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Cancer Biology & Immunology

Mitochondrial Stress Enhances PARP Inhibitor Effect in Ovarian Cancer Models

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Abstract - Homologous Recombination Deficiency is an important biomarker for advanced ovarian cancer, with half of HRD+ cases linked to BRCA1/2 mutations. PARP inhibitors (PARPi) have been especially effective in treating these tumors by blocking DNA damage repair resulting in synthetic lethality. We hypothesized that combining mitochondrial inhibitors such as metformin and CPI-613 with olaparib could enhances cancer cell vulnerability by causing metabolic shifts and increasing genomic and mitochondrial DNA damage. Metformin inhibits mitochondrial complex I, curbing ATP synthesis and AMPK activation. CPI-613 disrupts TCA cycle enzymes, hampering cancer cell metabolism and inducing apoptosis. This study investigates the effect of metformin and CPI-613 as mitochondrial inhibitors with different mechanism on olaparib-induced DNA damage and immune response in BRCA1/2-mutated ovarian cancer cells ID8p53+/+, ID8p53-/-, ID8p53-/-, BRCA1-/-, and ID8p53-/-, BRCA2-/- mouse ovarian cancer cells. All cell lines were

treated with olaparib (OLA, 5μM and 10μM), CPI-613 (75μM), and metformin (MET, 10mM and 20mM) alone or in combination with OLA. Cell viability was assessed by MTT assay, apoptosis by Annexin V-FITC/PI staining, and DNA damage via the comet assay and p-γH2AX. Metabolic changes were analyzed using the XF Seahorse analyzer. 2′,3′-Cyclic GAMP levels were quantified using ELISA. Mitochondrial function were determined by measuring mitochondrial membrane potential and mtDNA (mitochondrial DNA) release. STING response was measured using flow cytometry. Our results showed that combination of OLA + MET and OLA+ CPI-613 demonstrated comparable cytotoxicity across the ID8 cell line panel, indicating efficacy independent of genetic status. However, significantly higher cell death was observed in BRCA mutated cells as compared to wild type and ID8p53-/- cells, suggesting a heightened sensitivity in BRCA cells. OLA alone showed a significant increase in γH2AX ($p<0.001$) in the ID8 p53-/-, BRCA2-/- cells compared to ID8p53-/-, BRCA1-/- , which was further augmented by MET ($p<0.0001$); while minimum DNA damage was observed in wildtype cells ($p<0.001$). MET and CPI-613 both compromised mitochondria function as seen by decreased oxidative phosphorylation, ATP fall, decrease in membrane potential and increase in superoxide formation and release of mtDNA in the cytosol. We then postulated that increased nuclearDNA and mtDNA damage would result in enhanced STING response. Highest DNA damage was seen in ID8 p53-/-, BRCA2-/- cells which correlated with elevated STING by OLA alone and combination treatments ($p<0.0001$) as seen by increase in CD8+STING+, CD8+TBK1+ cells, CD8+IFNg+, CD8+perforin+ and CD8+granzymeB+ ($p<0.001$) T cells when co-cultured with treated tumor cells. Overall, our study demonstrates that combining mitochondrial inhibitors with PARPi significantly enhances treatment efficacy in BRCA1/2-mutated ovarian cancer by augmenting cytotoxicity and activating DNA damage mediated immune responses.

Exploring the PVT1 Exon 9-RSAD2 Signaling Axis as a Therapeutic Target in Neuroendocrine Prostate Cancer

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Abstract - Background: Plasmacytoma variant translocation 1 (PVT1) is an oncogene overexpressed in prostate cancer (PCa). It promotes oncogenesis by altering gene expression directly or acting as a noncoding RNA sponge. Our group has previously identified overexpression of PVT1 exon 9 in cancer models and PCa tissues. PVT1 exon 9 has been shown to exhibit pathogenic phenotype in vitro and induce neuroendocrine tumor (NEPC) formation in vivo from normal prostate epithelial cells. Further exploration is needed to understand the pathogenic activity of PVT1 exon 9.

Methods: We utilized genetic and biochemical techniques in distinct NEPC models to investigate our research question.

Results: We assessed underlying gene expression changes related to PVT1 exon 9 overexpression using RNA-sequencing. One of the top differentially expressed genes (p-value < 0.05, FC 4.89) was Radical S-adenosyl methionine domain containing 2 (RSAD2). We found significant (p-value < 0.001) sustained upregulation of RSAD2 in castrate-resistant prostate cancer and neuroendocrine (NEPC) patient tissues. In prostate cancer models, we noted RSAD2 overexpression in all NEPC models tested and acts as an essential gene. These findings led us to investigate the pathogenicity of dual PVT1 exon 9 and RSAD2 upregulation including their underlying mechanisms. We identified two distinct pathways of RSAD2 upregulation in NEPC related to and independent of PVT1 exon 9. These two models exhibited notable differences in Type I and II interferon signaling, AR expression and sensitivities to clinically relevant therapeutics such as enzalutamide. Knockdown of RSAD2 in NEPC led to reduced cell viability in all models while overexpression induced neuroendocrine marker expression, alterations in type I and II interferon signaling and enhanced cell proliferation in normal prostate epithelial cells.

Conclusion: Based on these results, we hypothesize both PVT1 exon 9 and RSAD2 are promising therapeutic targets in NEPC. There are no inhibitors that target RSAD2 or PVT1 exon 9 currently and further investigations will assess novel ways to target these molecular aberrations either with genetic or chemical manipulations.

Developing T-Cell and Organoid Co-Cultures Derived From Breast Cancer Patients of African Descent for Drug Discovery

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Abstract - Triple negative breast cancer (TNBC) is the most aggressive form of breast cancer. It is highly prevalent in women of West Sub-Saharan African ancestry (WSSA) and African American (AA) women, resulting in poorer survival outcomes compared to European American (EA) women. Studies have shown that immune cells in the tumor microenvironment (TME) contribute to the progression of TNBC by immune suppression, the reprogramming of macrophages into pro-tumorigenic subtypes and the release of inflammatory cytokines. A previous study by our laboratory revealed that the immune processes and the immune markers that are associated with TNBC are different in African and AA populations compared to EA, but the exact role of these immune components remains unknown. We hypothesize that, in TNBC of women with African ancestry, this differential immune signature can lead to the activation of specific immune modulators, thus enhancing immune suppression. To study the differential interaction between

immune cells, particularly T cells, and cancer cells in TNBC, we are currently establishing a drug-screening platform consisting of T cells co-cultured with organoids derived from WSSA, AA and European American (EA) patients. To do this, we first developed organoids, based on TNBC tissues from Ghana as part of the institution called Precision Medicine for Aggressive Breast Cancers (PMABC), and from AA and EA tumors at Henry Ford Hospital. We also collected T cells from these patients that are being expanded. We will test the extent of T cell activation by three different organoids from WSSA, AA and EA TNBC patients to enrich for tumor-reactive T cells. The activated T cells will be co-cultured with organoids to assess their efficiency in killing TNBC organoids and identify potential differences between co-cultures from diverse populations. These co-culture platforms will be used to accelerate drug testing for TNBC in women of African descent in the hope of bridging the gap in survival outcomes.

