UPMC Hillman Cancer Center

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University of Pittsburgh

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Poster No. 1
Cancer Biology Program

A novel function of paracaspase MALT1 : regulating immune evasion in TNBC

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TNBC represents the most aggressive subtype of breast cancer with limited therapeutic option and poor prognosis. Recent immunotherapy using immune checkpoint inhibitors (ICIs) has transformed treatment paradigm in many types of cancers. However, the response rate of anti-PD1/L1 therapy in TNBC is low, which is at least in part due to the immune evasion driven by cancer cells. The mechanisms underlying the immune evasion are poorly understood. Here, we demonstrated that deregulation of cancer cell-intrinsic protease-activated receptor 1 (PAR1), the key mediator of coagulation system, promotes immune evasion in TNBC. Mechanistical studies identified PD-L1 as the key mediator of PAR1-dependent immune evasion and tumor growth. Downstream of PAR1, we identified MALT1 as the mediator of PD-L1 induction. MALT1 depletion causes tumor growth inhibition through suppressing anti-tumor effector cells including $CD4^+$ T cells, $CD8^+$ T cells, and NK cells. Importantly, MALT1 expression well correlates with PD-L1 expression and immune evasion in $PAR1^{high}$ TNBC specimens from human patients. Finally, the suppression of anti-tumor immunity by PAR1/MALT1/PD-L1 cascade was proved in a co-culture system where cancer cells were co-cultured with primed immune cells. Taken together, our study revealed immune evasion as a novel mechanism for PAR1-driven malignancy in TNBC and provided mechanistic insight into how PAR1 promotes immune evasion. The uncovered role of cancer cell-intrinsic MALT1 in regulating PD-L1 expression and immune evasion would support an innovative concept for TNBC therapy by targeting MALT1 dual functions in cancer cells and Treg from tumor microenvironment (TME).

Poster No. 2
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 an ‘orphan’ 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. 3
Cancer Biology Program

Second generation probiotic Limosilactobacillus reuteri delivering IFN-β (LR-IFN- β) decreases radiation toxicity and improves survival after whole abdomen irradiation (WAI)

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WAI may be a valuable asset in the management of ovarian cancer except its use is limited by intestinal toxicity. Finding a strategy that overcomes toxicity is hence vital and can potentially transform standards of care. Our hypothesis is that the genetically engineered probiotic LR-IFN-β is a successful radiation mitigator that is rapidly cleared from the intestine, making it a novel candidate for intestinal radioprotection. Three C57BL/6J mouse models were assessed for overall survival, intestinal barrier integrity, and bacterial clearance. The first mouse model received LR-IFN-β 24 hours following 19.75 Gy WAI, the second model after 13.5 Gy PBI, and the third model after 9.25 Gy TBI. In each model, mice were divided into five subgroups: control (0 Gy), irradiation, IR + LR, IR + IFN-β, and IR + LR-IFN-β (109 bacteria in 100 mL of saline). Mice treated with LR-IFN-β gavage displayed significant improvement in overall survival ($p < 0.05$) and were noted to maintain intestinal barrier integrity by preserving Lgr5+ crypt stem cells and Occludin and ICAM levels ($p < 0.05$). Fecal matter analysis for LR-IFN-β clearance in irradiated mice (13.5 Gy PBI) was also observed by day three via colony assay and rt-PCR, with no detectable growth of LR-IFN-β in blood. We finally observed reduced levels of intestinal pro-inflammatory cytokines via Luminex assay of intestinal cells at day 5 in 13.5 Gy + LR-IFN-β mice, including IFN-γ ($p = 0.0261$), IL-3 ($p < 0.0020$) and IL-17 ($p < 0.0070$). We thus conclude that LR-IFN-β is a safe radiation mitigator that could possibly improve the management of ovarian cancer, and we anticipate that its combination along with WAI to current standard platinum/taxane-based chemotherapy will have a synergistic effect on reducing tumor burden while maintaining intestinal barrier integrity and improving survival.

Poster No. 4
Cancer Biology Program

Function and Mechanism of BCL2L14-ETV6 gene fusions in TNBC: chemotherapy resistance and immune evasion

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Triple-negative breast cancer (TNBC) is the deadliest breast cancer subtype accounting for 10-20% of breast cancer morbidity but a disproportionate large number of breast cancer deaths. Chemotherapy remained the mainstay of intervention due to the lack of well-defined targets, and recent deep sequencing studies have revealed a paucity of TNBC-specific mutations. Most recently immune checkpoint inhibition (ICI) emerged as an effective therapy for advanced TNBC expressing PD-L1 for which the responses appear to be durable. However, the ICI response rates for unselected TNBC are only 5-10%. Discovering the genetic aberrations driving TNBC immune evasion represents an unmet clinical need. Here we identified the first TNBC-specific recurrent gene fusion between BCL2L14 and the prototype cancer gene ETV6. BCL2L14-ETV6 is preferentially detected in ~19% mesenchymal-like TNBC tumors that exhibit more aggressive histopathological features. Our preliminary studies suggest that BCL2L14-ETV6 fusions modulate the target genes of NFκB, a central mediator of inflammation, orchestrate cytokine contexture, endow epithelial mesenchymal transition, confer paclitaxel resistance, and dictate an immune microenvironment that lacks immune infiltrates. Our study showed BCL2L14-ETV6 fusions orchestrate immunosuppressive and protumor cytokine contexture, and impair immune cell infiltration. Elucidating the role of BCL2L14-ETV6 fusions in TNBC immune evasion could reveal unique, exploitable vulnerabilities to tackle this devastating disease, and illuminate new immunotherapeutic strategies. As the incidence of BCL2L14-ETV6 is comparable to that of ICI responders in unselected TNBC, such development could substantially expand the patient cohort that could benefit from new immunotherapeutic strategies to tackle this devastating disease

Poster No. 5
Cancer Therapeutics Program

Small Molecule Allosteric Modulators of the AML-associated Src-family Kinase, Hck

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Hck and Fgr are members of the Src family of non-receptor tyrosine kinases expressed in myeloid hema-topoietic cells where high-level expression contributes to acute myeloid leukemia (AML). While ATP competitive inhibitors of Hck and Fgr show promise for AML therapy, emergence of resistance mutations may limit efficacy. Combination therapy targeting a single protein with allosteric and ATP-site inhibitors dramatically reduced resistance potential in various cancers and may have potential for AML as well. Our group has identified two small molecules with a shared pyrimidine diamine core (PDA1 & PDA2) that have potential as allosteric Hck inhibitors. Surface plasmon resonance (SPR) and NMR spectroscopy indicate that these compounds recognize a shared binding site involving the PPII-helix binding surface of the SH3 domain. In vitro kinase activity assays and hydrogen-deuterium exchange mass spectrometry (HDX-MS) reveal that despite the shared binding site, the compounds have opposite effects on overall kinase activity and dynamics. PDA1 stabilized overall Hck dynamics a nd did not affect kinase activity, while PDA2 disrupted the closed conformation of the kinase and stimulated kinase activity. In silico dock-ing and molecular dynamics simulations suggest that PDA1, but not PDA2, stabilizes the closed, inac-tive, conformation of Hck by bridging the SH3 and kinase domains. To test PDA1's efficacy in cells, we transformed the human TF-1 myeloid leukemia cell line with a chimeric protein that fused Hck to the coiled-coil (cc) domain of Bcr. The kinase-active cc-Hck fusion protein rendered the cells cytokine-independent and sensitized them to Hck ATP-site inhibitors. Treatment of TF-1/cc-Hck cells with PDA1 resulted in growth suppression which was significantly reduced with the introduction of PDA1-resistant mutants, providing evidence for on-target activity. Ongoing studies are exploring the structural basis of pyrimidine diamine interaction and the potential effects of combination therapy with PDA1 on acquired resistance mutations.

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Poster No. 6
Cancer Virology Program

Origin Melting by Tumor Polyomavirus Helicases is Independent of ATP-Dependent Helicase Activity

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Eukaryotic DNA replication is initiated by the minichromosome maintenance protein complex (MCM) helicase when it binds and melts the origin DNA, using ATP hydrolysis, during S phase. Merkel cell virus (MCV), a small DNA tumor virus related to the SV40 rhesus macaque polyomavirus, encodes a large tumor (LT) oncoprotein helicase that similarly binds to the viral origin (Ori) to melt and initiate viral genome replication. Both MCV and SV40 LTs form double-hexameric complexes on DNA at canonical G(A/G)GGC pentanucleotide sequences (pentads) at their respective viral Ori.

We visualized dynamic, real-time assembly of fluorescent MCV LT protein on single-molecule DNA replication origins using an optical tweezer-fluorescence microscope (C-Trap). We developed a modified Hidden Markov Model simulation (HMMs) to quantitate MCV LT binding on dsDNA, and showed that LT assembles as a highly-stable dodecamer on MCV origin DNA, as predicted from structural studies. By measuring single DNA strand binding by RAD51, RPA or by S1 nuclease we also visualized single molecule dsDNA origin melting by MCV LT protein. Surprisingly, both MCV and SV40 LT dsDNA origin melting occurred in the presence of the non-hydrolyzable ATP analog, adenylyl-imidodiphosphate (AMP-PNP), showing that this process is ATP energy independent. Origin DNA melting occurred for MCV LT proteins having successive helicase domain truncations or mutations to its Walker A box domain. These observations indicate that, unlike cellular MCM helicase, origin melting by MCV LT does not require helicase activity. Furthermore, our data demonstrates that LT invades and melts the viral Ori DNA as soon as it begins assembling and does not require full dodecamer multimerization. These data suggest that DNA tumor polyomaviruses use a novel mechanism to initiate viral DNA replication that is not licensed by the cell cycle, and confers an advantage to viruses in out-competing host cellular replication.

Poster No. 7

Cancer Immunology and Immunotherapy Program

Overcoming microenvironmental resistance to oncolytic virus immunotherapy with virus-encoded delivery of a potent TGFβ inhibitor

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Oncolytic viruses can induce immune infiltrate by generating a tumor specific immune response. This is especially important for patients with immune desert or excluded tumors or no tumor specific T cells. However, the mechanisms of resistance to oncolytic virotherapy are not well understood. To study this, we developed a derivative of the head and neck cancer line MEER, which is resistant to αPD1 therapy but sensitive to oncolytic vaccinia (VV) termed MEERvvS, and a VV-resistant line (MEERvvR). Using these, we determined that after VV treatment, there was increased T cell infiltration in both tumors however only MEERvvS had increased T cell function. We next compared the suppressive Treg cells between the tumors and found the Tregs in the sensitive tumors had lower Nrp1 and higher pSTAT1 signaling, suggesting that they may be primed for fragility. Indeed, Tregs in VV treated MEERvvS produced significant levels of IFNγ. We identified high intratumoral TGFβ as a driver of resistance to VV treatment in MEERvvR and found that TGFβ itself increased Nrp1 on the Treg cell surface and maintained Treg suppression in the presence of IFNγ. We then generated a VV that expressed a TGFβ inhibitor (VVTGFβi) which outcompetes TGFβ1-3 for receptor binding. When MEERvvR were treated with VVTGFβi there was a significant increase in tumor clearances and survival compared to VVctrl. We found that Treg cells in MEERvvR treated with VVTGFβi had lower Nrp1, higher pSTAT1, and lower surface LAP-TGFβ1. Our data suggest that a TGFβ rich TME is a main driver of VV resistance through the stabilization of Treg cells. Targeting TGFβ in combination with VV can overcome this resistance. Importantly, virally-encoded TGFβ inhibition carries no autoimmune or cardiac toxicity associated with systemic routes, suggesting this approach is effective and safe.

Poster No. 8
Cancer Biology Program

aKG-mediated carnitine synthesis promotes histone acetylation

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Wildtype isocitrate dehydrogenase 1 (wtIDH1) has been shown to be important for the proliferation of multiple cancer types at least in part through its production of alpha ketoglutarate (αKG) and subsequent histone demethylation. Interestingly, we found that wildtype IDH1 is upregulated specifically in cyclin E1-driven ovarian cancer cells and patient samples. Inhibition of wtIDH1 only in CCNE1-driven cells synergized with DNA damaging agents olaparib and cisplatin both in vitro and in vivo, which was rescued by αKG but not citrate, suggesting that IDH1-mediated production of αKG is required for resistance to DNA damaging agents in these cells. αKG is a co-substrate for various enzymes, termed αKG-dependent dioxygenases, that have pleotropic cellular roles. To identify the specific αKG-dependent dioxygenase that is required for the observed resistance to DNA damaging agents, we designed a CRISPR KO library targeting the 64 αKG-dependent dioxygenases and carried out a CRISPR KO screen in cells treated with olaparib. To our surprise, this screen identified TMLHE, the first enzyme in carnitine biosynthesis pathway, which dropped out only in CCNE1-driven cells treated with olaparib. Indeed, knockdown of TMLHE was highly synergistic with DNA damaging agents specifically in the context of CCNE1 overexpression, which was rescued by carnitine but not αKG, indicating that TMLHE lies downstream of the IDH1-αKG pathway in these cells. Consistent with recent literature demonstrating that carnitine promotes histone acetylation, we observed an overall increase in histone acetylation driven by IDH1, whereas histone methylation was less robustly affected. Using an unbiased mass spec approach, we identified 3 specific histone acetyl marks that are regulated in an αKG and carnitine-dependent manner. Interestingly, we observed that inhibition of IDH1 or knockdown of TMLHE in CCNE1 high cells increased DNA damage markers, which was rescued by carnitine, suggesting that IDH1 promotes DSB repair via TMLHE-dependent carnitine synthesis. Since carnitine is known to promote histone acetylation and histone acetylation enhances chromatin accessibility for DNA repair, ongoing studies are aimed at assessing the role of the IDH1-mediated carnitine synthesis pathway in promoting DSB repair in CCNE1-high cells. Together, these studies identified a novel histone acetylation pathway that is promoted by wildtype IDH1-mediated αKG production and suggest histone demethylation is dispensable for the observed resistance to DNA damaging agents.

Poster No. 9
Genome Stability Program

Radiation-induced pulmonary fibrosis in non-human primates is preceded by senescence and upregulation of tyrosine kinase Fgr.

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Radiation-induced pulmonary fibrosis (RIPF) is a severe side effect of radiotherapy, affecting up to 30% of lung cancer patients. There is increasing evidence that ionizing irradiation-induced senescence is associated with pulmonary fibrosis. We have recently reported that biomarkers of senescence and tyrosine kinase Fgr are induced in mouse RIPF, human idiopathic pulmonary fibrosis (IPF), and human RIPF. We also reported that Fgr inhibitor treatment significantly reduces fibrosis of the irradiated mouse lungs. Here, we investigated the association of senescence and tyrosine kinase Fgr to lung fibrosis in non-human primates (NHPs) and examined whether lung fibrosis can be predicted by analyzing the BAL cells and fluid after irradiation. We found that markers of senescence (p16, p21) and expression of Fgr are induced in the thoracic irradiated fibrotic NHP lungs and could be detected in the BAL cells of thoracic irradiated NHPs prior to the appearance of pulmonary fibrosis. In vitro, senescence and expression of Fgr were induced in irradiated normal human primary airway epithelial cells. In a trans-well system, senescent human airway epithelial cells induced fibrotic biomarkers Collagen1, Collagen 3, and Alpha-smooth-muscle actin in target human primary lung fibroblasts. Thoracic irradiated NHPs exhibited striking variation in lung fibrosis as measured by tomography and trichrome staining. In NHP bronchoalveolar lavage fluid (BALF) collected from thoracic irradiated NHP lungs, senescence-associated secretory proteins (SASP) were significantly induced, compared to the BALF collected from control NHPs. Moreover, the induction of Fgr and biomarkers of senescence were significantly higher in the severely fibrotic lungs compared to mildly fibrotic lungs. After radiation, the upregulation of proinflammatory SASP cytokines occurred in a temporal fashion that correlated with the extent of lung fibrosis. In summary, our results show senescence, induction of Fgr, and SASP cytokines, precede NHP RIPF. By analyzing these proteins, lung fibrosis and its severity can be predicted prior to the fully formed disease opening a window for therapeutic intervention.

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Poster No. 10
Cancer Biology Program

Genome-wide CRISPRa screening to functionalize lncRNAs in CDK4/6 inhibitor response

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Cyclin-dependent kinase (CDK) inhibitors (CDKi) has showed promising clinical significance treating ER+/HER2- breast cancer patients. However, there is a lack of clinically actionable biomarkers to predict patients' outcome of CDKi treatment overall. Moreover, the underlying mechanism of a highly heterogenous CDKi response among patients remains unclear. Recently, long noncoding RNAs (lncRNAs) have emerged as outstanding regulators and biomarkers for cancer therapeutic outcome. In this study, we performed a genome-wide gain-of-function CRISPR activation (CRISPRa) global screening targeting 9744 lncRNAs that potentially regulate cell proliferation and Palbociclib response in ER+/HER2- breast cancer. In our screening, we identified 144 and 152 lncRNAs that lead to Palbociclib sensitivity and resistance (fdr < 0.05), respectively. Meanwhile, we also screened for lncRNAs that play important roles in cell proliferation and observed a strong negative association between cancer cell proliferation regulation and Palbociclib responses. Further integration analysis with the pharmacogenomics data from 41 breast cancer cell lines suggested 34 lncRNAs as potential regulators for Palbociclib response ($p < 0.05$). The identified lncRNAs from our screening and pharmacogenomics analyses were further validated by a customized CRISPRa screening which includes 35 cell cycle-related protein coding genes (PCG) as controls. Experimentally validation of the customized CRISPRa screening demonstrated that activation of lncRNA TENM3-AS1, LINC01522 and LINC01117 and PCGs (e.g., CCND1 and CCND2) lead to the Palbociclib sensitivity. Mechanistically, RNA-seq analysis revealed that lncRNAs TENM3-AS1 and LINC01117 sensitizing Palbociclib response through promoting cell cycle progress while LINC01522 inducing Palbociclib sensitivity is cell cycle independent. These data also suggested that TENM3-AS1 and LINC01117 may regulate cell cycle proteins and lead to proliferation induction. In summary, our genome-wide gain-of-function CRISPR screening study demonstrated the important relationship between genes and cell cycle progression and further CDKi response. More importantly, our study identified novel lncRNA and PCGs biomarkers for CDKi treatment in cancer patients.

Poster No. 11
Cancer Biology Program

S100A11 Mediates Recruitment of Tumor-Associated Macrophages to Epithelial Cancer Cells in the Microenvironment of ER+ Breast Cancer: Insights from Single-Cell Analysis

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The tumor microenvironment (TME) plays a crucial role in tumor progression and consists of diverse cell types that can either promote (e.g., immunosuppressive macrophages) or inhibit tumor growth (e.g., cytotoxic T cells). Among the abundant cell types in the TME, tumor-associated macrophages (TAMs) are particularly influential in tumor growth, metastasis, and response to immune-based therapies, making them potential sources of diagnostic and prognostic biomarkers. Despite this potential, TAM-based biomarkers that can predict tumor aggressiveness and the complex interactions between TAMs, cancer cells, and other immune cells in the breast TME have not been fully explored. In this study, we performed an integrative scRNA-seq analysis of ER-positive breast tumors (n=25) from two independent public datasets to characterize TAM infiltration and identify gene signatures associated with tumor characteristics. We discovered that TAMs were enriched in patients with a high proportion of epithelial cancer cells (ECCs) but were depleted in those with high proportions of CD4+/CD8+ T-cells and B-cells, highlighting a distinct transcriptomic landscape. Further analyses allowed us to identify associations between TAMs and specific intracellular signaling transcripts or factors derived from cancer cells. In particular, we observed a positive correlation between TAM infiltration in ECCs and the expression of S100A11, a gene involved in cytoskeleton rearrangement, cell migration/invasion, and tumor progression. To validate these findings, we conducted experiments using ER-positive BRCA cell lines and organoid models in a 3D in vitro platform, demonstrating that S100A11 promotes monocyte/macrophage recruitment. This study provides valuable insights into the cell-type specific relationship between TAMs and S100A11 expression in human breast tumors. Additionally, it sheds light on the intricate interactions between TAMs and cancer cells or other immune cells, contributing to a better understanding of the TME and its potential implications for breast cancer treatment strategies.

Poster No. 12
Cancer Virology Program

CASTOR1 plays tumor suppressive function in a Kras-driven mouse model of non-small cell lung cancer through activating mTORC1 pathway

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Cytosolic arginine sensor for mTORC1 subunit 1 (CASTOR1) is a newly identified mTORC1 suppressor. Binding of arginine dissociates CASTOR1 from the GATOR2 complex resulting in mTORC1 activation. We have recently discovered that CASTOR1 is phosphorylated by AKT at S14, which targets it for ubiquitination and degradation by the E3 ligase RNF167. Thus, CASTOR1 behaves as a central mediator of mTORC1 integrating signals from both nutrients and growth factors. Importantly, we have shown that low expression of CASTOR1 predicts poor patient survival in a wide spectrum of cancer and CASTOR1 inhibits the progression of breast cancer in a xenograft model. To further evaluate CASTOR1's tumor suppressive function, we have generated a CASTOR1 knockout mouse and crossed it with a Kras (LSL-KrasG12D/+) mouse model of non-small cell lung cancer (NSCLC) to establish a novel mouse line with concurrent Kras and homozygous ablation of CASTOR1 (KC). While the number of visible tumor nodules was unchanged, loss of CASTOR1 significantly increased the sizes of lung tumor nodules along with increased proliferative index. Immunohistochemistry and immunoblot analysis revealed elevated the levels of p-4EBP and p-S6 in tumors without CASTOR1, indicating activation of the mTORC1 pathway induced by CASTOR1 ablation could be the driving force to promote tumor development. Furthermore, IHC staining of F4/80 revealed higher population of macrophage in KC lung, suggesting that microenvironment might be modulated to favor tumor development in the context of CASTOR1 depletion. In line with our speculation, p-CASTOR1(S14) level was elevated in tumor nodule comparing to adjacent normal lung, whereas the total CASTOR1 expression level was decreased, demonstrating that CASTOR1 is targeted for degradation to promote tumor growth. Taken together, our results demonstrated CASTOR1 could play a suppressive role in Kras-dependent NSCLC.

Poster No. 13
Cancer Virology Program

KSHV HIJACKS FAM50A TO ALTER RNA SPLICING AND INDUCE CELLULAR TRANSFORMATION

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Kaposi's sarcoma-associated herpesvirus (KSHV) is an oncogenic virus etiologically associated with Kaposi's sarcoma (KS) and primary effusion lymphoma (PEL). KS remains as the most common cancer in AIDS patients even in the era of highly active anti-retroviral therapy. To delineate the mechanism of KSHV-induced oncogenesis, we have developed a KSHV-induced cellular transformation model of primary cells. KSHV efficiently infects and transforms primary rat mesenchymal stem cells (MM). KSHV-transformed MM (KMM) cells can efficiently induce KS-like tumors in nude mice. In a CRISPR-Cas9 screening of MM and KMM cells, we have identified oncogenic drivers of KSHV-transformed cells including a group of splicing factors. Among them, family with sequence similarity 50 member A (FAM50A) is a splicing factor. We found that FAM50A knockout significantly inhibited the proliferation and cellular transformation of KMM cells but had less effect on MM cells. Mechanistically, FAM50A knockout differentially altered the splicing of a specific set of genes in KMM cells. Specifically, we found that SHP2 splicing was altered in KMM cells compared to MM cells, thus significantly upregulating a transcript isoform with a lower enzymatic activity, leading to a higher level of STAT3 Y705 phosphorylation. FAM50A knockout restored SHP2 splicing pattern to that similar to MM cells and reduced the level of STAT3 Y705 phosphorylation. We found that KSHV viral latent gene LANA interacted with FAM50A and upregulated FAM50A expression level to facilitate KMM cell proliferation and transformation. Both LANA and FAM50A showed direct interaction with histone variant H2AZ, indicating that LANA might recruit the splicing regulatory protein to the histones. These results indicate that KSHV hijacks FAM50A to induce cellular transformation by altering global transcript splicing. FAM50A-mediated SHP2 splicing activates STAT3, which is essential for KSHV-induced cellular transformation.

Poster No. 14
Genome Stability Program

Single-molecule visualization of single-strand break detection within nucleosomes by PARP1

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Poly[ADP-ribose] polymerase 1 (PARP1) acts as a damage sensor for multiple types of DNA damage, including DNA strand breaks and abasic sites. Inhibitors of PARP1 have already demonstrated success in the clinic, particularly for BRCA mutated cancers. Despite this clinical success, it is unclear how PARP1 detects DNA damage embedded in chromatin, in which DNA is packaged into nucleosome core particles (NCPs), compared to how PARP1 detects damage in duplex DNA. To better understand how PARP1 senses DNA damage, we utilized a new single-molecule approach to visualize YFP-tagged PARP1 from nuclear extracts binding single-strand breaks in real time on DNA substrates tethered to beads immobilized in optical traps. For nicks present in naked DNA, we observed high-affinity interactions, with a lifetime of 5.5 s and an on rate of 1.5×10^8 $M^{-1}s^{-1}$, yielding a K_D of 0.9 nM. In contrast, catalytically-dead PARP1 exhibited a 25-fold increase in lifetime and K_D of 0.4 nM. With a nick embedded in a fully-wrapped NCP at three different positions, the PARP1 binding affinity was lower compared to nicks on naked DNA, averaging 4.3 nM. However, all three interactions were of higher affinity than that of an undamaged NCP alone, which was 12.8 nM. We then increased DNA tension to unwrap one arm of the DNA around the NCP; this unwrapping increased the affinity of PARP1 for nicked and undamaged NCPs: the undamaged NCP affinity increased 4-fold to 3.0 nM. Thus, we find PARP1 exhibits high-affinity interactions for DNA damage in naked DNA and in chromatin, and that both the position of damage and nucleosome structure play key roles in DNA damage recognition.

Poster No. 15
Cancer Biology Program

Mesothelial cell motility and contractility promote ovarian cancer metastatic potential through a PKA-dependent mechanism

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Ovarian cancer is the leading cause of death among gynecological cancers, and metastasis to the peritoneum occurs in over 60% of patients. To form these metastatic implants, disseminated cancer cells attach and invade through the mesothelial barrier that lines the peritoneal organs in the abdominal cavity. The goal of our studies is to dissect how mesothelial cell contractility and barrier integrity impact the metastatic potential of high-grade serous ovarian cancer. We developed an imaging-based live cell assay that assesses the rate at which the ovarian cancer spheroids transmigrate across mesothelial monolayers (we termed this process “clearance”) and preconditioned the mesothelial monolayer with different pharmacologic agents. We discovered that treatment with forskolin, a cAMP activator, significantly reduced the number of clearing spheroids compared to the control untreated condition. Inhibition of protein kinase A (PKA) in mesothelial cells that were treated with forskolin reversed the anti-metastatic effects and restored clearance rates to baseline levels. Next, we analyzed mesothelial cell-cell junctions, cytoskeletal organization, traction forces and cell motility patterns. Compared to the control condition, forskolin treated mesothelial cells exhibited higher expression levels of ZO-1 and β-catenin junctions, as well as reduction in actin stress fibers. Consistent with these results, we found that forskolin reduced traction forces and mesothelial cell migration. To evaluate the impact of increasing mesothelial contractility, we treated mesothelial cells with calyculin A, a potent phosphatase inhibitor that elevated expression of phospho-myosin to promote traction forces and increase spheroid clearance rates. Our findings suggest that normalizing mesothelial cell contractility via a PKA-dependent mechanism enhances barrier integrity and limits the invasive potential of ovarian cancer spheroids. A better understanding of cancer-mesothelial paracrine signaling and environmental stressors in the peritoneal cavity that lead to mesothelial barrier dysfunction hold promise for the discovery of mesothelial-targeted therapies to reduce ovarian cancer metastatic dissemination.

Poster No. 16
Cancer Immunology and Immunotherapy Program

The functional role of sarcoma patient isolated CD91 SNPs

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Tumor immunosurveillance is critically dependent on many immune cell types and proteins, including CD91. CD91 is expressed on the surface of antigen presenting cells (APCs) and binds heat shock proteins (HSPs) released by dying tumor cells. These HSPs carry tumor antigens and are critical for initiating anti-tumor immune responses in de novo tumors. We have previously shown among multiple sarcoma types, osteosarcomas are poorly infiltrated by T cells and poorly immune edited. This correlates with the expression of CD91 on the surface of intratumoral APCs. CD91 is a highly polymorphic gene within the human population. Over 3500 single nucleotide polymorphisms (SNPs) have been identified, despite the relatively high conservation of CD91 throughout evolution. There are 155 SNPs within our sarcoma patient population, 11 of which are exonic and non-synonymous. We analyzed the immunologic data we obtained from patients with non-synonymous SNPs to patients with normal CD91 protein sequences. In addition, we used the computational modeling programs SIFT and PolyPhen-2 to predict the effects of these mutations on CD91 protein folding. SNPs rs746886209 and rs758644665 were indicated by both SIFT and PolyPhen-2 to have deleterious effects on CD91 folding and patients with these mutations had below average immune infiltration. While these data are correlative, there are many confounding factors within the patient population and the numbers of patients with these SNPs are minimal. Thus, we've developed an in vitro model to better determine the specific effects of these and other SNPs within our population. We are generating mutant THP1 cells containing single CD91 SNPs to determine how these mutations alter CD91 expression and function.

Poster No. 17
Cancer Biology Program

Metabolic Regulation of Quiescence Through Altered Acetyl-CoA Production

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Relapse occurs in most women diagnosed with tubo-ovarian high grade serous carcinoma (HGSC) and dramatically worsens their prognosis. A subpopulation of HGSOC cells are quiescent (transiently nonproliferative) and therefore not killed by existing chemotherapy regimens. Clinically, HGSC patients with higher expression of the cell cycle regulator *CDKN1C* have worse outcomes. Improving our very limited understanding of these cells could provide novel therapeutic targets to improve patient outcomes. Quiescent cells have been proposed to be metabolically inactive. However, recent data from our lab and others indicates that quiescent cells have an active metabolism that is distinct from proliferating cells. Whether and how these changes drive quiescence is underexplored. We found that HGSC cells upregulate ACSS2, which converts acetate to acetyl-CoA. Accordingly, we observed increased acetyl-CoA abundance in quiescent cells, with tracing studies confirming acetate as the predominant source. Both ACSS2 overexpression and acetate supplementation decreased proliferation without impacting viability, and acetate treatment increased the percentage of cells in G_0. These data suggest that this metabolic switch drives quiescence. Mechanistically, acetate treatment increased expression of the early-G_1 checkpoint inhibitor p57 (encoded by *CDKN1C*). Prior work has shown that acetate-derived acetyl-CoA influences histone acetylation and gene expression, and ongoing studies aim to interrogate this interplay in quiescent cells. Finally, given that quiescence is linked to chemoresistance, we aimed to determine whether this axis can be targeted as a therapeutic strategy. Indeed, pharmacological inhibition of ACSS2 in combination with standard-of-care cisplatin increased death of HGSC cells. Together our data suggest that nonproliferative stimuli increase the ACSS2-mediated conversion of acetate to acetyl-CoA and that this drives quiescence and chemoresistance. Future work will more fully characterize this metabolic/epigenetic cell-fate regulation and obtain the necessary preclinical data to advance this novel therapeutic approach.

Poster No. 18
Cancer Therapeutics Program

Mechanism of Inhibition of the AML-associated Src-family kinase Fgr by ATP-site inhibitors

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Development of acute myeloid leukemia (AML) is often associated with overexpression of non-receptor tyrosine kinases, including three members of the Src family: Hck, Lyn, and Fgr. Our group is actively investigating small molecule Src-family kinase inhibitors with significant anti-AML efficacy. These inhibitors include the pyrrolopyrimidine A-419259 and the N-phenylbenzamide TL02-59; the latter compound potently inhibits Fgr and Lyn in vitro and reverses bone marrow engraftment of the human AML cell line MV4-11 in a mouse model of AML. We recently solved X-ray crystal structures of near-full-length Fgr with both inhibitors. A-419259 bound to the Fgr ATP-site with the regulatory SH3 and SH2 domains packed against the back of the kinase domain, resulting in a closed conformation observed in previous structures of Hck with this inhibitor. However, while TL02-59 also bound to the Fgr ATP-site, it induced allosteric displacement of the SH3 and SH2 domains from their regulatory positions, resulting in an open conformation. To explore the effect of allosteric domain displacement on the inhibitory mechanism of each inhibitor, Fgr mutants were generated with enhanced SH3 domain interaction with the SH2-kinase linker (high-affinity linker or 'HAL' mutants). Fluorescence polarization assays and X-ray crystallography confirmed enhanced intramolecular SH3:linker interaction, thus favoring the closed conformation. To test the effect of HAL substitutions on Fgr sensitivity to TL02-59 and A-419259, we created an active form of Fgr by fusing it to the coiled-coil (CC) domain of the breakpoint cluster region protein (Bcr). We observed that stabilizing the closed conformation enhanced inhibitor potency in these cells, suggesting that both TL02-59 and A-419259 prefer a single Fgr kinase domain conformation. Ongoing work is directed toward combining allosteric modulators that lock a single Fgr conformation, which are predicted to synergize with TL02-59 and A-419259 to suppress the evolution of resistance mutations.

