TNBC, offering new insights for personalized treatment. Furthermore, some recurrent IGRs, such as RUNX1 and AUTS2, are preferentially detected in TNBC tumors lacking immune infiltrates, influencing the distribution and function of various immune cell types. Our study identifies the hidden pathobiological drivers and underscores the importance of integrating genomic data into clinical practice to improve patient outcomes in TNBC.

Poster No. 63

T cells in Malignant Pleural Effusion: Unlocking their Potential for Cancer Immunotherapy

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Introduction: Tumor-reactive T-cells are necessary for effective cancer immunotherapy with increased diversity associated with beneficial clinical outcomes. Malignant pleural effusions (MPE) contain exponentially greater T-cell numbers than solid tumors, representing a novel cell source for adoptive cell transfer (ACT) therapies. Nevertheless, the repertoire of MPE-derived T-cells (MPET), as well as their anti-tumor and immunosuppressive properties remain poorly defined.

Methods: MPE, solid tumor, and blood were collected from patients with heterogeneous primary tumors at UPMC. T-cell receptor (TCRα/β/γ/δ) sequencing was performed on paired MPE and blood samples (n=14). Single-cell RNAsequencing (scRNAseq; n=4) and flow cytometry (n=30) were used to phenotype MPETs. MPET tumor cell lysis, cytokine production, and proliferation were measured. Suppressive activity of MPE-derived regulatory T-cells (Tregs; n=8) with or without anti-PD-1 antibody was determined.

Results: MPEs contained distinct TCR repertoires compared to those in patient's blood, with 74%-87% of TCRα/β/γ/δ clonotypes found in MPEs only ($p = \leq 0.0005$). MPETs were capable of killing tumor cells from both MPEs and autologous solid tumors, when available, and produced multiple pro-inflammatory cytokines upon stimulation ex vivo, which was inhibited by addition of acellular MPE fluid. Cytokine production was restored upon addition of Metformin. MPE phenotyping identified significant upregulation of inhibitory checkpoint receptors of effector T-cells (Teffs) as well as elevated levels of highly immunosuppressive Tregs with 70% expressing CD39 and/or PD-1. PD-1+ Tregs from MPEs suppressed Teff proliferation to a greater extent than PD-1- Tregs, and Treg suppression was alleviated upon PD-1 blockade.

Conclusions: MPEs contain a distinct T-cell compartment that retains anti-tumor reactivity ex vivo. In conjunction, PD-1 blockade alleviates the suppressive activity of MPE-derived Tregs and may enhance MPET tumoricidal activity. These findings suggest that combining MPETs for ACT therapy with checkpoint blockade and Metformin may synergistically enhance treatment efficacy for advanced cancer patients with MPEs.

Poster No. 64
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 to recruit PRC2 enzymes SUZ12/EZH2 to chromatin to facilitate H3K27 methylation, a marker for transcription repression. PHF19 contains N-terminal Tudor and Extended Homology (EH) domains and a C-terminal domain (CD). CD is a region essential for PHF19-PRC2 interactions and Tudor and EH are reported to bind histone and DNA, respectively. Here, using CRISPR-Cas9 genomic editing method to disrupt PHF19, we found that PHF19 depletion significantly promotes EBV lytic replication. We further demonstrated that PHF19 is efficiently cleaved upon lytic induction and caspase-mediated cleavage separates Tudor and EH domains from CD, providing a way to disable PRC2 and facilitate viral gene expression. Together our study identifies PHF19 as a novel caspase substate 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 how PHF19 is disrupted by caspase-mediated cleavage to promote the EBV replication.

Poster No. 65
Cancer Immunology and Immunotherapy Program

Development of an in vivo model for CRISPR screening to uncover novel targets of tumor-infiltrating Treg cells

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Regulatory T (Treg) cells are abundant in tumor tissues and play key roles in facilitating immune evasion by tumor cells. Targeting tumor-infiltrating Treg cells specifically while sparing peripheral Treg cells act as promising strategy in immunotherapy. Here we aim to employ CRISPR screening to identify novel regulators of tumor-infiltrating Treg focusing on aspects such as recruitment, survival, and suppressive function. The enrichment of thymus-derived Treg cells from OT-II and SMARTA TCR transgenic mice were maximized through expressing antigen under Rip promoter as well as IL-2/anti-IL-2 complex treatments. We further optimized the in vitro culture, activation of Treg cells, and retroviral transduction. A co-transfer model was then established by transferring Treg cells and CD8+ T cells recognizing the same antigen. Transferred Treg cells were shown to be highly suppressive by rescuing tumor growth inhibited by transferred CD8+ T cells. From tumor-bearing mouse, we recovered a decent number of transferred Treg cells, enabling us to do multiple analysis following selection in tumor. To decide candidate genes for the screening, we integrated scRNA-seq datasets from various tumor tissues in both mouse and human. By filtering differentially expressed genes and machine learning-based analysis comparing tumor-infiltrating Treg to peripheral Treg cells, we selected 60 genes whose roles in tumor Treg cells have not been appreciated. The quality of the library was then validated by measuring coverage and uniformity. These efforts, together with primary analysis, lay the groundwork for in vivo single cell CRISPR screening.

Poster No. 66
Cancer Biology Program

Using Drug Therapy to Target Androgen Receptor and Aldehyde Dehydrogenase 1A1 in Metastatic Osteosarcoma In Vitro

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Background: Previous studies using the ALDH-inhibitor Disulfiram as a single agent to treat metastatic osteosarcoma (OS) in vivo and in vitro have been promising. However, combination therapy is the clinical standard to prevent treatment resistance and reduce effective doses. Previous pathway analyses predicted androgen receptor (AR) to directly activate ALDH1A1 in OS lung metastases. In the present study, we evaluated the combination of Disulfiram and Enzalutamide, an AR-inhibitor, in metastatic OS.

Methods: Metastatic OS cell line SaOS-LM2 was treated with Disulfiram and Enzalutamide alone and in combination. For combination matrix experiments, cell viability was measured after 72h treatment. Single treatment dose curves were created and SynergyFinder 3.0 was used to evaluate the combination matrix data. Promising combination treatment regimens were evaluated using growth curve analysis as well as clonogenic survival and migration assays.

Results: Less than 50% SaOS-LM2 cell viability was observed at 30 μM Disulfiram and 300 μM Enzalutamide when treated separately. Disulfiram and Enzalutamide had an average Bliss synergy score of -1.19 ± 2.98 (95% CI). The survival fraction of SaOS-LM2 was significantly reduced by 3 μM Disulfiram after 48- and 72-hour incubations, respectively. Enzalutamide significantly reduced the survival fraction at 33 μM for 48 hours and 11 μM for 72 hours. At 48 hours, the relative wound closure of SaOS-LM2 was significantly reduced with Disulfiram 1 μM and the combination treatment of Enzalutamide 11 μM and Disulfiram 0.5 μM. This same combination treatment significantly inhibited wound closure at 72 hours also.

Conclusions: Disulfiram and Enzalutamide display additive effects when used in combination to treat metastatic OS in vitro. However, the effective dose of Enzalutamide remains at least one order of magnitude greater than reported IC50 in cancers responsive to AR inhibition. Nevertheless, the development of combination schedules may further reduce effective doses and enhance additive effects.

Poster No. 67
Cancer Epidemiology and Prevention Program

The Epstein-Barr virus nuclear antigen 1 (EBNA1) variant associated with nasopharyngeal carcinoma defines the sequence criteria for serologic risk prediction

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Purpose: Antibodies to select Epstein-Barr virus (EBV) proteins can diagnose early-stage nasopharyngeal carcinoma (NPC). We have previously shown that IgA against EBV nuclear antigen 1 (EBNA1) can predict incident NPC in high- and intermediate-risk cohorts 4 years pre-diagnosis. Here, we tested EBNA1 variants, with mutants, to define the sequence requirements for an NPC risk assay.
Design: Mammalian-expressed constructs were developed to represent EBNA1 variants 487V and 487A which can differ by ≥15 amino acids in the N- and C-termini. Denatured lysates were evaluated by a refined IgA and IgG immunoblot assay in a case-control study using pre-diagnostic NPC sera from two independent cohorts in Singapore and Shanghai, P.R. China.
Results: At 95% sensitivity, 487V yielded a 94.9% specificity compared to 86.1% for 487A. EBNA1 deleted for the conserved glycine-alanine repeats (GAr) reduced false positives by 22.8%. NPC sera reacted more strongly to the C-terminus than healthy controls, but the C-terminal construct (a.a. 390-641) showed lower specificity (84.8%) than the EBNA1 GAr deleted construct (92.4%) at 95% sensitivity.
Conclusion: Although EBNA1 IgA was present in healthy sera, most epitopes localized to the immunodominant GAr. We conclude that a refined EBNA1 antigen deleted for the GAr but with residues consistently detected in Southeast Asian NPC tumors is optimal for risk prediction with an extended sojourn time of 7.5 years. Furthermore, distinct EBNA1 serologic profiles enhanced the utility of the EBNA1 IgA assay for risk stratification. This illustrates the importance of serologically relevant EBNA1 sequences for NPC risk prediction and early detection.

Poster No. 68
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. OVCA progression involves transcoelomic metastasis, where tumor cells disseminate into the peritoneal ascites. Malignant cells in the ascites adapt to survive under Anchorage-Independent (A-I) conditions by altering the expression of genes escalating anoikis (A-I cell death) 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 SRY-Box Transcription Factor 2 (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 the high-grade serous ovarian cancer cell line, OVCAR3, 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 is a G-protein coupled receptor known to modulate canonical Wnt signaling upon interacting with its ligand R-spondin1 (RSPO1). Time-course experiments in OVCAR3 cells revealed a striking increase in LGR5 expression that peaked after 24 hours in A-I. Furthermore, siRNA mediated knockdown of SOX2 downregulated LGR5 expression only in A-I, suggesting that LGR5 is a potential SOX2 target under A-I conditions. 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. Our current studies focus on understanding the molecular mechanisms behind SOX2-driven regulation of LGR5 and the role of LGR5 in driving OVCA A-I cell survival.

Poster No. 69
Cancer Biology Program

Functional characterization of the novel cell adhesion molecule MPZL3 in ovarian cancer

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Ovarian cancer (OVCA) is the most lethal gynecological malignancy in the United States often detected at advanced stages. OVCA progression involves transcoelomic metastasis, where cells disseminate into the peritoneal fluid, adhere to form multicellular aggregates that promote anchorage-independent survival and facilitate metastatic colonization of the peritoneum. To meet the requirements of each stage of this detachment, aggregation, and re-attachment cycle, OVCA cells have been shown to dynamically regulate the expression of multiple cell adhesion molecules (CAMs) throughout their progression. Moreover, targeting cell-cell adhesion molecules has shown to be an effective method to slow/inhibit the progression of OVCA.
Myelin protein zero-like 3 (MPZL3) is a transmembrane protein with homology to other immunoglobulin-like (Ig) family of CAMs. While it has been reported that altered Ig-CAM expression plays a role in ovarian cancer, the function of MPZL3 has not been investigated. TCGA data shows frequent chromosomal loss of the MPZL3 locus (11q23.3) in various cancers, including high grade serous ovarian cancers (HGSOC), suggesting that loss of genes located in this area has tumorigenic consequences.
To study the function of MPZL3 in OVCA, we used shRNA mediated MPZL3 knockdown in OVCAR4 human HGSOC cells and examined transcriptome-wide effects by RNA sequencing. We found that loss of MPZL3 resulted in decreased cell growth with a concomitant resistance to both Cisplatin and Olaparib treatments, both of which are commonly used for treating OVCA. Moreover, we demonstrated that knockdown of MPZL3 decreased the homotypic adhesive capacity and promoted invasiveness of OVCA cells. Ongoing studies are exploring the mechanistic link between MPZL3 loss and chemoresistance and determining the effects on in vivo tumor progression. Understanding the novel role of MPZL3 will provide further insight into OVCA progression, thus generating opportunities of developing new treatments for patients with low MPZL3 expression as an approach for precision cancer medicine.

Poster No. 70
Cancer Biology Program

RHOV: A Novel Driver of Ovarian Cancer Transcoelomic Metastasis, with Implications for Disrupting Early Adaptations to Impede Metastatic Fitness

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Ovarian cancer (OVCA) spreads via transcoelomic metastasis, where cells disseminate into the peritoneal fluid, resist anoikis, and form multicellular aggregates (MCAs) invading the peritoneum. Despite extensive study of MCAs as metastatic seeds, the molecular events immediately following cellular detachment, preceding MCA formation, remain unknown. This study hypothesizes that early molecular changes immediately following detachment facilitate OVCA cells' transition from an attached to a suspension state, enhancing metastatic fitness. Utilizing unbiased RNA-sequencing, we identified genes consistently upregulated shortly after matrix detachment in three OVCA cell lines and patient-derived ascites cells. Among them, RHOV, an understudied atypical Rho-GTPase, showed robust upregulation. Unlike typical Rho-GTPases, RHOV exhibits constitutive activity and its role, especially in cancer, remains poorly understood. Our investigations revealed, for the first time, that RHOV-knockout (KO) rendered OVCA cells susceptible to anoikis, impeded MCA formation, and hindered peritoneal colonization in both in-vitro mesothelial-clearance assays, simulating peritoneal colonization, as well as in-vivo using an Intraperitoneal (IP) xenograft model. Our current investigations are dedicated to uncovering the mechanism by which RHOV enhances metastatic fitness of cancer cells upon detachment. Additionally, we share unexpected findings demonstrating RHOV's involvement in mediating TGF-β signaling in disseminated ovarian cancer, diverging from the anticipated PAK signaling pathway. These results unveil a previously unrecognized function for this atypical GTPase. Our research sheds light on the novel pro-metastatic role of RHOV and offers a unique proof of concept: disrupting early adaptations following matrix detachment could impede the metastatic fitness of disseminated cancer cells, presenting a novel anti-metastatic therapy approach.

Poster No. 71

Optimizing Fc-engineered novel anti-MSLN VH-Fc fusion proteins through PET imaging

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Introduction: The protein mesothelin (MSLN) is overexpressed in various cancers, making it a promising therapeutic target. We recently developed and evaluated [^{89}Zr]Zr-2A10-VH-Fc, an anti-MSLN VH-Fc fusion protein, and compared it to the clinically relevant M912-IgG1 using [^{89}Zr]Zr-labeled PET imaging in a murine model of human colorectal cancer (CRC). Initial findings showed that [^{89}Zr]Zr-2A10-VH-Fc had higher tumor accumulation and penetration than [^{89}Zr]Zr-anti-MSLN IgG1, but also exhibited high spleen uptake due to Fcγ receptor binding. To address this, we compared the pharmacokinetics of Fcγ receptor-binding-deficient 2A10-VH-Fc mutants (Fc_{GRLR} and Fc_{LALAPG}) to wild-type (2A10-VH-Fc_{WT}) to optimize anti-MSLN VH-Fc fusion proteins for targeted imaging or therapy of MSLN-expressing tumors.

Methods: Mutant 2A10-VH-Fcs were engineered (Fc_{GRLR} and Fc_{LALAPG}) and modified to present the DFO chelator for subsequent labeling with Zr-89. *In vivo* stability studies were conducted in normal mice at 1- day post-injection (p.i.). PET imaging was performed in NCG mice bearing HCT116 tumors at 90 minutes; 1, 2, and 5-days p.i. and a biodistribution study was performed following the 5-day imaging timepoint.

Results: The [^{89}Zr]Zr-labeled VH-Fc mutants exhibited high *in vivo* stability (>90%) as compared to WT (>75%) at 1-day p.i. PET imaging revealed increased tumor accumulation for the both [^{89}Zr]Zr-2A10-VH- Fc_{LALAPG} and [^{89}Zr]Zr-2A10-VH-Fc_{GRLR} as compared to the [^{89}Zr]Zr-2A10-VH-Fc_{WT}. At 120 hours p.i., the VH-Fc mutants demonstrated a substantial reduction in spleen and liver uptake. The mutants exhibited increased kidney uptake, indicating greater kidney clearance than the WT. *Ex vivo* biodistributions demonstrated tumor uptake of [^{89}Zr]Zr-2A10-VH-Fc_{LALAPG} and -VH-Fc_{GRLR} (13.7 ± 0.6% ID/g, 10.5 ± 1.2%ID/g, respectively) and [^{89}Zr]Zr-2A10-VH-Fc_{WT} (4.2 ± 0.5% ID/g).

Conclusion: In conclusion, we successfully developed Fc-mutant anti-MSLN VH-Fc fusion proteins that effectively target MSLN-positive tumors while minimizing non-specific binding. The improved pharmacokinetics of the mutants support their potential as imaging and therapeutic agents.