Development of a Biomarker Test for an Immune Checkpoint Receptor Reveals TIGIT Expression Is Reduced in Black African American Pancreatic Cancer Patients

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Abstract - Purpose: Pancreatic ductal adenocarcinoma (PDAC) is a deadly malignancy with a 5-year survival rate of 13%. T cell immunoreceptor with Ig and ITIM domains (TIGIT) is an immune checkpoint receptor, which is highly expressed in PDAC tissues. The purpose of our study was to develop a biomarker screening test for all stages (by including fine needle biopsies and metastatic biopsies) on a racially diverse cohort of PDAC patients.

Experimental Design: We analyzed single-cell RNA sequencing (scRNAseq) data of 172 primary PDACs and 25 metastatic biopsies aggregated from publicly available datasets. We performed automated single-stain in situ hybridization staining for the immune checkpoint TIGIT on fine needle biopsies, metastatic liver core biopsies, and on treated and treatment naïve resected PDAC tissues. We utilized imaging analysis software HALO® to quantify overall TIGIT+ cells (1 or more copy of TIGIT) and TIGIThigh (3 or more copies of TIGIT) cells as a percentage of total cells and correlated these measurements with clinical metadata.

Results: Our scRNAseq atlas showed significant enrichment of TIGIT expression within T and NK cells in the PDAC TME. In our Henry Ford Health (HFH) PDAC cohort (n=75, 40% BAA), the percentage of overall TIGIT+ and TIGIThigh cells of total cells was increased in chemotherapy-treated resected PDAC tissues, compared to treatment naïve PDAC tissues. Overall TIGIT+ and TIGIThigh cells were significantly reduced in BAA PDAC tissues compared to White PDAC tissues, a finding that remained true after adjusting for disease stage.

Conclusions: Analysis of immune checkpoints from our scRNAseq atlas highlights the importance of individualized immunotherapy treatment approaches and underscores the critical need for rigorous biomarker tests that can be performed in all stages of PDAC patients. Our study is also the first to uncover the relative abundance of TIGIT+ cells in the TME is reduced in BAA PDAC compared to White PDAC, suggesting that the efficacy of immune modulatory therapies may differ across different race groups.

Dissecting the Phenotype and Function of an Elusive Cell Type in PDAC: γδ T Cells

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Abstract - Background: Pancreatic ductal adenocarcinoma (PDAC) is a lethal cancer with a 5-year survival rate of 13%. Its tumor microenvironment (TME) is characterized by dense stroma and immune suppression, making immune checkpoint inhibitor (ICI) therapies largely ineffective. A subset of T cells, γδ T cells, are believed to promote tumor growth in PDAC by inhibiting cytotoxic T cell function and enhancing immune suppression.

Hypothesis: γδ T cells in PDAC are polarized to an immune-suppressive phenotype, particularly in metastatic disease, through the downregulation of IL12A and CXCL10/11 signaling pathways, leading to decreased T cell activation.

Methods: Using single-cell RNA sequencing (scRNAseq) data from human PDAC samples, we analyzed phenotype of γδ T cells across 26 normal, 172 primary, and 21 metastatic liver core biopsy PDAC tissues. We performed differential gene expression analysis to compare γδ T cell profiles in the primary and metastatic setting. In a murine model of PDAC, we examined the functional role of γδ T cells by implanting orthotopic tumors into wildtype or Trdc knockout animals.

Results: We found a stepwise increase in γδ T cells from normal tissue to primary and metastatic PDAC. In metastatic tumors, γδ T cells showed significantly reduced expression of the transcription factor T-bet, effector molecule Granzyme B, and chemokine receptor CXCR3, indicating an immune-suppressive phenotype. Further analysis revealed downregulation of the ligands upstream of T-bet and CXCR3 signaling, IL12A and CXCL10/11, in metastatic PDAC,

compared to primary tumors. We find high expression of a scRNAseq-derived γδ T cell gene signature correlates with worse prognosis and worse overall survival in a cohort of patients with Stage II/II PDAC. Lastly, in an orthotopic mouse model of PDAC, deletion of Trdc reduced tumor burden, consistent with these cells playing an immune suppressive role in the primary setting.

Conclusion: Overall, our findings suggest that targeting the IL12-T-bet and CXCL10/11-CXCR3 pathways could enhance immunotherapy efficacy in PDAC by limiting γδ T cell-mediated immune suppression.

GPR68-ATF4 Signaling Is a Novel Prosurvival Pathway in Glioblastoma Activated by Acidic Extracellular Microenvironment

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Abstract - Glioblastoma multiforme (GBM), the most common brain cancer, is notable for its aggressive nature, limited treatment options, treatment resistance, and overall grim prognosis. A defining hallmark of GBM is altered tumor metabolism, involving a shift toward aerobic glycolysis, a phenomenon known as the Warburg effect. An important consequence of aerobic glycolysis is the acidification of the tumor milieu, which is thought to activate pro-tumorigenic signaling. Here, using an extracellular pH reporter, we show that glioblastoma cells acidify their microenvironment to activate GPR68, an extracellular proton-sensing G-protein-coupled receptor (GPCR). Additionally, using a novel small molecule GPR68 inhibitor, named ogremorphin (OGM), and genetic means, we show that blocking GPR68 signaling kills glioblastoma cells by selectively inducing ferroptosis in an ATF4-dependent manner without affecting non-malignant neural cells. Importantly, OGM induces robust cell death in all glioblastoma cell lines tested thus far, irrespective of genetic and phenotypic heterogeneity, or resistance to the mainstay GBM chemotherapeutic temozolomide. Moreover, OGM synergized with both temozolomide and ionizing radiation to induce ferroptosis in GBM cells. Our findings reveal GPR68 as a key mediator of an autocrine pro-tumorigenic signal activated in glioblastoma cells by extracellular acidification. Our study highlights GPR68 inhibition as a particularly promising therapeutic approach to induce ferroptosis selectively in glioblastoma cells while sparing normal neural tissue.

Beta-Catenin in Dendritic Cells Negatively Regulates CD8 T Cell Immune Responses Through the Immune Checkpoint Molecule Tim-3

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Abstract - Recent studies have demonstrated that beta-catenin in DCs serves as a key mediator in promoting both CD4 and CD8 T cell tolerance, although how beta-catenin exerts its functions remains incompletely understood. Here we report that activation of beta-catenin leads to the up-regulation of inhibitory molecule T-cell immunoglobulin and mucin domain 3 (Tim-3) in cDC1s (type 1 conventional DCs). Using a cDC1-targeted vaccine model with anti-DEC-205 engineered to express the melanoma antigen--human gp100 (anti-DEC-205-hgp100), we demonstrated that CD11c-beta-cateninactive mice exhibited impaired cross-priming upon immunization with anti-DEC-205-hgp100, resulting in much reduced memory responses. Further experiments revealed that treating CD11c-beta-cateninactive mice with anti-Tim-3 antibody upon anti-DEC-205-hgp100 vaccination leads to restored cross-priming of gp100-specific CD8 T cells, suggesting that anti-Tim-3 treatment likely synergizes with DC vaccines to improve their efficacy. Indeed, treating B16F10-bearing mice with DC vaccines using anti-DEC-205-hgp100 in combination with anti-Tim-3 treatment resulted in significantly reduced tumors, compared to treatment with DC vaccine alone. Taken together, we have identified the beta-catenin/Tim-3 axis as a novel mechanism to inhibit anti-tumor CD8 T cell immunity, and that combination immunotherapy of a DC-targeted vaccine with anti-Tim-3 treatment leads to improved anti-tumor efficacy.