Poster No. 19
Cancer Immunology and Immunotherapy Program

Myeloid cells correlate to differential responses to immune checkpoint inhibitor in responders versus non-responders of mouse HNSCC model

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While immune checkpoint inhibitors (ICIs) were approved for treating head and neck squamous cell carcinoma (HNSCCs), the response rate remains relatively low. Mechanisms underlying ICI unresponsiveness versus sensitivity are poorly understood. The role of myeloid cells in suppressing anti-tumor immunity is well-established. However, there are no effective agents targeting these cells in HNSCCs. Here, we employed a transplanted HNSCC mouse model, A223 harboring *Smad4* deletion and KrasG12D mutation, where tumor-bearing recipients diverged into responders (R) or non-responders (NR) upon anti-PD-L1 treatment. To distinguish the differences in the tumor microenvironment of R and NR tumors, we performed single-cell RNA-sequencing (scRNA-seq) using CD45+ tumor-infiltrating immune cells from R and NR. Our scRNA-seq data showed that R tumors were enriched with T and B cells, whereas NR tumors contained drastically expanded myeloid populations. By comparing differentially expressed genes between R and NR, we found that tumor-infiltrating myeloid cells from NR expressed a higher level of genes that may be involved in immunosuppression, such as LGALS1. Dual treatment of a LGALS1 inhibitor (OTX-008) and anti-PD-L1 significantly inhibited A223 tumor growth compared to single treatments. We will further test (1) if LGALS1 plays a role in promoting expansion of myeloid cells mediated by tumor cells; (2) if LGALS1 expression in peripheral blood myeloid cells correlates to ICI response; (3) validate other targets and test whether they can serve as novel target for combination therapy with ICI. Our study will help to identify predictive markers for ICI resistant HNSCCs and improve combinatorial immunotherapy.

Poster No. 20
Cancer Immunology and Immunotherapy Program

POSTPRANDIAL CHANGES TO SYSTEMIC METABOLISM IMPRINT DURABLE CHANGES ON T CELL IMMUNE RESPONSES

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While costimulatory and cytokine signals can shape an antigen-stimulated T cell immune response, it is now clear that nutrient availability and intrinsic metabolic pathways play key roles in T cell function and fate. Indeed, the effects of post-prandial metabolism on T cell function and fate are unstudied. Here we show that short periods of fasting and refeeding can have long-lasting effects on T cell immunity. Directly ex vivo, T cells from fed hosts have higher mitochondrial capacity, dense mitochiondrial cristae and volume compared to T cells from fasted hosts. Remarkably, these metabolic phenotypes persist after activation and 7 days of expansion in vitro. Further, when OT-I naïve T cells from congenically mismatched fasted and fed mice were co-transferred into VacciniaOVA-infected hosts, fasted T cells failed to fully engage an effector response and formed dramatically fewer memory T cells after viral clearance. Metabolomic profiling of serum revealed serum triglycerides as a likely culprit for postprandial metabolic reprogramming. Chylomicrons enriched from lymphatics of fed mice were sufficient to impart metabolic reprogramming on fasted T cells in a manner antagonized by the chylomicron protein apoCIII. T cells from LDL receptor (LDLR)-deficient mouse had the metabolic phenotype of fasted T cells and were insensitive to fed serum, confirming the role for triglyceride uptake in post-prandial T cell metabolic programming. Therapeutic T cells expanded in vitro from fasted mice failed to control tumor growth compared to those expanded from fed mice. Our data suggest that T cells are exquisitely sensitive to systemic metabolic changes in the postprandial period and inherit those metabolic programs for several generations after TCR signal. Further, our study highlights the need to consider diet content and timing as key factors in immunology, in immune cell analysis, vaccination strategies, and the generation of cellular therapies for disease including cancer.

Poster No. 21
Cancer Biology Program

Myocardin-related transcription factor activity in breast cancer cells promotes osteoclast differentiation and bone colonization in a paracrine signaling manner

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Bone is a frequent site for breast cancer metastasis. Conditioning of local tumor microenvironment through crosstalk between tumor cells and stromal cells in the metastatic niche is a major driving force for bone colonization of cancer cells. The objective of the present study was to determine the role of Myocardin-related transcription factor (MRTF) in bone colonization of breast cancer cells. We demonstrated either genetic depletion of MRTF isoforms or treatment with small molecules that interfere with MRTF function dramatically impairs bone colonization of breast cancer cells in experimental metastasis assays, thereby establishing the requirement for MRTF for bone colonization of breast cancer cells. We next studied the impact of loss of MRTF function in breast cancer cells on RANKL-induced osteoclast activation of primary bone-marrow derived mouse monocytes (BMDMs). In an indirect co-culture setting, tumor cell-derived conditioned medium enhances RANK-induced osteoclast differentiation in a paracrine manner. To gain additional insights into mechanistic understanding of MRTF's role in osteoclast differentiation process, we next performed RNA-sequencing analyses of 3D cultures of breast cancer cells under genetic and pharmacological perturbations of MRTF, and compared differentially expressed genes in these settings against a 21-gene signature specifically associated with bone metastasis of breast cancer cells. These studies identified CTGF (connective tissue growth factor), a cell-secreted factor known for promoting osteoclastic differentiation/activity and osteolytic lesions by tumor cells, to be positively regulated by MRTF in an SRF-dependent manner. Finally, we showed that exogenous CTGF supplementation partially rescues the defect in osteoclast differentiation imposed by MRTF deficiency suggesting that CTGF regulation is partly responsible for MRTF-dependent paracrine control of osteoclast differentiation. In conclusion, this study uncovers a novel MRTF-directed tumor-extrinsic mechanism of bone colonization of tumor cells and suggest that MRTF inhibition could be a novel strategy to suppress osteoclast activity and skeletal involvement in metastatic breast cancer.

Poster No. 22
Cancer Biology Program

Carcinoma-Associated Mesenchymal Stem/Stromal Cells Promote Ovarian Cancer Metastasis and Tumor Heterogeneity Through Direct Mitochondrial Transfer

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High-grade serous ovarian cancer (HGSC) is the most common and lethal form of ovarian cancer, accounting for over 70% of ovarian cancer cases. HGSC mortality is largely driven by early and diffuse transcoelomic spread from the primary site throughout the peritoneal cavity, resulting in roughly three-quarters of cases presenting with advanced stage disease at time of diagnosis. Following detachment from the primary tumor, disseminated ovarian cancer cells are spread by the peritoneal fluid and ascites where they must adapt to their new microenvironment to survive and successfully form metastases. Crucial to this spread is the formation of multicellular aggregates containing both cancer and non-malignant stromal cells, which enhance cancer cell survival and are associated with poor clinical outcome. While most studies on HGSC multicellular aggregates have focused on ovarian cancer cells alone, the interactions between ovarian cancer cells and other non-malignant cells have yet to be fully elucidated.

Recent work by our group demonstrates stromal-progenitor cells, termed carcinoma-associated mesenchymal stem/stromal cells (CA-MSCs), enhance HGSC metastasis through formation of heterocellular CA-MSC:cancer cell complexes. CA-MSCs enhance metastasis by preferentially donating their mitochondria to cancer cells with the least amount of endogenous mitochondria (‘mito poor’ cancer cells), increasing their ability to survive and driving tumor heterogeneity at the metastatic site in vivo. Mito poor cancer cells were shown to have decreased proliferative capacity, increased sensitivity to platinum-based chemotherapy and display decreased oxidative phosphorylation (OXPHOS) compared to ‘mito rich’ cancer cells, a vulnerable phenotype reversed by CA-MSC mitochondrial donation. Importantly, CA-MSC mitochondrial donation occurred in vivo and was associated with decreased survival in an orthotopic murine model. Using genomic barcoding to quantify clonal heterogeneity, we demonstrate CA-MSC mitochondrial donation significantly enhance tumor cell heterogeneity at metastatic sites in vivo. Collectively, we report CA-MSC mitochondrial transfer as a driver of HGSC progression, heterogeneity and metastasis.

Poster No. 23
Cancer Biology Program

A New Mechanistic Model of Profilin-Dependent Membrane Phosphoinositide Control

A New Mechanistic Model of Profilin-Dependent Membrane Phosphoinositide Control
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Downregulation of actin polymerization protein Profilin1 (Pfn1) increases breast cancer cell migration and invasion, contradicting its pro-migratory role in physiological contexts. Mechanistically, the pro-migratory phenotype of tumor cells induced by Pfn1-depletion is linked to increased plasma membrane accumulation of membrane phosphoinositide (PPI) PI(3,4)P2 (an understudied PPI generated downstream of activated PI3- kinase) at the leading edge resulting in recruitment of other pro-migratory protein complexes. The present study aimed to gain mechanistic insights into how Pfn1's action is coupled to cellular control of PI(3,4)P2. We utilized a highly comprehensive experimental approach combining lipid ELISA, immunostaining, high-resolution live cell imaging of PPI-specific reporters, and single molecule imaging to study the impact of Pfn1 perturbation on PI(3,4)P2 and its precursor PPIs. Biosynthesis of PI(3,4)P2 involves PI3K-mediated metabolic conversion of PI(4,5)P2 (PIP2) to PI(3,4,5)P3 (PIP3) followed by 5' lipid-phosphatase SHIP2-mediated dephosphorylation of PIP3. Our experimental findings collectively support a model that loss of Pfn1 increases the efficiency of PIP2's conversion to PIP3 and its subsequent dephosphorylation to PI(3,4)P2 but not as a secondary consequence of inhibiting alternate metabolic pathways of PIP2. In further support of PI(3,4)P2-related findings, we demonstrate that Pfn1 depletion enhances growth-factor induced membrane recruitment of SHIP2 and further discover a novel protein-protein interaction between Pfn1 and SHIP2. This experimental evidence leads us to propose a new model where the binding to Pfn1 competitively inhibits the plasma membrane recruitment of SHIP2 thereby diminishing the overall efficiency of metabolic conversion of PIP3 into PI(3,4)P2. This model provides a new mechanistic avenue of how cytoskeletal-regulatory protein modulation could potentially impact PI3K signaling in cells.

Poster No. 24
Cancer Virology Program

Membrane-bound Merkel cell polyomavirus middle T protein interacts with Src-family kinases to initiate PLCg1 and inflammatory cytokine signaling.

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Merkel cell polyomavirus (MCV or MCPyV) is an alphapolyomavirus that causes Merkel cell carcinoma (MCC) and encodes four tumor (T) antigen proteins: Large T (LT), small tumor (sT), 57kT, and a middle T (MT) or alternate LT open reading frame (ALTO) proteins. We show that MCV MT is a membrane protein that can be generated as multiple isoforms through internal methionine translational initiation. MCV MT self-oligomerizes in lipid-raft membranes and promiscuously binds to Src family kinases (SFK) through a Src homology (SH) 3 recognition motif as determined by surface plasmon resonance, co-immunoprecipitation and bimolecular fluorescence complementation assays. SFK recruitment leads to tyrosine phosphorylation of MT at an SH2 recognition motif that allows interaction with phospholipase C g1 (PLCg1). This leads to PLCg1 tyrosine phosphorylation that activates inflammatory signaling pathways including NF-kB. Mutations to either the SH2 or SH3 recognition sites in MCV MT abrogate activation of this complex signaling pathway and increase viral replication after MCV genome transfection into 293 cells. These findings reveal that MCV MT and murine polyomavirus (MuPyV) MT broadly act similarly on SFK signaling to the PLCg1 pathway but do so through different mechanisms.

Poster No. 25
Cancer Virology Program

Activation of glucocorticoid receptor signaling inhibits KSHV-induced inflammation and tumorigenesis

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Kaposi's sarcoma (KS), caused by Kaposi's sarcoma-associated herpesvirus (KSHV) infection, is the most common cancer in HIV-infected patients. KSHV-induced inflammation is a hallmark of KS. Interleukin-1 (IL-1) family represents a major mediator for inflammation and plays an essential role in both innate and adaptive immunity. Using a KS model of KSHV-induced cellular transformation, we screened for inhibitors that specifically inhibited KSHV-induced cell proliferation. Over half of the selected inhibitors had anti-inflammatory properties. One of these inhibitors is dexamethasone, a glucocorticoid receptor (GR) ligand and a commonly used anti-inflammatory corticosteroid. Treatment with dexamethasone inhibited cell proliferation, and colony formation in soft agar of KSHV-transformed KMM cells but had a minimal effect on the matched primary MM cells. In the xenograft mouse tumor model, dexamethasone suppressed the initiation and progression of KS tumors. Dexamethasone induced cell cycle arrest but did not increase apoptotic cells of KSHV-transformed cells. RNAseq analyses revealed that interleukin-1 alpha (IL1A) was upregulated by 5-fold while interleukin-1 receptor antagonist (IL1RA) was downregulated by 26-fold in KSHV-transformed cells compared to primary cells. Importantly, treatment with dexamethasone inhibited IL1A expression but increased IL1RA expression. Significantly, treatment with recombinant IL-1α protein or overexpression of IL1RA was sufficient to rescue or mimic the inhibitory effect of dexamethasone on KSHV-transformed cells, respectively. Together, these results reveal the important role of IL-1 signaling in KSHV-induced oncogenesis, which can be inhibited by dexamethasone-induced anti-inflammatory signaling through suppression of IL1A expression or induction of IL1RA expression. Dexamethasone and likely other anti-inflammatory drugs could be promising a therapeutic agent for KSHV-related cancers.

Poster No. 26
Genome Stability Program

An Unexpected Role for Centromeric Proteins in Telomere Maintenance in ALT Cancer Cells

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The Alternative Lengthening of Telomeres (ALT) pathway is a cancer-specific telomere extension mechanism observed in ~15% of cancer subtypes that occurs in a telomerase (TEL)-independent manner and is strongly correlated with the inactivation of ATRX-DAXX (**α**-thalassemia/mental retardation X-linked/ Death Domain Associated Protein) histone H3.3 chaperone complex. The absence of ATRX-DAXX disrupts H3.3 deposition at telomeres leading to heightened amounts of DNA double-strand breaks (DSBs) and replication stress. In response, ALT cancer cells hijack the homology-directed repair (HDR) factors to repair these DSBs, consequently elongating telomeres and achieving replicative immortality. We found that ALT cells engage the centromeric chromatin machinery including the centromere-specific histone chaperone HJURP (Holliday Junction Recognition Protein) to deposit the centromeric histone variant CENP-A (Centromere Protein A) at telomeres. Furthermore, we have found that HJURP-dependent CENP-A deposition at telomeres can be stimulated through the targeted generation of telomeric DSBs and is necessary for productive ALT-based telomere maintenance. Crucially, these atypical dynamics of HJURP and the telomeric deposition of CENP-A can be abolished when the default ATRX-DAXX-dependent histone H3.3 deposition pathway is restored. These data provide evidence of an epigenetic rewiring that occurs in ATRX-DAXX-deficient ALT cancer cells that promotes the interaction between centromeres and telomeres- two distinct chromosomal domains which normally do not interact

Poster No. 27
Cancer Biology Program

Opioid-mediated suppression of anti-tumor immunity via peripheral OPRM1 signaling in head and neck squamous cell carcinoma

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Head and Neck squamous cell carcinoma (HNSCC) causes severe pain. Opioids are the backbone of pain treatment but may be immunosuppressive in the presence of cancer. We recently demonstrated that opioids decrease the efficacy of anti-PD1 monoclonal antibody (mAb) treatment in recurrent/metastastic HNSCC patients; opioid usage was associated with significantly lower progression free survival, overall survival, and decreased CD8 T cells in the tumor microenvironment. We hypothesized that cancer immunosurveillance is impaired by the immunosuppressive actions of exogenous opioids via peripheral mu-opioid receptor (OPRM1) signaling, decreasing response to anti-PD1 mAb treatment. Mouse oral cancer cell line 1 (MOC1) tumor-bearing mice, a syngeneic oral cancer model, were treated with morphine (10mg/kg i.p., 2x daily for 4.5 days), followed by anti-PD1 mAb (250µg/mouse, 3x2 day interval), and the tumor-associated immune infiltrate was assessed using flow cytometry. Co-administration of methylnaltrexone (MNTX), a peripheral OPRM1 antagonist, was used to confirm specificity and site of action of morphine-induced effects. Finally, any direct effects of morphine on tumor cells were investigated in oral cancer cell lines using quantitative PCR and colorimetric proliferation assays. Morphine induced a significant reduction in CD4+FoxP3- T helper (75%) and CD8+ cytotoxic T (83%) cells, and significantly increased the exhausted CD8+aPD1+ phenotype (450%); immunosuppression was blocked with co-treatment of MNTX. Morphine administration also significantly decreased anti-PD1 treatment efficacy. Lastly, Oprm1 expression was not detected at the mRNA level in mouse oral cancer cell lines. In vitro, 10µM morphine for 48hrs did not induce cell proliferation (vehicle=100±8.3%, morphine=115.7±9.6%) or improve wound healing after 24hrs (vehicle=76.2±11.6%, morphine=78.5±11.4%). Taken together, our data suggest that morphine suppresses anti-tumor immunity via peripheral OPRM1 signaling in immune cells but not tumor cells. Further investigation is warranted to determine whether peripheral opioid blockade (i.e. MNTX) can slow tumor progression and improve response to cancer treatments while preserving centrally-mediated analgesia.

Poster No. 28
Cancer Biology Program

Pembrolizumab modulation of the Ewing tumor microenvironment: an analysis of SARC028 pre/post treatment Ewing sarcoma biopsy samples

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Objective: SARC-028 investigated the use of pembrolizumab in relapsed sarcoma, including 13 patients with Ewing sarcoma. Biopsies were obtained both pre- and post-pembrolizumab therapy. Given the paucity of data regarding the Ewing tumor microenvironment (TME), SARC-028 biopsy samples provide a unique opportunity to understand the impact of checkpoint inhibition on this rare tumor. We sought to determine the impact of pembrolizumab on immune cell infiltration, protein expression, and transcriptional signatures.

Methods: Tumor biopsy material from SARC-028 was obtained under an IRB-approved protocol. Tissue was available from all Ewing patients enrolled on SARC-028, with 7 available pre/post paired samples. A tissue microarray was constructed and analyzed utilizing Multiplex immunofluorescence (VECTRA Polaris), GeoMx DSP protein assay (Nanostring), and the CosMx Human Universal Cell Characterization RNA Panel (Nanostring). VECTRA data analyzed using INFORM software (Akoya), GeoMx protein expression data was normalized by library size across region and PCA was conducted to visualize differences between pre-/post- samples. RNA data were analyzed, mapped and compared pre-/post-samples. Patient disease response data was overlayed on cell, protein and transcriptional data.

Results: We successfully conducted multiplex immunofluorescence, protein, and transcriptional analyses on these rare specimens from patients with relapsed Ewing sarcoma treated on SARC-028. We note that pembrolizumab treatment remodels the Ewing TME. Total immune cell density did not change in pre-/post samples; however, there was a shift in individual immune cell populations. GeoMx protein data revealed increased in immunostimulatory factors post PD-1 inhibitor as well as concomitant increase in immunosuppressive markers such as FOXP3 and LAG3. Additional analyses including incorporation of tumor response data are ongoing.

Conclusion: On-treatment biopsy specimens for Ewing tumors are exceedingly rare. SARC-028 included pre-/post-pembrolizumab biopsy specimens from patients with Ewing sarcoma. Here we have maximized utilization of these samples through a multi-omics approach in order to advance the understanding of the Ewing TME and opportunities for future immunologic priming of the Ewing TME.

Poster No. 29
Cancer Biology Program

'scDeepJointClust jointly identifies cell types using biological condition information and gene expression

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With the recent advent of single-cell level biological understanding, there is a growing interest in identifying the cell states or subtypes that represent the molecular behavior in terms of gene expression the specific biological conditions. These conditions may include distinguishing between disease samples and normal samples, characterizing developmental stages among multiple stages, or differentiating experimental intervention samples. Due to the lack of methods in this direction, current approaches undertake a two-step process. In the first step, cell clusters of homogenous molecular behavior are identified based on gene expression information. From these clusters, condition-specific cell subtypes that show enrichment in the biological condition of interest compared to other conditions are then selected. However, the separate training in this approach can lead to suboptimal solutions due to three limitations: 1) it does not consider the impact of one criterion on another, 2) it disregards the dimensional differences in the criteria, and 3) the optimizations rely on linear modeling. To address the limitations and accurately identify such condition-specific cell subtypes, we present scDeepJointClust, the first method that addresses the limitations by jointly training on both types of information using a deep neural network (DNN) approach. We evaluated scDeepJointClust on both simulation data and real biological data and showed that scDeepJointClust outperform existing methods employing the two-step approach in terms of sensitivity and specificity. By outperforming existing techniques, scDeepJointClust exhibits significant promise in advancing our understanding of cellular states and their implications in complex biological systems.

Poster No. 30
Cancer Biology Program

Mathematical modeling of fibroblast-mediated drug resistance in HER2+ breast cancer

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Drug resistance is a major challenge for patients with HER2 amplified (HER2+) breast cancer, which accounts for 20% of all breast cancer cases. Fibroblasts are a prominent cell type in the breast tumor microenvironment and are associated with drug resistance and disease progression. Recent studies have found tumor cells cocultured with fibroblasts have reduced sensitivity to the HER2-targeted therapy lapatinib via reactivation of the PI3K/Akt/mTOR signaling axis in tumor cells. The number of fibroblasts, or fibroblast density, in the tumor microenvironment can vary patient-to-patient; however, the role of fibroblast density on tumor growth and lapatinib resistance has yet to be quantitatively explored.

Four HER2+ breast cancer cell lines were cocultured with mammary fibroblasts and subsequently treated with lapatinib at varying tumor and fibroblast seeding densities. The number of live/dead tumor cells were measured every 4 hours for 96 hours using time-lapse microscopy. The effects of fibroblasts on tumor cell growth dynamics were analyzed. An ordinary differential equation (ODE) mathematical model was calibrated using this experimental data to identify equations that describe tumor cell growth, predict conditions in which tumor cells stop responding to treatment, and simulate alternative treatments to target these drug-insensitive tumor cells. Model selection was performed using Akaike Information Criterion (AIC).

Each cell line exhibited different sensitivity to the effects of lapatinib and fibroblasts. Greater fibroblast densities increased the lapatinib IC50 for BT474 and EFM192 compared to monoculture Fibroblasts reduce lapatinib sensitivity by decreasing the growth-inhibitory effects of low lapatinib doses and cytotoxicity at high doses. Models containing parameters related to tumor-fibroblast bidirectional signaling fit experimental data best, suggesting tumor cells and fibroblasts may engage in bidirectional signaling (i.e., tumor cells re-educate fibroblasts). Using this best-fit model, we predicted targeting surviving tumor cells by treating for longer durations is ineffective, indicating alternative treatments may be necessary.

Poster No. 31
Cancer Immunology and Immunotherapy Program

Heritable effects of metabolic stress on CD8 T cell function

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CD8 T cells are critical for immune responses to cancer. In tumors, CD8 T cells are exposed to metabolically active environments where certain nutrients are scarce. The nutrient environment in tumors shapes T cell responses, but little is known about the long-term, heritable effects of tumor metabolic stress on CD8 T cells. To examine the heritable effects of metabolic stress, CD8 T cells were cultured in vitro in media deficient in different individual metabolites or Tumor Interstitial Fluid-like Media (TIFM), a cell culture medium that mimics the nutrient environment of tumor interstitial fluid of pancreatic ductal adenocarcinoma (PDAC). Naïve CD8 T cells were activated for 24 hours in control media, then transferred to metabolically stressful media for 48 hours. After metabolic stress, cells were returned to nutrient replete media for four days. On day seven, cells were restimulated with αCD3/αCD28. Cells treated with TIFM or arginine-deficient media had fewer TNFα+IFNγ+ cells, and cytokine-expressing cells produced less TNFα and IFNγ, respectively. IL-2 expression was also lower in T cells exposed to arginine-deficient media or TIFM. In these experiments, cells were removed from metabolic stress four days prior to restimulation. In that time, stressed cells expanded more than 10-fold after removal from stress. Thus, these cells are distant descendants of the cells exposed to metabolic stress. To further test the long-term effects of metabolic stress in vivo, stressed cells were adoptively transferred into Vaccinia-OVA infected mice and examined 11 days post infection. Stressed cells had fewer IFNγ+TNFα+ cells upon restimulation despite proliferating as well as control cells. Collectively, these data provide evidence that CD8 T cell responses are influenced by past metabolic stress, and these changes are heritable. Future work will identify mechanisms through which CD8 T cells “remember” past metabolic stress and the functional effects on metastatic disease.

Poster No. 32
Cancer Immunology and Immunotherapy Program

Sickeningly sweet: Glucose avidity in Treg cell fate and function

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Regulatory T cells (T_{reg}) are a specialized subset of CD4 T cells characterized by the expression of the transcription factor Foxp3. T_{reg} cells play a critical role in maintaining self-tolerance and immune homeostasis. T_{reg} cells are a heterogeneous population, and metabolic conditions strongly influence their differentiation and function. Our previous data have revealed the glucose avidity of T_{reg} cells was quite heterogeneous across different tissues, suggesting that glucose avidity may be associated with distinct T_{reg} subsets in different tissues. Using a novel glucose tracer called GlucoseCy5, we identified T_{reg} cells with differential glucose avidity, referring to them as Glucose^{lo} and Glucose^{h}i T_{reg} cells. Our findings indicate that Glucose^{hi} T_{reg} cells exhibited weaker suppressive capacity and were more prone to losing T_{reg} cell stability and functionality both in vitro and in vivo. Additionally, Glucose^{lo} and Glucose^{hi} T_{reg} cells remained stable phenotypes unaffected by extracellular glucose levels. To investigate whether altered glucose avidity can impact T_{reg} cell function, we used the Slc2a1$^{flox/flox}$ Foxp3$^{GFP\text{-}Cre\text{-}ERT2}$ model to knock out Glut1 expression in T_{reg} cells after tamoxifen treatment. Glut1^{ko} T_{reg} cells showed increased suppressive capacity, and there was a negative correlation between glucose avidity levels and T_{reg} cell function. Loss of Glut1 in T_{reg} cells accelerated tumor growth, suggesting Glut1^{ko} T_{reg} cells enhanced immunosuppression during tumorigenesis. Our current results demonstrate that increased glucose avidity is associated with reduced T_{reg} cell function. In the future, we hope to gain a better understanding of how glucose avidity influences T_{reg} cell biology and how Foxp3 is involved in mediating the effects of glucose avidity on T_{reg} cell function.

Poster No. 33
Biobehavioral Cancer Control Program

Oral squamous cell carcinoma induces nerve injury and adrenergic receptor plasticity resulting in sympathetically-maintained pain

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Head and neck squamous cell carcinoma (HNSCC) causes severe pain and stress, which exceeds other cancers. The effect of sympathetic neurotransmission on symptom burden remains unclear. We hypothesize oral tumor growth drives sensory and sympathetic nerve injury, resulting in sympathetically-maintained pain comprised of nociceptive adrenergic sensitivity and increased sympathetic tone.

We used a translational approach to test this hypothesis. A prospective analysis of 35 HNSCC patients was used to correlate pretreatment circulating norepinephrine (NE) as measured by ELISA with pain as measured on a visual analog scale. Using a syngeneic orthotopic transplant mouse model, tongue tumor-innervating nerves were identified using retrograde tracers and histology. Nerve injury, denoted by ATF3 expression and sympathetic sprouting, was assessed in trigeminal (TG) and superior cervical ganglia (SCG) using immunohistochemistry. Quantitative PCR and calcium imaging evaluated neuronal adrenergic receptor (ADR) gene expression and functionality, respectively.

Compared to healthy controls, we found a 2 and 4-fold increase in platelet NE concentration from HNSCC patients with early- and late-stage tumors, respectively. There was a significant positive correlation between circulating NE concentration and patient-reported spontaneous ($r=0.634$, $p=0.0001$) but not functional pain ($r=0.380$, $p=0.067$). In cancer mice, we found a 4-fold increase in both tongue-innervating ATF3+ SCG and TG neurons compared to sham. There was also a ncrease in trigeminal sympathetic innervation paired with a 2-fold decrease SCG Adra2a expression, an autoreceptor for NE release. We found a 20% increase in alpha1 ADR expression in tongue-innervating TG neurons from cancer mice compared to sham, and NE evoked a larger calcium transient in more tongue afferents from cancer mice (91%) compared to sham (9%); the evoked calcium transient was 350% larger in neurons from cancer mice; a pan-alpha1 ADR antagonist blocked this effect.
These results suggest tumor-induced nerve injury may underlie oral cancer pain and sensory and sympathetic ADR plasticity.

Poster No. 34
Cancer Immunology and Immunotherapy Program

Unveiling the Critical Role of Oxidative Pentose Phosphate Pathway in T Cell Proliferation and Function

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Effective cellular metabolism plays a pivotal role in sustaining mammalian cell functionality and proliferation. This study delves into the significance of the oxidative pentose phosphate pathway (oxPPP) in primary mouse T cells. Through targeted Crispr knockout, five pivotal enzymes within the oxPPP—hexokinase 1 (Hk1), hexokinase 2 (Hk2), glucose-6-phosphate dehydrogenase (G6pd), 6-phosphogluconolactonase (Pgls), and phosphor-gluconate dehydrogenase (Pgd)—are investigated. Intriguingly, the knockout of Hk1 and Hk2 surprisingly leaves T cell proliferation and function unscathed. In stark contrast, the knockout of G6pd, Pgls, and Pgd manifests a hindrance in T cell proliferation and cytotoxicity post-activation. Moreover, the deficiency of these three key enzymes correlates with heightened cytosolic reactive oxygen species (ROS) production, as evidenced by H2-DCFDA staining, along with increased mitochondrial ROS production, as indicated by Mito-SOX staining. Furthermore, our exploration of the LCMV chronic viral infections model and the murine B16 model reveals attenuated expression of oxPPP enzyme genes in exhausted T cells.

In summary, our findings underscore the indispensable role of the oxPPP in upholding optimal T cell function. We propose that harnessing the potential of the oxPPP could serve as a viable strategy to augment T cell functionality and counteract the onset of T cell exhaustion.

Poster No. 35
Cancer Immunology and Immunotherapy Program

The activity of tertiary lymphoid structures in high grade serous ovarian cancer is governed by site, stroma, and cellular interactions

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High grade serous ovarian cancer (HGSOC) patients need novel immunotherapeutic approaches as immunotherapies exhibit low response rates of only ~10%. Tertiary lymphoid structures (TLS) are recognized predictors of immunotherapy response and promote adaptive immune functions. TLS are present in about ~15% of HGSOC patients. These structures are inducible, and their development is regulated by multiple features of the TME. Stromal cells play a key role in the formation and maturation (architectural and germinal center development) of secondary lymphoid organs and TLS. However, stromal progenitor cells i.e. mesenchymal stem cells (MSCs) become "cancer-educated" in the ovarian TME, contributing to tumorigenesis. We hypothesized that cancer-reprogrammed MSCs (CA-MSCs) inhibit TLS formation and maturation in the ovarian TME, and that differences in stromal populations regulate development of TLS in ovary, fallopian tube and omentum.

Using a HGSOC cohort, we quantified the presence and maturity of TLS across anatomical sites (STIC, fallopian tube, ovary, omentum) using multispectral imaging, noting a striking loss of TLS number and maturity in the ovary compared with fallopian tube and omentum. We applied digital spatial profiling (DSP) to produce a prognostic, TLS-derived "TLS-DSP" signature in HGSOC. We identified transcriptional changes inherent to B cells and other immune populations when they reside in TLS versus outside, and demonstrated that ovarian TLS are less immunologically active compared with omental TLS. To identify stromal-associated pathways that influence TLS maturity, we used DSP to detect gene expression changes between TLS-adjacent vs. TLS-distal stromal regions. Using our CA-MSC signature, we determined that these populations are enriched in TLS-distal regions. Ex vivo, CA-MSCs were less efficient in interacting with B cells and may have reduced capacity to function as lymphoid stromal cells. Finally, we demonstrated that enrichment of a CA-MSC signature negates the prognostic benefits of TLS-predictive signatures in a large HGSOC dataset.