Poster No. 72
Cancer Biology Program

aKG-mediated carnitine synthesis promotes chemoresistance via enhancing histone acetylation

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Homologous recombination (HR) proficiency serves as a barrier to chemotherapy response. For example, cyclin E-driven tumors have a have a low response rate to DNA damaging agents due to elevated HR-mediated repair. The metabolic mechanisms underlying HR remain unclear. We found that alpha-ketoglutarate (aKG) is upregulated in cyclin E1-driven ovarian cancer cells, and suppression of aKG sensitized these cells to DNA damaging agents olaparib and cisplatin both in vitro and in vivo, demonstrating that aKG drives chemoresistance in these models. aKG is required for the activity of aKG-dependent dioxygenases. There are 64 known aKG-dependent dioxygenases, including those well known to promote demethylation reactions. Through a targeted CRISPR knockout library, we discovered that Trimethyllysine Hydroxylase Epsilon (TMLHE), the first and rate-limiting enzyme in de novo carnitine synthesis, is necessary for chemoresistance to DNA damaging agents in cyclin E1-driven cells. Unexpectedly, aKG-mediated TMLHE-dependent carnitine synthesis was required for histone acetylation, while histone methylation was affected but dispensable. The increase in histone acetylation via aKG-dependent carnitine synthesis promoted HR-mediated repair through site-specific histone acetylation. We found that the association of these marks at double stranded breaks are decreased by depletion of aKG and TMLHE and rescued by carnitine, indicating that this axis is critical for HR-mediated DNA repair. Additionally, analysis of patient samples demonstrated that TMLHE positively correlates with pan-acetyl H4 and both high tumor TMLHE or high serum acetyl carnitine are associated with worse progression free survival (PFS) in patients treated with DNA damaging agents. These data demonstrate for the first time that HR-proficiency in cyclin E1-driven cancers is mediated through aKG directly influencing histone acetylation via carnitine synthesis.

Poster No. 73

Integrating Single-Cell Technologies to Enhance Understanding of the Tumor Immune Microenvironment and Therapeutic Efficacy in HNSCC

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Understanding the tumor immune microenvironment (TME) is crucial for improving the efficacy of immune-checkpoint blockade (ICB) therapies, particularly in Head and Neck Squamous Cell Carcinomas (HNSCC), where response rates to ICB are suboptimal. Single-cell RNA sequencing (scRNA-seq) and spatial transcriptomics (ST) have emerged as powerful tools for dissecting cellular mechanisms in tumors, yet integrating these technologies to map cellular interactions within the TME remains underexplored. Via combining scRNA-seq with ST and multi-spectral imaging, we may elucidate the spatial and functional relationships between tumor and immune cells in HNSCC. We hypothesize that the spatial distribution and composition of key immune and TME components significantly influences tumor progression and patient outcomes to ICB. Our approach will facilitate the mapping of cell populations and their spatial contexts, allowing for the identification of distinct cellular neighborhoods through unsupervised clustering techniques. Utilizing this information, we will also explore neighborhood transcriptomic signatures, cell-cell interactions, and how the presence of distinct signatures of these neighborhoods influence patient outcome. Special attention will be given to tertiary lymphoid structures (TLS), which are associated with improved survival in many cancers, including HNSCC. By evaluating the presence and activity of TLS and their cellular composition, particularly germinal-center B cells, we aim to uncover how these structures influence the TME and therapeutic response. This research will enhance our understanding of HNSCC's complex cellular landscape, potentially leading to novel therapeutic targets and improved patient stratification for personalized treatment strategies.

Poster No. 74
Cancer Epidemiology and Prevention Program

Exploring early precursor lesions of ovarian cancer: insights from an in vitro model

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Ovarian cancer ranks as the fifth leading cause of cancer-related deaths in women, surpassing other cancers of the female reproductive system. Recent epidemiological and molecular studies highlight the significance of high-grade serous carcinoma (HGSC), the most aggressive subtype of ovarian cancer, constituting nearly 96% of cases with mutations in p53 (P53 signature). HGSC originates from fallopian tube secretory epithelial (FTSEC) cells through a progression of precursor lesions, ranging from p53 signatures to serous tubal intraepithelial carcinoma (STIC). The p53 signature involves a linear expansion of over 12 TP53 mutant FTSECs, morphologically normal and displaying no increase in proliferative activity.

In this study, we established a model system for P53 signature by either overexpressing p53 mutations or knocking down P53 expression in normal FTSECs maintained as 2D, 3D, or organoid cultures. We characterized the cells using RNA-seq, mass spectrometry, and functional assays. The functional assays revealed increased migration and proliferation, with no difference observed in apoptosis.

Despite the inability of p53 signature cells to proliferate or migrate in vivo, our hypothesis posited that clonal expansion of progenitor cells with a p53 mutation maintains plasticity by preserving the stemness of one or more cells capable of responding to cues from the microenvironment. Our experiments revealed heterogeneity in the model system, reflecting the composition of p53 signature in vivo. We also observed that stem-like cells tend to organize into clusters. Notably, stem-like cluster cells bearing a p53 signature exhibit migratory behavior, whereas clusters lacking p53 do not demonstrate such mobility. Given that most high-grade serous carcinoma (HGSC) cases occur in postmenopausal women, it will be interesting to see if the induction of proliferation and migration of p53 mutant stem cells in response to stimuli from aging stromal cells (fibroblasts and autologous immune cells) and to changes in hormonal background in our model.

Conclusion: This study illuminates a novel aspect of ovarian cancer development, underscoring the role of a distinct population of fallopian tube epithelial cells with stem-like properties within p53 signatures. Further investigations into the tumorigenicity of these cells hold promise for providing valuable insights into future therapeutic strategies.

Poster No. 75
Cancer Virology Program

HBc downregulates poly (ADP-ribose) glycohydrolase (PARG) expression via autophagy to potentiate HBx/DDB1-mediated SMC5/6 degradation

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HBx/DDB1-mediated degradation of SMC5/6 is crucial for HBV cccDNA transcription in hepatocytes. We previously reported that HBx alone cannot effectively degrade SMC5/6 in human hepatocellular carcinoma (HCC) cells primarily due to the downregulation of DDB1 by poly (ADP-ribose) glycohydrolase (PARG). Reducing PARG activity or expression enhances DDB1 PARylation and stability, thereby boosting cccDNA transcription. Further investigations revealed that during HBV infection or transfection in HepG2-NTCP cells, SMC5/6 degradation becomes significant along with a marked reduction of PARG expression. This observation led us to hypothesize that other HBV genes may downregulate PARG expression to benefit HBV replication. By surveying each individual HBV protein through transfection into HepG2 cells, we found that HBc can reduce PARG expression. Immunoprecipitation experiments confirmed a protein-protein interaction between HBc and PARG, specifically within the HBc N-terminal domain. Disruption of HBV capsid assembly by Y132A mutation or CAM-E treatment did not affect HBc-PARG interaction or PARG degradation, indicating an HBV capsid integrity-independent mechanism. We then compared the effects of proteasome inhibitors and autophagy inhibitors on HBc-mediated PARG downregulation and found that HBc lost its ability to degrade PARG following 3-methyladenine or Chloroquine treatment, but not MG132. Collectively, these findings suggest that in HBV-infected hepatocytes with a high level of PARG (ie: HCC cells), HBc interacts with PARG and induces PARG degradation via autophagy, thereby enhancing global PARylation including DDB1 PARylation, which in turn stabilizes DDB1 and facilitates HBx/DDB1-mediated SMC5/6 degradation. However, consistent with previous studies showing that HBc plays a dispensable role in HBV transcription, no upregulation of HBV transcription was found, which is probably due to the stabilization of cellular antiviral protein(s) upon the increase of global PARylation caused by HBc-mediated PARG degradation. Our study thus provides a comprehensive analysis of PARG-related virus-host interactions in HBV life cycle and hepatocarcinogenesis, offering insights for future clinical applications.

Poster No. 76
Cancer Immunology and Immunotherapy Program

Assessing the relative and synergistic contributions of PD1 and LAG3 on CD4+ T conventional cells in cancer

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Co-targeting of PD1/LAG3 has shown superior effects in cancer immunotherapy compared to PD1 alone in melanoma. However, little is known regarding mechanistic significance of PD1/LAG3 synergy on T cells. We recently reported that PD1/LAG3 synergize to drive $CD8^+$ T cell exhaustion (T_{EX}). However, recent evidence suggests that intratumor $CD4^+$ T conventional cells (T_{conv}) exhibit an exhaustion-like profile, including expression of PD1/LAG3, that correlates with worse outcomes. Despite increasing evidence for $CD4^+$ T_{EX}, their defining features, significance, and regulatory mechanisms remain unclear. We hypothesize that PD1/LAG3 exert differential yet synergistic effects on $CD4^+$ T_{conv} to drive their exhaustion and impaired anti-tumor responses. To study $CD4^+$ T_{EX}, we are utilizing tumor antigen-specific $CD4^+$ T cells isolated from Trp1*Tyrp1*$^{B\text{-}w}$*Rag1*$^{-/-}$ (Trp1) mice. Trp1 $CD4^+$ T cells recognize tyrosinase-related protein-1, a melanocyte differentiation antigen expressed by both normal melanocytes and melanoma. Our preliminary studies found that total-body irradiation of melanoma tumor-bearing recipient mice prior to adoptive transfer was required for successful Trp1 CD4 engraftment. Further, tumor-infiltrating Trp1 $CD4^+$ T cells upregulated PD1, LAG3, TOX, and CD39, suggestive of T_{EX} phenotype. Interestingly, adoptive transfer of Trp1*Pdcd1*$^{-/-}$ $CD4^+$ T cells led to tumor clearance, suggesting PD1 plays a crucial role in limiting tumor-specific $CD4^+$ T cell responses. Ongoing work is focused on assessing the impact of Trp1*Lag3*$^{-/-}$ and Trp1*Pdcd1*$^{-/-}$*Lag3*$^{-/-}$ $CD4^+$ T cells on tumor growth and survival. Future plans include quad-transfers to study cell-intrinsic effects of PD1/LAG3 across the same microenvironment. These studies aim to enhance mechanistic understanding of $CD4^+$ T_{EX} and may inform novel immunotherapeutic strategies.

Poster No. 77

Enhancing CAR-T cell Metabolism by Regulating AMPK Signal

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Metabolic modulation can optimize the *in vivo* performance of Chimeric Antigen Receptor (CAR) T cells and we have previously demonstrated that AMP-activated protein kinase (AMPK) overexpression (AMPK-$_{OE}$) increases oxidative capacity in human CAR-T cells. Here, we investigate the mechanism by which AMPK augments T cell metabolic performance.

AMPK$_{OE}$-CART cells were produced through lentiviral transduction of the regulatory AMPKγ2 subunit into human CD19-CART cells. AMPK$_{KO}$-(knockout) CART cells were generated via CRISPR-Cas9 deletion of the catalytic AMPKα1 subunit. CARTs were stimulated overnight in RPMI with 5.5mM glucose, no glucose, or 5.5mM glucose + 50μM metformin, followed by Seahorse analysis. AMPK$_{OE}$- and AMPK$_{KO}$- T cells were stimulated overnight in 2.25mM glucose RPMI, followed by bulk mRNA sequencing.

AMPK$_{OE}$-CART cells showed higher oxidative metabolism, with a 31.4% increase in spare respiratory capacity (SRC). AMPK$_{KO}$-CART cells exhibited the opposite trend (10.1% decrease in SRC) ($p<0.05$). Effects were more pronounced under glucose-free conditions. GSEA of RNA-seq data revealed equivalent enrichment of OXPHOS gene sets, suggesting that improved oxidative metabolism was not driven by transcriptional changes in OXPHOS genes. Notably, AMPK$_{KO}$-T cells had a significant upregulation in reactive oxygen species (ROS) gene sets, suggesting higher ROS burden in these cells despite a lower level of oxidative metabolism. Consistent with the idea that AMPK protects against mitochondrial dysfunction, AMPK$_{OE}$-CART cells were impervious to mitochondrial inhibition mediated by metformin treatment.

Here, we report that AMPK does not increase oxidative capacity through transcriptional control of OXPHOS genes, but rather improves functional mitochondrial capacity. Future studies will delineate the mechanisms underlying these AMPK-mediated changes.

Poster No. 78

Investigating Macrophage Populations in Metastatic Sarcoma Samples

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BACKGROUND: Sarcomas are tumors of the bone and soft tissues that display high metastatic potential to the lung. Although the specific mechanisms remain elusive, studies in other malignancies have emphasized the role of the tumor microenvironment in metastasis. Macrophages play an imperative role in immunity and tumor progression. Here, we investigate the role of M1 (tumoricidal) and M2 (tumor permissive) macrophages in a panel of metastatic sarcoma samples.

METHODS: This study utilized a set of 3 tissue arrays from 21 patients. From these patients, a total of 41 osteosarcoma samples and 7 chondrosarcoma samples were observed, including a cohort of patients (n=16) with multiple metastatic tumor samples. Vectra Polaris tumor microarrays were used to investigate immune subsets of macrophages (CD68+/HLADR+/CD163- for M1, CD68+/HLADR-/CD163+ for M2). Cell counts per core and antibody were collected for bioinformatics analysis using R software. M2/M1 cell density ratios were calculated, and three distinct groups were identified.

RESULTS: The M2/M1 cell density ratios found in our samples ranged from 0.0 to 37.5. The low (0.0-1.0) and medium (1.0-5.0) ratio groups were statistically significant from each other, with a p value <0.0001. The medium and high (above 5.0) ratio groups were statistically significant from each other as well, with a p value <0.001. Patient outcomes were identified and survival status, sarcoma type and location, comorbidities, and treatment were noted for each ratio.

CONCLUSION: With immunotherapy becoming more prevalent in cancer treatment, correct patient stratification is of the utmost importance. It is therefore imperative to understand the impact of the tumor microenvironment. The three distinct populations of patients observed can give an insight on macrophage population predominance and how that might affect sarcoma metastasis and disease progression.

Poster No. 79
Cancer Virology Program

Oncogenic KSHV Utilizes RNA Methyltransferase METTL16 to Enhance 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 several human cancers. Despite extensive research, the precise mechanisms underlying KSHV-induced oncogenesis remain unclear. METTL16 is a methyltransferase that specifically catalyzes the methylation of adenosine residues in RNA, particularly known for its role in N6-methyladenosine (m6A) modification—a key post-transcriptional modification involved in regulating RNA metabolism, including splicing, stability, and translation. METTL16 has been implicated in various cellular processes and plays a role in gene expression regulation. However, its role in KSHV-induced oncogenesis has not been previously explored. In this study, we report that METTL16 protein expression, but not its mRNA, is significantly upregulated during KSHV latent replication. Interestingly, KSHV latent infection enhances the stability of METTL16, as demonstrated by cycloheximide chase assays. Further, the proteasome inhibitor MG132 prevented METTL16 degradation, indicating that METTL16 is targeted by the ubiquitin-proteasome system. Through reverse genetic and overexpression studies, we discovered that the KSHV-encoded Latency-Associated Nuclear Antigen (LANA) directly interacts with METTL16 and reduces its ubiquitination, contributing to the upregulation of METTL16. Importantly, siRNA-mediated knockdown of METTL16 expression effectively inhibited cell proliferation and transformation. Moreover, METTL16-specific inhibitors significantly reduced cell proliferation and cellular transformation in KSHV-transformed cells. These findings suggest that KSHV exploits LANA to stabilize METTL16, promoting cell proliferation and transformation, which are essential for KSHV-induced oncogenesis. Our data propose METTL16 as a potential therapeutic target and METTL16 inhibitors as promising agents for the treatment of KSHV-associated cancers.

Poster No. 80
Cancer Immunology and Immunotherapy Program

TIM-3 upregulation by tumor-infiltrating NK cells may be associated with NK cell dysfunction in Head and Neck Squamous Carcinoma.

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Background: Despite significant advancements in the field, the responsiveness of head and neck squamous cell carcinoma (HNSCC) patients to the current generation of immune checkpoint inhibitor (ICI) is modest, with ~15% of patients benefiting from anti-PD-1 monotherapy. This has led to a search for novel druggable immune checkpoint receptors (ICR). Among these, T-cell immunoglobulin mucin 3 (TIM-3) has captured considerable attention due to its intricate immunomodulating mechanisms orchestrated by four distinct ligands.

Methods: To understand the impact of TIM-3 on NK cell anti-tumor immune responses, we leverage our neoadjuvant clinical trial designed to enhance anti-tumor immunity in subjects with resectable locally advanced HNSCC by combining anti-PD-1 with anti-LAG3 or with anti-CTLA-4 therapies. We generated and profiled single-cell RNA sequencing libraries for CD45+CD3- peripheral blood (PBL) and tumor-infiltrating leukocytes (TIL).

Results: Bioinformatic analyses revealed distinct signatures associated with NK cell phenotypes and highlighted the possible correlation between type I interferon and NK cell cytotoxic signatures with patient responsiveness. Notably, we observed an increase in TIM-3 gene (HAVCR2) expression in NK cells, both among TIL and PBL, in comparison to other lymphocytic populations. Furthermore, we observed an increase in HAVCR2 but not TIGIT or PDCD1 expression by tumor-infiltrating NK cells in non-responding patients. Our data from the murine model planted with murine tonsil epithelium MEER cell line indicates an increase in TIM-3 expression in tumor infiltrating NK cells in comparison with NK cell from spleen compartment.

Conclusions: The role of TIM-3 on tumor infiltrating NK cells remains elusive. Our data stress the importance of TIM-3 expression in the context of tumor infiltrating NK cells. Future mechanistic studies will provide better understanding of the role TIM-3 plays in ICI resistance.

Poster No. 81
Cancer Therapeutics Program

Soluble guanylate cyclase activator, cinaciguat, apoptoses radiation-induced senescent cells in prostate cancer.

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Irradiation is the therapy of choice for two thirds of men with localized prostate cancer. While irradiation destroys the majority of tumor cells, surviving ones can become senescent and resistant to treatment. Targeting senescent cells following radiotherapy may be a promising strategy to prevent cancer reemergence. Cinaciguat, a sGC heme-mimetic activator exhibits senolitic/senomorphic actions by decreasing the Bcl-2/BAX ratio and inflammation/NF-□□-mediated cytokine release. We testes the hypothesis that cinaciguat treatment following radiotherapy can prevent tumor reemergence and that it is preferable to apoptose the cells than promote mitophagy and recover them.