HPV Upregulates the Membrane-Associated Ubiquitin Ligase MARCHF8 and Promotes Head and Neck Cancer by Degrading MHC-I

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Abstract - Background: Major histocompatibility complex I (MHC-I) molecules are among the most common targets for cancer immune evasion. The loss of MHC-I impairs antitumor immune responses and attenuates immunotherapies that reactivate antitumor CD8+ T cells by immune checkpoint inhibitors (ICIs). Hence, understanding how cancer cells dysregulate MHC-I antigen presentation is critical. Membrane-associated RING-CH type finger8 (MARCHF8), one of the MARCHF family members, is highly upregulated in human papillomavirus-positive head and neck cancer (HPV+ HNC). MARCHF8 is the human homolog of Kaposi's sarcoma-associated herpesvirus (KSHV) K3 and K5, known to ubiquitinate MHC-I.

Hypothesis: MARCHF8 plays a crucial role in HPV+ HNC immune evasion by degrading MHC-I proteins.

Methods: We generated MARCHF8 knockdown (KD) and knockout (KO) HPV+ HNC cells and determined if MARCHF8 decreases MHC-I protein levels by western blotting and flow cytometry. We determined MARCHF8 and MHC-I interactions using immunoprecipitation and TurboID. We also investigated in vivo tumor growth using our syngeneic HPV+ HNC mouse model and analyzed immune cell profiles in the tumor microenvironment (TME) using high-dimensional flow cytometry and single-cell RNA-seq. We also performed T cell proliferation, IFNg production, and cytotoxic T cell assays.

Results: MARCHF8 KD and KO significantly increased MHC-I protein levels in HPV+ HNC cells, and MARCHF8 binds to and ubiquitinates MHC-I proteins. We found that Marchf8 KO considerably suppressed tumor growth in vivo and significantly increased CD4+ T, CD8+ T, and NK cells, particularly cytotoxic CD8+ T cells in the TME. Furthermore, Marchf8 KO markedly increased crosstalk between the cytotoxic NK cells and CD8+ T cells with macrophages and enhanced CD8+ T cell proliferation, activation, and tumor cell killing. Interestingly, Marchf8 KO, in combination with anti-PD-1 treatment, synergistically suppresses tumor growth in mice bearing ICI-refractory tumors.

Conclusions: Our findings suggest that HPV+ HNC evades antitumor immunity by MARCHF8-mediated MHC-I degradation. This provides new insight into virus-induced immune evasion in cancer progression with a promising target for novel immunotherapy.

A Subset of Glioblastoma Cells Is Sensitive to a Dual mTORtor and DNA-PK Kinase Inhibitor

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Abstract - Mammalian target of rapamycin (mTOR) and DNA-dependent protein kinase (DNA-PK) are members of the phosphatidylinositol 3-kinase–like protein kinases activated in various cancers. mTOR integrates extracellular and intracellular signals through protein complexes mTORC1 and mTORC2, to regulate several essential processes such as cell growth, proliferation, metabolism, and resistance to apoptosis. DNA-PK has a central role in the repair of double-stranded DNA breaks. Here we tested the efficacy of a double TORK/DNA-PK inhibitor (CC-115) as a therapeutic agent for glioblastoma, using a panel of patient-derived cancer stem cells (CSCs) and mouse xenografts. 10 genomically diverse glioblastoma CSCs were treated for 72h with both inhibitors, with concentrations ranging from 0 to 10 uM (n=5). Cell viability was determined using CellTiterGlo and area above the curve (AAC) and IC50 concentrations were determined from dose-response curves. A wide range of response to CC-115 was observed, with AAC varying from 0.4 to 0.6 for the 7 sensitive cells, and <0.25 for the 3 resistant lines. IC50 concentrations for the sensitive cells ranged from 0.16 to 0.70 uM.

Since response was not determined by genomic features, including PTEN status, we employed targeted proteomics (reverse phase protein array) to identify correlates of response in 5 sensitive CSCs and the 3 resistant ones (in triplicates). We observed a higher frequency of baseline mTOR activation and apoptosis markers in the sensitive lines. We observed that in response to 72h sublethal CC-115 treatment, phospho-mTOR and downstream targets of mTORC1 and mTORC2 were downregulated in all CSCs, as expected, while MAPK pathway and PDGFRa activation was observed only in the resistant CSCs. We then show that low doses of CC-115 (50 nM) can sensitize the sensitive CSCs to radiation. We conclude that CC-115 has efficacy as a single agent in a subset of CSC with higher levels of baseline mTOR and apoptotic markers, which are not determined by genomics, and potential for combination with radiation.

Elucidating the Autocrine and Paracrine Mechanisms of Gamma-Aminobutyric Acid Metabolism by Cancer Associated Fibroblasts in Pancreatic Cancer

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Abstract - Pancreatic ductal adenocarcinoma (PDAC) is a devasting disease with few therapeutic options. This is due, in large part, to the expansion of the non-tumor cellular compartment known as the stroma. Cancer-associated fibroblasts (CAFs) are one of the major cell types that populate the stroma of PDAC tumors and are extremely immunosuppressive because they produce large amounts of inhibitory cytokines, growth factors and metabolites. Thus, understanding how CAFs impart severe immunosuppression in the tumor microenvironment is critical to make PDAC amenable to immunotherapies. One key immunosuppressive metabolite detected in PDAC is gamma-aminobutyric acid (GABA).

Our preliminary data shows that that PDAC CAFs produce GABA, de novo, and that CAFs express all components of the GABA synthesis pathway and receptors, as determined by western blotting and from single cell RNA sequencing from over 120 patient tumors. Moreover, we could also detect GABA in the local fluid isolated from pancreatic tumors, referred to as tumor interstitial fluid (TIF), highlighting the potential clinical relevance of targeting GABA.

Here, our goal is to uncover the autocrine and paracrine mechanisms of GABA signaling in CAFs and evaluate how the modulation of GABA production and signaling affects immunosuppression in PDAC. We will take advantage of our novel 3D culturing system, advanced metabolomics platforms, and unprecedented access to patient samples to address our goals.

Translationally, we are analyzing the tumor interstitial fluid from PDAC patients to uncover secreted factors that can be correlated with patient parameters (e.g. overall survival, response to therapies) and immune cell activation and functionality. It is our hope that the insight provided by this study can lead to a novel set of prognostic or diagnostic factors to improve clinical outcomes in this devasting disease.