Poster No. 36
Cancer Immunology and Immunotherapy Program

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

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Tertiary lymphoid structures (TLS) are ectopic lymphoid structures that form in tissues with chronic infection, autoimmune disease, and cancer. The presence of TLS has been associated with improved patient prognosis in cancer, and with distinct gut microbiome taxa and pathways in cancer patients. Our group has shown that *Helicobacter hepaticus* (*Hhep*) colonization in mice supports TLS formation and maturation through lymphangiogenesis and immune cell recruitment; however, the initial signals that drive this process remain unknown. The *Hhep* colonization mouse model provides a novel system to elucidate the connections between lymphatic biology, the microbiome, and TLS. We hypothesize that colonization with *Hhep*, an immunogenic bacterium, alters local antigen presenting cells to support lymphangiogenesis and TLS development. We have shown that *Hhep* colonization induces a shift in macrophage fate and function, increases genes associated with lymphangiogenesis, such as *Lyve1*, *Pdpn*, and *Vegfc*, and an increase in lymphatic vessels surrounding mature TLS. Strikingly, single-cell RNAseq and immunofluorescent imaging indicate the presence of Lyve-1+ macrophage populations in *Hhep* colonized mice, suggesting a direct role for macrophage-supported lymphangiogenesis. Ultimately, we seek to understand the mechanisms promoting lymphangiogenesis and the formation and maturation of TLS in health and disease to improve the efficacy of immunotherapy.

Poster No. 37
Cancer Immunology and Immunotherapy Program

Obese, not starving: mitochondrial citrate export drives lipid accumulation and dysfunction in exhausted CD8 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, they are quickly rendered dysfunctional by the combination of chronic antigen stimulation and metabolic stress, resulting in an altered differentiation state termed exhaustion. T cell exhaustion remains a significant hurdle for immunotherapeutic success. We have shown exhaustion is driven by mitochondrial stress. 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 T cells accumulate large lipid stores as they become exhausted. It remains unclear whether lipid accumulation contributes to T cell dysfunction or represents an untapped source of fuel that may be the key to their reinvigoration. Citrate links mitochondrial metabolism and de novo fatty acid synthesis. Inhibition of mitochondrial citrate export via genetic deletion or pharmacologic inhibition of the citrate carrier results in reduced lipid accumulation and improved cytokine production in vitro. Additionally, genetic deletion of the citrate carrier improves cytokine production in adoptively transferred, tumor-specific T cells in tumor-bearing hosts. These results suggest a role for mitochondrial citrate export in the accumulation of cytosolic lipids and progression of T cell exhaustion. We propose that as T cells experience mitochondrial stress, they shuttle mitochondrial citrate to the cytosol, where it fuels lipid synthesis and accumulation. These data provide new insight into the metabolic mechanisms of T cell exhaustion and may inform future immunotherapeutic development.

Poster No. 38
Cancer Virology Program

Characterizing donor susceptibility to Epstein-Barr Virus infection in the nasopharynx using organotypic rafts

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Epstein-Barr virus (EBV) is a ubiquitous gammaherpesvirus that chronically infects humans. Although most humans are exposed to EBV by adulthood, some individuals are at elevated risk of developing an EBV-associated cancer. Approximately 95% of adults are seropositive for EBV but in some parts of the world (e.g. Southeast Asia) EBV-associated nasopharyngeal carcinoma (NPC) is endemic. Such NPC tumors harbor a latent and clonal EBV infection. IgA antibodies to several EBV lytic proteins spike several years prior to NPC diagnosis. Thus, heightened lytic infection at the nasopharyngeal mucosa is considered a risk factor. Conventional 2-D cell culture does not represent the diversity of cell types that exist in the nasopharynx. Furthermore, 2-D cell culture does not recapitulate the differentiation-dependent lytic infection program observed in stratified epithelium. Organotypic rafts derived from primary oral keratinocytes are known to be susceptible to EBV infection, yielding a lytic infection. Here, we have established 3-D organotypic rafts using conditionally reprogrammed cells (CRCs) from the nasopharynx to model EBV de novo infection in the nasopharyngeal epithelium. Such cultures are generated from primary nasopharyngeal cells collected from consenting adults undergoing skull-base surgery with no known nasopharyngeal co-pathology. Currently, we have established a nasopharyngeal CRC cryobank from 26 donors. Our hypotheses are that nasopharyngeal organotypic rafts can support EBV de novo infection, that across multiple donors cryopreserved CRCs can be thawed and differentiated, and that nasopharyngeal organotypic rafts can be used to identify host genes encoding EBV restriction factors. We have developed an immunostain molecular diagnostic panel to identify latent- or lytically-infected cells. 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. We are investigating variation in susceptibility to de novo EBV infection among different cell types and among donors.

Poster No. 39

Cancer Immunology and Immunotherapy Program

CD91 and its ligand heat shock proteins affect dendritic cell functions during immunosurveillance of emerging tumors

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During cancer immunosurveillance, dendritic cells (DCs) play a central role in orchestrating T cell responses against emerging tumors. Capture of miniscule amounts of antigen along with tumor-derived "danger" signals can drive maturation and activation of DCs for crosspriming. We have observed that these signals are transmitted through the cell surface receptor CD91. Tumor-derived heat shock proteins (HSP) in the extracellular environment can be endocytosed by CD91 on dendritic cells (DCs). Further, engagement of CD91 initiates signals for co-stimulation and cytokine production to prime anti-tumor responses. However, diverse DC populations can express CD91 and have the potential to prime T cell responses. Here we track, in vivo, the dispersal of tumor-derived HSPs within the myeloid compartment. We demonstrate that migratory DC subpopulations, particularly conventional type 1 (cDC1) dendritic cells acquire gp96 from the tumor microenvironment, while a different DC subset (CD11b+ CD11c+) confers protective antitumor immunity, following transfer of tumor-derived HSPs between these populations. We show that the CD91-dependent function of type 1 conventional DCs (cDC1) is necessary for their anti-tumor functions during cancer immunosurveillance. We further support our data using transcriptomic profiling of DC subsets which reveals expression of molecules tied to their respective function such as antigen capture, migration and/or T cell priming. These data support that antigen presenting cells vary in their antigen cross-presentation capacities, cytokine production and T cell priming capabilities. In particular, CD91 expression can affect the functional specialization of different DC subsets and determine immunological consequences.

Poster No. 40

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 cancer immunosurveillance is dependent on CD91, a receptor for heat shock proteins (HSPs), which is expressed on antigen-presenting cells (APCs). HSP-chaperoned tumor antigens are cross-presented following endocytosis by CD91. Binding of HSPs to CD91 also initiates an intracellular signaling pathway that is poorly understood. Two tyrosine residues on the intracellular domain (β-chain) of CD91 become phosphorylated following HSP binding, leading to downstream activation of NF-kB and STAT1. We found that the immunogenic HSPs differentially utilize these two phosphorylation sites on CD91 for downstream signaling resulting in unique cytokine profiles and co-stimulation capacity. We investigate here the CD91-interacting adaptor proteins and kinases by crosslinking and co-immunoprecipitation of CD91 and its signaling complex following stimulation with each immunogenic HSP (gp96, calreticulin or hsp70). Mass spectrometry studies with gp96 identified the adaptor protein Shc and two other receptor tyrosine kinase associated with CD91. Inhibition of these kinases leads to a significant decrease in gp96-mediated cytokine production. Further work is being done to elucidate the remainder of this signaling network for each HSP and examine any differences between APC types. This project will aid in better understanding of how CD91-mediated signaling differentially activates effector cytokine profiles in APCs. This knowledge can be used in the development of new therapeutic approaches to enhance immunosurveillance of cancers and to better eradicate established disease.

Poster No. 41
Cancer Virology Program

Inhibition of HBV transcription by interferon stimulated gene 12 (ISG12)

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IFNα is the only FDA approved immunomodulatory drug for treatment of chronic hepatitis B, and it possesses intracellular antiviral activity via induction of IFN-stimulated genes (ISGs). In order to identify the major antiviral ISGs against HBV replication, we preformed transcriptome analysis of HepG2-NTCP cells upon IFNα treatment and found that IFI27 was at the top list of differentially expressed genes. IFI27 is a small transmembrane protein of 12kD belonging to the FAM14 protein family. The high inducibility of IFI27 by IFNα was further validated in both HepG2 cells and PHH. Over-expression of IFI27 exhibited significant antiviral effect in HBV-transfected/infected cell cultures by reducing HBV total RNA, core DNA, and antigen levels. Furthermore, knocking down IFI27 markedly abrogated the antiviral effect of IFNα in HBV-infected HepG2-NTCP cells. Interestingly, IFI27 inhibited HBV transcription and replication derived from the replicon plasmid pHBV1.3 but not pCMVHBV, indicating an HBV promoter-specific transcriptional inhibition, which was further confirmed by a viral promoter reporter assay. Immunofluorescence microscopy revealed that IFI2z is predominantly localized in the cytoplasm, indicating that IFI27 indirectly inhibits HBV transcription in the nucleus. However, RNA-seq analysis demonstrated that IFI27 does not alter the expression of known HBV-related transcription factors at the RNA level. In line with this, our preliminary results showed that IFNα treatment or IFI27 overexpression promotes the degradation of the transcription factor C/EBPα, which is required for an optimal activity of the HBV core promoter. Furthermore, our results showed that the function of IFI27 in downregulating the protein expression of CEBPα occurs through ubiquitin-proteasome degradation, facilitated by the E3 ubiquitin ligase SKP2. As a result, the binding of CEBPα to the HBV promoter is impeded, leading to the inhibition of cccDNA transcription. Taken together, our study suggests that IFI27 mediates the antiviral activity of IFNα against HBV replication at the transcriptional level by downregulating cellular transcription factors exploited by HBV.

Poster No. 42
Genome Stability Program

Investigating how chemotherapeutic thiopurines inhibit telomerase elongation of telomeres

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Telomerase is an attractive target for cancer therapies because it is expressed in over 85% of cancer cells, while most adult somatic cells lack telomerase. Thiopurines are a class of nucleoside analogs used to treat leukemia and some pediatric cancers but are highly toxic. The prodrug 6-thio-2'-deoxyguanosine (6-thio-dG) was developed to reduce non-specific thiopurine toxicity. This drug can successfully reduce the growth of mouse tumor xenografts for multiple cancer types and is currently in phase 2 clinical trials for small-cell lung carcinoma. 6-thio-dG treatment also causes telomere shortening and dysfunction, however the mechanism was unknown until recently. We conducted a series of direct telomerase extension assays in the presence of the therapeutic 6-thio-dG metabolite, 6-thio-dGTP, on various telomeric substrates. Telomerase can continue DNA synthesis after inserting 6-thio-dGTP, but insertion inhibits telomerase repeat addition processivity by disrupting the translocation step. Furthermore, telomerase processivity factor POT1-TPP1 fails to restore processive elongation in the presence of 6-thio-dGTP. In addition, 6-thio-dGTP has a low micromolar IC50 for human telomerase due to telomerase's inability to discriminate between dGTP and 6-thio-dGTP but does not inhibit DNA polymerase progression, reducing its off-target impact In contrast, when a 6-thio-dG is present in the telomere 3' overhang, telomerase can bind to and extend the telomere. We found that telomerase-positive cell lines with critically short telomeres were the most sensitive to 6-thio-dG treatment. They had greater telomere fragility and loss since they rely more on telomerase to maintain their critically short telomeres in the short term. These results reveal the mechanisms by which 6-thio-dG targets telomerase in cancer therapies.

Poster No. 43

Cancer Immunology and Immunotherapy Program

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. Here, we aimed to directly compare the efficacy of commonly used T cell expansion conditions. Peripheral blood mononuclear cells were used to generate anti-CD19 CAR-T cells in different media formulations (standard RPMI, hyperglycemic RPMI, X-VIVO 15) in gas-permeable rapid expansion (G-Rex)R bioreactor or traditional flasks and analyzed for metabolic/functional parameters. X-VIVO15-expanded T cells showed decreased mitochondrial mass and glucose uptake compared to cells expanded in RPMI, indicative of metabolic insufficiency and poor in vivo persistence. Interestingly, CAR T cells expanded in 5mM glucose were more polyfunctional when activated with NALM6 compared to cells expanded in hyperglycemic R10 (50mM glucose) media. CAR-T cells expanded in hyperglycemic media also showed increased expression of the pre-exhaustion marker CD101. Our results indicate that hypermetabolic culture conditions impact metabolic and functional potential of therapeutic cells, representing a window of opportunity that can be harnessed to better equip these cells for success by modifying or engineering cell culture systems to more adequately mimic physiologic metabolic conditions to eradicate cancer in patients.

Poster No. 44
Cancer Therapeutics Program

Necroptosis Inhibitor Functions as a Ferroptosis Inducer in Drug-Resistant Myeloid Malignanc

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Introduction: Acute myeloid leukemia (AML) is associated with poor prognosis, especially in elderly patients unfit for intensive chemotherapy. AML chemotherapy agents, including venetoclax are designed to induce cell death through apoptosis. Unfortunately, AML cells eventually develop a resistance causing clinical disease recurrence. Alternative cell death pathways like ferroptosis and necroptosis are lytic forms of cell death mediated by plasma membrane lipid oxidation or MLKL phosphorylation (p-MLKL), respectively. Here, we investigated the role of necroptosis and ferroptosis in apoptosis-resistant AML cells.

Methods: We established venetoclax-resistant myeloid cell lines by exposing cell lines to increasing doses of venetoclax. We evaluated resistance to venetoclax by measuring IC50. We screened for basal activation of necroptosis by pMLKL or phosphorylated RIP3 (pRIP3) by western blotting. Necrosome inhibitor (Zharp-99) was tested to estimate IC50 for cell death induction in myeloid cell lines after Annexin-V/PI staining. Ferroptosis inhibitors (ferrostatin-1 and lipoxstatin-1) and apoptosis inhibitor (zVAD-FMK) were used for cell death rescue assay.

Results: We established venetoclax-resistant myeloid cell lines (THP1-VR and HL60-VR), which showed a significant shift in IC50 for venetoclax-induced apoptosis compared to parent cell lines. Venetoclax-resistant cell lines upregulated pRIP3 signal by western blot. Necrosome screening of primary AML samples revealed 73 AMLs (94%) showed basal necroptosis signal confirmed by pMLKL (Figure A). Necrosome inhibitor, Zharp-99 significantly induced cell death in venetoclax-resistant cell lines compared to parent cell lines. Cell death induced by Zharp-99 was through ferroptosis, confirmed by the venetoclax-resistant myeloid cells being rescued by ferroptosis inhibitor (ferrostatin-1 and lipoxstatin-1) (Figure B).

Conclusion: Necroptosis inhibitor Zharp-99 promoted cell death in necrosome-activated venetoclax-resistant cell lines. Our data suggest necroptosis inhibition targeting RIP3K activity has a novel function of sensitizing myeloid malignancies to ferroptosis. Targeting RIP3K activity in apoptosis-resistant myeloid cell lines opens a new therapy opportunity to treat apoptosis resistance in AML patients.

Poster No. 45
Cancer Biology Program

IL-6 axis in malignant effusions

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Many cancers metastasize to the pleura, resulting in effusions that cause dyspnea and discomfort. Regardless of the tissue of origin, pleural malignancies are aggressive, with no treatment shown to prolong life. The pleura form a contained bioreactor-like space, concentrating cytokines secreted by the mesothelium, tumor, and infiltrating immune cells. We measured cytokine/chemokine content of 262 malignant pleural effusion (MPE) samples across multiple cancers using a 40-analyte panel. Twelve analytes (7 chemokines, 5 cytokines) were consistently present in concentrations ≥ 0.5 pM. All are capable of mediating chemotaxis, promotion of epithelial to mesenchymal transition, or immunosuppression, and many of are reportedly downstream of a pro-inflammatory cytokine cascade mediated by cytokine IL-6 and its soluble receptor. The data support the contention that the pleural environment is the major factor responsible for the clinical course of MPE across cancer types and argue in favor of a therapeutic approach targeting the IL-6/IL-6R axis.

Poster No. 46
Cancer Biology Program

Hijacking a neurodevelopmental epigenomic program in metastatic dissemination of medulloblastoma

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Normal brain development relies on precise genetic and epigenetic spatiotemporal regulation of gene expression. How dysregulation of neurodevelopment relates to medulloblastoma, the most common pediatric brain tumor, remains elusive. Here, we uncovered a novel neurodevelopmental epigenomic program that regulates Purkinje cell migration in developing cerebellum is hijacked to induce tumor metastatic dissemination in medulloblastoma. Integrating publicly available datasets with our in-house data, unsupervised analyses revealed that BAF60C/SMARCD3, a subunit of SWI/SNF chromatin remodeling complex, promotes tumor cell migration in vitro and metastasis in vivo. Based on analyzing the single-cell RNAseq data of cerebellum developmental trajectory in mice and humans, aligning with the medulloblastoma patients' datasets, we found that BAF60C/SMARCD3 regulated DAB1-mediated Reelin signaling is involved in Purkinje cell positioning during cerebellum development and medulloblastoma metastasis by orchestrating the cis-regulatory elements (CREs) at the DAB1 gene locus. Moreover, analysis of spatiotemporal gene expression and chromatin architecture in the human and mouse cerebellum demonstrated that transcription activity of the BAF60C/SMARCD3-DAB1 circuit is downregulated in a mature state of cerebellar development, however, is upregulated in metastatic medulloblastoma. We further identified that a core set of transcription factors, enhancer of zeste homolog 2 (EZH2) and nuclear factor I X (NFIX), bi-directionally control BAF60C/SMARCD3 transcriptional regulation by coordinating with the CREs at the BAF60C/SMARCD3 gene locus to form a chromatin hub during developing cerebellar development and medulloblastoma metastatic dissemination. Highly expressed BAF60C/SMARCD3 activates the Reelin/DAB1 signaling pathway downstream Src kinase, which was validated in the pair-wised primary and metastatic tumors from medulloblastoma patients. Preclinical medulloblastoma mouse models revealed that inhibiting Src activity reduces tumor cell migration and metastatic dissemination at a lower and safer dose. Together, these data deepen our understanding of how the developmental program influences disease progression and provide an opportunity for the development of therapeutics for this devastating brain cancer in children.

Poster No. 47
Cancer Virology Program

Viral and Cellular N6-Methyladenosine (m6A) Epitranscriptomes During KSHV Primary Infection and Essential Roles in Viral Infection and Replication

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N^6-methyladenosine (m^6A), the most prevalent modification on messenger RNA (mRNA), plays an important role in all stages mRNA biogenesis and functions including pre-mRNA splicing, pri-miRNA processing, mRNA export, mRNA stability, translation modulation and mRNA degradation. We have previously mapped the viral and cellular m^6A epitranscriptomes during Kaposi's sarcoma-associated herpesvirus (KSHV) latency and reactivation, and observed abundant m^6A modifications on KSHV transcripts as well as global reprogramming of cellular m^6A epitranscriptome. In this study, we examined the kinetics of viral and cellular m^6A epitranscriptomes during KSHV primary infection of primary human umbilical vein endothelial cells (HUVEC). We found dynamic m^6A modifications on viral transcripts that were correlated with the expression of KSHV transcripts and replication. In addition, KSHV induced dynamic reprogramming of cellular epitranscriptome during primary infection, regulating pathways that control cell survival and viral replication. Knockdown of m^6A reader YTHDF2 reduced KSHV replication whereas overexpression of YTHDF2 enhanced KSHV replication during KSHV primary infection. RNA immunoprecipitation (RIP)- qPCR revealed direct bindings of YTHDF2 to KSHV lytic transcripts ORF45, ORF57 and ORF59. Knockdown YTHDF2 resulted in elevated expression level of interferon-stimulated genes including IFIT1, IFIT2, and IFIT3 upon KSHV infection. IFIT1, IFIT2, and IFIT3 transcripts stability were increased upon YTHDF2 knockdown, indicating YTHDF2 mediates their degradation during KSHV primary infection, which facilitating KSHV infection and replication. These results reveal a pivotal role of m^6A modifications in KSHV primary infection and provide rich resources for the community.

Poster No. 48
Cancer Biology Program

Myosin II mediated collagen matrix remodeling by breast cancer cells promotes macrophage recruitment

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Triple-negative breast cancer (TNBC) is associated with the poorest outcomes among all breast cancer subtypes. TNBC tumors are characterized by extracellular matrix remodeling at the invasive front of tumor which has been linked to increased cancer cell motility and metastatic progression. Cancer cells with elevated levels of myosin II, a key regulator of the cellular cytoskeleton, have been associated with a higher abundance of tumor-associated macrophages, creating an immunosuppressive microenvironment. However, the detailed relationship between myosin II activation in TNBC cancer cells and macrophage recruitment in 3D extracellular matrix remains poorly understood. To address this gap, we employed an innovative 3D microfluidic model of tumor-macrophage co-culture, allowing us to study macrophage migratory decisions in the presence of cancer cells in real-time. Using a panel of TNBC cell lines, we found that high myosin II expression in cancer cells correlated with increased macrophage infiltration in 3D collagen matrices. Pharmacologic or genetic inhibition of myosin II reduced cancer cell-induced matrix remodeling and the resulting macrophage recruitment. Furthermore, our microfluidic devices enabled us to control the spatial distribution of cancer cells and macrophages in the 3D matrix and dissect the contribution of paracrine factors compared to matrix remodeling on macrophage recruitment. In sum, our studies revealed the critical role of cancer cell-driven matrix remodeling in promoting macrophage infiltration in TNBC and how cancer cytoskeletal regulators can therapeutically targeted to reverse the establishment of an immunosuppressive microenvironment.

Poster No. 49
Cancer Immunology and Immunotherapy Program

Changes in the Microbiome After Treatment: Are We Setting Colorectal Cancer Patients Back Before they Begin?

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Colorectal cancer (CRC) remains one of the deadliest cancers in the US, with a concerning increase in the incidence of early-onset cases. Patients with metastatic disease tend to have a poor prognosis. Cancer treatments targeting the immune system (immunotherapy) have resulted in long-term durable responses for many across tumor types. However, only 10% of colorectal tumors (classified as MSI-H) respond to anti-PD1 therapy. Previous work has shown that the microbiome composition plays a large role in immunotherapy success, but the underlying mechanisms remain unknown. Given that CRC patients undergo numerous treatments prior to immunotherapy, we hypothesized that previous treatment with chemotherapy shifts the microbiome and reduces anti-PD1 efficacy.

We used a subcutaneous MC38 CRC model to measure the impact of chemotherapy on anti-PD1 effectiveness. Tumors were measured to track growth and calculate clearance rates. Lymphocytes were isolated from tumors and lymph nodes of mice 14 days post tumor injection to assess the changes in tumor infiltrating T cells through flow cytometry. Fecal microbiome transplants (FMT) were performed to directly test the impact of microbiome changes in immunotherapy success.

Mice who received prior chemotherapy (5-flurouracil, 5-FU) had lower tumor clearance rates with anti-PD1 compared to mice who received anti-PD1 treatment alone. Tumor infiltrating CD8+ T cells were more exhausted, as evidenced by a loss of polyfunctionality. In addition, 5-FU treatment was sufficient to cause a durable shift in the gut microbiome. Most notably, lack of response could be completely recapitulated in 5-FU naïve mice through an FMT from a 5-FU donor mouse.

Here, we have demonstrated that prior traditional cancer treatments, such as chemotherapy, significantly alter the microbiome and decreases the efficacy of anti-PD1 in a CRC mouse model. Interestingly, we have shown that chemotherapy-induced shifts in the microbiome are both necessary and sufficient to drive this phenotype.

Poster No. 51
Cancer Biology Program

Regulation of SIRT3 overexpression in Anchorage Independent Ovarian Cancer

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Ovarian cancer remains the most lethal gynecological malignancy, and late-stage diagnosis and significant metastatic spread are prominent contributors to its lethality. To achieve optimal metastasis, ovarian tumor cells must modify their response to heightened oxidative stress to ensure their survival. One crucial mechanism of this modification involves the scavenging of mitochondrial superoxide anions by superoxide dismutase (SOD2). Mitochondrial Sirtuin Deacetylase 3 (SIRT3) deacetylates SOD2 to enhance its antioxidant capacity, facilitating the conversion of superoxide (O2-) to H2O2. We have shown that this antioxidant mechanism helps to preserve mitochondrial function of high grade serous ovarian cancer cell lines, enhances cellular survival in anchorage-independence, and is necessary for in vivo metastatic spread to the omentum. Expression of both SOD2 and SIRT3 increase in ovarian cancer cells following matrix detachment, and we found that SOD2 is regulated by both transcription and translation, resulting in rapid and sustained increases in this mitochondrial antioxidant in response to matrix detachment. Presently, we are exploring the mechanisms of SIRT3 regulation, as this deacetylase is necessary for the maintenance of SOD2 activity in anchorage independent conditions. We find that active transcription is required for the increased SIRT3 expression observed in anchorage independence and are currently investigating the role NF-kB plays as a critical transcription factor of SIRT3 in this context. Understanding the regulation of SIRT3 will shed light on ovarian cancer cell survival adaptations during metastasis, and provide potential insights into new targets for therapeutic interventions.

Poster No. 52
Genome Stability Program

Deregulated DNA ADP-Ribosylation impairs telomere replication

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The recognition that DNA can be ADP-ribosylated has major consequences for our understanding of how ADP-ribosylation (ADPr) contributes to genome stability, epigenetics, and immunity. Yet, whether DNA-ADP-ribosylation (DNA-ADPr) contributes to genome stability and how it is regulated currently remains unknown. Here, we show that telomeres are subject to DNA-ADPr which is catalyzed by PARP1 and removed by TARG1. Mechanistically, we show that DNA-ADPr is coupled to lagging telomere DNA strand synthesis, forming at single-stranded DNA present at unligated Okazaki fragments and on the 3' single-stranded telomere overhang. Ultimately, persistent DNA-ADPr due to TARG1 deficiency leads to telomere shortening. Furthermore, utilizing the bacterial DNA-ADP-ribosyltransferase (DarT) toxin to directly target DNA-ADPr at telomeres, we demonstrate that unhydrolyzed DNA-ADPr compromises telomere replication and telomere integrity. Thus, by identifying telomeres as chromosomal targets of DNA-ADPr whose deregulation compromises telomere replication and integrity, our study provides fundamental knowledge of the critical importance of controlling DNA-ADP ribosylation turnover for sustained genome stability.

Poster No. 53
Cancer Biology Program

Investigating alterations in metabolic profile of ovarian carcinoma associated mesenchymal stem cells.

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Ovarian cancer causes more deaths than any other gynecologic cancer. Although aerobic glycolysis has been a hallmark of cancer metabolism, it has been found that ovarian cancer (OvCa) cells are metabolically plastic, also utilizing oxidative phosphorylation (OXPHOS) as an active metabolic pathway for their rapid proliferation and metastasis. A growing body of evidence also shows that cancer cells not only reprogram their own metabolism but also metabolically remodel the tumor microenvironment to meet their ever-increasing energy demand. Our lab has previously demonstrated that OvCa cells epigenetically reprogram their resident tissue mesenchymal stromal/stem cells (MSCs) to develop a cancer-supportive phenotype. These cancer-associated mesenchymal stem cells (CA-MSCs) support OvCa progression through several mechanisms, including enhanced metastasis. Recent work by our lab showed these CA-MSCs enhance metastasis by donating their mitochondria to metabolically vulnerable OvCa cells and increasing OvCa cell OXPHOS. While we have demonstrated the impact of CA-MSC mitochondrial transfer to OvCa cells, what changes are happening in the CA-MSC that allow them to transfer mitochondria to OvCa cells is still unknown. Gene set enrichment analysis performed on RNA sequencing data comparing patient derived CA-MSCs to normal MSCs revealed that OXPHOS is one of the top enriched pathways in CA-MSCs. Furthermore, cell mito stress test comparing mitochondrial respiratory profile of patient derived normal MSCs and CA-MSCs demonstrated that CA-MSC have significantly increased mitochondrial respiratory capacity compared to normal MSCs. Uncovering the metabolic profile of ovarian CA-MSCs for the first time, our data demonstrates that ovarian cancer alter the metabolism of the CA-MSC towards OXPHOS. Our current studies are focused on understanding the role of enhanced CA-MSC OXPHOS in mitochondrial transfer to OvCa cells and OvCa metastasis.

Poster No. 54
Cancer Biology Program

CCNE1 oncogene reprograms nuclear nucleotide metabolism.

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Cancers adopt unique metabolic adaptations to enhance their survival and support excessive cell division. For instance, cancers have increased nucleotide metabolism corresponding to increased replication. Nucleotide metabolism canonically occurs in the cytoplasm, with diverse metabolic pathways contributing to de novo nucleotide biosynthesis, including the pentose phosphate pathway (PPP) and methionine cycle. Recent evidence suggests that cancer cells have differential metabolism compartmentalization. For example, compartmentalized NAD+ metabolism, which occurs in the nucleus, mitochondria, cytosol, and extracellular space, influences cancer progression, whereas cytoplasmic and mitochondrial compartmentalization of reductive glutamine metabolism is modulated during hypoxia. In response to growth stimuli, de novo nucleotide synthesis is regulated by cell signaling pathway. Whether oncogenic stress that increases DNA replication promotes nuclear translocation of nucleotide biosynthesis enzymes and how this promotes nuclear DNA synthesis is unknown. To address this lack of knowledge about the reprogramming of subcellular metabolism, we decided to focus on a key oncogenic driver, CCNE1, encoding cyclin E1, which drives S phase progression and DNA replication. Moreover, CCNE1 is associated with chemoresistance and poor prognosis in high-grade serous ovarian carcinoma (HGSOC). Our preliminary data reveals metabolic reprogramming in CCNE1 amplification is partly specific to the nuclear subcellular compartment. Both ribose 5-phosphate isomerase A (RPIA), an enzyme in the PPP and the methionine enzyme methylenetetrahydrofolate reductase (MTHFR) are increased in the nucleus of CCNE1-driven cells, suggesting translocation from the cytoplasm to the nucleus under oncogenic stress that promotes aberrant DNA replication. To determine how generalizable this localization phenomenon is and what the system level impact of such compartment changes, proteomics, and metabolomics are being performed on isolated nuclei compare to whole cell lysate samples. Understanding nuclear metabolism reprogramming and enzyme translocation will enable the development of targeted therapies targeting nuclear enzymes or preventing their translocation in cancer cells expressing oncogenes causing chemoresistance in HGSOC.

Poster No. 55
Cancer Epidemiology and Prevention Program

Withaferin A suppresses the differentiation of osteoclasts induced by breast cancer.

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The bone is the most vulnerable site for breast cancer cell metastasis across all subtypes of the disease. Such metastasis leads to serious complications like severe pain and pathological fractures, negatively impacting the quality of life for patients. Currently available treatments, such as surgery, radiation, and bone-targeted therapies (like bisphosphonates), are expensive and often come with severe side effects like renal toxicity and jaw osteonecrosis. Consequently, there is a pressing need for a safe, cost-effective, and efficient agent to prevent breast cancer metastasizing to the bones.

Our previous RNA sequencing analysis showed that many genes associated with bone remodeling and breast cancer bone metastasis were significantly downregulated when exposed to withaferin A (WA), a promising chemopreventive compound derived from the medicinal plant Withania somnifera. This study aimed to determine whether WA could inhibit breast cancer-induced osteoclast differentiation (osteoclastogenesis). At doses achievable in the bloodstream, WA treatment effectively suppressed the differentiation of osteoclasts induced by three different breast cancer cell subtypes (MCF-7, SK-BR-3, and MDA-MB-231). Both WA and the root extract of Withania somnifera demonstrated equal effectiveness in inhibiting breast cancer-induced osteoclastogenesis. This inhibition was associated with a reduction in key osteoclastogenic cytokines, including interleukin (IL)-6, IL-8, and receptor activator of nuclear factor-κB ligand.

Furthermore, WA treatment resulted in decreased expression of runt-related transcription factor 2, nuclear factor-κB, and SOX9 transcription factors, all of which play a positive role in osteoclastogenesis. These findings collectively suggest that WA holds promise as an agent for preventing breast cancer-induced bone metastasis.