Luciferase expressing TRAMP-C1 cells in culture were subjected to 0 or 8 Gy irradiation. Four days later, cells were treated with cinaciguat, p62 mitophagy inducer or vehicle. Luciferin-induced bioluminescence was measured before and after irradiation and following drug treatment at different time points.

Adult male mice were anesthetized, the ventrolateral prostatic lobes injected with TRAMP-CI cells, tumors allowed to grow for 4–6 weeks and the prostates selectively irradiated. One week later, mice were implanted with osmotic pumps delivering cinaciguat or p62 mitophagy inducer for 2 weeks after which tumor weights were measured.

Cinaciguat-treated cells had the lowest survival (31±13%) in comparison to vehicle or drug-free (63±13% and 61±13%). In contrast, the p62 mitophagy inducer increased survival (107±15%). Five weeks after TRAMP-C1 cell injections, a solid tumor mass engulfed the ventrolateral lobes. Without intervention, there was a significant increase in tumor mass overtime. Irradiation alone significantly reduced prostate weight compared to non-irradiated controls. The p62 mitophagy inducer resulted in higher prostate tumor weights while cinaciguat caused a marked decrease compared to irradiation alone.

Both in vitro and in vivo data indicate cinaciguat promoted tumor cell death after irradiation. P62 mitophagy inducer increased cell survival indicating enhanced clearance of mitochondria is associated with recovery and subsequent regrowth of cancer.

Poster No. 82
Cancer Therapeutics Program

Senomorphic/senolytic action of soluble guanylate cyclase activator in irradiated prostate tumor to inhibit tumor reemergence

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Introduction and Aim. Biochemical recurrence of intermediate/high-grade prostate cancer following first-line treatments is a significant issue that can lead to treatment/castration resistance. Soluble guanylate cyclase (sGC) activators have been demonstrated to modulate malignant and aged (benign prostatic hyperplasia) prostate cells. Therefore, our goal was to examine the effect of cinaciguat, a heme-mimetic sGC activator, on TRAMP-C1 mouse prostate tumor cells following radiation treatment.

Methods. Orthotopic TRAMP-C1 tumors were implanted into C57Bl/6J mice. After 6 weeks, mice were subjected to fractionated irradiation (2 Gy/day, 5 consecutive days). The cohort was separated into vehicle, cinaciguat (1 mg/kg/day via osmotic pump) or p62 mitophagy inducer (1 mg/kg/day) treatment groups. After 28 days treatment, mice were sacrificed, tumors excised and examined by immunofluorescence and for localization of senescence associated beta-galactosidase activity.

Results. Orthotopic tumors following irradiation were significantly smaller than those from non-irradiated and irradiated tumors had greater numbers of beta-galactosidase mediated staining. Immunohistochemical localization of Bcl-2 (pro-tumorigenic marker), NF-kB (inflammatory marker) and sGCα subunit were all highly expressed in TRAMPC-1 tumors. The Cinaciguat + irradiation group showed a decrease in all three markers while p62 mitophagy inducer + irradiation did not alter their expression.

Conclusion. There is a significant risk of developing treatment resistant prostate cancer after initial interventions with limited options to prevent this from occurring. Our data suggests irradiation induced senescence is in part due to defects in the mitophagy pathway. Growth of prostate tumors was correlated with higher expression of sGCα subunit which could be reduced by treatment with cinaciguat, indicating a relationship between sGC activity, treatment induced senescence and prostate tumor reemergence. Thus, inclusion of sGC activators in irradiation treatments of prostate cancer may have beneficial effects to reduce the risk of recurrence.

Poster No. 83

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Poster No. 84
Cancer Therapeutics Program

Epigenetic screens reveal ARID1A as a therapeutic vulnerability in small cell lung cancer

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Small-cell lung cancer (SCLC) accounts for approximately 15% of all lung cancers and is characterized by an exceptionally high proliferative rate, a strong tendency for early metastasis and extremely poor prognosis. Despite its aggressive nature, current therapeutic options remain limited. To uncover novel therapeutic targets among epigenetic regulators in SCLC, we conducted pooled epigenome-wide CRISPR knockout screens both in vitro and in vivo, revealing the AT-rich interactive domain-containing protein 1A (ARID1A) as a potential therapeutic vulnerability. ARID1A expression is elevated in patients with SCLC and correlates with clinical outcomes. Genetic ablation of ARID1A significantly inhibited proliferation and colony formation in human SCLC cell lines. Moreover, ARID1A loss led to a marked reduction in human xenografts and a genetically engineered mouse model. Mechanistic studies demonstrated that ARID1A deficiency disrupted multiple signaling pathways essential for SCLC growth, including cGMP-PKG, neuronal development, and tyrosine kinase signaling. Comprehensive epigenetic profiling further confirmed a remodeled epigenetic landscape at key loci, underscoring ARID1A’s essential role in SCLC survival. In summary, our findings provide new insights into ARID1A's function in SCLC and support its potential as a therapeutic target.

Poster No. 85
Cancer Biology Program

ATR promotes mTORC1 activity via de novo cholesterol synthesis in p16low cancer

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Alterations in cellular metabolism are closely associated with various pathologies, including cancer. The tumor suppressor p16, a well-known regulator of the cell cycle, is often lost in many cancers. Our previous studies demonstrated that cells with low p16 expression (p16low) exhibit hyperactivation of mTORC1. However, the mechanism through which mTORC1 is activated downstream of p16 loss remains unclear. Interestingly, we found that tumors and cells with loss of p16 have increased DNA damage signaling, specifically activated ATR. While previous research has suggested that ATR acts upstream of mTORC1 during replication stress, the direct mechanism linking ATR to mTORC1 activation has not been elucidated. We found that ATR indeed promotes mTORC1 activity in multiple p16low models. Through an unbiased proteomics approach and by cross-referencing publicly available datasets of potential upstream mTORC1 regulators, we identified the de novo cholesterol synthesis enzyme lanosterol synthase (LSS) as a mediator between ATR and mTORC1 activation. Indeed, p16low cells exhibited increased LSS expression and elevated cholesterol levels in an ATR-dependent manner. Additionally, knockdown of either ATR or LSS reduced mTORC1 activity, which was rescued by cholesterol or lanosterol supplementation. Mechanistically, ATR knockdown decreased localization of mTOR at the lysosome, where mTORC1 is known to be activated, and this was rescued by cholesterol supplementation. Our findings reveal a mechanism of mTORC1 activation through ATR-mediated de novo cholesterol synthesis via LSS and establish a novel link between the DNA damage response and cholesterol metabolism. Excitingly, the observed cholesterol rewiring creates a metabolic vulnerability in p16low cells, increasing their sensitivity to therapeutic agents targeting cholesterol, including multiple statins. Future studies will focus on elucidating the precise mechanisms by which ATR regulates LSS and subsequent cholesterol synthesis. Additionally, we will explore whether sensitivity to ATR inhibitors is modulated by cholesterol availability, including how high dietary cholesterol may affect their efficacy.

Poster No. 86
Genome Stability Program

The zinc finger of DNA Ligase 3 is an autoinhibitory domain and binds to nucleosomes via an arginine anchor.

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Ligation of DNA single strand breaks (SSB) is critical for maintaining genome integrity during DNA replication and repair. X-ray cross complementing protein 1 (XRCC1) forms an important scaffolding complex with DNA Ligase 3 (LIG3α) during base excision repair (BER). To measure the dynamics of LIG3α-XRCC1 interactions with SSB in duplex lambda DNA or a single 601 nucleosome core particle (NCP), we used a real time single-molecule approach to visualize DNA binding events of Halo-tagged LIG3α with XRCC1-YFP from nuclear extracts. On duplex DNA, LIG3α and XRCC1 showed strong binding affinity with ligatable nicks, with K_d = 0.2 ± 0.007 nM & K_d = 1.6 ± 0.05 nM respectively, and 17.3% of events being co-localized. Domain analysis suggests that the unique N-terminal zinc finger (ZnF) of LIG3α acts as an autoinhibitory domain for long-lived stable interactions and had a strong effect on the binding affinities. By deleting the ZnF domain of LIG3α or just expressing ZnF-BRCT domain with XRCC1 construct made the lifetimes ~2 fold longer and has lower binding affinity when compared to the full construct. Surprisingly LIG3α and XRCC1 were found to bind to an undamaged 601 NCP with strong binding affinity, K_d = 0.07 ± 0.005 nM & K_d = 0.6 ± 0.05 nM, respectively, and colocalization frequency was 35.2%. Deletion analysis indicated that the N-terminal ZnF of LIG3α was responsible for nucleosome binding, and strikingly replacement of two key arginine residues in the zinc finger domain with alanine's abolishes the nucleosome binding. These single molecule studies have allowed us to uncover a novel arginine anchor of LIG3α for binding to an undamaged 601 NCP.

Poster No. 87
Cancer Biology Program

Revealing molecular vulnerabilities of immune suppression in breast cancer liver metastases

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Approval of checkpoint inhibitors in metastatic triple-negative breast cancer (TNBC) opened the door for use of immunotherapies in breast cancer. However, more than 50% of metastatic breast cancers recur in the liver, an understudied metastatic site that responds poorly to current immunotherapy approaches. To overcome this clinical challenge, we are assessing the effects of tumor secreted factors on the liver metastatic microenvironment. We showed that the heme metabolizing enzyme heme oxygenase-1 (HO-1) and its secreted metabolite bilirubin are an underappreciated immune modulatory axis in TNBC. In a TNBC-like liver metastasis model, HO-1 depletion nearly ablated tumor outgrowth. In human breast cancer liver metastases, tumor-HO-1 levels were associated with CD4+ T cell density, suggesting this pathway may regulate liver metastatic outgrowth and immune infiltration.

To reveal additional molecular vulnerabilities of breast cancer liver metastases, we profiled 21 human specimens using the NanoString nCounter system. This analysis revealed both immune infiltrated and immune cold or ignored liver metastases. Immune infiltrated tumors had increased expression of genes representing pathways such as cytokine signaling, immune infiltration, and antigen presentation. Confirmatory multispectral fluorescence demonstrated that immune infiltrated metastases had >2-fold increase in macrophage and T cell density, while ignored counterparts often had few immune cells present. Comparison of immune ignored to immune infiltrated metastases revealed anterior gradient-2 (AGR2), an understudied secreted factor that correlated with immune cell abundance in breast cancer, was the most highly upregulated gene in immune ignored tumors. Follow-up analysis using publicly available datasets suggests that AGR2 limits antigen presentation and is genetically altered (amplified or deleted) in >20% of metastases. These studies demonstrate that not all breast cancer liver metastases are immune ignored. Continued comparison of these immune phenotypes promises to reveal additional targetable tumor secreted factors that may enhance anti-tumor immunity at this metastatic site.

Poster No. 88

Centralization of care influences disparities in readmission patterns following radical cystectomy

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Introduction:
Patients undergoing radical cystectomy have been increasingly directed towards high-volume, regional referral centers. This process, known as centralization, has led to improved perioperative outcomes, but there is sparse evidence on how centralization has affected long-term readmission patterns. Patients readmitted to the original surgical hospital (index readmission) have improved outcomes compared with those readmitted to a secondary hospital (non-index readmission). We sought to determine whether receiving cystectomy at a regional referral center changes postsurgical readmission patterns.

Methods:
We analyzed inpatient records from the Pennsylvania Health Care Cost Containment Council dataset (PHC4) linked with the Pennsylvania Cancer Registry. We identified patients who underwent radical cystectomy between 2013-2020 and had at least 1 unplanned readmission within 90 days of surgery. We split this cohort dependent on whether cystectomy occurred at a regional referral center. Our primary outcome was readmission location. We used chi-square and Wilcoxon rank-sum tests to compare differences in patient, community, and hospital-level factors between those readmitted to an index versus non-index hospital.

Results:
We identified 2,585 patients who underwent radical cystectomy, of whom 1,475 (57%) had cystectomy at a regional referral center. Of these, 1,112 (44%) patients were readmitted within 90 days. The non-index readmission rate was greater for patients who had surgery at a regional referral center (31%) compared to patients who had surgery at a non-regional referral center (19%). Urban residents who had cystectomy at a regional referral center were more likely to experience index readmissions, while rural residents were more likely to experience non-index readmissions (p=0.002). Patients with Medicare originally treated at a regional referral center were more likely to experience non-index readmissions (p=0.009). These associations were not significant if patients had cystectomy at a non-regional referral center.

Conclusion:
Ongoing centralization of cystectomy may be associated with unfavorable readmission patterns for rural patients and Medicare enrollees.

Poster No. 89

Lymphoid infiltration and aggregation prevalence and prognostic value in cervical cancer

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The immune response in cervical cancer is of increasing importance with checkpoint inhibitors now standard of care therapy. However, there remains a significant lack of understanding of the immune landscape in cervical cancer hindering the development of novel immune therapeutics. This study investigated the immune microenvironment in early-stage cervical cancer with a focus on tertiary lymphoid structures (TLS), an understudied component of the anti-tumor immune response. We investigated a cohort of patients with clinical stage I squamous or adenocarcinoma of the cervix. Despite the presence of early-stage disease, recurrence was common at 37%, highlighting the need to identify patients at high risk for recurrence for future therapeutics. We demonstrated that high CD8+ T cell infiltration correlated significantly with improved overall survival (OS), particularly in patients with adenocarcinoma histology. Notably, CD8+ T cells colocalized with B cells, suggesting the eventual formation of TLS, which has prognostic benefit in other solid tumors, in particular HPV-driven head and neck cancer. CXCL13, a chemokine associated with TLS formation that can be generated by tumor-specific CD8+ T cells, correlated with improved recurrence-free survival. Validation in a larger cohort from The Cancer Genome Atlas (TCGA) illustrated similar trends in survival, highlighting the prognostic significance of immune infiltration and eventual TLS induction in cervical cancer. Previous investigations from our group also demonstrated that TLS with GC correlates with favorable outcomes in both HPV+ and HPV- HNSCC patients. Therefore, as a future direction, we plan to study the state and activity of TLS in our cervical cancer cohort. We expect that the enhancement of CD8+ T cell infiltration as well as the organization of lymphocytes into TLS could act as potential targets to improve outcomes in cervical cancer.

Poster No. 90
Cancer Immunology and Immunotherapy Program

The Role of Ly6C and Ly6A in the Activation of CD8+ T Cells Induced by Chemotherapy-Treated Cancer Cells Independent of MHC Class I

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CD8+ T cells are essential for anti-tumor immunity. The anti-tumor function of T cells is conventionally understood via the lens of T cell receptor (TCR) recognition of tumor antigens presented by major histocompatibility complex (MHC) class I. However, many cancers downregulate or lose expression of MHC class I, leading to worse responses to immunotherapies. While it remains poorly understood whether CD8+ T cells can recognize MHC class I null tumor cells, previous work in our lab has shown that a subset of CD8+ T cells can recognize MHC class I null tumor cells treated with chemotherapy. We have termed these chemotherapy-treated cancer cell-activated memory-like CD8+ T cells T_{CAMEL} cells. To elucidate the activation mechanism of T_{CAMEL} cells, we performed single cell-RNA- seq and found that Ly6C and Ly6A were the most significantly expressed genes in T_{CAMEL} cells that distinguish them from controls. Subsequently, we validated these results and showed that both Ly6C and Ly6A are significantly upregulated on CD8+ T cells activated by chemotherapy-treated β2M-KO tumor cells. We also show that the Ly6C agonist antibody HK1.4 enhanced T_{CAMEL} cells and expanded cells resembling VM CD8+ T phenotype. Likewise, we observed that the Ly6A agonist antibody D7 also enhanced T_{CAMEL} cells and improved CD8+ T cell cytotoxicity towards β2M-KO tumor cells. Lastly, we employed a PI3Kα inhibitor, A66, in our experimental system and found that it blocked the enhancement of T_{CAMEL} cells mediated by Ly6C or Ly6A agonist antibodies. In the future, we will delete Ly6C and Ly6A in CD8+ T cells to determine if they are necessary for T_{CAMEL} cell function. Our findings indicate that Ly6C and Ly6C may play a critical role in CD8+ T cell activation against chemotherapy-treated tumor cells independent of MHC class I.

Poster No. 91
Cancer Immunology and Immunotherapy Program

Enhancement of Antitumor CD4+ T Cell Responses by Engineered Antigen-Presenting Cells

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Immunotherapies such as immune checkpoint blockade, cancer vaccines, and adoptive cell therapies have revolutionized the treatment of cancers. Despite their promise, the efficacy of these therapies has been limited due to several factors including inefficient T cell infiltration, heterogeneity of tumor antigens, and dysfunction of antigen-specific T cells. In cases where the T cells fall short, such as exhaustion due to the tumor microenvironment, or inefficient infiltration due to lack of chemoattractant signals, they fail to control the tumors. To address this question, our lab has modified peptide-MHC complexes to incorporate intracellular signaling domains that will enable them to signal within an APC. These chimeric receptors, called Signaling and Antigen-presenting Bifunctional receptors (SABRs) can present a given antigen to T cells, and then generate a functional response that can alter the activity of that T cell. As a model system, we have utilized SABRs presenting Ovalbumin (OVA) peptides in mouse class II MHC and studying the interactions with respective TCRs. Currently, we have constructed SABRs incorporating signaling domains derived from derived from dendritic cells, macrophages, and B cells. From the initial screens, we have identified a candidate SABR-M containing CD28/CD3□ cytoplasmic domain that actively signals through the NFAT and NF-□B signaling pathways. Further, macrophages engineered with SABR-M demonstrated antigen-specific activation and secretion of proinflammatory cytokines including TNF□. The goal is to engineer professional APCs including Dendritic and B cells towards enhancing the efficacy of T cell-based therapies even in an immune suppressive tumor microenvironment.