Molecular Correlates of Response to Cyclin Dependent Kinase 4/6 Pharmacological Inhibition in Glioblastoma Cancer Stem Cells

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Abstract - Pharmacological inhibition of cyclin-dependent kinase 4/6 (CDK4/6) has potential to treat the 90% of glioblastoma patients bearing wildtype retinoblastoma (Rb). To address the lack of other predictive biomarkers, we integrated response to two CDK4/6i currently in clinical trials for GBMs with genomic and molecular landscape in a panel of 14 GBM patient-derived cancer stem cells (CSC) and addressed mechanism of resistance in longitudinal studies.

CSCs were treated with 0–2 uM concentrations of CDK4/6i for 4 and 7 days (n=5), cell viability measured using CellTiterGlo. Area above the curve (AAC) and IC50 values were obtained from dose-response curves. A RB1-null CSC used as control was resistant to the inhibitors as evidence for specificity, while the other CSC lines presented a wide range of AAC values, and the only genomic correlation we found was an increased frequency of MYC or MYCN amplification among the more resistant lines. Spearman correlation between RNAseq counts (RPKM) and AAC values from 7-day treatment (r>|0.7|) revealed that vesicle transport, mTOR and autophagy pathways were indicative of resistance to CDK4/6i, while E2F target genes and DNA-replication and repair were associated with sensitivity. By Western blot comparison of phospho-Rb levels between CSCs

treated with control or sublethal concentrations, we verified that all the inhibitors engaged the target. Because CDK2 activity is a potential mechanism of resistance to CDK4/6i, we tested the efficacy of a novel CDK2/4/6i (ebvaciclib), which was able to override resistance to CDK4/6i in only 2 CSCs, both bearing loss of p16ink, p14arf and p53 tumor suppressors, in combination with MYC/NMYC amplification. While 14-day treatment with all inhibitors in 3 CSC lines lead to 7 to 43% increase in apoptotic cells relative to control, as measured by FACS, senescence was only present in the treated ebvaciclib-responsive CSC line. We demonstrated the feasibility of employing integrative pre-clinical analysis to identify GBM patients that can benefit from CDK4/6i treatment and to anticipate and address mechanisms of resistance.

Understanding the Instructive Role of Keratinocytes in T Cell Mediated Tumor Surveillance

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Abstract - Keratinocytes (KCs) are highly innovative in providing barrier function against infection and tumor development. Previous studies have suggested a role for IFNGR and its ligand IFN-γ for tumor surveillance. IFNGR-deficient mice exhibited increased tumor formation. Moreover, IFN-γ is known to induce MHC Class II in KCs, an expression that is typically restricted to professional antigen-presenting cells. The findings suggest a role for KCs to instruct T cells for tumor immunosurveillance that is poorly understood. We hypothesize IFN-γ to induce a KC instructive tumor surveillance program with T cells via MHC II and co-stimulatory molecule expression in vitro. To test this hypothesis, we determined the induction of key transcriptional states in longitudinal profiles of normal KCs (N/TERT) and A431 epidermoid carcinoma cells in response to IFN-γ. Cells were harvested at 24, 48, and 72 hours post-IFN-γ treatment and specifically assessed for MHC II (HLA-DRA, HLA-DRB1), IFN-γ receptors (IFNGR1, IFNGR2), co-stimulatory molecules (CD58, ICAM-1, PDL-1), and positive controls (CXCL10, CXCL11) by qPCR and immunofluorescence (IF). CXCL10 and CXCL11 peak expressions associated with immune cell recruitment were observed at 24 hrs in both cell types demonstrating the efficacy of IFN-γ ($p < 0.05$) that was sustained only in N/TERT at 48 and decreased in both cells by 72 hrs. Furthermore, both HLA-DRA and HLA-DRB1 were induced in both N/TERT and A431 cells but were significantly higher and continued to be increased in N/TERT compared to A431 (all $p < 0.0001$). This higher fold induction in N/TERT was also confirmed by IF (57.4% vs. 13.1% [A431], $p < 0.05$). Furthermore, IFNGR1, IFNGR2, and CD58 were specifically downregulated in A431 yet significantly increased in N/TERT. Additionally, ICAM-1 and PDL-1 were also significantly decreased in A431 as soon as 48 hrs compared to N/TERT. Overall, our findings reveal that IFN-γ is sufficient to induce a KC-specific,

instructive program involving putative antigen presentation and co-stimulation that is compromised in A431 suggesting intrinsic cellular-mediated immune evasion in cutaneous tumors.

VPS72 Controls Regulatory T Cell Adaptation and Stability in the Tumor Microenvironment

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Abstract - Background: The tumor microenvironment (TME) harbors various stress factors that promote regulatory T cells (Tregs) adaptation, stability, and tumor progression. However, there remains a significant gap in understanding how TME stressors and related epigenetic networks control Tregs stability and their immunosuppressive activities within the TME. The vacuolar protein sorting-associated protein 72 homolog (VPS72), a chromatin remodeler via H2A.Z exchange, is highly expressed in various tumors and is positively correlated with tumor progression. Despite this, the role of VPS72 in the immune system, particularly in Tregs biology and their adaptation in TME remains largely unknown.

Hypothesis: We hypothesized that VPS72 controls Tregs stability and function.

Methods: To test this, we generated Treg-specific VPS72 knockout (VPS72-TregKO) and tamoxifen-inducible VPS72 knockout (VPS72-TregiKO) mice. Using the B16 melanoma model, we investigated the role of VPS72 in Treg functionality and tumorigenesis. Flow cytometry, bulk RNA-seq, scRNA-seq and CUT&RUN-seq were employed to evaluate the TME immune landscape, global transcriptomes and chromatin landscapes controlled by VPS72 in individual immune cells.

Results: Tumor infiltrating Tregs (tiTreg) from cancer patients expressed significantly higher VPS72 compared to peripheral blood Tregs, suggesting a role for VPS72 in tiTreg stability. In VPS72-TregKO mice, peripheral Tregs differentiation and suppressive function were severely disrupted, leading to lethal multi-organ autoimmune diseases. Bulk RNA-seq analysis confirmed the critical roles of VPS72 in Treg homeostasis and function. In the B16 melanoma model, VPS72-TregiKO

mice exhibited reduced tumor growth and progression. scRNA-seq analysis of the TME immune landscape revealed increased cytotoxic T lymphocyte infiltration and enhanced T cell anti-tumor activities, along with elevated dendritic cell antigen presentation and immunogenicity in VPS72-TregiKO mice. Additionally, low glucose levels, a key TME factor, induced the VPS72 expression in Tregs.

Conclusions: VPS72 play a central role in Treg stability and enhancing their immunosuppressive function in the TME, making it a potential novel target for cancer immunotherapy.

Detailed epigenetic mechanisms employed by VPS72 in Treg regulation and other TME factors regulating VPS72 expression are currently under investigation.