Poster No. 56
Cancer Biology Program

Paracrine effects of cancer therapy-induced senescence on metabolism

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High grade serous ovarian cancer (HGSOC) is the most common and lethal histosubtype of ovarian cancer. Standard-of-care therapies for HGSOC include platinum-based therapies (such as cisplatin) and poly (ADP-ribose) polymerase inhibitors (PARPi), each of which cause therapy-induced senescence in both tumor and normal cells. While the cell-intrinsic effects of senescence are tumor suppressive due to the proliferative arrest, the production of a senescence-associated secretory phenotype (SASP), composed of a variety of secreted factors, has context-dependent roles through its paracrine effects. Previous studies have shown that SASP from therapy-induced senescent cells can regulate metabolism. However, the precise mechanisms of metabolic reprograming due to the induction of senescence during chemotherapy treatment and the phenotypes induced by this reprogramming remain largely unclear. We analyzed gene expression of HGSOC patient samples from The Cancer Genome Atlas (TCGA) and found a significant positive correlation between the well-known SASP factor interleukin-6 (IL6) and expression of nicotinamide N-methyltransferase (NNMT), the main scavenger of nicotinamide (NAM) from the NAD+ salvage cycle. NNMT overexpression was further confirmed in the kidney of mice treated with cisplatin that showed increased accumulation of senescence. Using conditioned media, we confirmed this was a paracrine effect of chemotherapy-induced senescent tumor cell SASP on both cancer and nontransformed cells. Preliminary metabolomics experiments also indicate a decrease in NAD+ as well as other salvage cycle intermediates in cells treated with SASP-enriched conditioned media. These data suggest cancer therapy-induced senescence may promote a systemic decrease in NAD+. Interestingly, NAD+ decline is a hallmark of aging, and chemotherapy is known to promote aging phenotypes. Ongoing studies are aimed at interrogating whether systemic SASP drives chemotherapy-induced aging via an NNMT-mediated NAD+ decline. These studies will contribute to the fundamental understanding of how chemotherapy induced senescence contributes alters systemic metabolism and how this can impact patient outcomes and health spans.

Poster No. 57
Cancer Biology Program

Loss of E-cadherin Accelerates Post-translational Degradation of HER2 – Understanding the Mechanism

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Loss of E-cadherin (CDH1) is the pathognomonic feature of invasive lobular carcinoma (ILC). E-cadherin was thought to be mainly involved in structural features of epithelial cells, however, its role in intracellular signaling is now well appreciated. Recently, we identified E-cadherin as a regulator of HER2 stability. We observed accelerated HER2 protein degradation in heregulin (HRG)-stimulated ILC cells compared to invasive carcinoma of no special type (NST) cells. CRISPR-mediated knockout of E-cadherin in NST cells also accelerated HRG-stimulated HER2 protein degradation. Enhanced HER2 receptor internalization and degradation may have therapeutic implications as efficacy of HER2 antibody-directed conjugates (ADCs) is partly reliant on rates of HER2-ADC complex internalization and degradation in lysosomes. Thus, we hypothesize ILC cells have increased sensitivity to HER2 ADCs from higher rates of HER2 degradation due to loss of E-cadherin.

Protein expression may be reduced by inhibition of de novo synthesis from mRNA or by accelerated protein degradation. To decipher the mechanism underlying accelerated HER2 protein degradation upon HRG-stimulation in E-cadherin deficient cells, we assessed the time-course of HER2 mRNA expression upon HRG-stimulation in MCF7 and T47D wild-type and E-cadherin KO cells by RT-qPCR. We found HRG treatment did not downregulate HER2 mRNA levels significantly in wild-type and E-cadherin KO cells.

Based on our data, we propose decreased HER2 levels observed in E-cadherin KO cells result from enhanced protein degradation. Proteasomal and lysosomal degradation are two key protein degradation pathways. Our immediate future goal is determining which pathway mediates accelerated HER2 protein degradation in ILC and E-cadherin KO cells. We will then compare response to HER2 ADCs between ILC and NST.

Our studies provide critical understanding to the mechanism of action and potential differences in efficacy of HER2 ADCs between ILC and NST.

Poster No. 58
Genome Stability Program

Mechanisms of CENP-A-induced genomic instability acquisition

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CENP-A, a centromere specific histone H3 variant, is essential for faithful chromosome segregation. CENP-A overexpression serves as a poor prognostic marker in several cancers. When overexpressed, CENP-A is ectopically loaded onto non-centromeric transcriptionally active sites. Ectopic CENP-A are removed during DNA replication, as an error correction mechanism, to restrict CENP-A to the centromeres only maintaining genome stability. Here we aim to determine in near-diploid non-transformed cells if the error correction mechanism of ectopic CENP-A deposition is disrupted upon persistent overexpression of CENP-A, resulting in maintenance and reinforcement of ectopic CENP-A sites instead of their removal. Our overarching hypothesis is to determine if persistently overexpressed CENP-A at clinically relevant levels in two non-transformed cell lines, hTERT-RPE-1 and HPDE-c7 is sufficient to induce genome and chromosomal instability, ploidy levels, and cellular transformation. To induce CENP-A overexpression at different levels, we transduced hTERT-RPE-1 and HPDE-c7 cells with lentivirus expressing Doxycycline (Dox)-induced CENP-ALAP. Our preliminary data demonstrate that up to 20-fold or 10-fold CENP-A overexpression over the course of 14 days in hTERT-RPE1 or HPDE-c7 cells, respectively, is sufficient to induce ectopic CENP-A deposition and micronuclei formation within 2 days, suggesting a driving role for CENP-A overexpression in mitotic aberration. In addition, CENP-A overexpression at certain level enhanced cellular transformation and migration without having any effect on cellular growth. Besides, ectopic CENP-A can elevate DNA-damage marker proteins along with induction of aneuploidy observed through single-cell whole genome sequencing (scKaryo-seq) data analysis. Finally, we plan to determine whether a link be made between CENP-A overexpression and chromothripsis, the catastrophic shattering of one or few chromosomes, that can fuel tumorigenesis. Identifying mechanisms that maintain and reinforce ectopic CENP-A may reveal novel pathways used by certain tumor types to become progressively genetically unstable through increased chromosome segregation errors often lead to micronuclei formation, aneuploidy and chromothripsis.

Poster No. 59
Genome Stability Program

The processing of telomeric 8-oxoguanine by OGG1 and MUTYH glycosylases promotes cellular senescence

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Telomeres cap and protect linear chromosome ends and their integrity is crucial to preserve genome stability and ensure sustained cell proliferation. DNA damage caused by oxidative stress from environmental exposures and endogenous sources accelerates telomere shortening and dysfunction, which can contribute to tumorigenesis and aging. Base excision repair (BER) enzymes process the highly prevalent oxidative lesion 8-oxoguanine (8-oxoG), which forms preferentially at telomeric repeat sequences due to their susceptibility to oxidative damage. 8-oxoG DNA glycosylase (OGG1) excises 8-oxoG opposite cytosine, while MutY-homolog (MUTYH) removes the adenine misincorporated opposite 8-oxoG. By exploiting a unique tool that selectively targets telomeres with 8-oxoG formation, we demonstrated that acute telomeric oxidative base damage is sufficient to trigger premature cellular senescence in non-diseased cells without telomere shortening. Here we investigated the role of 8-oxoG processing by BER enzymes at telomeres in cellular senescence induction. We found that upon acute telomeric 8-oxoG formation, OGG1 or MUTYH knock-out partially, and double knock-out completely rescued telomeric 8-oxoG induced senescence, cytoplasmic chromatin fragments (CCFs) and proinflammatory response production, and DNA damage response (DDR) activation. BER deficiency also prevented replication stress and related telomere fragility. Moreover, treatment with Poly(ADP-ribose) polymerase (PARP) inhibitor Olaparib, to retain PARP at sites of 8-oxoG damage, revealed that BER proficient cells experienced an exacerbated senescence induction compared to the cells lacking one of the enzymes, whereas the double knock out cells were completely unaffected. However, after chronic telomeric 8oxoG formation, MUTYH activity promoted senescence to prevent chromosomal instability from persistent damage over time. Our studies reveal that inefficient completion of 8oxoG BER at telomeres triggers cellular senescence via SSB intermediates which impair telomere replication and stability.

Poster No. 60

Cancer Immunology and Immunotherapy Program

TCF-1 is required by CD8+ T cells for the maintenance of alloimmune responses in Graft-vs-Host Disease

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Graft-vs-host disease (GVHD) is a common complication of allogeneic stem cell transplant (alloSCT) wherein donor T cells target alloantigens on recipient tissues. It is unclear how alloimmune responses are maintained in GVHD despite abundant antigen, which causes T cell anergy, deletion and exhaustion. Previously, we identified alloreactive TCF-1hi T cells arising post-transplant that resemble exhausted progenitors (TEXP) capable of propagating immune responses in other chronic antigen models. Here, we sought to further characterize these cells in the B6→129 MHC-matched GVHD mouse model, in which 129 recipients express the immunodominant minor histocompatibility antigen (miHA) H60. As early as day +7 post-transplant, alloreactive CD8+ cells specific to H60 (as determined by MHC-I-tetramer staining; TetH60+) were nearly uniformly PD-1hiToxhi and contained TCF-1hiCD39lo cells, which is a canonical TEXP phenotype. To test if these CD39loTCF-1hi TEXP had proliferative advantages in GVHD, we sorted congenic TCF-1hiCD39lo and TCF-1loCD39hi CD8+ cells from recipient spleens and transferred them in competition (1:1 ratio normalized to TetH60+) into newly transplanted recipients. Among TetH60+ cells in all tissues at day 14 post-transfer, TCF-1hiCD39lo-sorted progeny greatly outperformed TCF-1loCD39hi-sorted progeny. We next tested whether TCF-1 itself is an important mediator of T cell fitness by competing congenic wild-type (WT) and Tcf7p45-/- (p45-/-) donor CD8 cells, which lack the N-terminal β-catenin binding domain of TCF-1, in allogeneic (129) and syngeneic (B6) recipients. Strikingly, p45-/- CD8 cells were greatly outcompeted by WT CD8 cells in 129 recipients in all tissues and at all times post-transplant. In contrast, in B6 recipients, WT and p45-/- cells remained evenly matched, suggesting that full-length TCF-1 isoforms are dispensable for lymphopenia-induced T cell expansion. Further, p45-/- cells were not disadvantaged when acutely challenged with H60 antigen by vaccination. Together, these results suggest TCF-1+ TEXP cells fuel alloimmune responses in GVHD and may prove to be useful therapeutic targets.

Poster No. 61
Cancer Therapeutics Program

Fluorescence Complementation Screening Assay for Allosteric Modulators of the Acute Myeloid Leukemia-Associated Src-family kinase, Fgr

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Acute myeloid leukemia (AML) is an aggressive hematologic cancer with limited treatment op-tions. Overexpression of the myeloid Src-family tyrosine kinases (SFK) Hck and Fgr is often ob-served in AML patients and ATP-site inhibitors of these kinases have demonstrated efficacy in AML mouse models. However, ATP-site inhibitor efficacy is limited by the emergence of acquired resistance through kinase domain active site mutations. Allosteric inhibitors may provide a new path to selectivity, and when combined with existing ATP-site inhibitors, are likely to prevent ac-quired resistance. Work with the structurally related Bcr-Abl tyrosine kinase associated with CML supports this view, where the combination of allosteric (asciminib) and ATP-site (nilotinib) inhibitors suppressed emergence of drug resistant disease. SFKs possess an N-terminal unique domain followed by regulatory SH3 and SH2 domains that allosterically control activity of the ki-nase domain. Small molecule ligands that bind the regulatory apparatus may allosterically sup-press SFK activity and signaling for therapeutic impact. Here we describe a novel high-throughput screening (HTS) assay to find small molecules that bind to the unique-SH3-SH2-linker (U32L) region of Fgr. This complementation assay is based on the fluorescence activating and absorption shifting tag (FAST) protein which fluoresces when a fluorogen is bound. The complementing partners include the N-terminal FAST residues 1-114 (nFAST) fused to the N-terminus of the Fgr U32L region and the C-terminal FAST peptide residues 115-122 (cF8) fused to the N-terminus of an SH3-binding peptide, VSL12 (cF8-VSL12). Reconstitution of the FAST protein from the binding interactions of the nFAST-FgrU32L and cF8-VSL12 components yields fluorescence in the presence of fluorogen. In the HTS, allosteric ligand binding will prevent cF8-VSL12 peptide interactions, reduce fluorescence, and be flagged active. Small molecule hits dis-covered in this assay are anticipated to cooperate with existing ATP-site inhibitors to enhance anti-AML efficacy and suppress acquired resistance.

Poster No. 62
Cancer Immunology and Immunotherapy Program

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

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The suppressive functions of regulatory T cells (Tregs) on effector T cells are integral in promoting tumor growth, as loss of Tregs in mice results in robust tumor clearance. However, these mice will quickly succumb to lethal autoimmunity, highlighting the importance for investigation into intratumoral Tregs and how to target them therapeutically. We have previously shown that Tregs can become fragile in the tumor microenvironment after exposure to IFNγ, a phenomenon in which they lose their suppressive capacity while maintaining Foxp3 expression. However, the exact mechanism that leads to Treg fragility remains unclear, as well as the ability of other cytokines that also skew T cells to a Th1 lineage to drive fragility, such as IL-12. Therefore, we have used a novel transgenic mouse with a conditional deletion of IL12Rβ2 in the Treg compartment to test the effect of IL-12 on Treg fragility induction after immunotherapy.

From our experiments we are able to show that using an IL-12 inducing therapy (anti-CD40 agonistic antibody) increased IFNγ and IL-12 levels drastically within the tumor as well as systemically compared to anti-PD1 treatment. However, mice with the Treg-conditional knockout of IL12Rβ2 did not show any change in tumor growth when treated with anti-CD40 compared to control mice. Additionally, flow cytometric analyses of these conditional knockout mice show that the Tregs remain sensitive to fragility after anti-CD40 treatment. These data suggest that IL-12 is not necessary for Treg fragility induction. However, due to the large increase in IFNγ levels after anti-CD40 therapy, we believe that this IFNγ pool may be circumventing the IL-12R loss in our model. Work is currently being done to understand how these two cytokines work together to drive Treg fragility, but these data suggest that IL-12 functions, in part, by increasing IFNγ levels and indirectly inducing fragility in Tregs.

Poster No. 63
Cancer Biology Program

Expression of ovarian cancer specific Drp1 splice variants regulate mitochondrial heterogeneity and cell plasticity

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Mitochondrial shape is integral for its proper function and is maintained by an active balance between the events of fission and fusion. Hence, an imbalance in mitochondrial fission/fusion dynamics can be detrimental and has been associated with multiple pathologies including tumorigenesis. We discovered that heterogeneous mitochondrial dynamics in ovarian cancer cells were associated with specific transcript variant signatures of the fission protein Drp1 (encoded by the gene DNM1L), the primary GTPase responsible for mitochondrial fission. While several Drp1 splice variants have been reported, few studies have linked the expression and potential interplay of splice variants of Drp1 on mitochondrial dynamics and function with pathophysiology, especially in ovarian cancer. We used RACE (rapid amplification of cDNA ends) and RT-PCR to establish the identity of the major Drp1 splice variants expressed in ovarian cancer. Cells derived from patient ascites, as well as TCGA ovarian cancer specimens, predominantly express two Drp1 variants: a transcript including both exons 16 and 17 (16/17) and a transcript lacking exon 16 (-/17). TCGA analysis of these variants highlighted a significant difference in the overall survival of ovarian cancer patients. Samples with high Drp1(-/17) expression were associated with poorer overall survival compared to those predominantly expressing Drp1(16/17). Overexpression studies and endogenous splice variant-specific knock-down demonstrated that Drp1 variants have unique splice-specific cellular localization and impact on mitochondrial morphology and function. Drp1(-/17) was associated with microtubules and to a lesser degree with mitochondria unlike Drp1(16/17), which had typical mitochondrial fission site localization. Additionally, Drp1(-/17) expression enhanced mitochondrial respiration, improved mitochondrial cristae organization, increased cellular proliferation, and decreased sensitivity to chemotherapeutics. Furthermore, metabolic profiling highlighted variant-specific alterations in total metabolite pools. Hence, the expression of distinct Drp1 splice variants may be a novel mechanism to regulate mitochondrial fission, and integral to ovarian cancer cell plasticity under different selection pressures during tumor progression.

Poster No. 65

Cancer Immunology and Immunotherapy Program

PIK3IP1/TrIP Immune Regulation on CD8+ T Cells Restricts Anti-Tumor Immunity

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The cancer immunology field has long been focused on understanding/optimizing T cell receptor (TCR) signaling to improve anti-tumor responses. Directly downstream of the TCR, the phosphoinositide-3-kinases (PI3Ks) play a major role in directing T cell responses [1]. Our lab has previously described a surface protein, TrIP(Transmembrane Inhibitor of PI3K, gene name: Pik3ip1), is capable of regulating PI3K signaling upstream of other known PI3K regulators [3, 4]. Additionally, we have shown that TrIP protein expression is distinctly high on T cells and can negatively regulate T cell immune responses via its modulation of the PI3K pathway [4]. These data have led us to propose that TrIP regulation in T cells suppresses their inflammatory activity and that targeting TrIP in CD8+ T cells may improve anti-tumor response.

Our data show that CD8+ T cell-specific TrIP knockout mice (TrIPfl/flE8icre) are resistant to syngeneic tumors. In addition to increased tumor resistance, we have also found that tumors harvested from our TrIPfl/flE8icre knockout mice contain twice as many infiltrating T cells compared to WT. The increased T cell infiltration is driven by the CD8+ compartment, and importantly don't display increased exhaustion. In our peptide studies, we have shown that TrIP expression loss from the surface of T cells is directly proportional to strength of stimulation (dose and/or affinity), whereby strong stim accelerated TrIP loss. Here we describe data demonstrating that TrIP, a PI3K inhibitor with uniquely high expression on T cells, plays a significant role in the antitumor immune activity of CD8+ T cells.

Poster No. 66
Cancer Biology Program

MALT1 protease is activated by doxorubicin and mediates treatment resistance in triple-negative breast cancer

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Background/Hypothesis: Breast cancer is the most commonly diagnosed malignancy in American women. The triple-negative breast cancer (TNBC) subtype has among the worst prognosis due to high rates of recurrence and metastasis. Since TNBC lacks targetable receptor proteins, treatment relies upon non-specific chemotherapy, which can become ineffective upon onset of resistance. One potential driver of TNBC treatment resistance is MALT1 protease, the effector component of the CARMA-BCL10-MALT1 complex which activates pro-survival signaling. Notably, breast cancer cells demonstrate increased sensitivity to chemotherapies such as doxorubicin and cisplatin when MALT1 is depleted. Thus, we hypothesize that MALT1 is a pharmaceutically targetable driver of TNBC treatment resistance.
Methods/Results: We analyzed RNAseq and proteomic data from TCGA and CPTAC and found that MALT1 is highly expressed in basal breast cancer (a subtype largely composed of TNBC) and its expression level in this context correlates with reduced treatment response and survival. We next developed TNBC cell models to evaluate the effect of MALT1 protease inhibition on chemotherapy sensitivity. First, we identified cell lines that were most resistant to doxorubicin using GDSC and CTRP databases. Results indicated that MDA-MB-231, BT20 and HCC1143 cells are highly resistant. We compared MALT1 protein expression and doxorubicin IC50s for these TNBC lines and found that MALT1 expression correlates with the degree of doxorubicin resistance. To determine if MALT1 blockade, via either siRNA-knockdown or MALT1 protease inhibitor treatment, increases doxorubicin sensitivity, we performed CellTiter-Glo and Incucyte Caspase-3/7 assays. We found that MALT1 protease is activated by doxorubicin and that MALT1 inhibition results in decreased viability and increased apoptosis in doxorubicin-treated TNBC cells.

Conclusions/Future Studies: Our studies suggest that MALT1 inhibition may be a promising approach to improving chemotherapy response in TNBC. We are currently assessing the effect of combination treatment with doxorubicin and MALT1 protease inhibitor in a TNBC xenograft model.

Poster No. 67
Cancer Immunology and Immunotherapy Program

Pipeline for Optimizing miHA Specific Adoptive Cell Therapy for the Treatment of Leukemia

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Adoptive T cell therapies (ACT), wherein T-cell receptors against minor histocompatibility antigens (miHA) are introduced into non-specific T-cells are being clinically investigated for the treatment of leukemia. A key question is what properties make for “ideal” or “adequate” TCRs. We compared the in vitro and in vivo properties of a panel of TCRs with distinct levels of expression and affinity in an ACT model of leukemia treatment, targeting the murine miHA H60. Using the high throughput TcXpress process we cloned TCRs from 2944 H60 multimer binding CD8+ T-cells sorted from H60-vaccinated mice. We observed no clonal dominance and randomly chose 178 TCRs for further study. Using retroviral vectors, we expressed the TCRs in a TCR-CD8+ NFAT-reporter cell line. We confirmed the specificity of the TCRs, and characterized the TCR expression and affinity of a smaller subset of T cells. Next, we compared the ability of a high or low affinity H60 specific TCRs to control H60+ blast crisis leukemia. The infusion of T cells transduced with anti-H60 TCRs coincident with efficient CRISPR-knock out of the endogenous TCR alpha/beta chains reduced the H60+ leukemia burden relative to treatment with T-cells transduced with irrelevant TCRs. However, mice treated with T cells expressing a low affinity TCR required 8x as many transduced CD8+ T-cells to reach the same level of leukemia control as product made with high affinity TCRs. Currently, we are exploring the role of redirected CD4+ T-cells in the control of leukemia and testing TCRs with a wider range of TCR properties.

Poster No. 68

Cancer Immunology and Immunotherapy Program

Personalized T-cell Therapy for the Treatment of Solid Tumors

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We are developing a method of personalized adoptive cell therapy (ACT) for the treatment of solid tumors called NeoXpress. We used a high-throughput TCR cloning method, TCXpress, to express the TCRs from 100's of tumor-infiltrating lymphocytes (TIL) in a "screening cell" line. Simultaneously, DNA sequencing identified tumor-specific mutations bearing informatically predicted neoantigen peptides. Using the MC38 tumor model, we cloned the TCR genes from single CD8+ T-cells sorted from the TIL. Sequencing identified over 3000 differences between the MC38 tumor line and the mouse's genome. Ranked on predicted MHC binding and gene expression levels, the top 212 mutated sequences were cloned into 28 tandem minigenes (TMG) and the TMG library transduced into fibroblasts. We screened 294 MC38 TIL associated TCRs from 5 different mice in TCR against the 28 TMGs cells and reactive TCRs identified by the upregulation of CD69. We found 24 unique TCRs responsive to MC38 mutations. Half of the responding TCRs recognized one mutation in the gene RPL18. We characterized the intrinsic properties of multiple RPL18 TCRs and used retrovirus coupled with CRISPR/Cas9-based KO of endogenous TCR loci to create a T-cell population with a high affinity (A09) or low affinity (G08) RPL18 specific TCR. Treatment of tumor-bearing mice with these T-cells significantly increased tumor regression with 76 and 74% of the tumors treated with A09 (n=34) or G08 (n=35) regressing and 37.5% (n=32) of the mice treated with controls regressing. We are currently testing TCRs specific for other MC38 antigens.

Poster No. 69

Cancer Immunology and Immunotherapy Program

Optimizing therapeutic T-cells for adoptive cell therapy: benefits of pretransfer 4-1BB stimulation

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Adoptive T-cell therapies (ACTs), wherein a single TCR against a defined antigen is introduced into cultured polyclonal T-cells and infused into patients with cancer, is a promising but thus far disappointing approach. To be effective, T-cells must survive, expand, infiltrate tumors and be capable killers. We established an ACT model using T-cells transduced with a TCR against OVA to treat B16-Ova melanoma. We improved the efficacy of redirected ACT by: 1) optimizing transduction methods by concentrating TCR-encoding retrovirus, infecting with a defined MOI and standardizing the time of retroviral TCR transduction post activation to ensure maximum and consistent TCR transduction; 2) designing a CRISPR-Cas9 system to knock out both endogenous TCR chains; and 3) generating a series of improved TCR retroviral vectors that produce high titer virus, CRISPR-resistant TCRs, and result in higher levels of vector-encoded TCR expression. We produced an OVA-specific ACT product which induced tumor regression in 43% (n=29 pooled from multiple experiments) of treated mice compared to 10% regression (n=29) in control mice. Because more than 50% of ACT-treated mice had progressive tumors we aimed to improve the cell product by testing various culture conditions. Cell products generated with a suboptimal anti-CD3 stimulation combined with a soluble anti-4-1BB mAb are more efficiently transduced and express higher levels of IL7R and TCF-1. This second-generation product more effectively controlled tumor with regression in 71% (n=31). Thus, the approach of suboptimal anti-CD3 plus 4-1BB agonist treatment during the production of cells creates a superior product.

Poster No. 70

Cancer Immunology and Immunotherapy Program

Exploring CD11b-targeted α-therapy for depletion of immunosuppressive tumor infiltrating myeloid cells: Optimization of [225Ac]Ac-DOTA-αCD11b dosing through PET imaging

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Introduction: Immunosuppressive, tumor associated Myeloid cells (TAMCs) in tumor microenvironment(TME) results in grime Glioblastoma(GBM) prognosis. The elimination of TAMCs may improve clinical outcomes in GBM; thus, TAMCs have become a potential target for radioimmunotherapy. The cell surface marker, CD11b, has been shown to be overexpressed in TAMCs.Targeted alpha therapy (TAT) exploits the high linear energy transfer of α-particles, providing a highly potent therapeutic effect. The focus of this study is to evaluate CD11b-TAT agent to eradicate CD11b+ TAMCs.

Material and methods: An anti-CD11b antibody(αCD11b) was radiolabeled with zirconium-89 for PET imaging and actinium-225 for TAT. The tumor accumulation of αCD11b was optimized through PET imaging in GL261 orthotopic tumor models. Biodistribution studies provided pharmacokinetic data of [225Ac]Ac-DOTA-αCD11b and its relocated decay daughters. Therapeutic efficacy of [225Ac]Ac-DOTA-αCD11b alone or in combination with immune checkpoint inhibitors (ICI) was evaluated as compared to a vehicle control and ICI alone.

Results. The αCD11b was radiolabeled with >95% yields. [89Zr]Zr-DFO-αCD11b PET demonstrated highest tumor uptake with 5mg/kg dose (2.57±1.9 %ID/g) as compared to 2.5 and 1.5 mg/kg (1.5±1.21, 1.5±0.96 %ID/g). PET findings were further corroborated with iQiD imaging of [225Ac]Ac-DOTA-αCD11b. Survival studies demonstrated significant improvements in survival with [225Ac]Ac-DOTA-αCD11b at 5 mg/kg(100µg) dose compared to control. The estimated maximum tolerated activity (eMTA) 0.5kBq was calculated. Therapeutic efficacy of [225Ac]Ac-DOTA-αCD11b(0.5 kBq) plus ICI demonstrated promising results with a median survival of 82 days, as compared to 13-days (Vehicle alone) and 20-days (ICI alone) with control groups at endpoint of 120 days.

Conclusions: The combination of ICI and TAT resulted in a dramatic improvement in overall survival in a GBM mouse model. The companion PET agent, [89Zr] Zr-DFO-αCD11b, helped optimize the therapeutic dose. These data validate the preclinical relevance of CD11b-TAT and highlights an image-guided therapeutic approach with potential to improve the treatment response in GBM.

Poster No. 71
Cancer Therapeutics Program

Conformational Analysis and Biological Evaluation of Meayamycin

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FR901464 is a cytotoxic natural product that binds splicing factor 3B subunit 1 (SF3B1) and PHD finger protein 5A (PHF5A), the components of the human spliceosome, to inhibit and alter the splicing of pre-mRNA. One thing that has halted its progression into the clinic is the poorly understood mechanism, or mode of inhibition, by this natural product. The left-hand tetrahydropyran ring of FR901464 binds SF3B1, and it remains unclear how the substituents on the ring contribute to the binding and three-dimensional conformation. We synthesized meayamycin D, an analog of FR901464, and three other analogs to probe the conformation through methyl scanning. Analogs Meayamycin D and meayamycin E show signs of cancer specificity. This poster outlines the synthesis, conformational analysis, and biological evaluation of meayamycin D and its subsequent analogs.

Poster No. 72
Cancer Biology Program

Investigating early events of ovarian cancer transcoelomic metastasis identifies the small Rho-GTPase RHOV as an essential gene for cellular detachment and aggregate formation

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A major mode of ovarian cancer (OVCA) metastasis is through transcoelomic spread, where cells disseminate into the peritoneal fluid, form multicellular aggregates (MCA), and proceed to invade the peritoneal organs. MCAs can be detected in ascites and are associated with reduced patient survival rates. The formation of MCAs enables OVCA cells to survive and spread in anchorage independent (A-I) conditions more efficiently. However, the key molecular events promptly following OVCA detachment, and preceding MCA formation, are unknown. This study focuses on identifying early gene expression changes that are driven immediately post-matrix-detachment to identify essential early response genes that drive anoikis resistance and MCA formation.

Using RNA-sequencing studies we uncovered a significant transcript upregulation of the RHOV gene in all cell lines tested. RHOV, a member of the Rho-GTPase family that controls cellular morphology, adhesion, and survival, was the only Rho-GTPase that showed differential upregulation during early detachment. Overexpression of RHOV has been documented in lung and breast cancer, yet its role in OVCA metastasis has not been studied.

To explore the potential impact of elevated RHOV transcription during early detachment, we used CRISPR-Cas9 to create RHOV-knock out (KO) clones in OVCA cell lines. RHOV-KO cells are more vulnerable to anoikis and lose their ability to aggregate under A-I conditions. RHOV-KO cells displayed abrogated migration, and RHOV-KO MCAs were less capable of clearing mesothelial cells in a co-culture model simulating peritoneal invasion. Furthermore, we show that RHOV-KO significantly reduced intra-peritoneal tumor spread of SKOV3 cells. Current work is investigating how RHOV regulates cellular adhesion and extracellular matrix-deposition in this context.

Our research offers valuable insights into the oncogenic role of the understudied RHOV gene and provides a unique proof of concept: Interrupting early adaptations proceeding matrix detachment could impede the metastatic cascade in OVCA patients diagnosed in metastatic stages.

Poster No. 73

Cancer Immunology and Immunotherapy Program

PD1 and LAG3 synergize on CD8+ T cells to drive T cell exhaustion and hinder autocrine IFNγ-dependent anti-tumor immunity

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Overcoming immune-mediated resistance to PD1 blockade remains a major challenge. Enhanced efficacy has been demonstrated in melanoma patients with combined nivolumab (anti-PD1) and relatlimab (anti-LAG3), the first in its class to be FDA- approved. However, little is known about how these two inhibitory receptors synergize to hinder anti-tumor immunity. Here, we show that PD1/LAG3-deficient CD8+ T cells, in contrast to CD8+ T cells lacking either, mediate greater tumor clearance and long-term survival in mouse models of melanoma. PD1/LAG3-deficient CD8+ T cells are transcriptionally distinct, with broad TCR clonality, and enrichment of effector-like and interferon-responsive genes resulting in enhanced IFNγ release indicative of functionality. PD1 and LAG3 combine to drive T cell exhaustion, playing a dominant role in the modulation of TOX. Mechanistically, autocrine, cell-intrinsic IFNγ signaling is required for PD1/LAG3-deficient CD8+ T cells to enhance anti-tumor immunity, providing insight into how combinatorial targeting of PD1 and LAG3 results in enhanced efficacy.

Poster No. 74

Cancer Immunology and Immunotherapy Program

Hyperglycemic culture conditions negatively impact therapeutic T cell function and signaling

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Adoptive cell therapy (ACT) is a mainstay treatment for many types of cancer and involves isolating T cells from a patient, genetically modifying or expanding them, and then transferring the cells back to the patient. While these therapies have shown remarkable success in some cancer types, many cancers have no response and relapses are common, due in part to an inability of transferred T cells to persist. We hypothesize that the hypermetabolic conditions used to expand T cells may favor proliferation but perhaps at the cost of cellular function and longevity. To assess this, we generated therapeutic T cells from both mouse and human donors, expanding them in different concentrations of glucose indicative of commonly used culture media. We found a bell-shaped curve of T cell functionality and metabolism, such that in very hyperglycemic conditions, cells bear a poorer metabolic profile and were less sensitive to T cell stimulation. Mechanistically, cells cultured in hyperglycemic conditions have elevated proteome-wide glycosylation, most notably O-linked B-N-acetylglucosamine (O-GlcNAc). These glycans are linked to serine and threonine residues which are also phosphorylation sites essential for many T cell signaling cascades. We thus hypothesize glucose-derived increases in O-GlcNAc thus blunts T cell signaling pathways, impacting their antitumor capacity. Future work will identify specific proteins most sensitive to O-GlcNAc-ation, and whether blocking this glycosylation during expansion enhances the efficacy of adoptive cell therapies for cancer.