Poster No. 92
Biobehavioral Cancer Control Program

Integrating Passive Sensor Data into the Clinical Workflow: A Qualitative Study

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Background: Throughout chemotherapy, patients experience a wide range of symptoms between office visits that continuous passive sensor data collected from wearables and patient smartphones can help capture. While this could assist with symptom management and improve patient-provider communication, provider preferences on how this would be integrated into their clinical workflow have not been assessed. The goal of this project was to gain insight into providers' preferences on using wearable device or other sensor data and the potential of sensor data metrics or predictions in clinical decision-making.

Method: Thirteen oncology care providers were enrolled (4 Physicians, 5 Physician Assistants, 1 CRNP, and 3 Nurses; M = 42 years old, range 25-61; 69% female; mean 9.7 years practicing, range 5 months to 22 years). Participants completed semi-structured interviews discussing the benefits and barriers to having access to sensor data and predictions. Providers were also provided with clinical vignettes from three patients with real patient sensor data and asked about how it influenced their clinical decision-making. Interview transcripts were transcribed and coded using an iterative thematic analysis approach

Results: Interviews revealed that providers considered sensor data as having the ability to support provider clinical care and actions, improve patient-provider communication, and complement other sources of information. Generally, providers preferred to be notified about changes in patient health via email (11/13), were most interested in viewing the dashboard when receiving calls from a patient (11/13) or before seeing a patient in clinic (9/13), and were very interested in receiving predictions about falls (11/13) or hospitalizations (9/13). Additionally, viewing sensor data made clinicians more confident in their decision-making when presented with the vignettes.

Conclusion: Preliminary findings suggest that mobile sensor tools and data will enhance clinicians' understanding of patient symptoms and concerns.

Poster No. 93
Cancer Biology Program

Targeting a novel TWIST1-Hexokinase II pathway in MET driven NSCLC

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Non-small cell lung cancer (NSCLC) has the highest incidence of brain metastases (BM) with ~40% of NSCLC patients developing BM. We identified a significant enrichment of *MET* amplification in LUAD-BM compared to primary LUAD and liver metastases. In addition, we found that *MET* amplified BM had a distinct transcriptional signature reflecting increased glycolysis. MET, a receptor tyrosine kinase, and its ligand, hepatocyte growth factor (HGF), promote proliferation, epithelial-mesenchymal transition (EMT), angiogenesis, and metastasis. We have found that TWIST1, an EMT transcription factor, is regulated by HGF/MET pathway and is essential for MET driven NSCLC and MET TKI resistance. Although MET tyrosine kinase inhibitors (TKIs) are approved for *MET* altered NSCLC and have CNS activity, many patients fail to respond and resistance is inevitable. Thus, novel therapeutic strategies are needed to prevent and overcome MET TKI resistance.

We hypothesized that MET driven LUAD-BM will have increased sensitivity to glycolytic inhibition. We observed a shift toward glycolysis in the *MET* amplified metastatic LUAD cell line (H1993) compared to the *MET* wild-type primary LUAD line (H2073) from the same patient. Furthermore, LUAD cell lines with high MET expression demonstrated increased expression and activity of glycolytic enzymes, and were more sensitive to glucose deprivation and glycolytic inhibitors both *in vitro* and in a novel *ex vivo* brain slice model. MET TKI treatment reduced glycolysis and oxidation phosphorylation; however, HGF or TWIST1 overexpression could restore expression of the key glycolytic enzyme, Hexokinase II (HK2) through a MYC-independent, TWIST-dependent pathway. Remarkably, in two novel *MET* altered models (cell line and patient derived xenograft) of acquired MET TKI resistance, TWIST1 and HK2 expression were increased, however, these resistant cell lines were less sensitive to glycolytic inhibition. In summary, these studies suggest a targetable metabolic reprogramming in *MET* altered LUAD-BM mediated through a novel TWIST1-HK2 pathway.

Poster No. 94
Cancer Biology Program

Targeting the HGF/MET/TWIST1 pathway in lung adenocarcinoma brain metastases

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Lung adenocarcinoma (LUAD) has the highest incidence of brain metastases (BM), portending a poor prognosis and highlighting the need for novel treatments. We identified a significant enrichment of MET amplification in LUAD-BM compared to primary LUAD and liver metastases. MET, a tyrosine kinase receptor, and its ligand hepatocyte growth factor (HGF), mediates proliferation, epithelial-mesenchymal transition (EMT), angiogenesis, and metastasis. We have found that MET tumorigenesis is dependent upon the EMT transcription factor, TWIST1, the HGF/MET pathway regulates TWIST1 expression and TWIST1 is overexpressed in LUAD-BM. We hypothesized that the HGF/MET/TWIST1 axis is a therapeutic target for LUAD-BM.
In ex vivo brain slice assays, HGF promoted brain colonization of MET wild-type (WT) H2073 cells, while the MET inhibitor capmatinib reduced colonization of MET amplified H1993 cells. Additionally, conditioned medium (CM) from H1993 cells increased astrocyte migration compared to CM from H2073 cells. Capmatinib reduced H1993 CM-mediated astrocyte migration, while HGF induced H2073 CM-mediated astrocyte migration. TWIST1 inhibition decreased brain colonization and astrocyte migration.
Using an intracardiac injection metastasis model, we found a higher frequency of BM in HGF-overexpressing mice compared to WT SCID mice in all cell lines. In WT SCID mice, time to BM was significantly shorter in mice injected with MET amplified cells compared to MET WT cells. Further, BM developed more quickly in HGF overexpressing mice injected with H2073 MET WT cells compared to WT mice. In syngeneic LUAD models, 40% of mice injected with HGF-overexpressing cells developed BM vs. 0% of mice injected with WT cells. Moreover, orthotopic injections showed a two-fold faster growth rate of HGF-overexpressing cells. Finally, capmatinib or harmine treatment in mice with BM led to BM disappearance, with harmine showing a sustained effect lasting over three months. These findings suggest that targeting the HGF/MET/TWIST1 may be effective treatment strategy for MET amplified LUAD-BM.

Poster No. 95
Genome Stability Program

Nucleosome unwrapping and PARP1 conformation drive affinities for chromatin and DNA breaks

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Poly[ADP-ribose] polymerase 1 (PARP1) detects DNA strand breaks that occur in duplex DNA and chromatin. We employed correlative optical tweezers and fluorescence microscopy to quantify how single molecules of PARP1 identify single-strand breaks (i.e., nicks), undamaged nucleosome core particles (NCP) and NCPs containing DNA nicks. Fluorescently-tagged PARP1 or PARP2 from nuclear extracts bound nicks with nanomolar affinity but did not engage undamaged dsDNA regions. In contrast, PARP1 avidly bound undamaged NCPs, and increasing DNA tension induced partial NCP unwrapping, which increased the on rate and affinity ~ten-fold. We further observed ADP-ribosylation of single NCPs in real time upon PARP1 binding. Catalytically dead PARP1 or EB-47 inhibition increased PARP1 affinity 20-100-fold to DNA nicks and undamaged NCP, implicating a mechanism where PARP1 reverse allostery regulates PARP1 retention to undamaged chromatin. These results provide key mechanistic insights into domain allostery and how pharmacological intervention alters PARP1 binding dynamics.

Poster No. 96
Cancer Immunology and Immunotherapy Program

CD8+ T cell infiltration into the tumor microenvironment

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Tumor infiltrating CD8+ T cells play a central role in many immunotherapeutic approaches against cancer. However, the processes that influence T cell infiltration into the tumor microenvironment (TME) remain incompletely understood. By identifying novel T cell intrinsic genes and pathways that modulate tumor infiltration, it may be possible to therapeutically target CD8+ T cells to increase their numbers within the tumor core and enhance tumor clearance. In this study, we utilized a spatial transcriptomics approach with human colorectal cancer samples to identify gene expression programs associated with the spatial distribution of CD8+ T cells both in and around tumors. We found that CD8+ T cells located outside of tumors were transcriptionally distinct from those that had infiltrated into the tumor core. Further, gene set enrichment analyses revealed that tumor infiltrating CD8+ T cells were enriched for biological processes associated with cell migration and locomotion. Interestingly, we found that SEPTIN1, a gene related to cytoskeletal stability and T cell motility, was expressed at a higher level in CD8+ T cells outside the tumor and may play a role in limiting T cell tumor infiltration. To determine whether SEPTIN1 and other differentially expressed genes modulate T cell infiltration into tumors we are developing a mouse tumor model where candidate genes can be deleting using a CRISPR Cas9 library approach and cells can be assessed for tumor infiltration.

Poster No. 97

Programming Universal Chimeric Antigen Receptor (CAR) T Cells Using Small Molecule 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, the therapy has failed against solid tumors due to several issues, such as the inability to identify tumor-specific antigens leading to on-target, off-tumor toxicities, and an immunosuppressive tumor microenvironment. To address these limitations, we are developing universal CAR T cells that target antigens on tumors and immunosuppressive cells via small molecule adaptors. Instead of directly binding to a target antigen, our universal CAR, SNAP-CAR, contains a mutated O6-alkylguanine-DNA alkyl transferase, SNAPtag. It is co-administered with one or more heterobifunctional small molecule adaptors containing a benzyl guanine (BG) motif which forms a covalent bond with the SNAPtag receptor. We generated small molecule adaptor variants targeting carbonic anhydrase IX (CAIX), folate receptor alpha (FOLR1), and beta (FOLR2), prostate-specific membrane antigen (PSMA) with different linker lengths. In vitro, we assessed the activity of CAIX, folate, and PSMA receptors targeting adaptors by co-culturing them with SNAP-CAR on antigen-positive/ negative cell lines or M2-polarized macrophages expressing FOLR2. We demonstrated potent activity at low nanomolar concentrations of the adaptors on cell lines. For M2-polarized macrophages, we demonstrated adaptor-mediated SNAP-CAR T cell proliferation and activation at 72 hours. To address on-target, off-tumor specificity issues for CAIX, we demonstrated that chemically caged BG reacts with the SNAPtag receptor, undergoes ROS-mediated decaging, and SNAP-CAR labeling occurs in the presence of ROS.

Poster No. 98
Cancer Therapeutics Program

Negative Contrast of Oxyhemoglobin Highlighted by ICE-MRI Differentiates Grade and Invasion of urothelial carcinoma

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Purpose: Diffuse reflectance and fluorescence cystoscopy and immunostaining reveal that the blood flow and hemoglobin is elevated in dysplastic and malignant regions of human bladder wall relative to normal areas. Given the narrow field of view and the absence of subsurface view in cystoscopy, we leveraged the lack of arterial phase of enhancement by intravesical contrast enhanced magnetic resonance imaging (ICE-MRI) for structural and functional imaging of urothelial carcinoma.
Methods: We consented 15 male and female patients (60-75 yrs) suspected of urothelial carcinoma on cystoscopy for a prospective study (NCT04369560) on ICE-MRI at 3T scanner. Dynamic T1 weighted time-resolved imaging with stochastic trajectories (TWIST) images with temporal resolution of 20s and spatial resolution of 0.55m were acquired pre and post-50mL transurethral instillation Gadobutrol 20mM and Ferumoxytol 0.1mM mixture. The maximum tumor signal in the first minute of TWIST was correlated with histopathology.
Results: The poor T1 contrast of tumor and urine in pre-instillation scans was improved upon instillation of Gadobutrol mixture. Tumor signal intensity on sixteen color coded signal intensity maps in the first minute of TWIST scans correlated negatively and positively with neovascularization and extracellular space, respectively of tumor grade and stage recorded on histopathology. Tumor signal rose from muscle invasive bladder cancer (MIBC) <non-muscle invasive (NMIBC) high grade < NMIBC low grade because diffusion of Gadobutrol in expanded extracellular space of NMIBC low grade exceeded the negative contrast of oxyhemoglobin, which was raised by angiogenesis in MIBC.
Conclusion: This is the first report on TWIST aiding ICE-MRI for pre-operative rapid recognition of MIBC and differentiation of BCG evoked inflammation from NMIBC recurrence. Unlike MRI with injected Gadobutrol, instilled Gadobutrol unmasks the signal of perfused oxyhemoglobin in tumor volume for virtual determination of grade and stage to guide chemoradiation or antiangiogenic therapy to extend the cystectomy-free survival span.

Poster No. 99
Cancer Immunology and Immunotherapy Program

Inhibition of DPP4 enhances chemotherapy by bolstering T-cell trafficking and potentiating antitumor immunity

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Chemokines such as CXCL10 play a critical role in controlling the infiltration of effector T cells into solid tumors and can be regulated by dipeptidylpeptidase 4 (DPP4), a protein enzyme capable of inactivating CXCL10. Here, we show that chemotherapeutic drugs, especially the drugs that induce immunogenic cell death (ICD), induce the expression of DPP4 at the transcriptional level in cancer cells, both in vitro and in vivo, which may limit the effectiveness of T cell infiltration in tumors. In addition, these chemotherapeutic drugs induce the expression of COX-2, another feedback mechanism that further contributes to immunosuppression. Based on these findings, we developed a 5-ASA (a COX inhibitor)-conjugated hyaluronic acid (HA)-based nanocarrier (HASA) that is highly effective in codelivery of 5-ASA, doxorubicin (DOX) and sitagliptin (a FDA approved DDP4 inhibitor) to tumors. HASA is highly selective in tumor accumulation through targeting CD44 that is overexpressed in both tumor cells and tumor endothelial cells. Intravenous delivery of the triple combination therapy using HASA nanocarrier leads to significant inhibition of tumor growth in breast cancer model along with increased antitumor immune response. Thus, targeting DPP4 in combination with chemotherapy may represent a novel strategy for cancer treatment.

Poster No. 100

CDKN2A-Low cancer cells outcompete macrophages for microenvironmental zinc driving immunotherapy resistance

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Approximately 50% of cancers have decreased CDKN2A expression (CDKN2ALow), which is associated with resistance to immune checkpoint blockade (ICB). The mechanism of ICB resistance in these patients is unexplored. To investigate it, we generated complementary syngeneic mouse models that demonstrate the sufficiency of CDKN2A loss for ICB resistance. Prior data from our lab and others shows that CDKN2Alow cells have dysregulated metabolism. Cancer cell metabolic reprogramming reshapes the compartmentalization of metabolites within the tumor microenvironment (TME), altering immune cell composition and activity and thereby affecting their response to ICB. Here, we performed a metabolism-focused in vivo CRISPR screen and cross-compared this to unbiased metabolomics of the tumor interstitial fluid to identify potential drivers of ICB resistance in Cdkn2ALow tumors. We identify the plasma membrane zinc importer SLC39A9 as a driver of anti-PD1 resistance. Mechanistically, we found that CDKN2ALow cancer cells outcompete other cells in the TME for zinc by increased plasma membrane SLC39A9 through increased cholesterol. Increased intracellular zinc in Cdkn2alow cells stimulated the production of PAI-1 which increased the recruitment of macrophages to the TME. Depleted zinc in the TME drives dysfunctional phagocytic and inflammatory activity of these macrophages. Recent studies suggest that ICB therapy can activate macrophages to enhance their anti-cancer activity. We found that macrophages, rather than T cells, drive ICB responses in Cdkn2alow tumors. Altogether, our findings suggest CDKN2Alow cancer cells increase zinc uptake leading to an increased macrophage recruitment and a zinc depleted TME promoting macrophage dysfunction and ICB resistance. Remarkably, a physiologically non-toxic and feasible threefold increase of dietary zinc in mice harboring Cdkn2alow tumors restored ICB responses. Excitingly, CDKN2Alow cancer patients with increased dietary zinc, showed better responses to ICB therapy. Ongoing research will investigate whether dietary zinc re-educates dysfunctional macrophages to boost ICB responses.

Poster No. 101
Cancer Biology Program

Investigating alterations in metabolic profile of ovarian carcinoma associated stroma

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Ovarian cancer (OvCa) is the deadliest gynecologic cancer with most of its lethality attributed to late diagnosis and early metastasis. Prior work demonstrates that carcinoma associated mesenchymal stem cells (CA-MSC) enhance OvCa metastasis by donating their mitochondria to metabolically vulnerable OvCa cells thus increasing OvCa cell oxidative phosphorylation (OXPHOS). Although we have shown a crucial role for these donated mitochondria in OvCa progression and metastasis, the functionality of CA-MSC mitochondria and how they differ from normal MSC (nMSC) mitochondria is not known. Preliminary data show that CA-MSC derived mitochondria persist in OvCa cells over multiple passages but fail to incorporate into the host mitochondrial matrix and instead take a donut or blob shaped morphology which is indicative of mitochondrial stress. Interestingly, CA-MSC, compared to their nMSC counterparts, are enriched in mitochondrial mutations. RNAseq data comparing CA-MSC to nMSC also show OXPHOS as one of the top enriched pathways in CA-MSC. Here we demonstrate that CA-MSC have altered mitochondrial functionality and morphology compared to nMSC. CA-MSC have increased mitochondrial respiration compared to nMSC as revealed by cell mito stress test. Confocal imaging shows that CA-MSC have more networked mitochondria consistent with increased OXPHOS. Along with networked filamentous mitochondria, we also see increased blob shaped mitochondria in CA-MSC similar to CA-MSC donated mitochondria seen in tumor cells. Importantly, CA-MSC have increased mitochondrial superoxide production compared to nMSC and CA-MSC increase mitochondrial ROS in tumor cells. Our data suggests that CA-MSC are enriched in oxidatively stressed mitochondria which are preferentially transferred to OvCa cells thus driving oxidative stress. Our data also strongly suggests that Wilm's Tumor 1 (WT1) gene drives the mitochondrial phenotype in CA-MSC. Our current studies focus on further elucidating the functional consequences of the unique mitochondrial phenotype of CA-MSC in OvCa and ways to target it to decrease OvCa burden.