Targeting Mucosal Associated Invariant T (MAIT) Cells Via the Gut Microbiome as a Novel Immunotherapy for Pancreatic Cancer Liver Metastasis

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Abstract - Pancreatic cancer liver metastasis (PCLM) is present in 50% of patients diagnosed with pancreatic ductal adenocarcinoma which leads to abysmal median survival. Here, we sought to identify options for treating PCLM by targeting mucosal associated invariant T (MAIT) cells, an invariant T cell restricted to MHCI related protein (MR1). MAIT cells are classified as protumor MAIT17 cells, induced by MR1 mediated activation, and antitumor MAIT1 cells. Interestingly, liver MAIT cells can respond to microbial antigens presented on MR1 that traverse from the gut. We hypothesize that the PCLM tumor microenvironment (TME) induces direct MAIT cell activation via MR1 which promotes MAIT17 cells and modulating the gut microbiome may therapeutically alter MAIT cells in the PCLM TME. In mouse models, we found that the PCLM TME is enriched for MR1 which promoted a MAIT17 phenotype. When comparing WT mice and MR1 KO (lacking MAIT cells), MR1 KO mice had reduced tumor burden. ScRNA-seq and flow cytometry revealed that the lack of MAIT cells promoted anti-tumor immunity. Furthermore, we found that the gut microbiome of MR1 WT PCLM mice had a distinct microbiome rich in microbial antigens that act as MR1 ligands which may traverse to the PCLM TME and promote direct MAIT cell activation and a MAIT17 phenotype. To reduce the presence of microbial antigens that traverse from the gut to promote liver MAIT17 cells, we subjected mice to oral antibiotics. This promoted MAIT1 cells in in the PCLM TME and reduced MAIT17 cells. Furthermore, we found that only antibiotic treated MR1 WT mice had reduced tumor burden via reduction in exhausted T cells while MR1 KO mice did not. Overall, our findings suggest that MAIT cells can be therapeutically modulated via the gut microbiome to yield anti-tumor immunity in PCLM.

DNA Methylation Regulation of PVT1 in Prostate Cancer

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Abstract - In the United States, prostate cancer (PCa) is the second most prevalent cause of cancer death in men. Chromosome 8q24 is a chromosomal region that has been linked to susceptibility to PCa. This chromosomal locus has frequent amplifications of the plasmacytoma variant translocation 1 (PVT1) gene, which is a non-protein coding gene with 12 exons and six annotated microRNAs. Our lab and others have previously uncovered the link between PVT1 gene and prostate carcinogenesis. Population-level genomic analysis of the PVT1 gene, from the 1000 Genomes Project, revealed genetic differentiation between African and non-African populations at PVT1 exons 4A and 4B. Additionally, PVT1 exon 4A and 4B showed increased expression in PCa tissues relative to normal prostate tissues and benign prostatic hyperplasia tissues. We also demonstrated that overexpression of PVT1 exon 4A, 4B and 9 caused increased migration and proliferation of prostate epithelial cells. Gene expression is modulated by DNA methylation. However, the role of DNA methylation regulation of PVT1 is unclear. We hypothesized that DNA methylation regulates PVT1 expression in PCa and we tested this hypothesis by treating five prostate epithelial cell lines (RWPE-1, WPE1-NA22, DU145, PC-3 and MDA-PCa-2b) with 5-azacytidine (global DNA demethylating agent) and performed real-time quantitative polymerase chain reaction analysis. We found that 5-azacytidine treatment resulted in significantly (p-value <0.05) increased expression of PVT1 exon 4A in DU145 and PC-3, while PVT1 exon 9 was significantly expressed in MDA-PCa-2b compared to non-tumorigenic prostate epithelial cells (RWPE-1) and cells that model indolent PCa (WPE1-NA22). Also, PVT1 exon 4B showed significantly increased expression only in PC-3 when compared to no treatment. When we compared the expression profile of these exons in the PCa cells versus RWPE-1 after treatment with 5-azacytidine, PVT1 exon 4A and 9 were significantly expressed in the PCa cells. These results show that PVT1 expression is regulated by DNA methylation and suggest that the expression of PVT1 exons maybe associated with distinct regulatory mechanisms in PCa.

MHC-I Degradation Via NDP52-Mediated Selective Autophagy in HPV-Positive Head and Neck Cancer

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Abstract - Recognition of tumor antigens presented by major histocompatibility complex class I (MHC-I) on cancer cells is critical for the antitumor T cell response. Accordingly, the process of MHC-I antigen presentation is frequently dysregulated in many cancers, including human papillomavirus-positive head and neck cancer (HPV+ HNC), as a strategy for immune evasion. It has been proposed that the downregulation of MHC-I is a major obstacle in HPV+ HNC treatment, particularly for non-responders to the current immunotherapies. However, the mechanisms of how MHC-I is downregulated in HPV+ HNC have yet to be elucidated.

To identify key regulators of MHC-I expression, we performed a genome-wide CRISPR/Cas9 screen in HPV+ HNC cells and identified genes involved in autophagy among the top negative regulators. Autophagy is a major catabolic process that recycles cellular components. A specific type of autophagy, selective autophagy, targets substrates via cargo receptors that recognize distinct ubiquitin chains. We recently found that membrane-associated RING-CH finger 8 (MARCHF8) induced by the HPV oncoproteins ubiquitinates MHC-I for degradation. Interestingly, while most ubiquitinated proteins are directed to the proteasome for degradation, MHC-I protein levels are restored in HPV+ HNC by inhibiting autophagy, but not the proteasome. Thus, we hypothesized that MHC-I ubiquitinated by MARCHF8 is degraded by selective autophagy in HPV+ HNC cells, and inhibition of autophagy will restore surface MHC-I to promote antitumor immunity.

To test this hypothesis, we determined specific autophagy cargo receptors required for MHC-I degradation by knocking out three major cargo receptors: NDP52, NBR1, or p62 in HPV+ HNC cells. Our results showed that depletion of NDP52 or NBR1 significantly increased MHC-I protein levels. Further, our immunoprecipitation analysis demonstrated that MHC-I protein binds to NDP52 but not p62 and NBR1. Taken together, our data suggests that MHC-I is degraded via the NDP52-mediated selective autophagy pathway in HPV+ HNC, and inhibition of autophagy initiation may restore MHC-I to potentiate the CD8+ T cell antitumor response.

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County and Regional Variation in HPV Vaccination Among Adolescent Girls in Michigan

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Abstract - Background. There are about 37,000 new cases of cancer attributed to human papillomavirus (HPV) each year in the United States. The HPV vaccine is highly effective in providing long-lasting protection against cancer-causing HPV infections. It is recommended that children receive the vaccine between 9 to 12 years of age. However, only 45% of adolescent girls have completed the HPV vaccination series in Michigan. This exploratory study examined geographic variability in HPV vaccination among adolescent girls in Michigan to identify potential hot spots in particular need of intervention.

Methods. We assessed the percentage of girls between 13 to 17 years of age who completed the HPV vaccination series in each county in Michigan. Data were drawn from the Michigan Department of Health and Human Services immunization reports, which provide aggregated immunization data from the Michigan Care Improvement Registry (MCIR). We first evaluated county trends in HPV vaccination from 2013 to 2023 by state region as defined by MCIR (Southeastern Michigan; Western Michigan; Central Michigan; Thumb of Michigan; Northern Lower Michigan; Upper Peninsula). We also visualized county data to examine vaccination coverage by state region.