Poster No. 75

Cancer Immunology and Immunotherapy Program

Disruption of murine LAG3 homodimers prevents its localization to the TCR at the Immune synapse

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The last decade has seen the use of immunotherapies targeting Immune checkpoint receptors in various cancers promoting anti-tumor response and consequently prolonged patient survival. The lymphocyte activation gene 3 (LAG3) immune checkpoint inhibitor Relatimab was approved in combination with Nivolumab, an anti-PD-1 antibody, to treat advanced melanoma. Additionally, over 40 clinical trials with LAG3 inhibitors are currently ongoing. Moreover, the mechanism of LAG3 inhibition with therapeutic antibodies remains unknown and analysis of LAG3 structure could elucidate domains to target for superior efficacy with lower toxicity profile. The extracellular structure of LAG3 consists of Immunoglobulin-like domains D1, D2, D3, D4, and a connecting peptide (CP) followed by the transmembrane domain. We previously showed that LAG3 forms homodimers and associates with the TCR independently of its canonical ligand MHC II indicating that TCR is a novel ligand to LAG3. Within the D2 domain is a hydrophobic region that is necessary for its dimerization. Here we mutate murine LAG3 D2 domain at W180E and L221E (LAG3mono) to disrupt homodimers and ectopically express LAG3mono in T cells. Flow cytometric analysis revealed anti-LAG3 antibodies specific for D1 (TKB58) and D3 (410C9) binding the monomer, however, D2 specific antibody (C9B7W) binding was significantly reduced. Using Total Internal Reflection Fluorescence Microscopy (TIRFM) and a lipid bilayer system, LAG3 monomers failed to associate in cis with the TCR in contrast to LAG3wt. Future studies will examine the expression of monomeric LAG3 in primary cells and its association with several other ligands.

Poster No. 76
Cancer Biology Program

MRTF-SRF interaction promotes breast cancer cell migration in a DIAPH3-dependent manner

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Breast cancer accounts for the second most cancer-related deaths in women in the United States. Mortality associated with breast cancer (BC) is due to the metastatic spread of tumor cells whose aberrant motility arises from a dysregulated actin cytoskeleton. Myocardin-related transcription factor (MRTF) belongs to a family of actin-regulated transcriptional co-factors of serum-response factor (SRF) that control gene expression in both SRF-dependent and –independent (through its SAP domain interaction) manners and is critical to invasion and metastatic outgrowth of breast cancer cells. In the present study, we sought to investigate the mechanistic underpinnings of how MRTF-SRF interaction regulates motility of breast cancer cells. Sub-lines of MDA-MB-231 cells, a triple-negative breast cancer cell line, that are genetically engineered to overexpress either wild-type (WT) or mutants of MRTF-A (one of the two major isoforms of MRTF) were used to investigate the role of specific functional interactions of MRTF-A on various modes of cell migration. We found that overexpression of WT MRTF-A significantly enhanced both 2D random migration and chemotactic migration of MDA-MB-231 cells. MRTF-A-induced increase in migration is reversed upon disruption of either SAP-domain or SRF's interaction with MRTF-A but more prominently by the latter, suggesting that SRF-mediated transcriptional arm plays the predominant role in MRTF-dependent control of cell migration. Further, decrease in migration speed upon MRTF-SRF interaction disruption was orthogonally validated by a competitive MRTF memetic peptide inhibitor. Kymography analyses showed that a distinguishing phenotypic feature of cells with disrupted MRTF-SRF interaction is dramatic suppression of the dynamic behavior of the leading edge. Consistent with this phenotype, IPA analyses of differentially expressed genes revealed the "Migration of Cells" and the "Invasion of Cells" functions to be significantly impacted by MRTF. In particular, we identified dramatic transcriptional repression of DIAPH3, a formin family of actin-nucleating and -elongating protein, in breast cancer cells, specifically induced by disruption of the MRTF-SRF interaction. Finally, we showed that transient knockdown of DIAPH3 alone was sufficient to prevent MRTF-A induced increase in cell migration, further suggesting that MRTF promotes breast cancer cell migration through transcriptional regulation of DIAPH3 in an SRF-dependent manner. In conclusion, this study links regulation of DIAPH3 to MRTF-dependent control of cell migration. These findings also pave the way for our ongoing effort to discover specific inhibitors of MRTF-SRF interaction as a novel pharmacological tool to curb migration and metastatic ability of breast cancer cells.

Poster No. 77
Cancer Biology Program

Betulinic Acid Targets Metabolic Reprogramming of Bladder Cancer Cells

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Introduction: Betulinic Acid (BA) derived from bark of white birch (Betula alba), is a tumor selective triterpenoid that underwent phase I clinical testing for melanoma (NCT00346502). Previous studies have conferred the selective entry of fluorescently labeled BA into human and mouse cancer cell lines in vitro. However, the mechanistic background of the selective entry of BA to the cancerous cells remain unsolved till date. Here we tried to examine whether selective uptake of BA into two fast replicating human bladder cancer cell lines, T24 and RT4 relative to two slow replicating benign urothelial cell lines HBDEC (human bladder epithelial cell) and TRTU-H1 cells is dependent on metabolic reprogramming fueled rapid cell growth amidst nutrient scarcity.

Materials and Methods: Cell lines T24, RT4, TRTU-H1 and HBDEC were grown in presence of high (10% increase in glucose) and normal glucose concentration in McCoy's5A, KSFM (Sigma) and BECB (ATCC) media respectively. Cell viability was assessed using MTT (Sigma) assay after treatment with increasing amounts of BA and Mitomycin C (control) for 18 hrs. RT-PCR experiment was done in T24 cell lines under high and normal glucose using standard protocol.

Result: High glucose concentration blunted the selective cytotoxicity of BA on urothelial carcinoma cell lines T24 and RT-4 than slow growing benign cell lines, while toxicity of Mitomycin C remained unaffected. In RT-PCR, experiment, the high glucose condition downregulated the expression of pro apoptotic genes like P53, Bax, Caspase3 and PTEN, while upregulating anti apoptotic XIAP and Bcl2 upon treatment with BA.

Conclusion: High glucose concentration reverts epigenetic changes in Cancer cells (Warburg effect) to weaken cytotoxicity of BA.

Poster No. 78
Cancer Immunology and Immunotherapy Program

Helicobacter hepaticus colonization endows immunotherapeutic sensitivity in refractory melanoma tumors

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While melanoma outlook has improved with the introduction of anti-PD1 immunotherapy, 50% of patients remain treatment resistant. The barriers to response remain largely unknown but recent studies have correlated the composition of the gut microbiome with response to immunotherapy in melanoma patients. Our group has shown that colonic colonization with *Helicobacter hepaticus* (*Hhep*) drives CD4+ T cell dependent anti-tumor immunity in a colorectal cancer model, but whether *Hhep* supports anti-tumor immunity at distant tumor sites is unknown. We hypothesize that cytotoxic *Hhep*-specific CD4+ T cells formed in response to *Hhep* colonization drive anti-tumor immunity in melanoma. Using the anti-PD1 resistant B16 mouse model of melanoma, we determined that *Hhep* colonization alone expedited tumor growth but it was slowed by combined *Hhep* colonization and anti-PD1 therapy. We transferred *Hhep*-specific and tumor-specific TCR transgenic T cells to assess each intratumoral population in these pro- and anti-tumor conditions. *Hhep*-specific CD4+ T cells were present intratumorally in *Hhep* colonized mice regardless of anti-PD1 treatment. However, both *Hhep*-specific and endogenous intratumoral CD4+ T cells shifted from a T follicular helper cell to a Th1 phenotype when colonized mice also received anti-PD1 therapy. Additionally, both *Hhep*-specific and tumor-specific T cells displayed lower levels of inhibitory receptors such as PD1 in dual treated mice. Ultimately, we show how the addition of a single bacterial species to the gut confers anti-tumor immunity in a distant, refractory tumor model, reinforcing the possibility of new microbiome driven immunotherapeutic targets in resistant tumors.

Poster No. 79
Genome Stability Program

IFDlong: an isoform fusion detector on long-read RNA-seq data

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Fusion transcripts are fused RNAs that can either translate into chimeric proteins or alter the gene expression. Studies show that fusion transcripts have high concurrence rate on multiple types of cancer samples and are closely correlated with cancer recurrence. Genes composing fusion transcripts are mostly oncogenes that can act as biomarkers for cancer prediction and therapeutic targets. To identify fusion transcripts, long-read sequencing technologies are developed to generate reads with length of several-thousand base pairs. These full-length or almost full-length transcripts promote the detection of various transcriptome structural variants.

In this project, we proposed IFDlong, a bioinformatic tool to detect isoform and fusion transcripts from long-read transcriptome sequencing data. Specifically, the software will first annotate the long reads with genes and isoforms and quantify isoforms expression by a novel estimation-maximization algorithm. Then the tool will discover and quantify fusion transcripts at isoform-level. For evaluation, our proposed IFDlong pipeline shows overall the best performance when compared with several existing tools on multiple in-silico simulation data sets, with different settings on sequencing error rate, read length, novel isoform and fusion combination. Next, novel fusion transcripts were detected by our pipeline when applied into in-house bulk long-read RNA-seq data with 8 colon cancer samples and single-cell long-read RNA-seq data on liver cancer samples. In addition, multiple public long-read data sets were employed and proved that our tool is compatible with multiple long-read platforms and its ability to accurately profile alternative splicing and fusion events. Novel isoforms and fusions detected by our pipeline will potentially serve as biomarkers for cancer diagnosis and therapeutic targets.

Poster No. 80
Cancer Biology Program

Functional characterization of EPHA3 exon 4-5 duplication (EPHA3d4-5) in high-grade serous carcinoma progression, and recurrence

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High-grade serous ovarian cancer (HGSC) accounts for 70-80% of ovarian cancer mortality, and overall survival has not been improved for decades. While standard therapy typically induces an initial response, most HGSC patients develop recurrent diseases following chemotherapy. The genetic aberrations that can be targeted to manage chemo-resistance remain ill understood. This suggests a desperate need to identity new therapeutic targets for the management of HGSC. Intragenic rearrangements (IGRs) leading to duplication or deletion of one or more exons have been sporadically reported to be cancer drivers. However, IGRs have not been rigorously studied in HGSC despite their potential significance as genetic drivers.

Our analysis of TCGA copy number data revealed that IGRs as a special type of genomic rearrangements may be far more frequent events than realized in HGSC. In addition, this analysis identified a duplication of exons 4-5 in the Eph-Like Receptor Tyrosine Kinase A3 (EPHA3) in 8.3% of HGSC tumors, which we termed EPHA3d4-5, and our recent data suggest that it may be far more frequent in recurrent tumors. Exon 4-5 duplicated EPHA3 transcript encode in an in-frame protein with an extra fibronectin type 3 domain, which we speculate could alter the function of EPHA3 protein. Indeed, specific knockdown of EPHA3d4-5 potently reduced the viability of the EPHA3d4-5 positive HGSC cell line OVCAR3 and DOV13, which is not observed in the EPHA3 wild-type cell line ES-2 and SKOV3, suggesting that EPHA3d4-5 may drive cancer cell growth in HGSC. Furthermore, transcriptome sequencing of genetic perturbation and ectopic overexpression models revealed that EPHA3d45 appears to activate cell cycle, oncogenic Rho signaling, and MYC pathways, and suppresses apoptosis, P53, TGF-β and interferon signaling.

We thus hypothesize that EPHA3 exon 4-5 duplication may play a key role in promoting HGSC progression and recurrence and thus constitute a viable therapeutic target. Successful completion of this project will qualify a novel genetic driver of recurrent and chemo-resistant ovarian cancer, and pave a way for a new precision medicine against EPHA3 exon duplications, which would benefit a substantial population of ovarian cancer patients, including patients with recurrent cancer.

Poster No. 81
Cancer Biology Program

Premature Aging and Reduced Cancer Incidence Associated with Near-Complete Body-Wide Myc Inactivation

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Dysregulation of the MYC proto-oncogene occurs in most human cancers and alters growth, metabolism, translation and other functions in ways that support tumorigenesis. However, little in known about the role of Myc in normal growth and development. Myc+/- mice are healthier and longer-lived than control mice but the long-term consequences of more complete Myc loss have not been investigated because Myc-/- mice die in mid-gestation. We now describe the life-long consequences of body-wide Myc inactivation initiated at the time of weaning. "MycKO" mice acquire numerous features of premature aging including altered body composition and habitus, metabolic dysfunction and hepatic steatosis. These mice also dysregulate numerous gene sets involved in functions that normally deteriorate with aging. These include those devoted to the maintenance of mitochondrial and ribosomal structure and function, translation, cell cycle control, regulation of reactive oxygen species, DNA damage recognition and repair, aging and senescence. Surprisingly, however, MycKO mice have extended life spans that are likely due to a 3-4-fold lower lifetime cancer incidence. Aging tissues from normal mice and humans also down-regulate Myc and gradually alter many of the same Myc target gene sets seen in MycKO mice. In normal humans and other animals, aging is the strongest predictor of cancer development. These two features cannot be separated in classical models of premature aging, the vast majority of which are based on extremely rare monogenic disorders of DNA damage recognition and repair. However, our results indicate that MycKO mice represent a more physiologic model of premature aging. Additionally, they identify a single gene, namely Myc, that links normal aging and cancer in both mice and humans. Targeted therapeutics currently under development and aimed at inhibiting Myc in tumors will need to be evaluated carefully in light of these findings indicating that such treatments may accelerate the aging process.

Poster No. 82
Cancer Biology Program

3D In-vitro Modeling of CAR-T Cell Infiltration in HER2+ Breast Tumors

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Introduction: The lack of immune-mediated cancer cell destruction is a dominant mechanism of immunotherapy failure. The role of HER2+ breast tumor cells in modulating CAR-T cell infiltration remains unclear. 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 micro treatments. We employed SNAP-CAR T cells, whose contacts with HER2+ breast tumor cells can be initiated by adding benzyl guanin-conjugated Herceptin (BG-Herceptin).

Results and Discussions: BT474 and HCC1954 cells recruited SNAP-CAR T cells at similar baseline levels. Upon adding BG-Herceptin, CAR-T cell infiltration increased significantly in the BT474 collagen droplets but remained unaffected in HCC1954 droplets. To investigate how tumor cell-derived chemokines affect CAR-T cell motility, we added TAK779, a CXCR3 and CCR5 inhibitor, to the 3D co-culture system. TAK779 significantly reduced the CAR-T cell infiltration in BT474 droplets to baseline levels, but not in HCC1954 droplets (Fig 1 C-D).

Cell motility patterns were further analyzed. In the BT474 droplet, the maximum step width of CAR-T cells was increased from 42.1um to 57.9um with BG-Herceptin, and the median track density increased from 2.8 to 25 within a 200um radius. With TAK779, the CAR-T cell maximum step width (39.4um) and track density (7) returned to baseline levels. However, similar trends of CAR-T cell motility in the HCC1954 3D collagen droplets are not shown.

Conclusions: Our findings suggest that CAR-T cell infiltration towards the solid tumor depends not only on the tumor cell secreted factors but also on tumor-CAR-T cell interactions initiated by BG-Herceptin, highlighting the importance of immunogenic cell death in CAR-T cell recruitment. This study demonstrated the effectiveness of simulating CAR-T cell infiltration in solid tumors using a 3D in-vitro collagen droplet model. Ongoing research is examining the cause of the tumor cell line-dependent T motility changes and the effects of immunosuppressive tumor microenvironment on CAR-T cell infiltration.

Poster No. 83
Cancer Therapeutics Program

A user-friendly R Shiny app for predicting drug response of cancer using deep learning

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Achieving the full potential of precision oncology requires precise prediction of treatment response. Previously, we developed DeepDR, a deep learning model that predicts drug sensitivity by integrating mutations and gene expression of a cancer sample. The model features a transfer learning design that captures two types of features: tumor relevant representations of mutation and expression data learned from tumors, and a predictive model for drug response learned from high-throughput drug screens of cell lines. Thus, DeepDR was applicable to both cell lines and tumors and achieved superior performance over conventional methods. To make DeepDR more accessible to biomedical researchers with limited programming skills, we present a user-friendly web server using an R Shiny framework. The web app allows users to upload mutation and/or gene expression profiles of a cancer sample (cell or tumor model) and predict the sample's response to 265 anti-cancer compounds. It provides an intuitive user interface to interactively visualize, search, and filter prediction results. Additionally, it enables various downstream analyses, including statistical tests, and provides links to external compound databases such as PubChem. We believe that the R Shiny app will foster accessibility of our deep learning prediction machine and facilitate the drug development process in cancer.

Poster No. 84
Cancer Virology Program

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

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Cytosolic arginine sensor for mTORC1 subunit 1 (CASTOR1) is a newly discovered arginine sensor, which negatively regulates mTORC1 activity. We have previously shown that CASTOR1 is tumor suppressive in KSHV-induced cellular transformation and breast cancer by regulating the mTORC1 activity and cell proliferation. However, the role of CASTOR1 in immune response and inflammation has not been investigated before. In this study, we generated a CASTOR1 knockout mouse model and used it to investigate the role of CASTOR1 in acute inflammatory bowel disease (IBD). We found that mice with CASTOR1 knockout were resistant to body weight loss, maintained intact intestinal barriers, and showed increased cell proliferation and decreased epithelial apoptosis during dextran sulfate sodium (DSS)-induced intestinal epithelial injury and acute colitis. Mechanistically, CASTOR1 knockout promoted intestinal crypt proliferation and regeneration by inducing interleukin-6-associated reparative inflammation and STAT3 activation. Consequently, treatment with berberine chloride induced mTORC1 activation and relieved IBD impairment while treatment with rapamycin exacerbated acute inflammation. Furthermore, recombinant IL-6 protein suppressed inflammatory immune response while stattic blocked IL-6/STAT3 signaling and exacerbated inflammation in DSS indued colitis mice. Together, these findings provided evidence that CASTOR1 deficiency and activation of mTORC1 could prevent and block intestinal inflammatory disorders. Our results indicate that CASTOR1 likely contribute to DSS-induced colitis, and that activation of the mTORC1 pathway could facilitate colon epithelial regeneration by activating the IL-6-STAT3 pathway, thus providing a new target for the treatment of colitis-related diseases.

Poster No. 85
Genome Stability Program

Single molecule studies of the assembly and dissociation of the LIG3α-XRCC1 complexes at sites of single strand DNA breaks

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The repair of DNA single strand breaks (SSB) is essential for human health. DNA ligation is critical for joining DNA ends in response to DNA damage that can occur within naked DNA or in chromatin in which DNA is wrapped around a histone octamer forming nucleosome core particles (NCPs). X-ray cross complementing protein 1 (XRCC1) forms an important scaffolding complex with DNA Ligase 3 (LIG3α) involved in base excision repair (BER). Previous biochemical studies showed that LIG3α & XRCC1 interacts with nicked DNA in a 5S rDNA NCP, and the ligation efficiency changes with the nick positioning. To test better understand the dynamics of LIG3-XRCC1 interactions with nicks in naked DNA or 601 NCP, we used a real time single-molecule approach to visualize Halo-tagged LIG3α with XRCC1-YFP, from nuclear extracts, binding single-strand nicks in real time. On naked DNA, LIG3α and XRCC1 showed strong association with ligatable nicks with lifetimes of 5.4s for and 6.9 s, respectively, with a 17.3% co-localization. We then designed non-ligatable nicks positioned in a fully wrapped 601 NCP at three different positions. LIG3α was found to bind to an undamaged NCP. With regard to a NCP containing a nick, LIG3α had higher affinity for a nick at SHL-4.5 then SHL -2.5 and SHL 0 (dyad). Furthermore, LIG3 and XRCC1 co-localized with high efficiency when nicks were located within nucleosomes (32-54%). These data suggests that both LIG3α & XRCC1, exhibits strong association for nicks in naked DNA and in chromatin, and the position of damage and nucleosome structure play critical role in DNA damage recognition.

Poster No. 86
Biobehavioral Cancer Control Program

Exploratory Analysis of the Relationship between DNA Methylation of BDNF and RASA2 Genes and Processing Speed in Women with Early-stage Breast Cancer Receiving Endocrine Therapy

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Purpose: This study aims to explore potential genetic predictors of decline in processing speed in breast cancer and its treatment and to elucidate the underlying biological mechanism of the decline. Design: This study is a candidate gene epigenetic association study with a longitudinal cohort design using data from a randomized controlled trial, The Exercise Program in Cancer and Cognition, which aims to examine whether aerobic exercise improves cognitive function in postmenopausal women with early-stage breast cancer receiving endocrine therapy. Method: We will use existing data collected at pre-randomization (T1) and after the six-month intervention (T2). Data include: 1) DNA methylation data for BDNF and RASA2 genes, collected with the Illumina Infinium Methylation EPIC Beadchip from peripheral blood samples, and 2) processing speed data measured with scores from the Grooved Pegboard and Digit Vigilance Tests. Data will be analyzed with descriptive and mixed-effect modeling. Results: The results will include 1) descriptions of processing speed and DNA methylation in BDNF and RASA2 genes at T1 and T2, and 2) the relationship between processing speed and DNA methylation in BDNF and RASA2 genes in 153 women with breast cancer receiving endocrine therapy. Conclusion: The results of this study will contribute to elucidating the biological mechanisms underlying changes in processing speed experienced by postmenopausal women treated for breast cancer. Relevance: Findings from this study may provide insights into genetic predictors of decline in processing speed, potentially informing nursing practice and future development of targeted interventions to improve processing speed in women with breast cancer.

Poster No. 87
Genome Stability Program

HIRA constrains telomeric R-loop formation via Histone H3.3

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The Alternative Lengthening of Telomeres (ALT) pathway is a mechanism of telomere extension that aids the proliferation of several aggressive cancers. Recurrent mutations inactivate the ATRX (alpha-thalassemia/mental retardation, X-linked)–DAXX (also known as death domain associated protein) chromatin remodeling complex, which coordinates histone H3.3 deposition at telomeres. Loss of this complex causes telomeric instability, chromatin decompaction, and elevation of the telomeric repeat-containing long noncoding RNA, TERRA, levels. ATRX-deficient cells have increased levels of TERRA which correlates with elevated telomeric R-loops and transcription-replication machinery collisions (TRCs). Recently, we showed that the HIRA-UBN-CABIN1 complex, the sole remaining H3.3 chaperone complex, remobilizes to ALT telomeres and maintains chromatin at telomeres to compensate for ATRX-DAXX inactivation. However, rather than fully rescuing ATRX-DAXX loss, HIRA permits stochastic DNA damage at telomeres leading to ALT telomere extension thereby ensuring the survival of ATRX-DAXX-deficient cancer cells. The reason for this is not known. We hypothesize that HIRA can step in to assemble telomeric chromatin but fails to maintain the optimal telomeric chromatin environment resulting in elevated TERRA levels and replicative stress thus augmenting ALT activity.

We observed a novel role for HIRA in ATRX-deficient cells, where it suppresses TERRA accumulation at damaged telomeres. This regulation is crucial to restrict excessive R-loops and deleterious TRCs that would disrupt ALT telomere maintenance and cause overwhelming DNA damage. HIRA interacts with UBN1 and UBN2 to deposit de novo histone H3.3 which is compulsory to block aberrant R-loop formation. This study delineates a unique mechanism by which HIRA-UBN-mediated chromatinization constrains TERRA R-loop homeostasis at damaged telomeres to prevent TRCs and ensure the survival of ATRX-DAXX-deficient ALT cancer cells.

Poster No. 88
Cancer Therapeutics Program

Platinum (II) nanocomplex targeting cancer stem cells for improved lung cancer therapy

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Cancer stem cells (CSCs), harboring stem cell-like properties involving self-renewal and aberrant differentiation potential, have been known to be one of the determining factors that contribute to therapeutic resistance and tumor recurrence. However, much remains to be understood about the reprogramming network leading to the generation of CSCs driven by chemotherapy. In this study, guided by bioinformatics study, we uncover and provide deeper insight into the CSC enrichment mechanism driven by cisplatin (CDDP) treatment. We discover that CDDP can repopulate the level of CSC by activating AKT1 oncogenic pathway that is further enhanced by COX-2 inflammatory signaling. Simultaneously blocking these two pathways can synergistically restrain the number of CSCs. Under the guidance of a series of advanced hierarchical computational modeling, including molecular docking, molecular dynamics (MD) simulation and binding free energy analysis, MK-2206 is selected as the AKT1 inhibitor to achieve optimal codelivery of CDDP, MK-2206 and 5-ASA (COX-2 inhibitor) through the use of 5-ASA-derivatized dual functional immunostimulatory nanocarrier (PASA). This triple combination (PASA/CDDP/MK-2206) significantly reduces tumor burden in both orthotopic and metastatic lung cancer model. Mechanistic studies show that this improved therapeutic activity is due to elimination of CSCs and reversal of the immunosuppressive tumor microenvironment. Our study suggests that PASA/CDDP/MK-2206 may represent a simple and effective lung cancer therapy via reversing CSCs-associated chemoresistance.

Poster No. 89
Cancer Immunology and Immunotherapy Program

Lymphoid Aggregates Dictate Immune Activity in Melanoma and Lung Brain Metastases

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Brain metastasis is the cause of death of more than 40% of all cancer patients and is five times more prevalent than primary brain tumors1. Melanoma, lung, and breast cancers are the three most common cancers metastasizing to the brain2. Currently, radiation and chemotherapy are the gold standard for the treatment of brain metastasis. However, despite the efficacy of current T cell-based immunotherapies in primary cancers, recent clinical trials have demonstrated little to no benefit in brain metastasis patients. Thus, we predict that tertiary lymphoid structures (TLS) could provide the cellular niches for increased T cell influx and activity. TLS are ectopic lymphoid structures consisting of clusters of B and CD4+ T cells with the presence of high endothelial venules (HEVs) and follicular dendritic cells (FDCs) as hallmarks of TLS formation. B cells and TLS correlate with superior response to immunotherapies and greater overall survival in solid tumors. Despite the positive prognostics of TLS and B cells in solid primary tumors, they are critically understudied in brain metastasis. However, the expression of CXCL13, a key initiating factor of TLS, has been shown to correlate with greater overall survival in melanoma brain metastasis patients3. In this study, we hypothesize that B cell infiltration and TLS signatures in brain metastases would correlate with improved anti-tumor immunity and better overall survival. Utilizing multispectral imaging, we demonstrate that despite lacking TLS with canonical hallmarks (HEVs and FDCs), brain metastasis patients have specialized lymphoid structures consisting of proliferating B and T cells, potentially early TLS. We observed that the presence of these lymphoid structures correlates with increased CD8+ T cell infiltration. More specifically, CD8+ T cells are more likely to localize intratumorally in melanoma brain metastasis and in non-tumor regions in LBM patients. Additionally, we identified more active lymphoid aggregates by Ki67 staining in melanoma-brain metastasis patients relative to lung-brain metastasis, suggesting that the primary tumor could influence the type of lymphoid structures in the brain. We also found a correlation between TLS at the primary tumor site and increased B cell infiltration and early TLS formation at the metastatic site in lung cancer patients. Utilizing spatial proteomics and transcriptomics, we aim to carry out an in-depth analysis of the immune activation of the lymphoid structures and key molecular pathways in the tumors of brain metastasis. In conclusion, uncovering differences in B cells and lymphoid aggregates will set the ground truth for structure formation in brain metastases, which will elucidate key immune mechanisms to target for TLS function in these immunologically unique tumors.

Poster No. 90

Cancer Immunology and Immunotherapy Program

Single-cell exploration of the impact of combined treatment with anti–LAG-3 and anti–PD1 on CD4+ regulatory T cells in patients with melanoma

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Recently, combination treatment with relatlimab (anti-LAG3) and nivolumab (anti-PD1) was approved as a first-line therapy for patient with advance metastatic melanoma due to improved survival outcome compared to nivolumab. While we know that this treatment regimen is clinically effective, we do not fully understand the mechanism(s) that underpin the enhanced antitumor immunity observed in clinical trials, especially the detailed consequences on immune cells. Consequently, we examined blood and tumor biopsies from 42 immunotherapy-naïve patients treated with a combination of anti–LAG-3 and anti–PD-1 in a phase II trial. Our evaluation involves using combined single-cell RNA and T cell receptor sequencing (scRNA+TCRβ-Seq). Initially, we noticed the highest expression of LAG3 and PD1 in CD8+ T cells, Tregs, and γδ T cells. Based on this finding and pre-existing knowledge that Tregs can dampen favorable immune responses in the tumor microenvironment (TME) that leads to antitumor immunity, we formulated an initial hypothesis suggesting that one plausible mechanism underlying the combination therapy's effectiveness is its impact on Tregs. This impact potentially fosters heightened activation of effector T cells, bolstering their ability to favor antitumor responses. Hence, we aimed to intricately explore the transcriptional shifts happening within Tregs before and after treatment with the combination therapy, contrasting them with individual monotherapies. This comprehensive analysis involved robust computational techniques alongside a rigorous series of experimental validations. We presume that this approach holds the potential to significantly enhance our comprehension of the precise mechanisms underpinning treatment response.

Poster No. 91
Cancer Biology Program

UPMC Hillman Cancer Center Academy

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The University of Pittsburgh Medical Center Hillman Cancer Center Academy (Hillman Academy) has the primary goal of reaching high school students from underrepresented and disadvantaged backgrounds and guiding them through a cutting-edge research and professional development experience that positions them for success in STEM. With this focus, the Hillman Academy has provided 676 authentic mentored research internship opportunities to 556 students from diverse backgrounds over the past 14 years, most of whom matriculated into STEMM majors in higher education. These efforts have helped shape a more diverse generation of future scientists and clinicians, who will enrich these fields with their unique perspectives and lived experiences. In this poster, we describe our award-winning program and the strategies that led to its growth into a National Institutes of Health Youth Enjoy Science-funded program including our unique multi-site structure, tiered mentoring platform, multifaceted recruitment approach, professional and academic development activities, and a special highlight of a set of projects with Deaf and Hard of Hearing students. We also share student survey data from 2016-2021 that indicate satisfaction with the program, self-perceived gains in key areas of scientific development, awareness of careers in STEM, and an increased desire to pursue advanced degrees in STEM.

Poster No. 92
Cancer Immunology and Immunotherapy Program

Systematic investigation of chemo-immunotherapy synergism to shift anti-PD-1 resistance in cancer

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Chemo-immunotherapy combinations have been regarded as one of the most practical ways to improve immunotherapy response in cancer patients. In this study, we integrated the transcriptomics data from immunotherapy-treated tumors and compound-treated cell lines to systematically identify chemo-immunotherapy synergisms and their underlying mechanisms. Through analyzing anti-PD-1 treatment induced expression changes in patient tumors, we developed a shift ability score that can measure whether a chemotherapy treatment shifts anti-PD-1 response. By applying the shift ability analysis on 41,321 compounds and 16,853 shRNA treated cancer cell line expression profiles, we characterized a systematic landscape of chemo-immunotherapy synergism and prioritized 17 potent synergy targets. Further investigation of the treatment induced transcriptomic data revealed that a mitophagy-dsRNA-MAVS-dependent activation of type I IFN signaling may be a novel mechanism for chemo-immunotherapy synergism. Our study represents the first comprehensive effort to mechanistically characterize chemo-immunotherapy synergism and will facilitate future pre-clinical and clinical studies.

Poster No. 93
Cancer Virology Program

Identifying Tissue Regions of Pathological Phenotypes from In Situ Spatial Single-Cell Transcriptomics Data Using Deep Graph Analysis

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Spatially resolved single-cell profiling with cellular or sub-cellular resolutions enables the measurement of gene expression of individual cells and their spatial locations within the tissue. Such technologies including in situ sequencing (ISS) and multiplexed error-robust FISH (MERFISH) are transforming the understanding of cellular mechanisms with spatial gene expression (SGE) patterns that are expected to define domains of tissue structures and pathology phenotypes. While several unsupervised machine learning algorithms have been proposed, delineating disease pathogenesis with tissue and spatial signatures such as cancer tumor microenvironments (TME) and COVID-19 lung infections remain challenging due to complex localized cell morphologies, interactions between multiple cell types, and limitations of existing spatial technologies such as low sequencing depth. In response, we propose a novel methodology that systematically organizes cellular spatial coordinates and corresponding transcriptional profiles into a cohesive graph-based representation, with nodes corresponding to individual cells and edges connecting nearby nodes. Node-level labels are meticulously derived from pathologists' expert annotations, leading to the development of partially labeled datasets involving severe SARS-CoV-2 infections and lung cancer tissues. To learn long- and short-range spatial features and disease-associated SGE patterns from partial labels, we introduce a novel semi-supervised graph neural network. This method achieves high accuracy in predicting COVID-19 pathology phenotypes and identifying tumor subtypes in the lung cancer dataset, demonstrating its effectiveness. Furthermore, we generate a novel 'spatial gene attribution map' illustrating the spatial importance of selected genes in making specific predictions. Analyzing SGE patterns and spatial gene attribution maps reveals crucial genes, spatial localization, and regulatory tendencies, offering a comprehensive view of the immune and structured cell landscape around the region of interest. This research propels spatially resolved single-cell profiling, holding potential for precise disease prediction and personalized treatment strategies by integrating machine learning with spatial gene expression data.