Poster No. 102
Cancer Biology Program

mDia2 is an important mediator of MRTF-A-dependent regulation of breast cancer cell migration

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Dysregulated actin cytoskeleton gives rise to aberrant cell motility and metastatic spread of tumor cells. This study evaluates the effect of overexpression of wild-type vs functional mutants of MRTF-A on migration and invasion of breast cancer (BC) cells. Our studies indicate that SRF's interaction is critical for MRTF-A-induced promotion of both 2D and 3D cell migration, while the SAP-domain function is important selectively for 3D cell migration. Increased MRTF-A activity is associated with more effective membrane protrusion, a phenotype that is attributed predominantly to SRF's interaction of MRTF. We demonstrate formin-family protein mDia2 as an important mediator of MRTF-stimulated actin polymerization at the leading edge and cell migration. Multiplexed quantitative immunohistochemistry and transcriptome analyses of clinical BC specimens further demonstrate a positive correlation between nuclear localization of MRTF with malignant traits of cancer cells and enrichment of MRTF-SRF gene signature in pair-matched distant metastases vs primary tumors. In conclusion, this study establishes a novel mechanism of MRTF-dependent regulation of cell migration and provides evidence for the association between MRTF activity and increased malignancy in human breast cancer, justifying future development of specific small molecule inhibitors of the MRTF-SRF transcriptional complex as potential therapeutic agents in breast cancer.

Poster No. 103
Cancer Virology Program

Building contextual knowledge graph of molecular regulatory pathway from literature with reguloGPT

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Molecular Regulatory Pathways (MRPs) are crucial for understanding biological functions, and Knowledge Graphs (KGs) are vital for organizing and analyzing these pathways by providing structured representations of complex interactions. Current tools for mining KGs from biomedical literature often fall short in capturing the intricate hierarchical relationships and contextual information inherent in MRPs. Large Language Models (LLMs) like GPT-4 offer advanced language capabilities, yet their full potential for end-to-end KG construction, particularly for MRPs, remains largely unexplored. In this study, we introduce reguloGPT, a novel GPT-4 based in-context learning prompt designed for the comprehensive extraction of regulatory graphs and context from sentences describing regulatory interactions and enabling end-to-end KG construction. ReguloGPT generates context-aware relational graphs that effectively capture the hierarchical structure of MRPs and resolve semantic inconsistencies by embedding contextual information within the relational edges. We evaluated reguloGPT using a benchmark dataset of 400 annotated PubMed titles related to N6-methyladenosine (m6A) regulations, demonstrating significant improvements over existing algorithms and other LLMs. Additionally, we developed a novel G-Eval scheme that leverages GPT-4 for annotation-free performance evaluation, which was consistent with benchmark results. Applying reguloGPT, we constructed the m6A-KG from 1,396 m6A-related titles, showcasing its effectiveness in elucidating m6A's regulatory mechanisms across various cancers. These results underscore reguloGPT's transformative potential for extracting and organizing biological knowledge from literature.

Poster No. 104

The effects of economic hardship and social demographics on overall dose intensity of early-stage breast cancer (ESBC) chemotherapy

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Background: Economic hardship (EH) can significantly impact cancer treatment outcomes, but this impact may vary by social demographics. This study investigated whether baseline EH predicted projected and actual receipt of chemotherapy dosage in women with ESBC and whether race and area socioeconomic deprivation moderated these effects.

Methods: A descriptive and correlational design utilized data from women with stage I-III breast cancer receiving chemotherapy in a multisite, longitudinal study (R01MD012245). EH was assessed by Psychological Sense of EH, which includes 4 subscales: financial strain, inability to make ends meet, not enough money for necessities, economic adjustments. An EH-composite score was created by summing weighted Z-scores of these subscales. Race was self-reported as Black or White. Area deprivation index (ADI) was calculated per patient's address (0=less deprived,100=more deprived). Chemotherapy dosage was measured as projected (received in prescribed timeframe) and actual (including delays/reductions), each dichotomized based on gold standard of receiving ≥85% of prescribed dose. Univariate and multivariate binomial logistic regression were used.

Results: Participants included 253 women (mean age=52.9 years; median ADI=63), with 95 (37.5%) Black and 158 (62.5%) White. By projected endpoint, each 1-point increase in EH-composite score was associated with 9.9% reduced odds of receiving ≥85% of prescribed chemotherapy (p=0.033). Specifically, each 1-point increase in inability to make ends meet score was associated with 26.1% decreased odds of receiving ≥85% of prescribed dose (p=0.028). In multivariate analysis, White women had 3.033 times higher odds of receiving ≥85% of prescribed chemotherapy than Black women by actual endpoint (p=0.009). However, no moderation effects of EH by race or ADI were detected at either endpoint.

Discussion: Assessing and addressing financial challenges before chemotherapy initiation is needed to intervene to ensure that women receive gold standard of chemotherapy. Further research is needed to understand and eliminate racial disparity during ESBC chemotherapy.

Poster No. 105

Increased Expression of Transcription Factor AP2B in Invasive Lobular Tumors

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Invasive lobular carcinoma (ILC) is responsible for 10%-15% of all invasive breast cancers. ILC has a distinctive single file discohesive growth and is associated with worse long-term outcomes compared to the most common no special type (NST). Despite the known differences between ILC and NST, the treatment for ILC is the same as that for stage matched NST. Therefore, identifying the drivers that are specific for ILC development and progression, and which can be therapeutically targeted is an urgent need. In this context, we recently identified the transcription factor, AP2β (TFAP2B), as one of the most hypomethylated genes in ILC compared to NST tumors and normal mammary tissues. Consistently, differential gene expression analysis in TCGA, METABRIC and SCAN-B datasets confirmed the upregulation of TFAP2B expression in ILC tumors. Here, we examined the expression and localization of TFAP2B in various normal breast tissue and breast tumors, as well as a series of rodent and human models including cell lines and patient derived organoids (PDOs) with a focus on comparing expression in NST and ILC. Immunoblotting and qRT-PCR confirmed the specific upregulation of TFAP2B in ILC compared to NST cell lines and PDOs. Additionally, immunohistochemistry revealed an enhanced expression in ILC clinical specimens. Immunofluorescence imaging showed a clear nuclear localization of this transcription factor in lobular cancer cells. Staining of normal human and mouse tissues showed TFAP2B expression in a subset of luminal epithelial cells in both ducts and lobules. Collectively, our data support the increased expression of TFAP2B in ILC compared to NST. Future studies will be conducted to uncover the potential role of TFAP2B in the development and progression of lobular tumors.

* = equal contribution

Poster No. 106
Cancer Biology Program

Destabilizing HER2 with Neratinib or Ganetespib Enhances Preclinical Efficacy of Trastuzumab Deruxtecan in HER2-mutant Invasive Lobular Breast Cancer

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Background: Activating mutations in HER2 (ERBB2) are enriched >4-fold in invasive lobular carcinoma (ILC) with a rate of >10% in metastatic ILC. Trastuzumab deruxtecan (T-DXd) is a HER2-targeted antibody drug conjugate (ADC) with reported single-agent activity against HER2-low and HER2-mutant breast cancer. Yet, their efficacy as a single agent or with other anti-cancer agents in HER2-mutant ILC is unknown and warrants investigation.

Methods: To model HER2 activating mutations as found in human breast cancer, we used CRISPR-based prime editing to generate a panel of isogenic ILC cell lines and patient-derived organoids (PDO) harboring HER2 wild-type (WT) or HER2 mutations (S310F or V777L). We then used them to test T-DXd alone or in combination with neratinib or ganetespib, which have been reported to destabilize HER2 and thereby increase internalization of ADCs.

Results: We successfully introduced single copy, heterozygous activating HER2 mutations (S310F or V777L) into two HER2-nonamplified metastatic ILC cell lines (MDA-MB-134 and SUM44PE) and one HER2-nonamplified metastatic ILC PDO (IPM-BO-053). Positive clones carrying the mutations were verified by Sanger sequencing and droplet digital PCR and subsequently pooled together. We further demonstrated that these mutations hyperactivate HER2 and downstream signaling pathways and enhance sensitivity to both HER2 ADCs and tyrosine kinase inhibitors (TKIs). Interestingly, we also observed accelerated HER2 protein degradation upon heregulin stimulation in HER2 mutants, suggesting activating HER2 mutations may enhance ADC/HER2 complex internalization, degradation, and release of payloads. Lastly, we explored drug synergy between T-DXd and neratinib or ganetespib and found that both combinations have additive anti-tumor effects in HER2-mutant ILC.

Conclusions: Our data warrant further testing of T-DXd alone or in combination with neratinib or ganetespib in vivo to better understand susceptibility to HER2 ADCs in HER2-mutant ILC. In future experiments, we will test if neratinib or ganetespib increases endocytic uptake of T-DXd using a fluorescently labeled endocytosis tracker. Lastly, an in-depth molecular characterization of our isogenic cell line and PDO models is ongoing to gain mechanistic insights into how activating HER2 mutations increase HER2 internalization and degradation.

Poster No. 107
Cancer Biology Program

An Unconventional Role of Actin-Binding Protein Profilin1 in Phospholipid Maintenance in Cancer Cells

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Background: Phospholipid dysregulation is a major contributor to promoting oncogenesis, malignant behavior, therapeutic resistance, and metabolic plasticity in cancer. The present work explores the unconventional role of profilin1 (Pfn1), an actin monomer-binding protein whose expression is altered in various human cancers (including breast cancer), in the regulation of phospholipid content in cancer cells.

Methods and Results: We used both gain- and loss-of-function (overexpression, RNAi, CRISPR/Cas9-mediated gene deletion) and orthogonal strategies (mass-spectrometry lipidomic analysis, immunostaining, live-cell imaging, and biochemical assays) in various cell lines to demonstrate that Pfn1 plays a key role in controlling $PI(4,5)P_2$ (PIP_2, the most abundant of the phosphoinositide family of phospholipids) levels in cancer cells. We first show that gene knockout of other major promoters of actin polymerization (Mena/VASP/Evl) are not associated with PIP_2 alteration, suggesting that changes in PIP_2 are specific to the action of Pfn1 and not a secondary consequence of reduced F-actin content in cells. We further show that loss of Pfn1 does not affect the cellular levels of precursor phosphoinositides for PIP_2 synthesis (i.e. PI, PI(4)P), nor does it impact plasma-membrane (PM) recruitment of PIP5K. Although Pfn1 depletion does not affect phospholipase Cg (PLCg)-mediated PIP_2 hydrolysis, Pfn1-dependent changes in PIP_2 are reversed when phospholipase Cß (PLCß) activity is reduced. These findings, together with additional experimental evidence of elevated diacylglycerol (DAG – an immediate byproduct of PIP_2 hydrolysis) levels in cells in a Pfn1-depleted setting, lead us to propose a novel mechanistic model where Pfn1 plays a key role in PIP_2 maintenance by protecting it from PLCb-mediated hydrolysis. Additionally, our lipid mass-spectrometry analyses reveal that loss of Pfn1 expression also leads to a dramatic decrease in phosphatidylcholine (PC - the most abundant cellular phospholipid) content in cancer cells.

Conclusion: These findings for the first time uncover a previously unrecognized role of Pfn1 in phospholipid maintenance.

Poster No. 108

Examining antitumorigenic effects of Ruminococcus gnavus

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Melanoma is one of the most commonly occurring cancers in the world, with increasing occurrence over the past several decades. Common treatment regimens for melanoma include immune checkpoint inhibitor (ICI) therapy, which enhances interferon-gamma production in CD8 T cells (Tc1). Despite showing efficacy in some patients, a majority of melanoma patients are resistant to ICI therapy. Prior studies have demonstrated the ability of the gut microbiota to impact Tc1 antitumor responses and ICI therapy efficacy. In analyzing top gut bacteria enriched in ICI-responders, Ruminococcus gnavus (R. gnavus) is a commonly identified species. Along with being ICI-responder associated, R. gnavus has been shown to catabolize dietary tryptophan into aryl hydrocarbon receptor (AHR) ligands. Here we demonstrate the tumor restraining properties of R. gnavus in subcutaneous, spontaneous, and metastatic cancer models. Further, gnotobiotic monocolonization experiments demonstrate the ability for this bacterium to restrain tumor growth independent of a complex microbiome. Analyzing the mechanism behind how R. gnavus causes melanoma growth suppression in our model, we have demonstrated requirements of bacterial viability and AHR activation. Colonization with R. gnavus at early, middle, and late-stage tumor timepoints was able to induce a significant Tc1 response locally in the tumor and systemically in tumor-draining lymph nodes and spleen. Finally, we found R. gnavus colonization to facilitate ICI efficacy in our model. This study uncovers a potential mechanism of how a common ICI-response associated bacterium enhances ICI efficacy in melanoma.

Poster No. 109
Cancer Immunology and Immunotherapy Program

The Dual Role of TLR2 in Lung Cancer: Promoting Early Tumor Growth and Modulating Inflammation and Differentiation in KRAS Mutant Mice

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Lung cancer is one of the leading causes of cancer death. In addition to its high mortality rate, the disease is characterized by complex tumor progression and immune evasion mechanisms, which complicates treatment. *KRAS* mutation is one of the most commonly mutated oncogenes. Toll-like receptor 2 (TLR2) is well known for its role in the innate immune system, recognizing pathogen-associated molecular patterns and initiating inflammatory responses. However, its role in tumor development remains unclear, especially in genetically susceptible settings such as KRAS mutations. In this study, we explored the role of TLR2 in lung cancer development using mice with *Kras* mutation and TLR2 knockout (KO). Our results showed that TLR2 KO mice with *Kras* mutation formed significantly larger tumors at early stages than *Kras*$^{+/-}$, TLR2 wild-type (WT) controls, suggesting that loss of TLR2 may promote early tumor growth. Surprisingly, tumor development in TLR2 KO mice slowed down at later stages, indicating that TLR2 plays a complex role in promoting initial tumor growth and regulating later-stage progression. Building on this foundation, we analyzed RNA expression levels and conducted histological analysis further to understand the role of TLR2 in lung cancer development. Our results revealed that TLR2 KO mice exhibited significantly lower levels of inflammatory cytokine expression, suggesting that the absence of TLR2 may lead to a reduced inflammatory response in the lung cancer microenvironment. Additionally, histological analysis showed that tumors in TLR2 KO mice had a higher degree of differentiation, which aligns with the slower tumor progression at later stages. These findings indicate that while TLR2 promotes early tumor growth, it regulates inflammation and differentiation during later stages. This further underscores the potential of targeting TLR2 in lung cancer therapy, where fine- tuning its activity could help balance the immune response and improve treatment outcomes.

Poster No. 110

Roles of Toll-like receptor 2 on lung tumorigenesis in mice carrying KRAS mutations.

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Mutations in KRAS, a critical oncogene that regulates cell growth and survival, are present in about 25% of all lung cancer incidences. KRAS mutation carries a poorer prognosis on survival outcome in Non-small cell lung cancer (NSCLC), highlighting the need for KRAS-targeted therapy. While activating KRAS mutations are important in initiating lung tumor growth, additional factors may contribute to the progression to malignant adenocarcinoma. Inflammation is among several key factors that can promote the progression of KRAS-initiated lung tumorigenesis. While respiratory infections can induce inflammatory conditions within the lung, KRAS mutations may promote an inflammatory response through downstream pathways. It is essential to investigate which molecules downstream of KRAS could be involved in the inflammatory response to introduce targeted therapies. Toll-like receptor 2 (TLR2) is an extracellular receptor whose activation leads to the synthesis of several inflammatory cytokines. We hypothesize that KRAS mutations associated with NSCLC tumor progression are potentiated through TLR2 signaling-regulated inflammation. We have generated mice with KRAS mutation (G12D) in the TLR2 knockout (TLR2-/-) genetic background to test the hypothesis. These mice's lung tumor burden and characteristics were compared to KRAS-bearing mice without TLR2 ablation (TLR2 +/+). Our results indicated that tumor burden increased in TLR2 knockout mice compared to wild-type mice 18-20 weeks old. Tumor burden in both genders of TLR2 +/+ mice was similar, but was greater in males than females for the TLR2-/- mice. Although interconnected, these results contradict the original hypothesis, indicating that the relationship between the TLR2 pathway and KRAS-initiated inflammatory response may be more complicated than predicted. Further research would improve the conditions of this experiment, specifically by increasing the sample size and extending the ages of the mice. In addition, the potential anti-tumor effects of TLR2 could be explored by targeting specific cell types instead of global knockout.

Poster No. 111

Co-delivery of Nebl siRNA via a tumor-targeting hybrid nanoparticle to improve the therapeutic efficacy of azacitidine treatment in non-small cell lung cancer

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NEBL, a gene encoding protein LASP2, is critical in cell adhesion and actin filament architecture. It has recently been identified as an oncogene in NSCLC, facilitating tumor metastasis, invasion, and progression. However, the role of *NEBL* in drug resistance and immune modulation remains elusive.