Results. Nearly all counties demonstrated an increase in vaccination from 2013 to 2023. The rate of change was similar across counties within each respective region, although there were some county outliers in Southeastern Michigan and Northern Lower Michigan. Geographic clusters also emerged, wherein HPV vaccination coverage in 2023 tended to be highest among counties in Western Michigan. This is in contrast to 2013 when vaccination coverage tended to be highest among counties in Northern Lower Michigan and the Upper Peninsula.

Conclusions. Findings add to existing literature demonstrating geographic variability in HPV vaccination and illustrate the need for geographically focused HPV vaccination efforts in Michigan. Future research should build upon the present findings and seek to identify the underlying factors that contribute to regional disparities, which can inform region-targeted vaccine interventions and initiatives.

Impact of Social Economic Status on Patient-Reported Outcomes (PROs) in Non-Small Cell Lung Cancer (NSCLC)

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Abstract - Introduction: Patient-reported outcomes (PROs) are a powerful method to assess a patient's well-being as reported directly by the patient without any provider interpretation. PROs in lung cancer have been shown to improve patient quality of life and survival, while decreasing health care utilization. Low socioeconomic status is associated with 13% more advanced lung cancer at diagnosis and carries an inferior prognosis.

Methods: We conducted a retrospective study of patients diagnosed with NSCLC between 2020 and 2023 who completed their initial PRO survey within 180 days of their diagnosis. NIH PROMIS computer adaptive testing was used as the PRO survey in the cancer clinic. PRO surveys included four domains- physical function, fatigue, pain interference, and depression. Patient demographics including race, gender, ethnicity, marital status, and cancer staging were obtained through electronic medical records. State Area Deprivation Index (ADI) rank was used to assess neighborhood socio-economic status. Statistical analysis was performed using Spearman's correlation test to assess the correlation between ADI and PRO scores.

Results: 491 patients completed their first PRO surveys within 180 days of lung cancer diagnosis. 48.9% of the patients were female (n=194). 21.4% of patients were African American and 71.3% were Caucasian. 48.9% were married. About 40% of the patients in the cohort had a high ADI Rank. Most of the patients had advanced disease (Stage III- 20.4% and Stage IV- 34.8%). The mean PRO scores were 52.8 (depression), 55.6 (fatigue), 57.7 (pain interference), and 37.8 (physical function). Patients with high ADI rank were found to have significantly worse pain scores (p-value-0.0118) and depression scores (p-0.045).

Conclusion: Socioeconomic status, as measured by ADI, appears to negatively impact PRO scores around the time of diagnosis in NSCLC patients. These findings underscore the importance of considering socioeconomic factors in assessing patient-reported outcomes and developing targeted interventions to address disparities in healthcare access and outcomes among lung cancer patients.

Lung Cancer Risk, Incidence, and Mortality Disparities in Rural, Persistent Poverty Counties

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Abstract - Introduction: In the US, lung cancer burden is greater in counties that are either rural or in persistent poverty. This study examined lung cancer risk (e.g., smoking), incidence, and mortality across four county types defined by cross-classification of rurality and persistent poverty.

Methods: We conducted a secondary analysis of county characteristics and lung cancer outcomes. We used data from USDA to classify counties according to rurality (using rural-urban continuum codes) and persistent poverty (i.e., 20%+ of residents living below the poverty line for 30+ years). We used publicly-available data to calculate mean county-level prevalence of smoking among adults (in 2019), lung cancer incidence (2015-2019), and lung cancer mortality (2015-2019) across county types. Linear regression models assessed differences in smoking, lung cancer incidence, and lung cancer mortality by rurality and persistent poverty.

Results: Among U.S. counties, 1,115 were urban, non-persistent poverty, 1,675 were rural, non-persistent poverty, 52 were urban, persistent poverty, and 301 were rural, persistent poverty. Smoking, lung cancer incidence, and lung cancer mortality were higher in rural counties and in persistent poverty counties than in their comparison counties. Counties that were both rural and persistent poverty had the highest rates of smoking, lung cancer incidence, and lung cancer mortality. Persistent poverty and rurality interacted in their relationship with smoking prevalence ($p<.01$), and lung cancer mortality ($p<.10$).

Conclusion: Smoking, lung cancer incidence, and lung cancer mortality are highest in counties that are both rural and persistent poverty, suggesting an urgent need to develop targeted lung cancer interventions in these communities.

Molecular Features Associated with Estimated Genetic Ancestry of PDAC Patients

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Abstract - Pancreatic cancer is the third leading cause of cancer-related death in the US, and the incidence rates of this disease have continued to rise over the past decade. Epidemiological analyses have revealed that different racial groups are affected by this cancer disproportionately, with the Black or African American population being the most affected. Currently, it is unclear to

what extent biological pathways contribute to race-associated disparities in pancreatic cancer development. Research efforts to address this topic require analyses of patient-derived clinical data and face many challenges, such as low enrollment rates of minority patients and incomplete records of self-reported race information. In this project, we utilized DNA and RNA sequencing data generated by the Know Your Tumor Program from the Pancreatic Cancer Action Network (PanCAN) to: 1) classify each patient into their most similar genetically assessed population (GAP) based on continent-level ancestry using targeted region sequencing of tumor genomes and 2) identify gene expression differences between GAPs. Our sample size consisted of 409 individuals with confirmed pancreatic cancer and targeted tumor DNA sequencing, 226 of whom also had tumor RNA-Seq data. Reference GAPs were defined as the five 1000 Genome Project (1KG) super-populations, and each PanCAN subject was assigned to one of these reference GAPs based on their top three genome-wide principal components and k-nearest neighbors clustering, where k=5 1KG reference subjects. Gene expression differences between pairs of GAPs was performed using a negative binomial model implemented in DESeq2, and differential expression of splice variants was performed using suppa2. Both gene expression analyses accounted for pathologic stage of disease and adjusted for multiple comparisons using the false discovery rate (FDR). For the 409 PanCAN participants, the GAP assignments to the five 1KG super-populations were as follows: Africans/African Americans (AFR, n=19), Admixed Hispanic/Latino Americans (AMR, n=22), East Asians (EAS, n=17), South Asians (SAS, n=7), and Europeans/European Americans (EUR, n=344). Among the 184 participants (45%) that provided self-reported race information, GAP assignment was in high agreement (99%)with their self-reported race. Due to sample size, gene expression analyses were restricted to the AFR, EAS, and EUR GAPs. At an FDR<0.05, 605, 128, and 695 differentially expressed genes were identified for the AFR/EUR, EAS/EUR, and AFR/EAS pairwise comparisons, respectively. In particular, differentially expressed genes between AFR and EUR GAPs were significantly enriched for multiple immune related pathways that regulate B cell functions. For this GAP comparison, RNA splicing patterns of multiple genes, such as CDK4, were also differentially expressed. The RNA transcript variants differentially expressed between these groups can be candidates for future studies to understand their roles in driving biological changes that contribute to racial disparities in pancreatic cancer.