Poster No. 94
Cancer Biology Program

ADVANCEMENTS IN POST-MORTEM TISSUE COLLECTION FOR BREAST CANCER RESEARCH: INSIGHTS FROM THE "HOPE FOR OTHERS" PROGRAM

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BACKGROUND INFORMATION: Rapid Autopsy (RA) programs offer a unique resource for tissue collection from cancer patients. Our study focuses on the UPMC/Pitt RA group's program "Hope For Others" program for advancing breast cancer research.
OBJECTIVES: The study aims to provide an update on the operations, challenges, achievements, and future plans of the RA program in the context of breast cancer research.
METHODS AND/OR INNOVATION: Autopsies are performed by the Autopsy and Forensic Pathology Center of Excellence/Decedent Affairs Service of UPMC, with samples banked in the Pitt Biospecimen Core (PBC). Improvements to the program include the incorporation of patient advocates and a dedicated research coordinator, as well as the establishment of innovative tissue processing techniques.
RESULTS/DISCUSSION: As of June 2023, 30 autopsies were completed, with an additional 44 patients having consented. The average disease-free survival and overall survival were 76.1 and 123.6 months, respectively. Data showed that 83.33% of patients passed outside the hospital. The most common metastatic sites for specimen collection were liver, lung, and lymph nodes, with an average of 13.4 different tumor specimens collected per patient. The inclusion of patient advocates has significantly improved the program's approach to patient recruitment and handling of logistical challenges. Preliminary analysis has yielded insights into intra- and inter-patient and intra-tumor heterogeneity, and ongoing molecular studies are expected to offer more findings regarding the transformations involved in cancer metastasis.
CONCLUSION: Through innovative strategies and a multidisciplinary approach, the UPMC/Pitt RA program has successfully enhanced post-mortem tissue collection for breast cancer research. The focus now lies on expanding omics studies and ensuring diversity in patient enrollment, ultimately driving the development of similar successful RA programs both nationally and globally.

Poster No. 95
Cancer Immunology and Immunotherapy Program

SNAP CAR T cells for programmable antigen targeting

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Universal chimeric antigen receptors (CARs) are synthetic receptors that instead of directly binding to an antigen, recognize one or more adaptor molecules that bind to target antigens. Universal CARs are of high clinical interest due to their abilities to be tuned by adaptor dose and to be targeted toward multiple antigens, potentially avoiding toxicities and relapse respectively. We developed a universal CAR, "SNAP-CAR," that carries out a self-labeling reaction to covalently attach to adaptor antibodies conjugated to a benzylguanine (BG)-tag. In vitro experiments showed potent and specific SNAP-CAR function with co-administered adaptors targeting HER2, EGFR, and CD20 including activation of CD69 and CD107a markers, specific target cell lysis, and IFN-gamma production. To assess the in vivo activity of SNAP-CAR, NSG mice were challenged with HER2+ or CD20+ human leukemia or ovarian tumor xenografts. Mice were then treated with SNAP-CAR T cells and adaptor injections, and tumor size was measured by IVIS imaging. In the human leukemia tumor xenograft NSG mouse model targeting HER2, we observed that SNAP-CAR T cells were able to significantly reduce tumor burden, leading to a lack of detectable tumors in most mice. In another leukemia model targeting the CD20 antigen, SNAP-CAR T cells showed significant inhibition of tumor growth. While tumors in these mice relapsed, investigation demonstrated that cancer cells were CD20 negative, suggesting the importance of future multi-antigen targeting. Finally, evaluating two anti-HER2 adaptors with distinct binding epitopes in a human ovarian cancer xenograft model, we observed a significant tumor reduction with both adaptors compared to adaptor only and SNAP-CAR T cell only controls. Overall, these data demonstrate the potent and versatile antigen targeting abilities of SNAP-CAR T cells both in vitro and in vivo in human tumor xenograft models, suggesting future potential for treating liquid and solid tumor malignancies.

Poster No. 96
Cancer Immunology and Immunotherapy Program

LncRNA EPIC1 is a suppressor of cytoplasmic dsRNA-type I interferon signaling and a therapeutic target to enhance tumor immune response

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Dysregulation of cytoplasmic double-stranded RNAs (dsRNAs) has emerged as a key factor contributing to the immune-suppressive tumor microenvironment (TME). However, the mechanism by which dsRNAs are dysregulated in tumors remains unclear. In this study, we mechanistically characterized the lncRNA EPIC1 as a master suppressor of cytoplasmic dsRNA accumulation in tumor cells. EPIC1 inhibition of cytoplasmic dsRNA suppresses cancer cell mediated type I interferon (IFN) secretion, which remolds the TME by regulating cancer cells, macrophages, and T cells. Further RNA-seq and ChIP-seq analyses revealed that EPIC1 epigenetically silenced endogenous retrovirus (ERVs) expression. Using an hCD34+ humanized mouse with human TNBC tumor model, we showed that targeted delivery of EPIC1 interfering oligoes in vivo can activate dsRNA accumulation in cancer cells and boost the therapeutic effect of pembrolizumab. Further single-cell sequencing analysis revealed that in vivo knockdown of EPIC1 enhanced T cell and macrophage infiltration by activating type I IFN signaling. Collectively, our findings suggest that EPIC1 plays an important epigenetic role in dsRNA-MAVS-mediated type I IFNs production in tumors and may be explored as a therapeutic target for immunotherapy.

Poster No. 97
Cancer Immunology and Immunotherapy Program

Kinetics of neoantigen-specific T cells in post-transplant relapse

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Introduction: In allogeneic stem cell transplantation (alloSCT), donor-derived T-cells mediate graft-versus-leukemia effect (GVL) by recognizing antigens expressed by leukemia cells. However, the role of neoantigens remains unknown in GVL. Here, we screened potential neoantigens and identified neoantigen-reactive T-cells in patients who developed post-transplant relapse.

Materials and methods: Leukemic blasts from patients in relapse were FACS-isolated from bone marrow aspirate or peripheral blood mononuclear cells (PBMCs). DNA/RNA from these specimens were analyzed for whole exome sequencing, paired with RNAseq. Somatic mutations with variant allele frequency >20% were subjected to pVAC-seq for prediction of neoantigen likely presented on recipient HLA (IC50 < 500nM). PBMCs were obtained before, during, and after post-transplant relapse. For HLA-A*02:01-restricted neoantigens, PBMCs were stained with HLA-A*02:01-tetramers loaded with neoantigens or CMVpp65 (control). Purified CD3 cells were stimulated with neoantigens for 10 days and tested for cytokine production in response to target/control peptides.

Results: Eight patients with relapsed acute leukemia after alloSCT and their transplant donors were screened. Potential neoantigen candidates and donor: recipient miHAs were identified in seven and two subjects, respectively. CD8 T-cells from a responder to donor lymphocyte infusion (DLI) showed dominant CMVpp65-specific T-cells during relapse and persistently low-frequency TP53- or HA-2-specific T-cells after relapse by HLA-A*02:01 tetramer staining. HA-2-specific T-cells upregulated PD-1 expression at the time of graft-versus-host disease (GVHD), indicating activation of T-cell clones during GVHD. In two subjects with long-term remission after DLI, CD8 T-cells reactive to neoantigens were detectable by IFN-γ/CD107a production specific to mutant peptides (SDE2.pK123X and TP53.pR248W).

Conclusion: This study suggests somatic mutations in acute leukemia may serve as putative neoantigens in alloSCT. Although the sample size is small, we demonstrated the feasibility of studying neoantigen-specific T-cells in post-transplant specimens. Further investigation is needed to test if neoepitope burden and miHA disparity could correlate with post-transplant relapse outcomes.

Poster No. 98

Cancer Immunology and Immunotherapy Program

Ovarian cancer-induced ascites fluid promotes differentiation of antibody secreting plasma cells

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High grade serous ovarian cancer (HGSOC) is the most common and aggressive type of ovarian carcinoma, accounting for almost 70% of all cases and a 5-year survival rate below 50% to immunotherapy. One of the hallmarks of HGSOC is the build-up of fluid in the peritoneal cavity, known as ascites, which is associated with advanced tumor stage, increased metastasis and decreased survival. Patient derived ascites contains cellular and acellular immunosuppressive cues, however, B cell function within ascites fluid is understudied. Thus, we aimed to understand B cell function in patient derived ascites and link this function to B cells and tertiary lymphoid structures (TLS) in ovarian tumors. B cells can differentiate into plasma cells to produce cancer-specific antibodies and help in the formation of TLS in solid tumors, correlating to better survival after immunotherapy. Our studies initially aimed to test the impact of patient derived ascites on B cell differentiation to antibody-secreting plasma cells. We isolated naïve and memory B cell populations from healthy donors and cultured them in acellular patient-derived ascites fluid. We noted an increase in viability, activation, and differentiation of B cells to antibody-secreting plasmablasts and plasma cells compared to culture conditions without acellular fluid. Based on this preliminary data, we hypothesize that patient-derived ascites fluid promotes the activation of functional antibody-secreting plasmablasts and plasma cells. We are now assessing antibody amount, isotype, and effector function. Further, because TLS can be a source of antibody-secreting plasma cells, we will correlate TLS formation and maturation in ovarian tumors with B cell function in matched ex vivo ascites fluid. Further, we will test if antibodies from ascites fluid can extracellularly bind matched ovarian tumors. These studies will aid in our understanding of the link between B cells in the ovarian tumor and patient derived ascites, and, if all plasma cells are differentiating through a GC or extrafollicular cues.

Poster No. 99
Cancer Immunology and Immunotherapy Program

AMPKγ2 Overexpression Protects CD19-CAR T Cells From Metabolic Insults

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Acute lymphoblastic leukemia (ALL) is the most common leukemia found in children. Chimeric Antigen Receptor (CAR) T cells induce high response rates against relapsed/refractory B-cell ALL, but 40% of CAR-T recipients suffer disease relapse. Metabolic exhaustion has been proposed as a major barrier to optimal CAR-T cell performance. AMP-activated protein kinase (AMPK) is a cellular energy sensor which promotes mitochondrial health and oxidative metabolism. We have demonstrated that overexpressing the regulatory subunit AMPKγ2 increases AMPK signaling in human T cells, and hypothesized that this overexpression would enhance CAR-T metabolic fitness and improve anti-leukemia activity.

We used lentiviral transduction to introduce a CD19-reactive, CD28 CAR into human T cells, followed by transduction of either AMPKγ2 or an empty vector (EV). AMPK-CART cells showed higher oxidative metabolism, with a 29% increase in basal oxygen consumption rates (OCR) and a 45% increase in maximal OCR ($p<0.001$) following overnight stimulation by NALM6 leukemia cells. In addition, co-culture with Zs-Green+ NALM6 cells revealed greater cytotoxicity by AMPK-CART cells ($p<0.001$) and by a 35% increase in CD25 expression ($p<0.05$). To further investigate the impact of AMPKγ2 overexpression on cellular metabolism under adverse conditions, we treated CAR-T cells with the mitochondrial inhibitor metformin or the glycolysis inhibitor 2-deoxy-d-glucose (2-DG). Intriguingly, 50μM metformin treatment lowered OCR of EV-CART cells by 23% ($p<0.001$) without impacting AMPK-CART cell metabolism. Similarly, 5mM 2-DG treatment decreased viability of EV-CART cells by 21%, more than twice as much as AMPK-CART cells.

Here, we report that AMPKγ2 overexpression in CD19-CAR T cells improves in vitro cytotoxicity and oxidative metabolism. Future work will focus on studying their anti-tumor capacity in vivo using mouse models.

Poster No. 100
Cancer Biology Program

EstroGene 2.0: Transcriptional spectrum in antiestrogen treated and endocrine resistant breast cancer cell lines

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ER+/HER2- is the most common subtype of primary breast cancer, and hormone therapy has long been the standard treatment. However, endocrine resistance eventually occurs in a subset of patients, and mechanism of resistance and evolution has been extensively studied, especially with transcriptomic analysis in cell lines. However, discoveries from single experiments may be limited, due to specific selection of cell lines, antiestrogen drugs, or temporal variability of observations. We previously developed the EstroGene database (Li et al, 2023) and user-friendly browser (https://estrogene.org/) consisting of unified processing of data from 246 experiments including 136 transcriptomic, cistromic, and epigenetic datasets focusing on estradiol (E2)-triggered ER activation across 19 breast cancer cell lines. To extend this, we here performed collective analysis of antiestrogen-treated and endocrine resistant breast cancer cell lines from 109 RNA-seq (66) and microarray (43) experiments, in four specific antiestrogen conditions: transient treatment, long-term estradiol deprivation (LTED), tamoxifen-resistant (TamR), and ESR1 mutation models. Our integrated dataset mostly contains MCF7 and T47D as models, tamoxifen and fulvestrant as transient treatment drugs, 6 months or longer as TamR selection period, and Y537S and D583G as major ESR1 mutation models. Using our previous work of collective data in estrogen transcriptomic response from EstroGene we discovered a strong positive correlation between estrogen stimulated gene expression and that seen in ESR1 mutant models (R=0.46), and a strong negative correlation with short-term endocrine treatment (R=-0.51) or LTED models (R=-0.35). Of note, association of TamR RNA expression (long-term endocrine treatment) is weak compared to both estrogen (R=-0.026) and short-term antiestrogen (R=0.169, both significant), which suggests major roles of alternative pathways other than estrogen signaling contributing to eventual resistance development. Further inspection of genes upregulated in both transient and long-term antiestrogen treatment showed enrichment in autophagy pathways, a gene signature derived from which signifying poor prognosis in ER+ primary breast cancer patients. Also of note are genes most consistently upregulated in ESR1 mutant models, which highlights GPCR ligand binding, including NPY1R and NPY5R. Future steps will include investigation of clinical significance and bench validation for identified signatures, as well as integration of current data into EstroGene browser (https://estrogene.org/) in constructing an easy-accessible estrogen and endocrine resistance-centered public resource.

Poster No. 101
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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Promising data have reported a correlation between B cells and tertiary lymphoid structures (TLS) with superior patient outcomes and immunotherapeutic response. B cells are the second most abundant tumor-infiltrating lymphocyte in the tumor microenvironment (TME) and direct the antitumor immune response through the production of antigen-specific antibodies and long-lived memory B cells (MBCs). MBCs undergo affinity maturation in either germinal center (GC)-dependent (within a TLS), or GC-independent responses at extrafollicular sites. We have previously shown that MBC phenotypes differ in head and neck squamous cell carcinoma (HNSCC) by etiological agent. HNSCC tumors that are human papilloma virus positive (HPV+) are enriched with GC associated B cells and TLS with GCs, while carcinogen-induced (HPV-) tumors show expanded extrafollicular MBCs. The presence of GCs in TLS varies by maturation and tumor type; thus, defining the phenotype of extrafollicular-derived MBCs and the antigen stimuli that promote their production is crucial. Here, investigate if HPV+/- tumors differentially educate B cells by evaluating B cell receptor mediated signaling of naïve B cells in the presence of tumor cell line conditioned media. We found in co-culture with HPV+ tumor cell line conditioned media, naïve B cells are hyporesponsive to BCR crosslinking, suggesting tumors regulate MBC maturation in a paracrine manner. We evaluated the expression of toll-like receptors (TLRs) in extrafollicular-derived MBCs, as a balance of TLR7 and TLR9 signaling prevent pathological responses in activated B cells. Moreover, TLR7 stimulation is a critical stimulus for extrafollicular responses. We demonstrated that intratumoral and circulating extrafollicular MBCs express higher levels of TLR7 relative to GC-derived counterparts. Expression of TLR9 differs between extrafollicular-derived subsets, suggesting a role for TLR9 regulation in their differentiation. Together, these data highlight a role for BCR-TLR signaling in MBCs and support further investigation of TME regulation of MBC maturation.

Poster No. 102
Cancer Virology Program

Deletion of the Gly/Ala repeat from Epstein-Barr virus nuclear antigen 1 (EBNA1) improves specificity of the EBNA1 IgA biomarker for nasopharyngeal carcinoma risk prediction

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Early-stage Epstein-Barr virus (EBV)-associated nasopharyngeal carcinoma (NPC) has a favorable prognosis. Since 2022, early detection screening programs have become available in some NPC-endemic regions such as Hong Kong. Measurement of plasma cell-free EBV DNA levels can diagnose early-stage NPC with 97% sensitivity and 99% specificity. Those that test negative are expected to retest every 1-3 years. Repeat screening of large populations can be cost prohibitive and implementing a more targeted screening strategy is warranted. Alternative to cell-free EBV DNA which reflects release of EBV from apoptotic/necrotic tumor cells, antibodies against EBV proteins are known to spike several years before NPC diagnosis. This can be exploited to identify high-risk groups for follow-up screening and may also extend the time interval for retesting. Although EBV seropositivity is ubiquitous, our serologic survey of healthy individuals that later developed NPC revealed IgA against EBV nuclear antigen 1 (EBNA1) identified 100% of incident NPC cases within a 4-year time frame prior to diagnosis in a case-control study from two independent prospective cohorts (Singapore and Shanghai, China). Across all years pre-diagnosis, specificity was 92.9% in the Singapore and 67.7% in the Shanghai cohort. Previous studies detecting EBNA1 IgA have not achieved such accuracy. We hypothesize that IgA cross-reactivity with a conserved iterative repeat region in EBNA1 (Gly/Ala repeat) found in multiple circulating EBV strains can contribute to false-positives. An individual can carry multiple EBV strains, but one strain is invariably detected in NPCs from endemic areas. We aim to improve assay specificity by optimizing the EBNA1 target sequence using truncation mutants in a multiplex (IgA, IgG) serology screen. Preliminary data shows that sera from healthy individuals does indeed test positive when presented with an EBNA1 target containing for the Gly/Ala repeat. We are currently testing additional polymorphic residues that may alter specificity.

Poster No. 103
Cancer Therapeutics Program

Pharmacokinetics, Dosimetry and Therapeutic Efficacy of [225Ac]Ac-DOTA-TDA-Lipiodol® in a Murine Cancer Model: The importance of considering the actinium-225 in vivo generated decay daughters for accurate treatment dosing

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Background: Interest in targeted-alpha-therapy (TAT) has increased since the approval of Xofigo® against mCRPC. A prominent α-particle-emitting radioisotope in TAT is actinium-225, which has a net emission of a total of 4 α-particles and 2 β-particles in its decay scheme. TAT-agents utilizing actinium-225 have shown a higher efficacy compared to single α-particle-emitting radioisotopes. [225Ac]Ac-DOTA-TDA-Lipiodol® is an α-particle-emitting transarterial radioembolization (αTARE) agent developed in the Nedrow lab, and has shown promising results in pre-clinical studies against hepatic tumors. Hepatic tumors are supplied through the hepatic artery while the liver is supplied by the portal vein. TARE exploit these arterial routes to selectively deliver therapeutic radioisotopes (e.g., yttrium-90) to the tumors. The main focus of this study was to investigate the pharmacokinetics of [225Ac]Ac-DOTA-TDA-Lipiodol® and its daughters, and their impact on the radiation absorbed dose (AD). Furthermore, we investigated the therapeutic efficacy of the αTARE-agent [225Ac]Ac-DOTA-TDA-Lipiodol® in a murine colorectal cancer model.

Methods: Mice were administered the αTARE-agent [225Ac]Ac-DOTA-TDA-Lipiodol® intratumorally. Pharmacokinetic studies were performed for [225Ac]Ac-DOTA-TDA-Lipiodol® and the in vivo generated daughters. To assess therapeutic efficacy the mice were treated with [225Ac]Ac-DOTA-TDA-Lipiodol®.

Results: We demonstrated that when only considering [225Ac]Ac-DOTA-TDA-Lipiodol® and not accounting for the relocating decay daughters the tumor AD was overestimated by 37% and the kidneys AD was underestimated by 76%. Significant improvement in survival compared with controls was observed when treated with [225Ac]Ac-DOTA-TDA-Lipiodol®.

Conclusions: The in-depth pharmacokinetic analysis of [225Ac]Ac-DOTA-TDA-Lipiodol® and the free daughters highlights their impact on the AD in the selected organs/tissues (e.g., tumors and kidneys). The inaccurate estimation of the AD when using actinium-225 labeled TAT-agents may lead to undertreatment of tumors and/or causing unwanted toxicities in normal organs. [225Ac]Ac-DOTA-TDA-Lipiodol® is a promising αTARE-agent for treatment of hepatic tumors and the understanding of the impact of the relocating decay daughters will help optimize the treatment dosing.

Poster No. 104
Cancer Biology Program

DEDIFFERENTIATED AND HIGH GRADE CHONDROSARCOMAS: HOW DOES ISOCITRATE DEHYDROGENASE STATUS APPRISE PROGNOSIS AND THERAPY?

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Objective: High grade and dedifferentiated chondrosarcomas (CSAs) are frequently associated with isocitrate dehydrogenase (IDH) mutations and often exhibit a poor clinical outcome. Treatment is limited mainly to surgery. Defining IDH status {wild type (WT) and mutant} and the associated transcriptome may prove useful in determining other therapeutic options in these neoplasms.
Methods: Formalin fixed paraffin embedded material from 69 primary and recurrent grades 2, 3 and dedifferentiated (CSAs) was obtained. DNA sequencing for IDH1 and IDH2 mutations (n= 47) and RNA sequencing via Nextseq 2000 (n=14) were performed. Differentially expressed genes (DEGs) were identified and used to predict aberrant biological pathways with Ingenuity Pathway Analysis (IPA) software (Qiagen). Gene Set Enrichment Analyses (GSEA) using subsets C3, C5 and C7 were performed. Differentially expressed genes WT1, AR and SATB2 were validated by immunohistochemistry. Outcome analysis was performed using the Wilcoxon Test.
Results: A set of 69 (CSAs), 28 females, 41 males, average age 65, distributed among femur, pelvis, humerus, and chest wall were identified from available clinical material. After further selection based on available IDH status, we evaluated 15 IDH WT and 32 IDH mutant tumors as part of this dataset. 7 of 15 IDH WT tumors involved the chest wall/scapula, while 1 of 32 mutants arose in the scapula. There were far more genes overexpressed in IDH WT tumors compared to IDH mutant tumors. Furthermore, IDH WT and IDH mutant tumors were transcriptomically distinct in the IPA and GSEA with IDH mutant tumors showing increased activity in methylation pathways and endochondral ossification, while IDH WT tumors showed more activity in normal matrix development pathways. Validation immunohistochemistry demonstrated expression of WT1 and AR in IDH WT tumors, but not in IDH mutants. SATB2 was expressed in IDH mutant tumors and not in WT tumors. Outcome analysis revealed differences in overall survival between mutant and WT tumors ($p=0.04$), dedifferentiated mutant and high grade mutant tumors ($p=0.03$) and dedifferentiated mutant and high grade WT tumors ($p=0.03$). Longest survival times were observed in patients with high grade WT tumors while patients with dedifferentiated mutant tumors showed lowest survival. Generally, patients with IDH WT tumors displayed longer survival in both high grade and dedifferentiated groups.
Conclusions: High grade and dedifferentiated chondrosarcomas are further characterized by IDH status which in turn informs transcriptomic phenotype and overall survival. The transcriptome is distinct depending on IDH status and implies different treatment targets.

Poster No. 105
Cancer Virology Program

Phosphorylation Status of Tumor Suppressor CASTOR1 is a Prognostic Marker for Male Lung Cancer Patients with KRAS Mutations

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Lung cancer is the most common cause of cancer-related mortality accounting for about 25% of all cancer-related death worldwide. We have previously shown that the cytosolic arginine sensor for mTORC1 subunits 1 (CASTOR1) involved in regulation of mTORC1 pathway is a novel tumor suppressor. Overexpression of CASTOR1 attenuates mTORC1 activation in KSHV-transformed cells, and in breast and lung cancer cells, leading to the suppression of tumorigenesis in xenograft and genetic mouse models. A lower expression level of CASTOR1 predicts poor survival in a wide variety of cancer. Our recent work has shown that CASTOR1 integrates signals of both nutrients and growth factors to regulate mTORC1 and cell proliferation. Specifically, AKT-mediated phosphorylation of CASTOR1 at S14 (CASTOR1-pS14) leads to CASTOR1 ubiquitination and degradation, resulting in mTORC1 activation, cell proliferation and tumorigenesis. In this study, we examined the potential of using CASTOR1-pS14 as a prognostic marker in lung cancer. Using an in-house specific antibody, we performed immunohistochemical (IHC) staining of lung cancer tissue microarrays (TMAs) composed of tumor and adjacent non-tumor tissue cores from lung cancer patients. Semi-quantitative analysis of CASTOR1-pS14 level showed that male patients with a higher level of CASTOR1-pS14 had significantly worse overall survival (OS) and relapse-free survival (RFS), compared to those with a lower level of CASTOR1-pS14, giving rise to more than 40% difference in both 5-year OS and RFS. Significant difference is also found in stage I-II male lung cancer patients harboring KRAS mutations. Among these patients, a higher level of CASTOR1-pS14 confers significantly worse OS and RFS compared to patients with a lower expression of CASTOR1-pS14, with 5-year OS and RFS similar to male patients with stage III-IV lung cancer. These results indicate that CASTOR1-pS14 is a prognostic marker for male lung cancer patients with KRAS mutations, which could be useful for guiding their precision therapy.

Poster No. 106
Cancer Therapeutics Program

TWIST1 mediates de novo and acquired resistance to tyrosine kinase inhibitors in MET altered non-small cell lung cancer.

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Background: *MET* amplification/mutations are important targetable oncogenic drivers in non-small cell lung cancer (NSCLC), however, acquired resistance is inevitable, the majority of patients with targetable *MET* alterations fail to respond to MET TKIs and a subset of *MET* alterations are untargetable with MET TKIs. Furthermore, *MET* amplification is a common mediator of TKI resistance for oncogenic drivers such as EGFR. Here we show that a novel TWIST1-p27 pathway mediates resistance to MET TKIs and targeting TWIST1 can overcome resistance.

Methods: TWIST1 expression was measured in cell lines, in presence and absence of HGF and in patient derived xenografts (PDXs). TWIST1 was inhibited with shRNA and harmine. *MET* altered NSCLC cell lines and PDXs were utilized and *in vitro* and *in vivo* MET TKI resistance models as well as a novel transgenic mouse model were derived and generated to evaluate role of TWIST1 in MET-driven tumorigenesis and MET TKI resistance.

Results: We found that the HGF/MET pathway induces TWIST1 expression and TWIST1 is required for *MET* altered NSCLC *in vitro* and *in vivo*. TWIST1 co-operated with Hgf in a novel mouse transgenic CCSP-Hgf (CH) model that constitutively overexpresses Hgf in lung and develops NSCLC after treatment with the tobacco carcinogen, 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone (NNK). We demonstrated that the Twist1 overexpressing CHT (CCSP-rtTA/CCSP-Hgf/Twist1-tetO-luc) mice developed significantly larger and more aggressive tumors in response to NNK as compared to CH mice and Twist1 was required for tumorigenesis in this model. Furthermore, TWIST1 is increased in both *in vitro* and *in vivo* models of MET TKI resistance and TWIST1 expression led to MET TKI resistance through bypass of a p27-dependent cell cycle arrest. Critically, TWIST1 inhibition overcomes HGF and MET mediated EGFR and MET TKI resistance.

Conclusions: Our findings suggest that targeting TWIST1 may be an effective therapeutic strategy to overcome resistance in MET-driven NSCLC.

Poster No. 107
Cancer Biology Program

Landscape of intragenic rearrangements in triple-negative breast cancer reveals RUNX1 exon aberrations driving tumor immune evasion

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Triple-negative breast cancer (TNBC) is the deadliest breast cancer subtype, accounting for 10-20% of breast cancer morbidity. Chemotherapy remained the mainstay of intervention for TNBC due to the lack of well-defined genetic targets, and recent genomic sequencing studies have revealed a paucity of TNBC-specific mutations. In this project, our landscape study of genomic rearrangements in TNBC revealed that recurrent intragenic rearrangements that result in one or more exons being duplicated or deleted may constitute a major TNBC-specific genetic landscape that may contribute to its pathobiology. As a proof of concept, we discovered novel intragenic rearrangements (IGRs) involving RUNX1, a prototype cancer gene, which dictate an immune contexture in TNBC tumors that lack lymphocyte infiltrates. RUNX1 IGRs are preferentially detected in approximately ~7% of TNBC, which result in in-frame rearranged proteins that disrupt the RHD domain required for DNA binding and interaction with the CBFβ regulatory protein. RUNX1 is a master regulator that plays key roles in hematopoiesis, epithelial cytokine production, and induction of immune response. Our data suggest that RUNX1 rearrangements lead to potent repression of RUNX1 and NFκB target genes, resulting in upregulation of immunosuppressive cytokines such as CCL5 and repression of key proinflammatory cytokines such as CXCL10, suggesting its role in dictating tumor immune contexture. RUNX1 rearranged tumors are more aggressive showing larger tumor sizes, geographic necrosis, relative cold immune microenvironment that lack interferon γ signature and lymphocyte infiltration (especially CD8+ and CD4+ T cells), and a devastating clinical outcome. To date, this is the first report of somatic RUNX1 exon rearrangements in solid tumors, and the first study of their functions on regulating cancer immune landscape.

Poster No. 108
Cancer Biology Program

Role of stromal CD10 expression in modulating the immune microenvironment of ovarian clear cell carcinoma

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Ovarian clear cell carcinoma (OCCC) is the most aggressive and chemo-resistant subtype with a very poor prognosis compared to all subtypes of ovarian cancer. This is due to its unique cell of origin which is thought to arise from endometriosis. While endometriosis is a known chronic inflammatory condition, the molecular mechanisms for the malignant transformation of endometriosis is unknown. Mesenchymal stem cells (MSC) are a critical component of the ovarian cancer microenvironment. While the immune microenvironment in endometriosis has been well documented, the role of the endometriotic stroma in regulating the immune microenvironment remains largely unexplored. We recently identified a subset of benign endometriosis-derived MSCs (enMSCs) that are characterized by loss of CD10 expression, a known endometrial stromal marker, and this loss is correlated with the acquisition of tumor-promoting properties. Thus, we hypothesized that stromal CD10 expression loss modulates the endometriosis immune microenvironment toward OCCC progression. EnMSCs were isolated from primary human benign endometriosis deposits. We also obtained human endometriosis samples and stained serial sections with CD10 and multispectral immunofluorescence (mIF) of human immune cell markers.

RNA sequencing of CD10 negative enMSCs identified upregulation of retinoic acid receptors and Matrix Gla protein (MGP) which suggested an anti-inflammatory role of CD10 negative enMSCs. RNA sequencing of CD10 positive enMSCs identified upregulation of genes associated with increase immune infiltration (including 2B EVI2B, ADAMTS, TGM2 and ROBO4). Further, our image analysis showed a positive correlation between stromal CD10 levels and immune cell populations, especially CD4 T cells. Our preliminary data showed a three-fold increase in CD4 T cells infiltration in area with high CD10 stromal expression compared to low CD10 area. Our results indicate there is an association between stromal CD10 and immune cell infiltration within the endometriotic microenvironment, which may be critical to the formation and propagation of OCCC.

Poster No. 109
Cancer Immunology and Immunotherapy Program

Circulating immune biomarkers of response in neoadjuvant pembrolizumab for cutaneous squamous cell carcinoma

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BACKGROUND: Cutaneous squamous cell carcinoma (cSCC) is the second most common skin cancer, with more than 2 million new cases yearly and an increasing incidence. Anti-PD-1 inhibitors have been approved in metastatic and non-operable locally advanced cSCC. To evaluate circulating immune biomarkers of response to therapy, we conducted a phase II single-arm neoadjuvant trial of pembrolizumab in patients with PD-1 naïve high-risk resectable cSCC.

METHOD: Patients with AJCC/UICC ≥T3 or N+ disease received pembrolizumab 200mg Q3W twice before surgery. We collected PBMCs pre- (D0) and on-treatment (D21 and D45) to evaluate peripheral immune biomarkers with multiparameter spectral flow cytometry (Cytek Aurora®). The preliminary findings included eight responders with pathological complete response and four non-responders (3 patients with pathologic non-response and one with pathological partial response).

RESULTS: Responders exhibited a transient increase (D21) of proliferating Ki67+CD8+ and Ki67+CD4+ T cells but not Ki67+Tregs compared to non-responders. Ki67+CD8+ and Ki67+CD4+ T cells upregulated multiple markers of T cell activation, including ICOS, Granzyme B, Perforin, and TIGIT. As compared to non-responders, Responders exhibited higher baseline frequency of Perforin+CD8+ T cells, higher on-treatment (D21) frequencies of ICOS+CD8+, ICOS+CD4+ T cells, terminally differentiated effector (TEMRA) CD8+ T cells. We also measured higher frequencies of CD14loCD16hi non-classical monocytes in responders compared to non-responders. No significant difference in the frequency and phenotype of circulating NK cells was observed.

CONCLUSION: Response to neoadjuvant anti-PD-1 in cSCC is associated with peripheral immune activation of CD8+ and CD4 +T cells and increased frequency of non-classical monocytes. Single-cell RNA-seq of PBMCs, tumor-infiltrating lymphocytes, and neoepitope discovery are ongoing.