DNA hypermethylation plays a crucial role in 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 single-agent therapy, Aza shows limited efficacy in solid tumors. To further understand the mechanism of Aza treatment and drug resistance, we performed an RNA-seq analysis on Aza-treated 3LL tumors. We found that *Nebl* was significantly upregulated by Aza treatment. We hypothesize that *NEBL* knockdown shall improve Aza-based therapy for lung cancer.

MTT and wound healing assays show that Aza synergized with *NEBL* siRNA (siNEBL) knockdown to inhibit tumor cell proliferation and invasion. Furthermore, *NEBL* knockdown led to a RIPK3/MLKL-dependent necroptosis and significant induction of CXCL10, a chemokine critically involved in T cell trafficking. This is consistent with the analysis of GSCA data showing a negative correlation between *NEBL* expression levels and T cell infiltration in several tumor types, including NSCLC. To facilitate the therapeutic translation of these findings, we developed an Aza prodrug-based nanocarrier that is highly effective in selective siRNA delivery to tumor tissues. Combining Aza and siNebl in a nanocarrier dramatically increased the total and IFN-γ-positive CD8+ T cell infiltration in tumors, leading to significantly improved antitumor activity in both subcutaneous and orthotopic models of murine NSCLC (3LL) models. Targeting *NEBL* in combination with Aza may represent a new and improved treatment for NSCLC.

Poster No. 112

"Cancer Care Crossroads": The Effect of Rurality on Patient Attitudes and Decision-Making Related to Surgical Prostate Cancer Care

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Background:
Previous studies have shown that rurality significantly impacts treatment choice for prostate cancer (PCa), with patients in rural areas less likely to opt for surgery — citing distance from a treatment facility as a key factor in decision-making. However, the specific factors that influence patients' decision-making for PCa treatment choice during the narrow window of diagnosis to when a treatment plan is made, as well as patients' own meta-perceptions of rurality's impact on barriers to PCa surgical care, is not well understood. We aim to use qualitative methodology as a more flexible, granular research instrument to gain insight into rural-urban patient perspectives related to PCa surgical decision-making.

Methods:
Patients receiving surgical PCa care at a single National Cancer Institute Comprehensive Cancer Center, serving both urban/rural populations across Western Pennsylvania and its four neighboring states, were recruited. We conducted 20 semi-structured interviews with 10 urban and 10 rural patients according to U.S. Census Tract/RUCA data. The interviews were then transcribed, de-identified, and coded with two independent coders using qualitative analysis software (NVivo 14.0), with thematic analysis utilizing a hybrid deductive-inductive approach. Coding is ongoing with final thematic analysis pending.

Results: Preliminary themes included: 1) 'transfer of trust' from community to centralized providers; 2) patients becoming used to receiving care at centralized locations, and 3) the limited impact of travel distance on decision-making: "I'm fine with driving two hours, even six, if it's somebody I trust is going to do a good job." (P11)

Impact: This is the first study, to our knowledge, that aims to understand qualitatively the impact and nuances of rurality and geography on PCa patient attitudes and surgical decision-making, laying the groundwork for exploring broader themes of inter-provider transfer of trust, sources of anxiety from time of diagnosis until a treatment plan is executed, and provider referral patterns, among others.

Poster No. 113
Cancer Biology Program

Preclinical discovery and characterization of Immune privileged transcriptional regulon for therapeutic precision in cancer treatment

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Cancer immunotherapy, particularly immune checkpoint blockade (ICB), faces challenges such as low patient response rates, severe side effects, and the absence of reliable predictive biomarkers. Although transcriptional dysregulation is known to play a role in cancer progression and immune regulation, its impact on immunotherapy response and prognosis is not fully understood. To bridge this gap, we conducted an extensive analysis of bulk and single-cell RNA sequencing data from clinical trials across various cancers and healthy tissues. Our focus was on transcriptional regulon signatures, defined by the expression of their target genes.
We identified a novel transcriptional signature, IMPREG, characterized by neuronal, endothelial, and fibroblastic cell states, crucial for immune-privileged sites. Importantly, IMPREG was absent in T-cell engaging tissues and immune regulatory processes. Validation through bulk-RNA sequencing and multiplex immunohistochemistry in triple-negative breast cancer (TNBC) cohorts confirmed the existence and significance of the IMPREG signature.
The IMPREG signature not only predicts immunotherapy efficacy across multiple cancers but also indicates sensitivity to anti-angiogenic therapy and EGFR tyrosine kinase inhibitors. These findings provide a new approach to overcoming immunotherapy resistance and enhancing the precision of immune-modulating treatments, paving the way for personalized treatment strategies and improved clinical outcomes

Poster No. 114

Exploiting Genomic Instability-Induced Vulnerabilities for Precision Breast Cancer Treatment

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Breast cancer is characterized by fundamental genomic instability, leading to progressive gene loss during disease evolution. This cumulative gene loss creates specific vulnerabilities in breast cancer cells that are absent in normal tissue. We propose that these 'pair-wise' vulnerabilities, arising from the depletion of essential genes, present a novel opportunity for precision breast cancer treatment. By identifying and targeting these cancer-specific 'synthetic lethalities', it may be possible to develop therapeutic strategies that effectively combat tumor growth while minimizing adverse effects on healthy patient tissue.

We present an analytical pipeline that takes a patient-to-benchside approach using the Hope for OTHERS Organ Donation Program, where breast cancer tissue can be rapidly biopsied, sequenced, and analyzed. This pipeline integrates multiple computational tools including Sarek for variant calling, TUSV-ext for phylogenetic reconstruction and subclone assignment, and DepMap data for identifying potential vulnerabilities.

To validate our findings, we employ patient-derived breast cancer organoids to test identified vulnerabilities through shRNA knockdown experiments. We also analyze data from the US AURORA study to confirm trends in genomic alterations between primary tumors and metastases, and conduct in vitro experiments using breast cancer cell lines to validate the synthetic lethality of identified gene targets.

This integrated computational and experimental approach aims to reduce the timeline for treatment target selection on a patient-by-patient basis in breast cancer. By exploiting the unique genomic landscape of each patient's tumor, we hope to pave the way for more effective and personalized breast cancer therapies that minimize collateral damage to healthy tissue.

Poster No. 115
Cancer Immunology and Immunotherapy Program

Tumor-derived nutrient stress induces lasting effects on CD8+ T cell function and epigenetic remodeling

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In tumors, CD8 T cells are exposed to metabolically active environments where certain nutrients are scarce. The tumor nutrient environment shapes T cell responses, but little is known about the long-term, heritable effects of tumor nutrient stress on CD8 T cells. To examine the heritable effects of nutrient stress, CD8 T cells were cultured in Tumor Interstitial Fluid-like Media (TIFM), a cell culture medium that mimics the nutrient environment of the tumor interstitial fluid of pancreatic ductal adenocarcinoma (PDAC). Naïve CD8 T cells were activated for 24 hours in control media, then transferred to nutrient stress for 48 hours. After stress, cells were returned to nutrient-replete media for four days. On day seven, cells were restimulated. Cells exposed to tumor nutrient stress had fewer TNFα+IFNγ+ cells, and cytokine-expressing cells produced less TNFα and IFNγ, respectively. To test the long-term effects of metabolic stress in vivo, stressed cells were adoptively transferred into Vaccinia-OVA-infected mice and examined 11 days and 30 days post infection. Fewer stressed cells were IFNγ+TNFα+ upon restimulation despite expanding equally to controls. ATAC-Seq and RNA-Seq of cells collected 4 days after removal from stress in vitro had persistent changes in chromatin accessibility, indicating a potential epigenetic mechanism driving the changes in functional phenotype. Collectively, these data show that CD8 T cell responses are influenced by past nutrient stress, and these changes are heritable. Future work will identify mechanisms by which T cells “remember” past nutrient stress.

Poster No. 116
Cancer Therapeutics Program

Rational development of 'POEM' nanomaterials for co-delivery of SERPINB9 siRNA and gemcitabine to overcome chemo-immune-resistance

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SERPINB9 (SPB9), an endogenous inhibitor of granzyme B (GzmB), has emerged as a critical factor in the resistance to immunotherapy, attributed to its protective mechanism against GzmB-mediated killing of cancer cells. However, its role in chemosensitivity remains unknown. In this study, we show that gemcitabine (GEM) treatment leads to significant upregulation of SPB9 in vitro and in vivo through ATF-3 transcription factor, consistent with its known role in inhibiting GzmB-mediated antitumor immune response. Interestingly, GEM also induces the expression of GzmB and knockout (KO) or knockdown (KD) of SPB9 results in enhanced response of tumor cells to GEM, suggesting a new role of GzmB/SPB9 axis in regulating chemosensitivity. To facilitate the therapeutic translation of these findings, we have engineered a 'POEM-like' nanocarrier that is highly effective in codelivery of built-in GEM and loaded SPB9 short interfering RNA (siSPB9). Incorporation of GEM introduces an additional mechanism of carrier/siRNA interaction, which reduces the toxicity through decreasing the amounts of positively charged materials needed for effective i.v. delivery. Such design can be applied to the development of similar siRNA nanocarriers modified with other nucleosides or their analogues. Codelivery of GEM and siSPB9 leads to significantly enhanced antitumor activity and improved tumor immune microenvironment in several pancreatic cancer models. Targeting SPB9 in combination with chemotherapy may represent a new and improved immunochemotherapy for the treatment of various types of cancer including pancreatic cancer.

Poster No. 117
Cancer Therapeutics Program

Knocking Down of Xkr8 Enhances Chemotherapy Efficacy Through Modulating Tumor Immune Microenvironment

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Scramblase Xk-related protein 8 (Xkr8) regulates the externalization of phosphatidylserine (PS) during apoptosis and holds a pivotal role in fostering tumor immunosuppression. Targeting Xkr8 in conjunction with chemotherapy demonstrated a novel avenue for amplifying antitumor immune response and overcoming chemo-immune resistance. Here we further evaluated this strategy by using a clinically relevant orthotopic model and elucidated the mechanism through in-depth single-cell RNA sequencing (scRNA-seq). We found that Xkr8 knockdown exhibited the potential to lead to immunogenic cell death (ICD) by impeding the normal clearance of apoptotic cells. Co-delivery of Xkr8 small interference RNA (siRNA) and a prodrug conjugate of 5-fluorouracil (5-Fu) and oxoplatin (FuOXP) showed remarkable therapeutic efficacy in an orthotopic pancreatic tumor model with increased infiltration of proliferative NK cells and activated macrophages in the tumor microenvironment (TME). Single-cell trajectory analysis further unveiled that tumor infiltrating CD8+ T cells are differentiated favorably to cytotoxic over exhausted phenotype after combination treatment. Our study sheds new light on the impact of Xkr8 knockdown on TME and solidifies the rationale of combining Xkr8 knockdown with chemotherapy to treat various types of cancers.

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Poster No. 118

Chemotherapy induces a pro-metastatic secretome through cellular senescence

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High grade serous ovarian cancer is the most lethal gynecological malignancy, with 70-80% of patients having the disease recur withing 12 months of treatment with first line therapies. A potential factor in ovarian cancer recurrence is that these chemotherapies induce senescence, a stable cell cycle arrest that is characterized by a unique secretome (termed the senescence-associated secretory phenotype- SASP). Prior work found that senescent cells can promote tumor progression and metastasis; however, the direct contribution of the SASP to metastatic recurrence has not been deeply explored. Interestingly, we found that the SASP is sufficient to drive increased ovarian cancer metastatic tumor nodules in vivo. Mechanistically, we found that naïve cancer cells cultured in conditioned media from senescent cells expressed lower levels of multiple extracellular matrix and adhesion related proteins, including fibronectin and integrins. Indeed, the decrease in adhesion factors corresponds to lower cell adhesion and increased cell detachment in 3D conditions. Unexpectedly, our experiments suggest a metabolic component of the SASP contributes to the increased metastasis through loss of adhesion factors. Using a metabolic CRISPR knockout screen to identify genes required for decreased adhesion after exposure to the SASP, we identified several genes in the electron transport chain, including NDUFA5, a subunit of complex I. Consistently, ovarian cancer patients with low NDUFA5 expression have upregulated adhesion gene signatures, and NDUFA5 knockdown both increases cell adhesion and rescues fibronectin and integrin levels. Together, our data demonstrate that the chemotherapy-induced SASP may have a paracrine effect on mitochondrial metabolism in tumor cells, which decreases cell adhesion. We hypothesize this decrease in adhesion will lead to an increase in tumor metastasis, which contributes to recurrence. Future studies will identify the SASP component that leads to paracrine metabolic reprogramming, the mechanism behind NDUFA5's regulation of adhesion, and its role in SASP-induced dissemination in vivo.

Poster No. 119
Cancer Immunology and Immunotherapy Program

Elucidating the role of LGALS1 in sensitizing HNSCC to immune checkpoint inhibitor and assessing its prognostic value in predicting ICI responses in HNSCC

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Immune checkpoint inhibitors (ICIs) were approved for treating head and neck squamous cell carcinoma (HNSCCs); however, the response rate remains relatively low. Thus, it is vital to identify new targets that sensitize HNSCC to ICI treatment. The role of myeloid cells in tumor immunosuppression is well-established. However, effective agents targeting these cells in HNSCCs are still lacking. We employed a transplanted HNSCC mouse model, A223 harboring *Smad4* deletion and $Kras^{G12D}$ mutation, where tumor-bearing recipients diverged into responders (R) or non-responders (NR) upon anti-PD-L1 treatment. Our single-cell RNA-sequencing data of $CD45^+$ tumor-infiltrating cells from R and NR mice showed that NR tumors contained expanded myeloid populations, while R ones were enriched with T cells. Differential gene expression analysis showed that NR tumor-infiltrating myeloid cells expressed a higher level of LGALS1. LGALS1 expression is also elevated in our ICI-resistant SCC. We found that a higher level of intracellular LGALS1 in $CD11b^+$ PBMC samples and lower level of plasma LGALS1 correlate with response to anti-PD-L1. Dual treatment of LGALS1 inhibitor (OTX-008) and anti-PD-L1 significantly inhibited A223 tumor growth compared to single agents and substantially prolonged recipient survival. Using a unique co-culture system, we found that OTX-008 treatment did not affect the percentage of tumor-associated macrophage (TAMs) but altered their phenotypes. OTX-008 treatment reduced the expression of LGALS1 in tumor cells. We will elucidate the underlying mechanisms of the enhanced efficacy of combo treatment and assess the prognostic value of LGALS1 for ICI treatment in HNSCC patients. Our study identified LGALS1 as a potential target to improve efficacy of combinatorial immunotherapy for HNSCC by targeting both tumor and tumor-infiltrating myeloid cells. We envision LGALS1 expression level may serve as a correlative marker for therapy responses in HNSCC patients.

Poster No. 120
Cancer Immunology and Immunotherapy Program

Programming T cell targeting through antibody-lipid conjugates

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Chimeric antigen receptor (CAR) T cell therapy is a successful example of synthetic immunology showing effectiveness treating hematological cancers. It consists of patient T cells that are transduced with a viral vector encoding the CAR which mediates antigen recognition and tumor cell killing in a non-MHC dependent manner. A major limitation of this therapy is the complex, expensive, and time-consuming manufacturing process. Here we provide a novel method to program T cell targeting through non-genetic means, anchoring tumor-targeting antibodies onto T cell surfaces via conjugated lipid moieties. In one embodiment, antibodies are first fused with DBCO molecules, followed by cholesterol-azide, which produces the lipid-antibody conjugates through click chemistry. Notably, the amount of DBCO molecule per antibody is controlled by using specific mixing time and concentration of DBCO. We quantified conjugation through mass spectrometry thus preventing excessive tight labeling of antibody surface, which may occupy its antigen recognition site and interfere with its function. Application of conjugates to Jurkat T cell line culture leads to efficient and stable surface display of the antibody over 5 days, as shown by fluorescence microscopy and flow cytometry. Furthermore, surface-modified Jurkat cells with a cholesterol labeled anti-CD3/anti-CD20 bi-specific antibody, monsuntuzumab, allows for potent and antigen specific T cell activation up to 5 days post labeling. Overall, these data provide a proof-of-concept for programming cell function through non-genetic surface modification for which further development aims to dramatically lower the complexity and cost of cell therapy manufacturing.

Poster No. 121
Cancer Biology Program

RET and HER2 Crosstalk: Implications for Therapy of Breast Cancer Brain Metastasis

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Breast cancer brain metastasis (BrM) has become a major contributor to mortality among breast cancer patients, yet current therapeutic strategies are limited. The identification of novel molecular targets involved in BrM is crucial for the development of effective treatments. Recent studies have identified RET and HER2 as two of the most upregulated genes in BrM, with a co-occurrence rate of 62.5% (5 out of 8). And their ligands, glial cell-derived neurotrophic factor (GDNF) and heregulin, are abundantly expressed in the brain. These suggest potential crosstalk between RET and HER2 in breast cancer cells, leading us to hypothesize that dual inhibition of RET and HER2 could synergistically inhibit tumor growth and brain metastasis.

To test this hypothesis, we utilized Selpercatinib and Tucatinib, small molecule inhibitors for RET and HER2 that can penetrate the blood-brain barrier. Our studies demonstrated a synergism of the two inhibitors in vitro, where they significantly suppressed tumor cell growth, migration, and invasion. Notably, the synergistic inhibition was enhanced in the presence of HER2/RET ligands, implying an interaction between the two receptors. Co-immunoprecipitation assays confirmed the physical interaction between RET and HER2 proteins in MCF-7 and SUM44 cell lines. Further molecular pathway analysis showed that selpercatinib and tucatinib effectively inhibited the activation of HER2 and RET signaling pathways induced by heregulin and GDNF, respectively, with dual inhibition synergistically suppressing downstream signaling.