Formative Evaluation of a Patient-Reported Outcomes Program Implementation in a Tertiary Cancer Center: The Provider Perspective

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Abstract - Background: Patient-reported outcome measures (PROMs) are standardized instruments used to collect and measure any reports on a patient's health condition (including social and physical functioning) that comes directly from the patient without any provider interpretation. PROMs-generated scores can enhance provider clinical decision making. Despite their widespread integration in ambulatory oncology care, several barriers to PROMs adoption in diverse clinical settings remain. Limited incorporation of PROMs in clinical care delivery can undermine patient outcomes, particularly for patients with poor symptom management.

Methods: A cross-sectional formative evaluation was conducted in 2024 to examine providers' opinions on factors impacting the implementation of a PROMs program at Henry Ford Cancer (HFC). The evaluation consisted of a semi-structured phone interview designed using the Health Equity Implementation framework and the Technology Acceptance Model. Providers were recruited using email invitations from all 5 HFC clinics and across cancer specialty services. Interview transcripts were analyzed using thematic analysis.

Results: Of the total 180 providers, nine completed the interviews. A total of four overarching themes were identified: 1) Physicians are aware of the literature and benefits of PROMs in the general cancer care environment; 2) Despite implementation of PROMs at HFC, there is variable knowledge about its details; 3) Providers desired practical improvements to increase uptake among providers and patients (e.g. increased education around PROMs, increased visibility and accessibility of scores); and 4) Physicians identified patient-driven factors that impede consistent PROMs completion (e.g., limited technology literacy, flexibility of PROMs frequency).

Conclusions: While PROMs can streamline patient symptom reporting and guide clinical care, its utility can be undermined if not efficiently adopted by providers. Findings from this study will inform future adaptations to the PROMs program, ensuring a more user-engaged implementation process and potentiating positive downstream impacts on patient outcomes.

Leveraging Multifaceted Patient Data and Patient-Reported Outcomes for Prediction of Survival

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Abstract - Understanding when patients enter into their terminal stages of life is important, especially in cancer. Health care utilization near the end of life increases astronomically and frequently results in medical decisions that are not concordant with patients' end-of-life wishes. However, clinical assessment alone often falls short in understanding when patients are near death. This study aims to assess the value of phenotypic data, cancer risk factors, Social

Determinants of Health (SDOH), and Patient-Reported Outcome Measures (PROMs) in predicting survival among patients with head + neck and lung cancers.

In a retrospective IRB-approved study, we curated a dataset on N=4259 patients diagnosed with head + neck and lung cancer. The dataset included variables such as patient-level SDOH, Charlson Comorbidity Index, cancer diagnosis and staging, PROMs using the Patient Reporting Outcome Measuring Information System (PROMIS) domains such as depression and fatigue, and demographic information. Various classification models, including non-parametric models (Random Forest, Categorical Boosted Trees (CatBoost), and K-Nearest Neighbors) and parametric models (TabNet and Logistic Regression) were employed to evaluate their performance in predicting 120-day survival.

Among the models, CatBoost demonstrated the strongest performance, achieving 79% accuracy and an AUC of 89% in predicting 120-day survival on the test set. Subsequent feature ablation analysis revealed that SDOH variables were the most significant single predictors, accounting for a substantial 10% of the predictive performance as measured by AUC. The incorporation of PROMs features accounted for 3% of the baseline AUC. However, after SDOH was factored out, PROMs accounted for a 6% difference in predictive performance for 120-day survival. This consistent pattern across several ablative permutations suggests that PROMs serve as a valuable integrative predictor of survival likely due to significant interaction effects with other features.

These findings underscore the importance of integrating multifaceted data, particularly SDOH and PROMs, in enhancing survival predictions in head + neck and lung cancer patients. This approach needs to be validated in broader cancer populations in future studies.

Analysis of Causal Relationship Between Smoking and Head and Neck Cancer

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Abstract - Background. Head and neck cancer (HNC) accounts for approximately 4% of all cancers in the United States, with over 68,000 new cases diagnosed each year. Smoking is a major risk factor for HNC, contributing to a significant portion of these cases. A recent genome-wide association study (GWAS) using UK Biobank data, involving 1,106 HNC patients, identified single nucleotide polymorphisms (SNPs) associated with HNC. However, no studies to date have thoroughly explored the causal relationship between smoking and HNC development.

Hypothesis. We hypothesize that smoking is causally linked to an increased risk of HNC.

Methods. Using a large biobank dataset, we analyzed both genomic and health data from individuals diagnosed with HNC and matched controls. SNPs identified in previous GWAS studies were utilized to explore their role in the causal relationship between smoking and HNC. Two-sample Mendelian randomization algorithm was employed to assess the causal impact of smoking on HNC risk.

Results. No significant causal relationship between smoking and HNC was identified in this analysis. The SNPs associated with smoking behavior did not demonstrate a strong link to an increased risk of HNC in the study populations, and the genetic factors examined did not explain the relationship between smoking and HNC development.

Conclusions. Despite the established link between smoking and head and neck cancer, this study did not find significant evidence to support a causal relationship mediated by the genetic variations analyzed. Further research may be needed to explore alternative genetic or environmental pathways contributing to smoking-induced HNC risk. These findings suggest that genetic predispositions identified in previous studies may not fully explain the association between smoking and HNC, highlighting the complexity of cancer development in smokers.

Can New Onset Depression and Anxiety be Utilized to Assist with Early Diagnosis of Pancreatic Cancer? A Review of Prodromal Psychiatric Symptoms among Pancreatic Cancer Patients

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Abstract - Background: There are high rates of psychiatric comorbidity among patients with pancreatic cancer. Though depression and anxiety are often conceptualized as a response following a cancer diagnosis, literature suggests that some patients begin to experience psychiatric symptoms prior to a pancreatic cancer diagnosis.

Methods: The authors conducted a literature review examining the relationship between prodromal psychiatric symptoms and pancreatic cancer diagnosis and treatment outcomes.

Results: Studies suggest that 20 to 40 percent of patients with pancreatic cancer experience symptoms of depression and/or anxiety that began in the year prior to their cancer diagnosis. Prodromal psychiatric symptoms appear to be distinct from the early physical symptoms of pancreatic cancer such as fatigue or gastrointestinal issues. Furthermore, prodromal psychiatric symptoms are associated with a lower likelihood of undergoing chemotherapy and a lower survival rate among those with metastatic disease. While the relationship between new onset psychiatric symptoms and the development of pancreatic cancer is not fully understood, theories suggest inflammatory, hormonal, immunological, and biochemical contributing factors.

Conclusions: A significant proportion of patients will experience new onset depression and anxiety, independent of early physical symptoms, in the year prior to a pancreatic cancer diagnosis. Prodromal psychiatric symptoms may negatively impact treatment adherence and survival rates. Future research should seek to understand the mechanisms underlying this relationship and effective treatment strategies for prodromal psychiatric illness. Given the poor prognosis of pancreatic cancer, early identification and treatment is critical; inclusion of new onset psychiatric symptoms in a diagnostic screening algorithm should be explored.