Poster No. 110
Cancer Biology Program

Describing a 3D Model for Osteosarcoma Research: From Spheroids to Beyond

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INTRODUCTION: The use of 3D models ("spheroids") for clinical investigation have demonstrated to be a more accurate representation of tumor biology in vivo and possible tumor response to drugs and treatments. Nevertheless, spheroids have rarely been used in sarcoma. Our group has shown promising results using chondrosarcoma (CSA) primary cell populations in 3D culture where this model may better represent the patient tumors. This project aims to use spheroid culture in osteosarcoma.

METHODS: Five human osteosarcoma (OS) cell lines, 3 commercial (SaOS2, LM2, and MG63), and 1 patient-derived (OS15b) were cultured in their appropriate media. Cells were then plated in 96-well ultra-low attachment plates at 1x10^4 concentration and cultured for 7 days to create spheroids. On day 7, spheroids were prepped and Cell-Titer-Glo® 3D Cell Viability Assay solution was added. Luminescence was then recorded using a luminescence plate reader. Photographs of some of the spheroids were taken to assess quality, morphology, and volume of spheroids using the microscope imaging software.

RESULTS: All cell lines used yielded a 3D spheroid. LM2 demonstrated the highest luminesce compared to other cell lines. The human-derived spheroid (OS15b) produced spheroids with viability comparable to established cell lines. After 7 days of culturing and microscope evaluation, SaOS2 spheroid coincided with a gradual, stepwise increase in both spheroid viability and spheroid volume across 7 days. LM2, on the other hand, demonstrated a more disorganized increase in both viability and volume of the spheroids.

DISCUSSION: To our knowledge, the formation of spheroids with primary patient-derived OS cells has not been described. As we uncover the biochemical processes that drive metastatic disease in OS, novel, targeted, anti-metastatic therapies are a huge unmet need. Building novel 3D models of OS could provide more accurate preliminary data and a more precise depiction of the biology of OS, fueling future clinical trials.

Poster No. 111
Cancer Therapeutics Program

Targeting Xkr8 via nanoparticle-mediated in situ codelivery of siRNA and chemotherapy drugs for cancer immunochemotherapy

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The exposure of phosphatidylserine (PS) to the cell surface is regulated by the downregulation of flippases and the activation of scramblases, which plays an important role in tumor immunosuppression. As an important scramblase, Xkr8 has long been believed to be regulated at the posttranscriptional level and is activated by caspases during apoptosis. Here we report that Xkr8 was significantly induced in cancer cells at the transcriptional level by chemotherapeutic agents in vitro and in vivo. To facilitate the therapeutic translation, we developed a nanocarrier that is effective in codelivery of siRNA and chemotherapeutic drugs. Importantly, this nanocarrier is highly effective in tumor targeting through both EPR and targeting of tumor vasculature. Codelivery of Xkr8 siRNA and FuOXP (a prodrug conjugate of 5-FU and oxoplatin) led to significant improvement in antitumor activity in colon and pancreatic cancer models along with increase in CD45+ cells, decrease in Treg cells, and reversal of FuOXP-induced decrease in M1/M2-like ratio. Targeting Xkr8 in combination with chemotherapy may represent a novel strategy for the treatment of various types of cancers.

Poster No. 112
Cancer Biology Program

A novel algorithm for full-resolution registration of giga-pixel images with sub-cellular accuracy

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A comprehensive understanding of the pathobiology of cancer development is essential for its early detection and effective treatment. While two-dimensional (2D) analysis of H&E-stained whole slide tissue sections is the gold standard in anatomic pathology, three-dimensional (3D) analysis is necessary for providing a more holistic understanding of the tumor microenvironment. By reconstructing the tumor and its surroundings in 3D, complex patterns and interactions between cells can be identified, which may be difficult to discern in 2D. In addition, 3D analysis can assess spatial relationships between different cellular and acellular components of the tumor microenvironment which can inform treatment decisions.

3D reconstruction of tumor microenvironment is typically done by registering down-sampled whole slide images of its 2D serial sections. Although downsampling provides good registration, the resulting low-resolution reconstruction results in information loss and a less accurate characterization of the 3D architecture of the tumor microenvironment. Ideally, we would like to reconstruct images at the full resolution they were acquired. However, such full-resolution registration of gigapixel images remains a challenge due to memory and computational constraints. To address this challenge, we have developed a new approach that utilizes a sparse representation of the whole slide tissue sample to align serial sections at full resolution and achieve their accurate sub-cellular registration without compromising speed and efficiency.

Poster No. 113
Cancer Therapeutics Program

Magnetic Hyperthermia Therapy in Combination with Chemoradiation for the Treatment of Glioblastoma

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INTRODUCTION: Glioblastoma (GBM) is an aggressive primary brain cancer with significant resistance to the current therapeutic approach of chemotherapy and radiotherapy, jointly known as chemoradiation (CRT). Magnetic hyperthermia therapy (MHT) is a promising therapy for GBM that can be used to perform multiple sessions of non-invasive, localized hyperthermia by activating locally delivered magnetic iron oxide nanoparticles (MIONPs) with an external alternating magnetic field (AMF). In this study, MHT-mediated enhancement of CRT was evaluated in murine and human glioma cell lines both in cell culture and in rodents.

METHODS: The heating profile of MIONPs was assessed in the test tube and mouse brain in vivo. Computed tomography scan and magnetic particle imaging were used to confirm intracranial MIONP localization after convection enhanced delivery. Cell viability assays were performed following treatment with MHT and/or radiation. MHT-induced alterations to the tumor microenvironment were assessed in a syngeneic murine glioma model, and a survival study was performed in a GBM patient-derived xenograft (PDX) model to investigate synergism between MHT and CRT.

RESULTS: Significantly increased survival was observed in mice treated with MHT+CRT compared to CRT alone in a therapy-resistant GBM PDX model. In vitro studies demonstrated that MHT with radiation was more cytotoxic than radiation or MHT alone. Additionally, MHT with CRT significantly increased tumoral expression of biomarkers for DNA double-strand breaks (□-H2AX), CD8+ T cell recruitment (CD8), and inflammation (P-selectin) compared to CRT alone, suggesting MHT-mediated radio-sensitization and immune cell recruitment to the tumor. MIONP heating was confirmed in the test tube (93.3 oC) and intracranially (50.7 oC) within minutes of AMF exposure, and localization of MIONPs to the delivery site was verified with imaging.

CONCLUSIONS: Adjuvant MHT may induce tumor radio-sensitization, immune cell recruitment, and survival benefit when combined with CRT. Optimization of the combination therapy treatment scheme is required.

Poster No. 114
Cancer Immunology and Immunotherapy Program

Prognostic Impact of Statins for Early-Stage Non-Small Cell Lung Cancer Patients following Image-Guided Radiofrequency Therapy

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Background: Lung cancer is the leading cause of cancer-related mortality worldwide. NSCLC accounts for 85% of lung cancers.[1] Surgical resection is not feasible for 25% of early-stage NSCLC patients.[2] Image-guided RFA is a well-recognized option for these patients.[3] There is an interest in the use of statins for their antitumor effects particularly in high-risk NSCLC patients.[4,5] We investigated whether statin use was associated with overall survival (OS), progression free survival (PFS), and response following RFA.

Methods: Patients with biopsy proven stage I NSCLC who underwent image-guided RFA from 2001-2018 were reviewed. Primary outcomes were OS, PFS, and response at 3-6 months post-RFA (RECIST criteria).[3] Statin use at surgery, 1-year postop, and any time 1-year preop to last follow-up was collected. Univariable analyses were performed by Wilcoxon rank sum tests for continuous variables, and Chi-square and Fisher's exact tests for categorical variables. Unadjusted survival models were performed by the Kaplan-Meier method.

Results: 111 patients (60 females; median age 74) underwent RFA for NSCLC. Pack years at surgery was a significant predictor of OS (HR:1.01, CI: 1.00-1.01, p=0.015). Medication data was unavailable at time of surgery for 1 (0.9%) patient and at 1-year follow-up for 30 (27%) patients. At surgery, 49 (44.5%) patients were on statins. Twenty-two (45%) of these patients had squamous cell carcinoma (SCC) versus only 16 (26%) patients not on statins (p=0.009). Body mass index (BMI) was 29 kg/m^2 (IQR:24-33) for patients on statins compared to 26 (IQR:22-30) for patients not on statins at surgery (p=0.059). Statins were not significantly associated with OS, PFS, or response.

Conclusions: Statin usage was not significantly associated with outcome. Patients on statins had more SCC tumors which are associated with worse outcomes. Smoking history at surgery was associated with oncologic outcomes. Further investigation with a larger cohort and adjusted for confounding variables is planned.

IRB / IACUC / CORID / QA approval number or exemption: 20050098

Poster No. 115
Cancer Immunology and Immunotherapy Program

CD8+ T Cell Landscape in Neoadjuvant TLR9 Agonist and Anti-PD1 Antibody for Melanoma

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BACKGROUND: TLR9 activation induces type I IFN production, which can enhance T cell function. Adding a TLR9 agonist to existing anti-PD1 agents may overcome some of the treatment resistance seen with anti-PD1 alone. We conducted the first neoadjuvant clinical trial with Type A TLR9 agonist Vidutolimod and anti-PD-1 Nivolumab in patients with resectable melanoma. How the intra-tumoral and peripheral CD8+ T cell landscape changes during this combination treatment is currently unknown.

METHOD: To fill this void, we conducted scRNAseq on tumor and matched PBMC samples collected pre- and post- treatment from patients on this trial. All samples underwent scTCR enrichment for clonotype analysis. The PBMC scRNAseq samples included a panel of over 200 oligo-tagged surface antibodies to improve cell type identification and to investigate protein-level changes.

RESULTS: A major pathologic response (mPR) rate of 55% was observed. In the tumor, responders show increased cytotoxic and MHC class II expressing CD8+ T cells, while non-responders show a more naïve and terminally exhausted profile. In the blood, responders show increased central and ki67+ effector memory CD8+ T cells, while non-responders show more naïve CD8+ T cells. A treatment effect post- vs pre- treatment is present in intra-tumoral and peripheral CD8+ T cells in responders but is absent from non-responders.

CONCLUSION: Combining the TLR9 agonist Vidutolimod with the anti-PD1 agent Nivolumab leads to an improved mPR over what has been reported with anti-PD1 alone. Responders to the combination treatment show an activated CD8+ T Cell profile that is distinct from non-responders. This work gives us insight into how a combination TLR9 agonist and anti-PD1 antibody leads to better outcomes than anti-PD1 alone.

Poster No. 116
Cancer Immunology and Immunotherapy Program

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 and 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 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 motif. This adaptor forms a covalent bond with the SNAPtag receptor, which leads to binding and effector functions targeting a tumor cell antigen. We generated several small molecule adaptor variants targeting carbonic anhydrase IX (CAIX), folate receptor alpha (FOLR1) and beta (FOLR2), programmed-death ligand 1 (PD-L1), and prostate-specific membrane antigen (PSMA) with different linker lengths. In vitro, we co-cultured SNAP-CAR cells with a dose titration of CAIX targeting adaptors on a glioblastoma cell line, U87-MG, which expresses CAIX at physiological levels, and measured T cell activation and tumor cell lysis via flow cytometry. We demonstrated robust dose-dependent CAR T-cell activation and specific cytotoxicity where different linker lengths played a significant role. We designed caged adaptors reactive to reactive oxygen species (ROS) to address on-target and off-tumor toxicity. We synthesized a caged BG precursor that shows SNAPtag binding in the presence of H202 (ROS). This shows promise for generating conditional targeting for SNAP-CAR.

Poster No. 117
Cancer Immunology and Immunotherapy Program

Reprogramming T cell Glycosylation: a novel immunotherapeutic strategy in Colorectal Cancer treatment.

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Colorectal cancer (CRC) is the second leading cause of cancer related death worldwide, remaining a serious public health problem in developed regions. Despite the clinical success of immunotherapy, only a minority of CRC patients benefit from this therapeutic modality, highlighting the urgent need for identifying novel mechanisms underlying cancer immunoregulation envisioning the improvement of CRC immunotherapies.

Changes in glycosylation are a hallmark of cancer. Glycans play a pivotal role in each pathophysiological step of malignant transformation including in cancer immunoediting. Recent evidence highlights the importance of glycans as relevant immune checkpoints in cancer immunosurveillance and immunoediting. Moreover, glycans have been shown to play a key role in the regulation of T cell-mediated immune response.

In this study, we investigated the impact of the reprogramming of T cell glycosylation in CRC interception and treatment. Using colorectal cancer mouse models, we observed that along tumor progression there is a gradual increase in the expression of complex N-glycans, that directly correlates with expression of exhaustion markers in tumor infiltrating T cells. Furthermore, we used CRISPR-Cas9 to alter the glycoprofile of therapeutic T cells by deleting crucial glycosyltransferases. After confirming the effects of these deletions on the glycoprofile of the cells, we used them to treat MC38-bearing mice. This revealed a decrease tumor growth due to an increased anti-tumor immune response of these T cells. Taken together, these results suggest the key role of glycans in cancer immunosurveillance, supporting the importance of T cell glycosylation as a promising target for novel immunotherapeutic strategies.

Poster No. 118
Cancer Biology Program

Identifying genes signatures associated with inflamed and remission status in patients with inflammatory bowel disease

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Introduction. Inflammatory bowel disease (IBD) – ulcerative colitis (UC) and Crohn's disease (CD) – is a chronic gastrointestinal (GI) inflammatory disorder with relapsing-remitting symptoms. It is characterized by abdominal pain, bloody diarrhea, and risk of CRC development, and is growing in incidence and prevalence worldwide. Given its heterogeneous etiology and relapsing and remitting nature, most patients require long-term therapy to induce and maintain remission. However, due to its heterogeneity, there is considerable patient to patient variability in response to therapeutic treatments in individual patients. Therefore, a major challenge in designing therapeutic treatments and developing new ones is the robust and objective characterization of this variability in patient's remission status and its prognosis. To overcome this challenge, we have begun to systematically study the inflamed and remission status of patients with IBD, including UC and CD, and identify gene expression signatures uniquely associated with each. Materials/Methods. Here, we present our preliminary results of RNA-seq based analysis of 24 UC and 24 CD patients – each comprising 12 inflamed and 12 remission patients – along with 6 healthy patients as controls (HC). Results/Conclusion. Based on our analysis we identified distinct gene profiles for CD and UC concordant with inflammation and remission status. For UC patients we found that overexpression of CSF3, SERPINB3, SERPINB7, TCN1, and TNIP3 was strongly associated with inflamed status, while overexpression of ANXA10, BPIFB1, CSF3, CXCL8, CXCR1, FPR2, MUC6, and PGC was strongly associated with inflamed status in CD patients. Interestingly, we found MUC5AC and NPSR1 to overexpress in UC patients experiencing inflammation and remission. Importantly, we also identified differential gene signatures separating inflamed and remission status in UC (CXCL5, KLK6, SERPINB4) and CD (AQP9, GKN2, PROK2) patients. Visualization of these gene signatures showed a gradual transition from HC to inflammation via remission suggesting that our signatures capture the IBD disease trajectory.

Poster No. 119
Cancer Virology Program

Methyltransferase-independent function of Ezh2 maintains tumorigenicity induced by human oncogenic polyomavirus and papillomavirus

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Merkel cell polyomavirus (MCV) and high-risk human papillomavirus (HPV) are human tumor viruses that respectively cause Merkel cell carcinoma (MCC) and oropharyngeal squamous cell carcinoma (OSCC). The HPV E7 and MCV large T (LT) oncoproteins both target the retinoblastoma tumor suppressor protein (pRb) through the conserved LxCxE motif. Previous results indicated that enhancer of zeste homolog 2 (EZH2) is a host oncoprotein that is activated by both HPV E7 and MCV LT through the pRb binding motif. EZH2 is a catalytic subunit of the polycomb repressive complex 2 that trimethylates histone H3 and lysine 27 (H3K27me3). MCC tissues expressed EZH2 at high levels regardless of MCV status. Loss-of-function studies also showed that viral HPV E6/E7 and T antigen expression are required for Ezh2 mRNA expression and that Ezh2 is necessary for continued HPV(+)OSCC and MCV(+)MCC cell growth. Ezh2 protein degraders were found to significantly reduce cell viability and rapidly in both HPV(+)OSCC and MCV(+)MCC cells, whereas EZH2 histone methyltransferase inhibitors did not affect cell proliferation or viability within the same treatment period. These results suggest that there is a methyltransferase-independent function of EZH2 the contributes to tumorigenesis downstream of the pRb binding ability of two viral oncoproteins, and that direct targeting of EZH2 protein expression could be a promising therapeutic strategy for inhibiting tumor growth in HPV(+)OSCC and MCV(+)MCC patients.

Poster No. 120

Cancer Immunology and Immunotherapy Program

The temporal contribution of interferon-γ in driving T-cell exhaustion and response to immune checkpoint blockade.

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Background: Interferon-gamma (IFN-γ) drives protective T cell responses and augments anti-tumor immunity yet also promotes T cell exhaustion. Such dichotomy impacts immunotherapeutic responses. However, how IFN-γ modulates immune responses on exhausted T (TEX) cells remains unanswered. We hypothesize understanding temporal expression profile of IFN-γ on Lag3+ TEX cells will help address mechanistic insights into diverse responses to immune checkpoint blockade (ICB).

Methods: We established unique murine models, to assess tamoxifen induced temporal expression of IFN-γ on Lag3+ cells (Lag3iCreERT2 IfnγYFP Rosa26LSL-tdTomato) and temporal genetic deletion of IFN-γ and IFN-γR1 on Lag3+ cells (Lag3iCreERT2 IfnγL/L Rosa26LSL-tdTomato and Lag3iCreERT2 IfnγR1L/L). These models were used to evaluate tumor growth and survival kinetics, TEX profile, response to ICB therapy and memory phenotype in melanoma and adenocarcinoma.

Results: Transcriptional profile illustrates, LAG3+ NK and NKT cells as major contributors of IFN-γ at progenitor TEX state and CD8+ T cells at terminal-TEX. Lag3iCreERT2 IfnγL/L Rosa26LSL-tdTomato model shows temporal deletion of IFN-γ in time-dependent manner from LAG3+ cells may not effect tumor growth and survival in B16 and MC38 models. Deletion of IFN-γ in LAG3+ cells from progenitor TEX state (TCF1+ TIM3-) leads to loss of CD8+ T cells and CD8+ PD1high population. IFN-γ+ LAG3+ cells are required for reinvigoration and maintenance of central memory phenotype.

Conclusions: This study highlights distinct temporal response and exhaustion profile with Ifnγ deletion (pre-exhausted vs terminally exhausted Lag3+ cells). IFN-γ from LAG3+ cells is a key mediator of anti-tumor immunity and memory response.

Poster No. 121
Cancer Biology Program

Ovarian Cancer Cell Fate Response to Pharmacologic Treatment

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Chemotherapies available for oncologic treatment often cause two types of responses within treated cancer cells: cell death or cell cycle arrest. Though both responses are favorable compared to active proliferation, cell cycle arrest can lead to re-entry into the cell cycle and continued growth or relapse of tumors. Cis-diamminedicholoroplatinum II (cisplatin) treatment in ovarian cancer is very effective in initial treatment, but is also associated with high risk of relapse. Multimodal investigation into ovarian cancer cell fate response was conducted, consisting of timelapse analysis of live cells and multiplex immunofluorescence imaging.

Timelapse experimentation was conducted using the OVCAR-8 cell line with DHB mvenus and 53BP1 mapple biosensors. Initial testing showed arrested cells shut down cyclin-dependent kinase 2 (CDK2) activity; these cells became abnormally large, with some re-entering the cell cycle to become polyploid. In apoptotic cells, CDK2 activity was highly upregulated. Subsequent experiments showed that less cell death was observed in the presence of Wee1, a CDK2 inhibitor. Likewise, when Wee1 was inhibited, more cell death was observed.

Multiplexed immunofluorescence imaging was also conducted, providing detailed molecular signatures of over 40 cell cycle effectors within each cell. This high-dimensional dataset was reduced into 2- and 3-dimensional structures showing the progression of the cells through the cell cycle. Distinct cell cycles were observed between cisplatin-treated and control populations. These structures suggested arrest of treated cells occur in late S phase or in G2 phase. Highly similar molecular signatures between mitotic cells and therapy-induced apoptotic cells were also observed, suggesting cell death may be associated with failed division after cisplatin treatment.

Poster No. 122
Cancer Biology Program

MET-abolism: Targeting glycolysis in MET-driven non-small cell lung cancer brain metastases

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Non-small cell lung cancer (NSCLC) is the most common primary tumor to metastasize to the brain, with up to 40% of NSCLC patients developing brain metastases (BM). While novel drugs and immunotherapies have improved lung cancer survival, there is a need for therapies that specifically target NSCLC BM. Our lab identified a significant enrichment of *MET*-amplification in lung adenocarcinoma (LUAD) BM (16%) compared to primary NSCLC (3%) and liver metastases (5%). MET, a receptor tyrosine kinase, and its ligand, hepatocyte growth factor (HGF) are involved in cell proliferation, epithelial-mesenchymal transition (EMT), angiogenesis, and metastasis. MET tyrosine kinase inhibitors (TKIs) are approved for use, but half of patients with *MET* alterations fail to respond or develop resistance. We found that *MET*-amplified BM had distinct transcriptional signatures reflecting increased glycolysis. A *MET*-amplified metastatic LUAD cell line (H1993) showed higher metabolic activity, ATP production, and glycolytic flux than a *MET*-wild type LUAD line (H2073) derived from the same patient at the primary site. Furthermore, LUAD cell lines with *MET*-amplification or high MET expression demonstrated increased expression and activity of glycolytic enzymes, as well as increased susceptibility to glucose deprivation and glycolytic inhibitors. Conversely, *MET*-amplified cells treated with MET TKIs showed a metabolic phenotype comparable to non-*MET*-amplified lines. Activation or inhibition of the MET pathway primarily increased or decreased respectively, the mRNA and protein expression of the key glycolytic enzyme, Hexokinase II (HK2). We have found that the HGF/MET pathway upregulates expression of the EMT transcription factor, TWIST1 and TWIST1 is required for upregulation of HK2 on the transcriptional level. Ongoing experiments are exploring whether HK2 is a direct TWIST1 transcriptional target and if inhibiting TWIST1 and the glycolytic pathway is efficacious in *MET*-amplified LUAD BM. In summary, these studies suggest a targetable, metabolic reprogramming undergone by *MET*-amplified LUAD BM.

Poster No. 123
Cancer Immunology and Immunotherapy Program

γδ T cells support pancreatic cancer immunity by enhancing αβ T cell activation

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The prognosis for pancreatic ductal adenocarcinoma (PDAC) remains dismal due to limited options for surgical resection and minimally effective treatments. The value of patient-derived organoids (PDOs) centers on their ability to recapitulate the parental tumor. We aim to improve outcomes in patients with PDAC by advancing combination cell immunotherapies, utilizing PDOs to assess tumor-specific reactivity of expanded TIL. PDOs and TIL were simultaneously expanded from resected primary PDAC tissue. γδ TIL were negatively selected from harvested bulk TIL and further expanded in separate cultures, followed by phenotyping, TCR sequencing, and immunoassays. Whereas bulk TIL consisted primarily of αβ TCR+ cells, γδ-enriched TIL were comprised of varying proportions of NK, αβ TCR+, and γδ TCR+ cells. γδ TIL appeared to play an important helper role, as αβ TCR+ cells expanded in the presence of γδ TIL demonstrated an enhanced activation profile. PDOs exhibited the tissue architecture and cellular morphology of ductal adenocarcinoma on H&E staining, namely, ductal structure and prominent nucleoli. Both TIL cultures were functionally active, producing IFNγ following mitogenic stimulation; however, the γδ-enriched population was more potent than αβ TIL in recognizing and lysing autologous PDOs. TCR sequencing showed that γδ-enriched cultures were dominated by fewer, more clonally expanded uCDR3s in the Vβ and Vδ repertoires. Importantly, infiltration of γδ T cells within pancreatic tumor tissue, assessed in the TCGA database, is associated with improved overall survival. Together, these findings highlight the potential benefit from adoptive cell transfer of expanded γδ-enriched TIL in patients with PDAC.

Poster No. 124
Cancer Biology Program

Spatial landscape of malignant pleural and peritoneal mesothelioma tumour immune microenvironment

Presenter: Elena S Kim (1,4)

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(Original order of authors is: Xiaojun Ma, David Lembersky, Elena S Kim, Tullia C Bruno, Joseph R Testa, Hatice U Osmanbeyoglu)

Malignant mesothelioma is an aggressive cancer with ~3'000 new cases diagnosed in the U.S. yearly. The most common, plural mesothelioma comprises 80% of cases and has the 5-years survival rate <10%. The general treatment, immunotherapy has shown modest clinical benefit with extending overall survival for several months only. A deeper understanding of composition of the tumor immune microenvironment (TIME) with spatial distribution of the immune cell within is needed to identify interactions between tumor and different immune cell types that might help to revisit and improve the efficacy of currently used and potential immunotherapy strategies.

To elucidate the spatial distributions of major immune cell populations in patients with malignant peritoneal (n=25) and pleural (n=88) mesotheliomas (MPM and MPeM, correspondingly), clinical tissue microarrays (n=3) were characterized with high throughput multiplex immunofluorescence. The assessed TIME cells spatial distributions were analyzed in their association with tumor suppressor genes LAG3, BAP1, NF2, and MTAP expression as a proxy to CDKN2A. We further analyzed the relationships between the spatial distribution of different immune cell subpopulations with MM patient survival prognosis and clinical features.

The distribution of immune cells within TIME was shown to be similar between MPM and MPeM. However, there was a higher level of interaction between immune cells and tumor cells in MPM than MPeM. Within MPM tumors, there was a higher level of interaction between immune cells and CD8+ T cells in "BAP1 high" tumors compared to "BAP1 low" tumors. The identified patterns of cell-to-cell interaction could potentially be implicated in the immune response against the tumor and could be the factors in the different behaviors of these two types of mesotheliomas.

Our study exemplifies the utility of spatial resolution within single-cell analyses in translational research and provides a valuable resource for finding strategy for personalized medicine and targeted therapy of mesothelioma.

Poster No. 125
Cancer Biology Program

Differences in real-world pembrolizumab dosing patterns (every 3 weeks versus every 6 weeks) across a large cancer center network.

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Pembrolizumab (pembro) is an intravenous immune checkpoint inhibitor used for anti-cancer treatment, with equivalent monotherapy dosing options of 200 mg every 3 weeks (Q3W) or 400 mg every 6 weeks (Q6W). Pembro Q6W dosing was approved for use in April 2020 after the Q3W dose had already been established. As pembro dosing patterns in real-world practice are not well-described, we evaluated this in a large cancer center network consisting of a central site (CS) and 36 community network sites (NS).

We retrospectively reviewed the charts of all patients who received pembro monotherapy for cancer treatment between 4/2020 – 4/2022 at UPMC Hillman Cancer Center sites across the state of Pennsylvania, as well as sites in New York and Ohio. We performed statistical comparisons using the Fisher's exact test and Wilcoxon rank-sum test.

We identified 1,640 patients who received pembro monotherapy, including 377 patients at the CS and 1,263 patients in the NS. Pembro Q6W dosing was significantly more common at the CS than in the NS (42% vs. 15%, $p<0.0001$). When analyzed by tumor type, pembro Q6W dosing was significantly more common at the CS than in the NS for head & neck (53% vs. 11%, $p<0.0001$), thoracic (49% vs. 15%, $p<0.0001$), skin (60% vs. 19%, $p<0.0001$), and genitourinary (30% vs. 16%, $p=0.015$) cancers, but not for other tumor types. Within the NS, 30% (11/36) of sites only utilized pembro Q3W dosing and never administered Q6W dosing to patients.

In our large cancer center network, pembro Q6W dosing was significantly more common at the CS than in the NS. Further work is needed to clarify the patient, provider, and institutional factors influencing differences in pembro dosing patterns in real-world practice, as well as the impact of dosing patterns on care delivery outcomes such as cost and accessibility.

Poster No. 126
Biobehavioral Cancer Control Program

Implementing Research Electronic Data Capture (REDCap) platform in a multi-site randomized clinical trial enrolling individuals with serious illnesses: A practical example

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Background: Maintaining personalized and tailored communication presents a substantial challenge in randomized clinical trials (RCT) involving adults with serious illnesses. The STRONG RCT, which aims to test a serious game teaching self-advocacy skills to women with advanced cancer, uses the Research Electronic Data Capture (REDCap) platform to collect patient-reported outcomes, track and enhance participant engagement, monitor intervention fidelity, and facilitate communication between team members and sites.

Purpose: The purpose of this presentation is to review our team's approach to harness REDCap for RCT engagement and retention among seriously ill, diverse participants.

Methods: We utilize the various capabilities of REDCap for electronic consent and stratified randomization, automated survey invitations, reminders, and alerts, and develop forms to track intervention fidelity. REDCap can build trust and facilitate engagement by accommodating various means of communication (i.e., emails, phone calls, text messages, and mails) and allowing at-home data collection (i.e., by phone or mail). Automated alerts prompt timely attention from the research team on adverse events, including clinically significant anxiety/depression, and inactive engagement in the intervention.

To facilitate seamless coordination and enhance the RCT recruitment and management process between diverse stakeholders and the research team, the clinical research coordinators and regulatory managers from collaborating clinical sites are granted access to REDCap. Communication logs are established to record questions or concerns from these seriously ill participants. This practice ensures a shared understanding of the participants' conditions between team members and sites, prevents redundant questions, and alleviates potential participant burden.

Conclusion: REDCap is a multifaceted and effective tool for personalized, tailored engagement within RCTs involving adults with serious illnesses and for communication between research team members and partners from multiple clinical sites. Utilizing REDCap helps our RCT conduct effective trial management and manage a balance between participant engagement and participation burden with an emphasis on inclusivity.

Keywords: Research Electronic Data Capture, REDCap, randomized controlled trials, intervention, serious game, data collection, data management.

Poster No. 127
Cancer Therapeutics Program

Image-Guided Radiofrequency Ablation for Treatment of Limited Pulmonary Metastases

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Objective: Surgical resection is the preferred treatment in patients with limited pulmonary metastases (LPM). In high-risk patients, image-guided radiofrequency ablation (RFA) may offer alternative treatment option for aggressive local control. Our objectives were to evaluate the outcomes after RFA for LPM.

Methods: We retrospectively reviewed outcomes of 71 patients treated with image-guided RFA for LPM at our center. Patients were selected using a multidisciplinary approach and were subsequently followed in clinic. Primary endpoint was overall survival. Complication rates were also assessed.

Results: Seventy-one (37 men; 34 women) with limited metastatic lesions to the lung were treated with RFA. Median age was 68 years (range 23-84 years). Primary tumor histology included colorectal (n=34), head and neck (n=8), breast (n=3), sarcoma (n=10), and other (n=16). During follow-up, four patients are alive at times from 25 to 221 months after treatment. No perioperative deaths occurred. Median survival after treatment was 27 months (95% confidence interval=21-39 months). Estimated 2-year and 3-year, and 5-year overall survival was 55%, 42%, and 23%, respectively.

Conclusions: Surgical resection remains the standard for resectable LPM patients. However, image-guided RFA may offer an alternative in selected patients with comorbidity precluding operative intervention. In this study, we demonstrate safety of RFA in selected patients for treatment of LPM. Survival after treatment with RFA was reasonable in this high-risk cohort with metastatic disease. Future studies are needed in larger cohorts of patients to evaluate optimal role of RFA compared to other ablative therapies and surgery in aggressive local control of pulmonary metastases.

Poster No. 128
Biobehavioral Cancer Control Program

Memory and Attention Adaptation Training for Patients with Gastrointestinal Stromal Tumor: Case Studies

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Cancer-related cognitive impairment (CRCI) is well-documented among cancer survivors and can lead to disruptions in health-related quality of life. CRCI is also prevalent among survivors of gastrointestinal stromal tumor (GIST), a disease commonly treated with tyrosine kinase inhibitors (TKIs; e.g., imatinib mesylate or Gleevec). In our previous study (Cancer 2022; 128:4017-4026), a majority of GIST patients (64%) reported cognitive symptoms that negatively impact quality of life, more frequently reported among those on long-term TKIs. Memory and Attention Adaptation Training (MAAT) is an evidence-based short-term cognitive-behavioral therapy (CBT) shown to improve cognitive function and quality of life for survivors with CRCI. MAAT is delivered via telehealth in 8 weekly 45-minute visits and as a CBT reimbursed by health insurance payors. Here, we report cognitive function and quality of life outcomes of 2 patients with GIST on long-term TKIs. Standardized telephone-based neuropsychological tests were administered at pre-treatment and post-treatment, along with validated online patient-reported outcome measures (PROs): perceived cognitive impairments, fatigue, mood, anxiety, pain, and quality of life. Patient alertness, medication use, alcohol consumption, and caffeine intake were recorded at each timepoint, and demographic data was collected at baseline. Both patients demonstrated improved processing speed (Symbol Digit Modalities Test; SDMT) and verbal fluency (Controlled Oral Word Association; COWA) at post-treatment but no improvement in verbal memory (California Verbal Learning Test; CVLT). Both patients made clinically significant improvements in PROs of perceived cognitive impairments and fatigue. These pilot case data suggest MAAT is a practical, effective CBT for GIST survivors with CRCI. Research is needed with more GIST survivors to test MAAT efficacy, ideally with randomized clinical trials. Although GIST is a rare tumor, MAAT can be tested with telehealth technology and remote data capture, allowing for the numbers of GIST survivors necessary to achieve adequate power for a valid clinical study.