Our preliminary findings suggest that dual inhibition of RET and HER2 represents a promising strategy for the treatment of breast cancer brain metastasis. Currently, in vivo studies are underway, where breast cancer cells are orthotopically injected into the mammary fat pads of mice to assess the efficacy of the dual inhibitors on brain metastasis. Additionally, we are conducting ex vivo co-culture experiments with brain slices to evaluate the potential of the two inhibitors in blocking breast cancer cell invasion into the brain.

Poster No. 122

Trajectories of symptom severity in patients receiving treatment for head and neck cancer

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Background/Significance: Patients receiving treatment for Head/Neck Cancer (HNC) experience many symptoms; however, their longitudinal experiences have not been well described. The purpose of this study was to identify trajectories of groups of patients with similar symptom severity experiences in individuals with HNC undergoing radiation using group-based trajectory modeling (GBTM).
Methods: This secondary analysis of patients with squamous cell HNC recruited in the UPMC HNC Survivorship Clinic used a prospective, repeated measures design. Symptoms were assessed twice a week and averaged weekly using the MD Anderson Symptom Inventory Head and Neck Module (MDASI-HN). We used GBTM to group patients with similar symptom severity trajectories using two subscales of the MDASI-HN. We defined low symptom severity as <5, and moderate to severe as ≥5, per previous work with the MDASI.
Results: Patients (N= 176) were on average 62±17years old, white (85.7%) males (74.0%), with tumors of the oropharynx (46.7%), oral cavity (47.1%), and larynx/hypopharynx (19.3%), and stages I-II (47.2%) and III-IVc (52.8%) disease. For the MDASI Core symptom severity, the model identified a: low stable (42%), a low increasing (38%), and a moderate symptom severity group (20%). For HN module symptom severity, the best model included a low stable symptom severity (34.5%), a low linear increasing to moderate (45%) and a low non-linear increasing to moderate group (20.5%).
Discussion: Our analysis demonstrated that the two MDASI-HN subscales generated different groups of symptom severity trajectory profiles. Each subscale covers a wide range of symptoms which may be mechanistically unrelated. Future work should include a more granular examination at the individual symptoms in each subscale to better understand the longitudinal experiences of patients with HNC undergoing treatment. These results lay the foundation for future work that could investigate predictive factors associated with group membership to better predict patients at high risk for symptom severity.

Poster No. 123
Cancer Biology Program

MitoChontrol: Adaptive mtRNA filtering of single cell RNA sequencing data for enhanced data quality

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We propose a novel method, adaptively tuned to cell type and origin tissue, for identifying and removing compromised cell populations in single cell RNA-sequencing (scRNA-seq) data, thereby improving data quality for downstream analysis. scRNA-seq quantitatively measures gene expression profiles of individual cells by counting mRNA transcripts, including those from mitochondrial genes. High proportions of mitochondrial RNA (mtRNA) can indicate apoptosis or cellular distress, which introduce noise into the data and bias downstream analysis. Filtering cells with disproportionately high mtRNA eliminates these complications.

The current filtering standard applies an arbitrary mtRNA-proportion threshold across the entire scRNA-seq sample, removing all cells exceeding the threshold. However, mtRNA levels fluctuate with metabolic demand, which is tied to cell type and tissue of origin. Fixed thresholds disregard this diversity, risking including compromised cells and/or excluding intact cells. Therefore, there is need for robust, adaptive mtRNA filtering methods which ensure observed scRNA-seq gene expression profiles accurately reflect the sample's biological state without bias from compromised cells. Existing adaptive methods fail to appropriately segregate cell populations without bias. To address these limitations, we are developing a filtering approach which independently identifies the distribution of healthy mtRNA expression for each cell population in different tissue types to generate an adaptive threshold for filtering compromised cells.

We collected 50 scRNA-seq samples per tissue type from publicly available datasets to represent each tissue's full distribution of mtRNA expression levels. Cell typing is performed uniformly for samples within each tissue type via the batch-independent method, TOSICA. Using expectation maximization, the distribution of mtRNA reads for each cell type is fit to an mtRNA mixture model. The cell population represented by each mixture component is characterized, and filtering rules are established to encapsulate the expected distribution of healthy mitochondrial expression. Our method results in adaptive mtRNA filtering which distinguishes between intact and compromised cells accurately and efficiently.

Poster No. 124
Cancer Epidemiology and Prevention Program

Identifying and Addressing Disparities in Head and Neck Cancer Clinical Trial Enrollment

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Improving diversity in cancer clinical trials is essential for enhancing the generalizability of findings and expanding access to novel therapies. Older patients, women, and those from lower socioeconomic backgrounds are typically underrepresented in trials. Few studies have explored the participation of head and neck cancer patients in clinical trials. This study investigates sociodemographic and geographic factors associated with lower participation rates among head and neck cancer (HNC) patients in clinical trials. We conducted a retrospective analysis of HNC patients treated within the UPMC system from 2012 to 2022 (n = 2312), including those enrolled in any of 43 HNC clinical trials. The outcome of interest was on participation in clinical trials in addition to the Head and Neck Specialized Program of Research Excellence (SPORE). We incorporated the area deprivation index (ADI) and rural-urban commuting area code (RUCA) based on patient addresses for geospatial context. A fixed effects generalized linear model was used to assess associations with trial participation, supported by chi-squared and Fisher's exact tests for demographic analyses. Out of 2312 patients, 282 (12.2%) participated in a trial beyond SPORE, with 4.6% being Black and 80.9% male. Patients in the lowest three ADI quintiles were less likely to participate compared to those in the highest quintile (Odds Ratios (OR) 0.592, 0.486, 0.515; 95% Confidence Intervals (95% CI): 0.377-0.922, 0.304-0.767, 0.316-0.828). Medicare patients were less likely to participate compared to those with private insurance (OR 0.651; 95% CI 0.439 – 0.959). HPV-positive Oropharynx cancer patients were more likely to participate than those with oral cavity cancer (OR 1.99; 95% CI 1.39-2.87). Significant differences were observed based on demographics and cancer diagnoses. Barriers such as geographic access, insurance coverage, and trial availability may impact participation. Strategies to enhance access, provide social services, and reduce travel burdens could improve participation rates.

Poster No. 125

Engineering sustained and tumor-specific gene expression by CAR T cells via a synthetic feedforward gene circuit

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One previous strategy to enhance chimeric antigen receptor (CAR)-T activity in solid tumors, "TRUCK T cells" add a therapeutic gene such as IL-12, under the control of the NFAT promoter, leading to therapeutic gene expression upon CAR activation. While showing some therapeutic benefit, gene output relies on continuous NFAT stimulation, and there are potential safety concerns regarding toxicity of the gene payload. To improve TRUCK activity, we constructed a feedforward gene circuit that enables sustained tumor-specific therapeutic gene expression, with drug safety switch control. Upon T cell activation, NFAT drives the transcription of the rtTA transcriptional activator, which, with doxycycline, drives the expression of both the therapeutic gene and additional rtTA on second loop, thereby sustaining gene expression even after NFAT signaling ceases. We engineered primary human T cells with the feedforward gene circuit driving GFP expression and activated them with anti-CD3 agonist, anti-CD3/CD28 "TransAct" reagent, G4S antibody or Her2+K562 with varying concentrations of doxycycline. Reporter gene expression was tracked for 7 days in a time course experiment. We found that activated CAR-coupled memory loops showed sustained GFP expression comparable to that observed with OKT3 activation. Doxycycline could effectively sustain gene expression, showing 75% of maximum gene expression level at 7 days post activation compared to only 6.5% without the feedforward circuit. Importantly, GFP expression was strictly doxycycline-dependent, displaying no significant expression in the absence of doxycycline, even with CD3 stimulation. This preliminary data supports the new approach to improve TRUCK T therapy with sustained and drug-controllable tumor-specific gene expression.

Poster No. 126
Genome Stability Program

Probing the PARP1 Allosteric Network Via the WGR Domain

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Poly(ADP-ribose) polymerase 1 (PARP1) plays an important role in base excision DNA repair. Upon recognizing DNA strand breaks via several domains, including the WGR domain, PARP1 is catalytically activated producing chains of ADP-ribose (PAR) from NAD+ at a site on the protein about 45 angstroms away. It has been suggested that an allosteric network connects these two domains allowing coupling of SSB detection with ADP-ribose synthesis. Inhibitors of PARP (PARPi) are used in cancer treatments bind to the NAD+ binding site. Some of PARPi alter the conformation of PARP1 promoting long retention or release from DNA nicks. While EB47 is not used clinically, it promotes reverse allostery to cause PARP1 to be retained longer on DNA nicks. In this study we replaced five highly conserved amino acids with alanine in the WGR domain that are suspected to be important in the allosteric network and studied these variant proteins utilizing a technique termed SMADNE (single molecule analysis of DNA binding proteins from nuclear extracts). Single molecule studies employing the LUMICKS C-trap where uninhibited binding affinities of WT PARP1 and chosen PARP1 variants were compared to those in the presence of EB47. Experiments measured how these variants altered the capacity for EB47 to “trap” PARP1 on DNA or a decrease in ADP-ribose synthesis providing new information on the allosteric network. These five PARP1 variants all showed some degree of weaking the allosteric network. We found that amino acid K621 plays a key role in the allosteric network and substituting alanine at this site decreases EB-47 induced affinity over 100 fold and decreases PAR production by 4 fold. Mapping the allosteric network provides a beneficial framework for what mutations would cause a cancer patient to be PARPi resistant as well as to improve the search for a more efficacious PARPi.

Poster No. 127
Cancer Virology Program

Interrogating patient susceptibility and resistance to Epstein-Barr virus infection in the nasopharynx using organotypic cultures

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Epstein-Barr virus (EBV) is a ubiquitous gammaherpesvirus that chronically infects most humans. Approximately 95% of adults become seropositive for EBV by age 18, however, despite its prevalence, a subset of individuals are at elevated risk of developing an EBV-associated cancer. In specific regions of the world (e.g. Southeast Asia), epithelial pathologies like EBV-associated nasopharyngeal carcinoma (NPC) are endemic and far more common. EBV-associated NPC is characterized by a latent and clonal EBV infection, where oral IgA antibodies to EBV lytic proteins are observed to increase several years prior to NPC diagnosis. Thus, increased lytic burden from EBV at the nasopharyngeal mucosa is considered a risk factor. Conventional 2-D cell culture cannot replicate the tissue microenvironment that exists in the nasopharynx and does not recapitulate EBV's differentiation-dependent lytic infection program observed in stratified epithelium like organotypic rafts from oral keratinocytes. Here, we have established 3-D organotypic rafts using conditionally reprogrammed cells (CRCs) from patients to model EBV de novo infection in the nasopharyngeal epithelium. Primary nasopharyngeal cells were collected from consenting adults undergoing skull-base surgery without nasopharyngeal co-pathology. We have established a nasopharyngeal CRC cryobank from 42 total donors to further characterize the EBV life cycle within the nasopharynx. Here we demonstrate that nasopharyngeal organotypic rafts can support EBV de novo infection, that multiple donor cryopreserved CRCs can be thawed and differentiated, and that nasopharyngeal rafts can be used to identify host genes encoding EBV restriction factors. We have developed a molecular diagnostic panel to identify cells undergoing lytic or latent EBV replication. Using permissive cell line as benchmarks, we have optimized single cell RNA-sequencing (scRNA-seq) analyses to profile the spectrum of EBV genes expressed among different cell types in the natural host. We are investigating variation in susceptibility to de novo EBV infection among different cell types.

Poster No. 128
Cancer Virology Program

Interactive Spatial Transcriptomic Conversational Agents for Cancer Discovery

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Spatial transcriptomics (ST) provides powerful insights into the tumor microenvironment by systematically mapping spatial distribution gene expression across these heterogeneous tumor landscapes, facilitating a better understanding of tumor biology, cellular interactions, and heterogeneity, which can inform novel diagnostics and treatment strategies. However, the specialized bioinformatics skills required to analyze ST data pose significant challenges for biologists to effectively interpret complex spatial datasets, thereby hindering the discoveries.

To address this challenge, we introduce an interactive conversational agent designed to make ST data analysis more accessible to biologists and clinicians, especially those with limited coding expertise. By integrating large language models (LLMs) with intuitive visualization tools, our platform democratizes access to spatial transcriptomics, enabling researchers to perform complex analyses on various tumor types without the need for extensive computational training. This approach facilitates faster discovery, enhances collaboration, and allows for the generation of novel hypotheses in oncology research.

Our system is built on a multi-agent framework integrated into the open-source Napari visualization tool, which supports natural language interactions. This setup allows users to query sophisticated bioinformatics questions and automatically generate and execute code. By broadening the accessibility of advanced data analysis, our tool empowers a wider range of scientists and clinicians to contribute to KSHV research and beyond.

We validated our tool on a benchmark dataset of 100 question-answer pairs and applied it to single-cell spatial transcriptomic data from 49 Kaposi’s Sarcoma patients and 3 healthy controls. The tool accurately captured cell type distributions and expression profiles, offering insights into KS tumors and Kaposi’s sarcoma herpesvirus infection stages. Additionally, we identified novel spatial features associated with different KS stages, highlighting stage-specific spatial heterogeneity and interactions with immune cells. These findings provide a deeper understanding of the tumor microenvironment's role in disease progression.

Poster No. 129
Cancer Virology Program

Deep learning-based prediction of immune checkpoint inhibitor responses in cutaneous squamous cell carcinoma from multiplex immunofluorescence images

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Response to PD-1 inhibitors in cutaneous squamous cell carcinoma (cSCC) is limited to a subset of patients, and the biomarkers indicative of response to this therapy are not well-established. To identify novel treatment response biomarkers, we studied multiplex immunofluorescence (mIF) imaging of 30 patients with high-risk unresectable cSCC. Patients received two cycles of Pembrolizumab prior to surgery and underwent surgical resection. Patients received 15 further cycles of Pembrolizumab over 45 weeks after the adjuvant phase. In this study, we analyzed mIF images of a subset of 10 of the patients, of which 6 had a pathologically complete response (pCR) and 4 had a pathological non-response (pNR). In total, images of 435 regions-of-interest (ROIs) were analyzed across the 10 patients. Cell type density, cell-cell interactions, and cellular neighborhoods were computed within each ROI to identify potential biomarkers of response to PD-1 inhibitor therapy. Contrasting these features between ROIs from pCR and pNR showed a high density of B cells (AUC=0.697, P<0.0001) and macrophages (AUC=0.766, P <0.0001) associated with ROIs from pCR patients. In contrast, PD-L1+ macrophages (AUC=0.732, P<0.0001) were overrepresented in ROIs from pNR patients, suggesting immunosuppressive microenvironments. Cellular neighborhood analysis revealed 10 unique cellular neighborhoods. Of particular interest was the cellular neighborhood primarily composed of a combination of B cells and T cells was associated with the pCR status (AUC=0.66, P<0.0001). Visual inspection of the tissue revealed that this neighborhood identified tertiary lymphoid structures, highlighting a potential biomarker.

Additionally, we trained an attention-based multi-instance learning model on 222,206 image patches taken from the ROIs to differentiate pCR from pNR. Cross-validation resulted in 0.96 AUC on holdout data. Our results demonstrate clinically relevant biomarkers for PD-1 inhibitor therapy responses. Furthermore, deep learning models trained on imagery alone can outperform individual spatial features without requiring costly cell segmentation or cell typing.

Poster No. 130

Defining the role of AhR and Nrf2 in commensal immunomodulation of antitumor immunity

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Fecal microbiota transplants can re-sensitize patients to immune checkpoint inhibitor (ICI) therapy, yet the mechanisms these commensals use to impact antitumor immunity remain understudied. Microbial tryptophan catabolites acting as aryl hydrocarbon receptor (AhR) agonists are highly immunomodulatory, providing a potential mechanism for commensals to impact ICI outcomes. Microbial tryptophan catabolites acting as AhR agonists can drive cytotoxic effector CD8 T cell function (Tc1) to enhance antitumor immunity, but it is not known if this mechanism extends to all tryptophan catabolites. Additionally, AhR ligands can signal through both AhR and nuclear factor E2-related factor 2 (Nrf2), a transcription factor recently highlighted for its ability to bolster CD8 T cell immunity in a murine melanoma model, suggesting that both transcription factors are implicated in microbial immunomodulation.

Here, we screened all 12 known microbial tryptophan catabolites for their ability to promote Tc1 immunity and activate AhR and/or Nrf2. We uncovered that each catabolite had ligand-specific effects on antitumor immunity, AhR/Nrf2 activation and tumor burden. Indole-3-aldehyde, tryptamine, and indole-3-propionic acid directly enhanced Tc1 fate ex vivo. In a murine melanoma model, while many tryptophan catabolites restrained B16F10 tumor growth, others worsened tumor burden. Global transcriptomic profiling revealed that AhR ligands drive Tc1 immunity via distinct pathways that can act synergistically to enhance Tc1 immunity. While our studies confirm the ability of microbial tryptophan catabolites to impact antitumor immunity, future investigations are needed to determine the functional and transcriptional underpinnings of microbial AhR ligand-enhanced Tc1 immunity, and if these effects are AhR and/or Nrf2 dependent.