Poster No. 129
Biobehavioral Cancer Control Program

Trajectories of symptom burden increase throughout treatment in patients with head and neck cancer

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Significance: Increased treatment intensity and multimodal therapies are associated with severe symptoms in patients with head and neck cancer(HNC). Little is known about the trajectories of these symptoms over time.

Purpose: The purpose of this secondary analysis was to characterize the average weekly symptom severity and interference trajectories of patients with HNC and explore potential predictors.

Methods: This study used a prospective repeated measures design involving patients with squamous cell HNC, recruited in the HNC Clinic of the Department of Otolaryngology in the UPMC Ear and Eye Institute. Participants were enrolled prior to radiation and followed through 3-months post-treatment. Symptoms were measured using the MD Anderson Symptom Inventory Head and Neck Module. Due to variability in assessment timing, symptom scores were averaged weekly.

Results: Patients (N= 176) were on average 62±17years old, white(85.7%), and male(74.0%), with tumors of the oropharynx(46.6%), and oral cavity(21%), and stages III-IVc(52.9%) HNC. Most did not have surgery(53.4%) and received cisplatin chemotherapy(84.5%). Random coefficient modeling revealed that symptom interference and severity, and HNC severity differed significantly from pretreatment($p<.05$). The only predictor that improved the model significantly was HPV+ status in the symptom interference model($p=.021$). Cisplatin chemotherapy vs any other regimen significantly moderated the time effect in the linear model for Core HNC symptom severity ($p<.001$). ADI national moderated the time effect in the linear model for symptom severity score($p=.039$). Early stage($p<.001$) and HPV+ status($p\leq.011$) moderated the time effect in the linear model for all three symptom scores.

Discussion: Symptom severity and interference may be most severe and persist throughout treatment. Predicting symptom severity may mitigate the problem before their peak severity.

Poster No. 130
Cancer Immunology and Immunotherapy Program

Development and Modulation of Tertiary Lymphoid Structures in lung cancer for improved immunotherapeutics

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Tertiary lymphoid structures (TLS) are lymphoid aggregates that often form locally in tissues with chronic infection, autoimmune disease, and cancer. They provide optimal crosstalk between B and T cells in cancer patients. TLS correlate with favorable prognosis and survival in patients with solid tumors, including non-small-cell lung cancer (NSCLC). Further, TLS have recently been associated with superior response to immune checkpoint blockade (ICB). Despite the therapeutic potential of TLS, TLS have not been targeted in the tumor microenvironment due to the complexity of these ectopic structures in cancer patients. To that end, TLS have varying cellular states with mature TLS having dense immune aggregates and an active germinal center (GC). Moreover, the mechanisms of TLS development remain poorly understood due to paucity of murine models with spontaneous TLS development as in humans. Therefore, our project utilizes a tobacco carcinogen (4-(methylnitrosamino)-1-(3- pyridyl)-1-butanone; NNK) induced murine model of lung adenocarcinoma (LUAD) that not only recapitulates human LUAD but also spontaneously develops TLS at a similar frequency as in humans. This model enables us to assess the temporal evolution of TLS and garner a mechanistic understanding of TLS development. In parallel with our mouse studies, our lab also evaluates the complexity of TLS within human NSCLC using multispectral imaging and spatial transcriptomics. We have discovered that TLS in patients can have incomplete expression of inducing factors such as LIGHT/LTβ, CXCL13, CD40 ligand (CD40L), and IL-21. Thus, we have generated an oncolytic virus (OV) to deliver these factors, create immunogenic antigens, and generate stromal space for TLS to thrive. We will utilize two syngeneic lung cancer murine models to test our OV; (1) FVBW-17, derived from NNK induced LUAD murine model, and (2) lewis lung carcinoma (LLC), which was established from a spontaneously developed lung carcinoma that does not form TLS. According to our preliminary data, we have observed increased formation and improved maintenance of TLS upon treatment with an OV that delivers LIGHT, CXCL13, IL-21 and CD40 ligand, compared to an empty vector in FVBW-17 subcutaneous model. At a cellular level, there was an increase in the number of effector T cells and B cells and a decrease in the number of Foxp3+ regulatory T cells in tumors treated with OV plus all four TLS-inducing factors. These studies will increase our mechanistic understanding of TLS development for improved immunotherapies in NSCLC patients and will potentially provide therapeutic interventions to treat patients.

Poster No. 131
Cancer Immunology and Immunotherapy Program

Speculating the response to neoadjuvant immunotherapy using microbiome signatures accompanying formation and maturation of Tertiary lymphoid structures (TLS)

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Immune Checkpoint Inhibitors have revolutionized cancer treatment in patients since FDA approval. However, majority of the population doesn't respond to anti-PD1 treatment. Although PD-L1 expression status and microsatellite instability provide some assistance in predicting anti-PD1 response, the need for predictive biomarkers with increased proficiency is necessary. Recent studies have shown certain gut microbiome and TLS formation to be beneficial markers for positive prognosis. However, whether the microbes or TLS drive the outcome of immunotherapy remains unknown. In the current study, we sought to assess the presence of microbe-containing TLS in melanoma patients receiving Pembrolizumab and high dose interferon to determine if bacteria are a driving force of TLS maturity and potentially immunotherapy response. Tumor biopsies were collected before administration of the treatment (Baseline) and after surgery (Post), and the tumor sections were analyzed for presence of TLS using CD20 staining. The sections were then stained using Fluorescence in situ hybridization (FISH) and examined for microbial signatures localized near or within TLS. Finally, comparative development and maturation of TLS from baseline to post-surgery samples were correlated with microbiome signatures and the patient response to immunotherapy. Patients who had a poor immunotherapy response and had a disease recurrence post-surgery were found with very little to no bacteria associated TLS before surgery. Whereas most of the patients found with helicobacter hepaticus associated TLS before or after surgery had a complete or partial response to immunotherapy with no further reports of disease recurrence post-surgery. Altogether, these results indicate that presence of immune modulating microbiome might be responsible for TLS generation and positively driving immunotherapy response.

Poster No. 132
Biobehavioral Cancer Control Program

Social Determinants of Sleep Quality in Patients Diagnosed with Cancers Affecting the Hepatobiliary System

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Background: Social determinants of health (SDOH) and sleep quality have both been associated with morbidity and mortality in patients with cancer, but few studies have examined the relationships between SDOH and sleep quality in this population. The aims of this study were to examine (1) the rates of SDOH indicators in patients diagnosed with cancers affecting the hepatobiliary system, and (2) the associations between Electronic Health Record (EHR)-based SDOH and sleep quality.

Methods: Patients completed a battery of questionnaires which included the Pittsburgh Sleep Quality Index (PSQI) and 10 EHR-based SDOH. Linear regression was used to examine the association between total SDOH risk scores and sleep quality. Independent samples t-tests were used to examine the between group differences in sleep quality by each SDOH risk factor.

Results: A total of 339 patients were enrolled in the study (mean age=61.52; SD=11.44). The majority of patients were male (59.0%) and white (non-Hispanic; 90.3%). The average PSQI score was 7.57 (SD=4.06). On average, patients reported 2.47 SDOH risk factors (SD=1.84). After controlling for age and disease-specific predictors, patients' total number of SDOH risk factors significantly predicted PSQI scores [β=1.05, p<.001]. After adjusting for multiple comparisons (set at p=.005), between group differences were observed with poorer sleep quality in patients endorsing SDOH risk-factors including depression [t(318)=-8.19, p<.001], high perceived stress [t(326)=-6.41, p<.001], financial resource strain [t(67.07)=-5.57, p<.001], housing instability [t(326)=-4.38, p<.001], transportation problems [t(327)=-4.24, p<.001], social isolation [t(315)=-4.82, p<.001], tobacco use [t(336)=-3.47, p<.001], and drug use [t(333)=-3.69, p<.001]. There were no significant differences in sleep quality by alcohol use [t(333)=1.17, p=.24] or physical inactivity [t(337)=-1.61, p=.11].

Conclusions: These findings indicate SDOH are associated with poorer sleep quality in patients diagnosed with cancers affecting the hepatobiliary system. Additional research is needed to develop effective and scalable interventions for SDOH risk factors in outpatient oncology settings.

Poster No. 133
Cancer Epidemiology and Prevention Program

Modeling connectivity with non-stationary resting state fMRI data in patients with brain tumors

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Resting state fMRI can be used to assess brain connectivity and the potential impact of brain tumor surgical resection. For instance, it is important to identify connectivity to peri-humoral language and motor regions, so that essential functions are preserved during resection. Here, we use multi-band EPI and sliding-window seed-based correlation analysis with seeds in the healthy hemisphere to assess peri-tumoral connectivity . Accurate statistical inference related to correlation must take into account temporal non-stationarity of resting state fMRI connectivity. Dynamic sliding windows have been used in correlation point estimation. We demonstrate their use in estimation of autocorrelation and lagged cross correlation structures, and the impact on connectivity inference. Early stopping of resting state experimentation based on the proposed estimation approach also is considered. This approach is suitable for real-time resting state fMRI in intra-operative settings.

Poster No. 134
Biobehavioral Cancer Control Program

The feasibility, acceptability, and preliminary efficacy of a self-advocacy serious game for women with advanced breast or gynecologic cancer

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Background: Patient self-advocacy is a skill set that allows patients with cancer to fully engage in patient-centered care to ensure their needs are met when experiencing challenges in their cancer care. Yet, effective, theory-based interventions are lacking for promoting self-advocacy skills in patients with an advanced cancer diagnosis.

Purpose: This study examined the feasibility, acceptability, and preliminary efficacy of Strong Together, a low-intensity, real-world self-advocacy serious game (a tablet-based educational video game) intervention designed to teach self-advocacy skills in women with advanced breast or gynecologic cancer.

Methods: Women with recently diagnosed (<3 months) metastatic breast or advanced gynecologic cancer were randomized 2:1 to receive Strong Together (n = 52) or enhanced care as usual (n = 26). Feasibility was based on recruitment, retention, data completion, and intervention engagement. Acceptability was assessed via a postintervention questionnaire and exit interview. Preliminary efficacy was assessed on the basis of change scores from baseline to 3 and 6 months in self-advocacy (Female Self-Advocacy in Cancer Survivorship Scale) using intention-to-treat analysis.

Results: Seventy-eight women (55.1% with breast cancer; 44.9% with gynecologic cancer) were enrolled. Feasibility was demonstrated by satisfactory recruitment (69% approach-to-consent rate; 93% enroll-to-randomize rate), retention (90% and 86% at 3 and 6 months, respectively; 85% data completion), and intervention engagement (84% completed ≥75% of the game). Participants endorsed the intervention's (75%) and trial's (87%) acceptability. Participants in the intervention group experienced significant improvements in overall self-advocacy, informed decision-making, and effective communication from baseline to 3 and 6 months compared to participants in the enhanced care as usual group.

Conclusions: Strong Together is feasible and acceptable among women with advanced breast or gynecologic cancer. This intervention demonstrates promising evidence of clinical efficacy. A confirmatory trial is currently in process to test the efficacy of the intervention for patient and health system outcomes.

Poster No. 135
Cancer Epidemiology and Prevention Program

Body Tissues Affect Lung Nodule Malignancy and Growth

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Introduction: Indeterminate pulmonary nodules (IPNs) are common findings on low-dose computed tomography (LDCT) and often lead to unnecessary follow-up procedures. This study aims to investigate the potential impacts of LDCT-derived body tissues on lung nodule malignancy and growth.

Methods: A cohort of 159 subjects (75 with non-small cell lung cancer) was created, including baseline and follow-up LDCT scans, patient demographics, and clinical information. Each subject was found to have lung nodules on the baseline and had undergone multiple scans. Five different body tissues on baseline scans were segmented, including skeleton muscle (SM), subcutaneous fat (SAT), visceral fat (VAT), intramuscular fat (IMAT), and bone. The nodule and body tissue characteristics (e.g., volume and density) were computed along with their relative change (%) between the baselines and follow-up scans. Uni- and multivariate statistical analyses were performed to identify variables significantly associated ($p<0.05$) with doubling time (DT), relative tumor growth, and tumor malignancy. Variables with a variance inflation factor (VIF) larger than 3.0 were removed. Logistic regression models were evaluated through five-fold cross-validation using the Area Under the Receiver Operating Characteristic Curve (AUC-ROC).

Results: Body tissue features in the baseline scans, including SAT density, IMAT density, SM density, IMAT volume, and their changes, including % SM density, and % bone density, were significantly associated ($p<0.05$) with % tumor growth metrics. These associations were found only in cancerous subjects. Body tissue features in the baseline scans, including IMAT density, SM density, and their changes, including % VAT density, % IMAT volume, and % IMAT density, were significantly associated with tumor malignancy. The prediction model incorporating the significant body tissue features identified in the baselines achieved an AUC of 0.781 (95% confidence interval (CI): 0.710-0.851).

Conclusions: CT-derived body tissues are significantly associated with lung nodule malignancy and growth and can serve as novel image biomarkers for assessing IPNs.

Poster No. 136
Cancer Biology Program

MUSCULOSKELETAL ONCOLOGY VIRTUAL EVALUATION STUDY (MOVES)

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BACKGROUND: Improvements in pain and function are the key indication of prophylactic femoral intramedullary (IM) nailing for metastatic bone disease (MBD), but there is limited prospective research characterizing the impact of surgery on activity levels and patient-reported quality of life. This lack of objective and patient-recorded data represents a tremendous gap in our knowledge.

METHODS: Patients undergoing IM nailing procedures due to MBD at our institution will be provided with a Fitbit Inspire 3 and asked to wear it continuously for at least three days prior to surgery and 12 weeks post-surgery. A demographic questionnaire will be completed at baseline that includes information on gender, age, race, and education. Participants will also be asked to complete a brief PROMIS measure once per week via an online platform, capturing information on physical function, pain interference, sleep disturbance, and depression. Recruitment is currently ongoing.

RESULTS: Of five patients enrolled to date, 001 passed away within a month after enrollment, and data collection for 005 is ongoing. Complete sets of step count and PROMIS score data have been collected for participants 002, 003, and 004.

CONCLUSION: Our understanding of the efficacy of prophylactic IM nailing is limited despite nailing being considered standard-of-care in the MBD setting. This study will further our understanding of the physical and psychological effects on patient quality of life and aid informed decision making.

Poster No. 137
Cancer Biology Program

Comprehensive Analysis of Metrics Affecting Glioblastoma Randomized Controlled Trials Robustness

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Introduction: Randomized controlled trials (RCTs) are the gold standard for evaluating the effectiveness of oncological interventions. RCTs are influenced by various factors, including trial design, outcome measures, sample size, recruitment, and retention. These factors can impact the robustness of a trial.

Objective: We comprehensively evaluate the characteristics and robustness of RCTs supporting current GBM management by evaluating trial design, treatment effects, reporting strategies, primary outcome description, patients' loss to follow-up as well as author and patient demographics.

Methods: We conducted database searches and all GBM RCTs with significant outcomes published between 2010-2022 were included. Trial characteristics, survival times, the fragility index (FI-minimum number of patients needed to switch to nullify trial outcome) were assessed. Multivariable regression was performed to assess association of trial characteristics with robustness.

Results: We identified 68 trials eligible for inclusion and found that, on average, approximately 88.1% of screened patients were enrolled, with a median sample size of 64 and a median follow-up time of 2 months. Only about 2.2% of patients were lost to follow-up. Commercial sponsorship was associated with 39.7% of trials, and 30.8% of primary investigators reported conflicts of interest. The median Fragility Index (FI) was 3.5, with 33.8% of trials having an FI less than or equal to the number of patients lost to follow-up, indicating low robustness. Multivariable regression showed that commercial sponsorship, larger sample sizes, and the use of an intention-to-treat protocol were associated with higher FIs, suggesting greater robustness in these cases.

Conclusion: Contemporary RCTs in GBM have low robustness and majority of the trials lose statistical significance with a change in the outcome of fewer than 4 enrolled patients.

Poster No. 138
Biobehavioral Cancer Control Program

Exploring the Meaning of "A Good Day" for Individuals Living with Advanced Cancer

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Background: Most stage IV cancer cases are incurable, making prioritizing quality of life essential for these patients. This study aims to characterize the factors that contribute to day-to-day quality of life for individuals living with advanced cancer.

Methods: Twenty participants with stage IV cancer were recruited from the Pitt+Me registry and UPMC Hillman Cancer Center. Participants completed semi-structured interviews followed by two weeks of nightly surveys rating and characterizing each day. Interview transcripts and survey responses were reviewed to identify common themes across participants, and daily experiences were correlated with quality of life.

Results: As of August 1, 15 of the 20 participants had completed interviews and 156 nightly surveys (87% mean survey completion rate). Mean age was 63 (range 41-75), 33% (N=5) were black, and 67% (N = 10) were female. These first interviews reveal that feeling in control or like oneself, maintaining normalcy in the face of cancer, feeling connected to or making a positive impact on others, and feeling present were common experiences on “good days” while loss of control, uncertainty/unpredictability, disruptive physical symptoms, negative experiences with health care, and inability to reach one’s goals were common on “bad days.” In nightly surveys, joyfulness, calmness, and feeling like oneself were factors most strongly correlated with good days ($p < 0.001$). Feelings of helplessness were most strongly correlated with bad days, followed by stress, sadness, and thinking about health ($p < 0.001$). Almost universally, participants reported feeling most grateful for simply living another day, good or bad.

Conclusions: While common threads exist, a good day takes on different meanings for different individuals with stage IV cancer. It is invaluable for health care providers to understand individual patient values so that they can guide patients toward their personal definitions of well-being as they navigate treatment decisions together.

Poster No. 139
Biobehavioral Cancer Control Program

Perceived Cognitive Function is Associated with Adherence to an Aerobic Exercise Intervention for Women with Early-Stage Breast Cancer

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Perceived cognitive problems are frequently reported during endocrine therapy for early-stage breast cancer (ESBC). Aerobic exercise has been shown to enhance cognitive function in healthy older adults and may be beneficial for women treated for ESBC. We sought to test the efficacy of moderate-intensity aerobic exercise to improve perceived cognitive function over a 6-month period of endocrine therapy in women with ESBC. We employed a blinded, pre-test, post-test randomized controlled design (Exercise Program in Cancer and Cognition; NIH R01CA196762; NCT02793921), and randomly assigned postmenopausal women (N=153) within two years of hormone-receptor positive ESBC diagnosis to either aerobic exercise (n=77) or usual care (n=76). The intervention consisted of ≥150 minutes/week of moderate-intensity aerobic exercise, delivered in a community setting over 6 months. Perceived cognitive function was measured using the total score from the Patient Assessment of Functioning Inventory (PAOFI) at pre-randomization and at intervention completion with similar timing for usual care participants. Higher PAOFI total scores indicate poorer perceived cognitive functioning. Adherence to the exercise intervention was assessed as the mean percentage of recorded minutes to the prescribed 150 minutes/week. Analyses entailed linear mixed-effects modeling following an intention-to-treat approach and considering exercise adherence. Participants were on average(±SD) 62.1±8.2 years old, white (91.5%), well-educated (mean±SD: 15.9±3.0 years) and had stage I breast cancer (64.1%). Treatment groups were similar at baseline (p≥.05). Intention-to-treat analyses revealed no significant group by time interactions or main effects for the PAOFI total score (p≥.05). Although the exercise group was on average adherent to the intervention (mean±SE: 101.3±6.8%), 42.7% had <100% adherence. PAOFI total scores were negatively associated with exercise adherence (b= -0.043; p=.040) and intervention participants with ≥100% adherence had the lowest PAOFI total scores (p=.046). In conclusion, aerobic exercise may improve perceived cognitive function in women treated for ESBC, especially if exercise adherence is maintained.

Poster No. 140
Cancer Immunology and Immunotherapy Program

TRAF2/3 deficient B cells resist DNA damage-induced apoptosis via NF-B2/XIAP/cIAP2 axis and IAP antagonist sensitizes mutant lymphomas to chemotherapeutic drugs

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Deletion of TRAF2 or TRAF3 in B cells prolongs their survival. However, it remains unknown whether deletion of such factors affects B cells' ability to tolerate DNA damage, which can be induced by chemotherapeutics and cause apoptosis. Genetic alterations of TRAF2 or TRAF3 are observed in subsets of human B-cell lymphomas. However, it remains unknown whether double deficiency of TRAF2 and TRAF3 accelerates B-cell lymphomagenesis. Here, we showed that B cell-specific TRAF2/3 double deficient (B-TRAF2/3-DKO) B cells were remarkably more resistant to DNA damage-induced apoptosis via upregulating cIAP2 and XIAP, which in turn attenuates caspase-3 activation. Mechanistically, resistance to DNA damage-induced apoptosis required NF-B2, which effects by upregulating XIAP and cIAP2 transcription. B-TRAF2/3-DKO mice exhibited a shorter lifespan. Unexpectedly, the incidence of B-cell lymphoma development in B-TRAF2/3-DKO mice was relatively rare (10%). Sequencing B cell receptor repertoire of diseased B cells revealed that TRAF2/3 deficiency caused abnormal oligoclonal or clonal expansion of B cells. While a fraction of mutant B cells (25-43%) from aged diseased mice harbored recurrent chromosomal translocations, primary B cells isolated from young B-TRAF2/3-DKO mice had no detectable chromosomal alterations, suggesting that TRAF2/3 deficiency does not cause evident genomic instability in B cells. Chemo-resistant TRAF3-deficient B-cell lymphomas were sensitized to chemotherapeutic drugs by blocking IAP activity using IAP antagonist. We conclude that double deficiency of TRAF2 and TRAF3 does not accelerate B-cell lymphomagenesis. Our studies provide insight into mechanisms regulating DNA damage-induced apoptosis and may help develop effective therapies targeting mutant B-cell lymphomas using IAP antagonist.

Poster No. 141
Biobehavioral Cancer Control Program

Identifying the Prevalence of the ICCTF Guidelines in Cancer Related Cognitive Impairment Research: A Systematic Review of Published Research

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This review examines the prevalence of published research utilizing the International Cognition and Cancer Task Force (ICCTF) recommended criteria for defining cognitive impairment in cancer populations. Published in 2011, the ICCTF recommendations were intended to identify a psychometric standard that would facilitate harmonization of neuropsychological test data across studies for improved accuracy of estimating prevalence or incidence of cancer-related cognitive impairment (CRCI). The ICCTF recommended standards could help decipher variability or stability of CRCI prevalence across various cancer groups, such as individuals within a diagnostic classification (e.g., lung cancer) or type of cancer treatment (e.g., immunotherapy). Using Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines, we screened (145) manuscripts published since 2011 that utilized neuropsychological testing to evaluate CRCI among patients with cancer, 92 of which fulfilled eligibility criteria. Of those 92 manuscripts, 35 (38%) incorporated the ICCTF guidelines in their methodology and data analysis. Eligible manuscripts published between 2011 and 2023 demonstrated a notable increase in the use of ICCTF recommendations with greatest use seen in 2022. Percentages of CRCI among samples studied range from 16% to 70%. ICCTF guidelines were most commonly used to study breast cancer (n=17). This review demonstrates there has been a growing use of the ICCTF recommended definition of CRCI in neuropsychological test data, but further use, especially in cancer populations other than breast cancer is encouraged.

Poster No. 142
Biobehavioral Cancer Control Program

Does Work-related Psychological Stress Increase Systemic Levels of Oxidatively Damaged DNA in Healthy Premenopausal Women?

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When oxidative stress exceeds the capacity of antioxidant defense mechanisms to maintain intracellular equilibrium, DNA damage can occur. Repeated cellular experiences of such damage increase the likelihood of introducing errors into DNA during normal attendant DNA repair processes and increase the risk of introducing somatic mutations that heighten the risk of cancer development over the long term. Acute psychological stress responses have been reported to increase oxidative stress and DNA damage levels in previous experimental studies. Since the work environment is a common source of repeated stresses in daily life, we used a within-subject study design to explore the hypothesis that urinary levels of 8-hydroxy-2-deoxyguanosine (8-OHdG), a biomarker of excisional repair of oxidative damage to DNA, would be higher on a routine workday than on a weekend day. Healthy, working (day shift), nonsmoking, premenopausal women were recruited from the community. Participants (n=50) collected timed urine samples during a workday and a weekend day. Current negative moods were assessed hourly by self-report with an ecological momentary sampling procedure. 8-OHdG levels were determined in frozen urine samples using a commercially available ELISA. Participants' workday and weekend urine samples were assayed concurrently by staff blinded to the study source of the samples. Participants' ratings of negative moods were significantly higher ($p<0.05$) on their workdays compared to their weekend days, as expected. Levels of urinary 8-OHdG were also significantly higher on workdays, consistent with the study hypothesis. Exposure to work stresses appears to result in increases in DNA damage. However, experimental studies are needed to confirm causal relationships. The public health significance of stress-induced increases in DNA damage and the underlying psychobiological mechanisms should be investigated in future research.

Poster No. 143
Cancer Epidemiology and Prevention Program

The Prognostic Role of Eosinophil Count on Mortality Rates in NSCLC Patients Treated with ICI Therapy

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Objective: To determine whether mortality rates are different for subjects with different levels of blood eosinophil count in non-small cell lung cancer (NSCLC) patients under immune checkpoint inhibitors (ICIs).

Methods: Using a prospective cohort study design and electronic medical records, a cohort of NSCLC patients undergoing ICIs at the Wilmot Cancer Center in Rochester, New York from April 2015 to June 2020 was established, and followed until February 2022. Using Cox proportional hazards models (N=208 patients), we estimated the risk of mortality associated with a high or low maximum baseline blood absolute eosinophil count (AEC; ≥300 cells/μL or ≤100 cells/μL, compared to AEC=200 cells/μL) measured at ICI initiation, adjusting for concurrent treatments (radiotherapy, chemotherapy, steroids, antibiotics), sex, smoking, sex-chemo interaction, smoking-chemo interaction, pre-existing lung diseases, prior thoracic radiotherapy, expressed programmed death ligand 1, Eastern Cooperative Oncology Group performance status, liver metastasis, cancer stage, neutrophil count, and body mass index.

Results: Compared to AEC=200 cells/μL, AEC≤100 cells/μL was associated with a 138% increased risk of mortality (hazard ratio [HR], 2.38; 95% confidence interval [CI], 1.46 to 3.90), and AEC≥300 cells/μL was associated with a 202% increased risk of mortality (HR, 3.02; 95% CI, 1.66 to 5.49). A series of sensitivity analyses incorporated various combinations of confounders and extreme value exclusion, demonstrating a consistent non-linear pattern.

Conclusion: Baseline blood eosinophil count was non-linearly associated with overall survival in this patient population, with both high AEC and low AEC associated with an increased risk of mortality in NSCLC patients treated with ICIs.

Poster No. 144
Biobehavioral Cancer Control Program

Exploring symptom burden, financial hardship due to medication cost, and self-reported medication adherence among patients receiving oral anticancer medications for multiple myeloma

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Background: Multiple myeloma (MM) causes burdensome symptoms and requires long-term oral anticancer medications (OAMs). Side effects and cost of OAMs can reduce adherence. Predictors of OAM adherence are not well known. We aimed to explore relationships between symptom burden, financial hardship, and self-reported OAM adherence.

Methods: Secondary analysis (N=80) of an ongoing study (K23NR019296) of patients, age ≥18 years, prescribed OAM maintenance therapy for MM. Measures: ESAS (symptom burden); PROMISÒ Medication Adherence Scale ([PMAS]; OAM adherence); sociodemographics; and medical records. A single item assessed financial hardship due to medication cost. Descriptive statistics, Pearson correlation, and linear regression characterized variables and assessed correlations.

Results: Participants' average age was 63.9 years (SD=10.0), with 29.0 (IQR=13.0-69.8) months since diagnoses. Most were male (56.3%) and non-Hispanic white (83.8%) or Black (13.8%). Average total ESAS score was 16.6 (SD=13.2), with fatigue (M=3.0, SD=2.6), sleep (M=2.6, SD=2.3), and pain (M=2.5, SD=2.5) rated most burdensome. Average financial hardship and PMAS scores were 1.4 (SD=0.8) and 43.3 (SD=3.6), respectively. Symptom burden and financial hardship were negatively correlated with PMAS ($r = -0.317$ and $r = -0.378$, respectively); financial hardship was the strongest predictor ($B= -2.117$, $p<0.001$) in step-wise regression.

Conclusions: Participants generally had low symptom burden and financial hardship and high self-reported adherence. Moderately strong correlations existed between greater symptom burden and financial hardship and lower adherence. Future research should determine the extent and equitability of poor adherence in this sample and elucidate specific mechanisms for how symptom burden and financial hardship contribute to poorer self-reported OAM adherence.

Poster No. 145
Biobehavioral Cancer Control Program

Symptom Management Self-Efficacy in Patients Undergoing Chemotherapy

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Background: Perceived symptom self-efficacy for cancer patients refers to their ability to prevent, recognize, and relieve symptoms they may experience throughout treatment. Previous studies have found mixed results on changes in self-efficacy over time, but they have varied in the ways self-efficacy has been measured and the populations being investigated. The goal of this presentation is to examine how self-efficacy changes over three months in a sample of cancer patients enrolled in an ongoing study monitoring symptoms during chemotherapy and the factors that may influence self-efficacy.

Method: Participants completed the PROMIS Self-Efficacy for Managing Symptoms short form at study enrollment (baseline), 45 days (midpoint), and 90 days (end of study). To determine whether there was a significant effect of time on self-efficacy t-scores, a repeated measures ANOVA with post-hoc pairwise paired t-tests was performed.

Results: 139 participants had complete data for all three time points. The mean age of participants was 59.75 years (SD=11.6, range 29 – 92) and the majority were female (60%), White/Caucasian (85.6%), and had Stage IV cancer of varying types (59%). There was a statistically significant effect of time on self-efficacy t-score ($p<0.001$). On average, self-efficacy t-score significantly increased from baseline to 90 days by 2.777 points ($p<0.001$) and from 45 days to 90 days by 1.476 points ($p=0.012$). Although self-efficacy t-score also increased from baseline to 45 days by 1.301 points, this difference was not statistically significant.

Conclusion: High symptom self-efficacy has been associated with a range of positive health benefits during cancer treatment. Results suggest that self-efficacy increases over time during outpatient chemotherapy, as patients learn to anticipate and manage their side effects. Future analyses will include examining the sociodemographic and clinical factors associated with changes in self-efficacy.

Poster No. 146

Cancer Epidemiology and Prevention Program

Lifetime Ovulation Years and Breast Cancer Risk: Is there a Link?

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Background: Previous studies have identified risk and protective factors for breast cancer, but how these factors collectively influence breast cancer has not been fully characterized. Many of these factors play an important role in ovulation, either promoting or reducing the amount of anovulation that a woman experiences. For endometrial and ovarian cancer, researchers have used lifetime ovulation years (LOY) as a metric for the amount of ovulatory cycles showing that increased LOY is associated with an increased cancer risk. Therefore, we hypothesized that increased LOY will be associated with breast cancer risk.

Methods: In this study, we analyzed data from the Mammograms and Masses Study (MAMS) in which patients at UPMC Magee-Women's Hospital completed a questionnaire about their medical and social history. Of the original 1,072 patients, 1,062 patients had adequate data to be analyzed and were categorized by case-control status. LOY was calculated as the difference between menstrual span and anovulatory years. Age at menarche was recorded in the questionnaire and age at menopause was estimated following an algorithm that considered reproductive surgeries and hormone replacement therapy. Years of anovulation included time during pregnancies, oral contraceptive use, and breast feeding. Kolmogorov-Smirnov test and multivariate logistic regression were used to calculate the odds ratio for the association between LOY and breast cancer.

Results: Increased LOY was not associated with breast cancer risk. The Kolmogorov-Smirnov tests for LOY in all controls and all or just invasive cases were not statistically significant while Kolmogorov-Smirnov tests for LOY in healthy controls and all or just invasive cases was significant. Both the crude and adjusted logistic regression calculations produced either statistically insignificant results or statistically significant odds ratio of 1.00 ± 0.05.

Conclusion: Our results suggest that previously identified risk and protective factors for breast cancer do not convey their effects via anovulation.