Poster No. 131
Cancer Biology Program

In-vitro modeling of cognate interaction-induced CAR-T cell infiltration dynamics

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The lack of immune-mediated cancer cell destruction is a dominant mechanism of immunotherapy failure. The characteristics and mechanisms of cognate recognition-induced CAR-T cell infiltration have yet to be revealed. In this study, we developed an in-vitro 3D collagen droplet co-culture system to simulate CAR-T cell infiltration in the tumor microenvironment and track T cell motility and interactions with tumor cells in real-time under different microphysiological environments. We employed CAR-T cells overexpressing the SNAP tag (SNAP-CAR T cells), where the contacts between CAR-T cells and HER2+ breast tumor cells can be initiated by adding benzyl guanin-conjugated Herceptin (BG-Herceptin).
Quantification of infiltrating cell numbers showed that BT474 and HCC1569 cells recruited SNAP-CAR T cells significantly at baseline levels. Upon adding BG-Herceptin, contacts between HER2+ breast tumors and SNAP CAR-T cells were activated. CAR-T cell infiltration was increased significantly in both cell lines. To investigate how cognate interaction-induced chemokine secretion affects CAR-T cell motility, we inhibited chemokines that showed changes in a chemokine array assay. By inhibiting CCL2, CAR-T cell infiltration was successfully inhibited in the +BG-Heceptin groups in both cell lines, which indicates CCL2 secretion is the mechanism of cognate-induced CAR-T cell infiltration.
By analyzing CAR-T cell motility patterns, we found that CAR-T cell infiltration and interaction with tumor cells are dependent on cognate recognition. With BG-Herceptin, CAR-T cell interaction and swarming around HER2+ breast tumor cells were significantly higher. By inhibiting CCL2, CAR-T cell motility was significantly impaired to baseline.
Our findings suggest that CAR-T cell infiltration towards the solid tumor depends on tumor-CAR-T cell interactions initiated by BG-Herceptin, highlighting the importance of immunogenic cell death in CAR-T cell recruitment. Furthermore, this study demonstrated the effectiveness of simulating CAR-T cell infiltration in solid tumors using a 3D in-vitro collagen droplet model.

Poster No. 132
Cancer Biology Program

The Impact of EPHA3 Intragenic Rearrangements on Immune Evasion and Immunotherapy Efficacy

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The advancements in immunotherapy have brought about a remarkable transformation in the field of cancer treatment. This has resulted in notable reduction of tumors and extended periods of remission for a specific group of patients. Nevertheless, the rate of patients achieving complete cure remains relatively low, typically ranging from 10-20% across different types of cancer. The limited success achieved so far can be partially attributed to the unique characteristics of certain tumors, which can impede the effectiveness of immune responses. These characteristics include being immune-deserted, immune-excluded, or immune-inflamed. New studies indicate that certain genetic rearrangements, like the ones observed in the EPHA3 gene, might have a significant impact on facilitating immune evasion in these types of tumors. This study examines the potential of EPHA3 intragenic rearrangements (IGRs) to affect immune evasion mechanisms and how they may impact the effectiveness of immunotherapy. We performed an extensive transcriptomic analysis using RNA-seq to investigate changes in gene expression related to the immune system in connection with EPHA3 IGRs in various types of cancer, such as kidney renal clear cell carcinoma (KIRC), bladder cancer (BLCA), and breast cancer. Our research suggests that individuals with higher levels of EPHA3 IGR expression may have a reduced response to immune therapy, specifically in cases of KIRC. It seems that the expression of EPHA3 has an impact on the immune context score (ICS) and treatment response in BLCA. This suggests that it could be used as a biomarker to identify therapeutic resistance. In the context of breast cancer, we have discovered that EPHA3 IGRs play a crucial role in controlling the positioning of immune cells at the tumor margin. Additionally, these IGRs have been found to impact the expression of immune checkpoint molecules like PD-L1 and CD8, which ultimately leads to the creation of an immunosuppressive environment within the tumor. In addition, the presence of activated fibroblasts was found to be correlated with EPHA3 IGRs, which in turn facilitated immune evasion. However, no significant correlation was observed with endothelial cells, macrophages, or epithelial cells. Exploring the potential of targeting EPHA3 IGRs could offer a fresh perspective in tackling immune resistance and enhancing the overall efficacy of cancer immunotherapy.

Poster No. 133

Who is Left Behind by Centralization? A Characterization of Patients Not Receiving Care at Centralized Cancer Centers

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Background: To improve cancer care, treatment has shifted towards a centralized model in which patients with cancer requiring multi-disciplinary care selectively receive treatment at regional referral centers. Considering regional referral centers are associated with improved outcomes, a disparity arises between patients with and without access to such centers. As such, we aim to characterize the patients receiving surgical cancer care outside of referral centers.

Methods: We used the Pennsylvania Cancer Registry linked to the Pennsylvania Health Care Cost Containment Council database to identify major cancer surgeries. Referral centers were defined as Commission on Cancer/National Cancer Institute-designated centers. We included adults with surgery in 2013-2020 for bladder, brain, breast, colorectal, esophageal, liver, lung, pancreatic, and prostate cancers. Patient demographics, with a focus on race and ethnicity, area deprivation index as a surrogate for socioeconomic status, and rurality, were abstracted and compared between patients undergoing surgical care at referral versus non-referral centers.

Results: A total of 115,437 patients underwent surgery across all cancer types, with 44,376 (38%) of patients receiving care at referral centers. Among racial and ethnic minorities, 60% received surgery at referral centers versus 40% at non-referral centers, compared to 35% and 65%, respectively, of non-minorities. Among the most deprived patients, 36% had surgery at referral centers and 64% at non-referral centers. Finally, for residents of urban areas, 41% had surgery at referral centers, 59% at non-referral centers. Among residents of rural areas, 22% had surgery at referral centers, 78% at non-referral centers.

Conclusions: A higher proportion of racial and ethnic minorities receive care at referral centers, compared to non-minorities. However, the most deprived patients and those from rural areas, are less likely to receive care at referral centers. Characterizing these groups will aid in understanding how the centralization of cancer care impacts healthcare access for traditionally underserved patients.

Poster No. 134
Cancer Biology Program

INVESTIGATION OF HETEROGENEITY IN HIGH-GRADE SEROUS CARCINOMA

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High-grade serous carcinoma (HGSC) is not only the most common form of ovarian cancer, but also the deadliest. Initial treatments for HGSC can be successful in minimizing the original tumors during treatment, but they do not eradicate all tumor cells. This residual cell population causes relapse in a large portion of HGSC patients. Thus, it is important to analyze how treatments impact different populations of HGSC cells in order to make treatments more effective for heterogeneous tumors.

HGSC model cell line OVCAR-3 and OVCAR-8 were treated with four front-line chemotherapies, emulating some variety of HGSC cells with various treatment. After treatment and incubation, samples underwent EdU treatment, fixation, and permeabilization. Samples underwent Hoechst stain and EdU activation, then were imaged using the Leica Biosystems high-resolution THUNDER Imager. DAPI images were analyzed to produce a metric of total genetic content within each cell. EdU and genetic content were analyzed using IC-50 curves.

Palbociclib, a CDK4/6 inhibitor, showed different responses between cell lines. While OVCAR-8 dropped in proliferation, OVCAR-3 increased in EdU positivity. This may be due to its cycE1 amplification; with a higher level of cycE1, the cell does not need the effects of CDK4/6 to activate its transcription through the RB pathway. This leads to continued proliferation as cycE1 and CDK2 come together to progress the cell through proliferation.

The DNA content of OVCAR-3 and OVCAR-8 cells vary. One variation occurs in PF-068700, where OVCAR-3 cells accumulate gradually to a bulk G2-state, while the OVCAR-8 cells acclimate mostly in a 2n state indicating pre-S phase.

Poster No. 135

The role of cell cycle plasticity in the drug resistance of pancreatic ductal adenocarcinoma (PDAC)

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Pancreatic ductal adenocarcinoma (PDAC) is renowned for its aggressive nature, delayed detection, and limited treatment options. Frequently harboring KRAS mutations, a driving genetic alteration, PDAC employs the MAPK pathway as a proliferative mitogenic mechanism, akin to a cellular gas pedal accelerating the cell cycle. Attempts to induce cell cycle arrest via ERK/MAPK inhibition, unveiled resistance in several PDAC cell lines, suggesting alternative pathways through the cell cycle that circumvent ERK-mediated effects.

To reveal potential alternative pathways through the cell cycle, we performed cell cycle mapping, which combines single-cell multiplexed imaging with machine learning to directly visualize and investigate the dysregulated phenotype in cancer, the cell cycle .

Cell cycle mapping revealed an alternative cell cycle trajectory in all cell lines exhibiting resistance to the drug. These alternative trajectories exhibited low levels of the retinoblastoma (RB) protein, an inhibitor of G1/S progression, potentially allowing cells to proliferate with much lower mitogenic signaling. Using machine learning, we have isolated cells along this alternative trajectory and show its hallmark is the degradation of RB in the G1 phase. The next steps are to investigate potential combination therapies to block these alternative trajectories and induce arrest in otherwise resistant PDAC cells.

Poster No. 136

High intake of non-nutritive sweeteners AceK and sucralose are associated with resistance to PD-1 based immunotherapy in melanoma and non-small cell lung cancer.

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Background: Non-nutritive sweeteners (NNS) are becoming more common as a healthier alternative to sugar in diet and sugar-free foods and beverages. Response to immune checkpoint inhibitors (ICI) is directly associated with the gut microbiome, and how the diet and its downstream effects on the gut microbiome and thus response to ICI therapy in cancer remain unclear.

Methods: In a prospective cohort study of n=152 advanced cancer patients treated with anti-PD-1 ICI therapy enrolled in HCC 20-019 (n=102 advanced melanoma, n=50 advanced NSCLC) completed the NCI's Diet History Questionnaire (DHQ III), a validated food frequency questionnaire (FFQ). The DHQ III generates an average daily intake for food groups, macronutrients, and micronutrients including NNS. From this output, we analyzed five NNS (sucralose, aspartame, acesulfame potassium (ace-K), saccharin, and xylitol). We normalized NNS intake relative to pre-treatment weight to permit evaluation per FDA intake cutoffs.

Results: We observed that higher normalized intake of sucralose and acesulfame was associated with significantly lower overall response rate (ORR) in PD-1 treated melanoma and NSCLC patients. Concordantly, we also observed that higher normalized intake of sucralose and acesulfame was associated with poorer progression-free survival (PFS) in PD-1 treated melanoma and NSCLC patients.

Conclusions: Our data suggests that higher intake of select NNS is associated with attenuated outcomes in PD-1 treated advanced melanoma and NSCLC.

Poster No. 137
Cancer Biology Program

Whether αKG promotes homologous recombination via histone acetylation in high MYC models

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Homologous recombination (HR) deficiency enhances sensitivity to DNA damaging agents commonly used to treat cancer. The metabolic mechanisms driving resistance to DNA damaging agents in HR-proficient cancer is still under research. We recently discovered that depletion of α-ketoglutarate (αKG) sensitizes cells with high endogenous *CCNE1* expression, which are HR-proficient, to DNA damaging agents by metabolic regulation of histone acetylation. To further investigate the generality of the pathway that αKG mediates homologous recombination through histone acetylation, we decided to look into cell with MYC amplification, which is also found in HR-proficient cancer and known to have increased αKG. Interestingly, similar to cells with high CCNE1, we observed histone hyperacetylation level and increased DNA damage foci in cells with MYC overexpression. Current experiments are aimed at investigating whether αKG drives HR-proficiency in MYC overexpressing cells and whether the mechanism underlying this is similar to CCNE1-driven cells. This research is important for generalizability of our findings on how HR-proficient cancers acquire resistance to DNA damaging reagents. Moreover, this research will provide a metabolic avenue to induce HR-deficiency and sensitivity to DNA damaging agents in HR-proficient tumors especially with high *MYC* expression.

Poster No. 138

Cancer Immunology and Immunotherapy Program

CDKN2A loss is associated with resistance to PD-1 based immunotherapy in melanoma.

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Background:

CDKN2A encodes two INK4 family members - p16 and p14arf – that act as tumor suppressors by regulating cell cycle progression. Somatic mutations of CDKN2A are common in the majority of human cancers, and inactivating mutations are observed in ~25% of cutaneous melanoma. Loss of function mutations in the CDKN2A gene result in loss of CDK4/6 inhibition, increased RB protein phosphorylation, and abnormal cell cycle progression. Prior work has provided conflicting evidence regarding the association between CDKN2A loss of function (LoF) mutations and response to immune checkpoint inhibitors (ICI) in cancer. We aimed to evaluate the link between CDKN2A status and ICI response in ICI-treated melanoma.

Methods:

We systematically evaluated the association between loss CDKN2A LoF alterations and the ICI response in melanoma across multiple cohorts including: adjuvant PD-1 (n=28), advanced melanoma treated with PD-1 ICI (n=19), advanced melanoma treated with PD-1/LAG-3 ICI (n=16), and advanced melanoma treated with PD-1/CTLA-4 ICI (n=36). CDKN2A LoF status was inferred from targeted Oncomine next generation sequencing (NGS) performed on pre-treatment tumor tissue.

Results:

We observed that CDKN2A LoF was associated with significantly reduced objective response rates (ORR) and significantly diminished relapse-free (RFS) or progression-free survival (PFS) across multiple cohorts of ICI treated high-risk resected and advanced melanoma.

Conclusions:

Our data show that CDKN2A LoF alterations were associated with reduced benefit from ICI therapy in ICI-treated melanoma patients across a range of indications.

Poster No. 139
Cancer Epidemiology and Prevention Program

Disparities and trends in human papillomavirus (HPV) pathologic testing among oropharyngeal cancer patients in the National Cancer Database

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Background: Since 2012, human papillomavirus (HPV) testing has been recommended for all incident cases of oropharyngeal squamous cell carcinoma (OPSCC), as knowledge of HPV status has clinical and prognostic value. There is limited data on utilization of HPV testing and disparities in OPSCC after 2018.

Methods: Our analysis included 5489 patients in the National Cancer Database diagnosed with OPSCC between 2013 and 2021. The proportions of patients who received HPV testing were compared across year of diagnosis, race, facility type, insurance status, and overall stage. Multivariable logistic regression analyzed the impact of all variables on HPV testing status.

Results: 73.8% of patients diagnosed with OPSCC in 2013 were tested for HPV, compared to 91.2% in 2018 and 95.9% in 2021. The proportion of patients tested between 2018 and 2021 differed significantly by facility type ($p = 0.034$). In a mutually adjusted analysis, patients diagnosed between 2013 and 2017 in community (OR: 1.59; 95% CI: 1.04, 2.38) or integrated network cancer programs (OR: 1.79; 95% CI: 1.40, 2.30) were more likely not to be tested for HPV compared to academic/research programs. From 2013 to 2017, patients diagnosed with stage IV OPSCC were less likely not to be tested compared to stage I (OR: 0.65; 95% CI: 0.43, 0.9995). From 2018 to 2021, patients diagnosed with stage III (OR: 5.72; 95% CI: 2.73, 12.52) or stage IV (OR: 9.02; 95% CI: 4.32, 19.76) OPSCC were more likely not to be tested compared to stage I.

Conclusions: HPV testing rates for OPSCC patients increased over time and with the implementation of the AJCC 8th edition staging manual in 2018. However, testing was still substandard in later years, with differences by overall stage and facility type. Further research is needed to investigate whether non-uniform testing practices contribute to these

Poster No. 140
Cancer Biology Program

Betulinic Acid Targets Metabolic Reprogramming of Urothelial Carcinoma

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An innately high metabolic rate of healthy bladder lining (urothelium) is verified by an early entry of fludeoxyglucose (FDG) during PET scan, which gets further accelerated in urothelial carcinoma owing to the aberrant expression of genes involved in glycolysis and fatty acid metabolism. Moreover, glycogen depletion together with fatty acids accumulation in cancer cells isolated from urine of patients motivates the hypothesis that if the cancer cell accumulation of fatty acid mimetic, Betulinic Acid is dependent on glucose (monomer of glycogen) scarcity driven metabolic reprogramming to fuel the proliferation of urothelial carcinoma via metabolism of fatty acids, then glucose supplementation should rewire metabolic reprogramming and blunt the anticancer action of Betulinic Acid derived from the bark of white birch (Betula alba). Betulinic Acid is shown to induce apoptosis, cell cycle arrest and depolarization of mitochondrial membrane potential in melanoma cells, which is recognized by NCI. Here, we probed whether accumulation and IC50 of Betulinic acid on urothelial carcinoma cell lines T24 and RT-4 compared to benign human urothelial cells HBDEC and TRT-HU1 is sensitive to glucose supplementation. While all cell lines have glucose insensitive IC50 for mitomycin, two-fold lower IC50 of 18.50-25.8µM in T24 and RT-4 cells vs 32.8- 45.3µM in TRT-HU1 and HBDEC of Betulinic acid is abolished by glucose supplementation. Indeed, compared to TRT-HU1, confocal microscopy of T24 revealed glucose scarcity dependent mitochondrial localization of fluorescein tagged Betulinic acid was blunted by glucose supplementation and stunted glycogenesis is accompanied with downregulation of pro-apoptotic genes PTEN, p53 and Caspase 3 and upregulation of anti-apoptotic XIAP gene. These findings support our hypothesis that glucose supplementation of T24 rewires metabolic reprogramming, targeted by Betulinic acid but not by mitomycin. Therefore, cancer cell-selectivity of Betulinic acid could replace FDG based diagnosis and reduce toxicity of chemotherapy in urothelial carcinoma.