Fibroblast Growth Factor-1 Modulates Kynurenine Uptake in an In Vitro Model of Estrogen Receptor Positive Tamoxifen Resistant Breast Cancer

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Abstract - Resistance to anti-endocrine therapies, including tamoxifen, remains the major clinical cause of estrogen receptor positive (ER+) breast cancer mortality. Approximately 40% of women treated with tamoxifen will develop resistance, a statistic that is even greater in women with obesity. Research from the Bernard and Wellberg laboratories demonstrates that adipose tissue-derived fibroblast growth factors (FGF) 1 and 2, which both activate fibroblast growth factor receptor-1 (FGFR1), contributes to obesity-associated breast cancer in both in vitro and in vivo preclinical models. However, the specific role FGFR1 signaling plays in tamoxifen resistance remains unknown. Utilizing an untargeted metabolomics assay of tamoxifen resistant (TAMR7) ER+ human breast cancer cells, of the 277 metabolites measured, FGF1 significantly increased the intracellular concentration of only one metabolite: kynurenine. An endogenous ligand of the aryl hydrocarbon receptor, kynurenine has been shown to modulate immune function. Our published research demonstrates that mice fed a high-fat diet have elevated levels of circulating kynurenine, as compared to low-fat diet fed mice. This is further supported by epidemiological data illustrating that kynurenine levels are elevated in obese individuals. Therefore, we hypothesized that FGFs promote tamoxifen resistance in ER+ breast cancer through the enhanced uptake of kynurenine from the microenvironment. We treated sensitive (MCF7) and resistant (TAMR7) ER+ breast cancer cells with FGF1 and performed RNA-sequencing, flow cytometry, and qRT-PCR. Using a flow cytometric monitoring system, we demonstrated that FGF1 increases the rate of kynurenine uptake into TAMR7s, as compared to an untreated control. Additionally, FGF1 increased the gene expression of SLC7A11, a kynurenine transporter, in TAMR7s. However, we found little gene expression of the tryptophan metabolizing enzyme indoleamine 2,3-dioxygenase in untreated and FGF1-treated MCF7s and TAMR7s, demonstrating that the increased intracellular kynurenine in FGF1-treated TAMR7s was likely through increased uptake and not increased synthesis.

Therefore, uncovering the role of FGFR1-driven kynurenine uptake in tamoxifen resistance will provide a greater understanding of potential targets to combat the reduction of efficacy of endocrine therapies.

Disparities in Psychological Distress Among Colorectal Cancer Survivors: Impact on Emergency Room Usage and Overall Mortality

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Abstract - Introduction: Cancer survivorship often entails considerable psychological distress. Colorectal cancer (CRC) presents distinct stressors like colostomy use and multimodality treatments leading to side effects like social isolation, body image issues, intimacy problems, and marital strain which affect the mental health of CRC survivors. However, information on the prevalence of psychological distress and its link to clinical outcomes is limited. We examined the prevalence of psychological distress and its association with emergency room usage (ER) and all-cause mortality among CRC survivors.

Methods: We utilized data from the 2000-2018 National Health Interview Survey (NHIS) and the NHIS linked mortality files. The main exposure was psychological distress, assessed with the six-item Kessler Psychological Distress Scale (K6) and classified as (no/low, moderate, severe). The outcomes were ER usage during the past 12 months and all-cause mortality. Multivariable logistic and Cox proportional hazards models were used to examine the associations between psychological distress, and ER usage and all-cause mortality, respectively. The models were adjusted for age, gender, survey year, race/ethnicity, marital status, education, smoking, geographic region, visit to mental professionals, general health status, comorbidities, and time since cancer diagnosis.

Results: A total of 3198 CRC survivors were included in the study, of whom 4.1% and 19.6% reported severe and moderate psychological distress, respectively. In the 12 months preceding the survey, 29.8% of CRC survivors had ER usage and 41.5% deaths occurred with a median follow-up of 84 months. In the adjusted model, compared to CRC survivors with low/no psychological distress, those with severe (aOR=1.86; 95% CI, 1.11–3.10) or moderate (aOR=1.60; 95% CI, 1.22–2.11) psychological distress had high odds of reporting ER use. Similarly, after adjusting for covariates, CRC survivors with severe (aHR =1.32; 95% CI, 1.30–1.34) or moderate (aHR =1.02; 95% CI, 1.01–1.02) psychological distress had higher risk of all-cause mortality compared with survivors experiencing low/no psychological distress.

Conclusion: CRC survivors with severe or moderate psychological distress have higher ER usage and all-cause mortality.

Methylation Patterns Define Meningioma Subtypes with Distinct Survival and Faster Recurrence Rates

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Abstract - Background: Meningiomas, common CNS tumors ranging from World Health Organization (WHO) grades 1-3, can exhibit malignant behavior and resist treatment, leading to recurrent interventions. While DNA methylation is linked to meningioma behavior, its role in tumor progression remains unclear. We hypothesize that molecular changes in primary tumors can predict recurrence risk by exploring shifts in DNA methylation patterns between primary and recurrent meningiomas and their impact on clinical outcomes.

Methods: We profiled genome-wide CpG methylation levels of primary-recurrent meningioma pairs (n=40 specimens) using the Illumina EPIC v1.0 array from 20 patients (median age: 52 years; 65% female; 85% Caucasians, median follow up: 26.5 months). Methylome data were pre-processed with the Sesame pipeline and downstream analysis of processed data was performed in R Studio (v4.4.1). Unsupervised analyses, including K-means clustering and principal component analysis, were performed and integrated with clinicopathological features. The optimal number of DNA methylation clusters was determined using appropriate parameters of consensus. Recurrence-free survival analyses were conducted using Kaplan-Meier curves for each distinct cluster.

Results: Analyzing the primary-only and the full primary-recurrent paired cohorts, we identified four DNA methylation subtypes with distinct patterns, each associated with specific

clinicopathological features. The most hypomethylated subtype showed significant enrichment in WHO grade 2, non-skull base location, and the majority deceased. In contrast, the most hypermethylated subtype exhibited enrichment in WHO grade 2, non-skull base location, and majority alive. Survival analysis revealed that the hypermethylated subtype exhibited significantly shorter time-to-recurrence compared to the hypomethylated counterpart (p=0.0043). In recurrent samples, 15% and 30% change WHO grade and methylation subtypes, respectively.

Conclusions: We observed infrequent changes in WHO grade and DNA methylation groups during the progression of meningiomas. However, distinct DNA methylation patterns are associated with different clinicopathological features and outcomes, including a DNA methylation subtype with significantly faster recurrence, indicating that these profiles could be valuable predictors of tumor behavior and help refine treatment strategies.

T cell expression in Benign Prostate and Subsequent Prostate Cancer Risk

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Abstract - Background: Prostate carcinogenesis is often heralded by infiltration of immune cells including regulatory Cd4+ T cells, a subpopulation of T cells that may have a role in promoting tumor growth and Cd8+ T cells, which may have specificity for prostate-derived antigens. Studies of T cell infiltration in prostate have focused on the tumor microenvironment, and heretofore not investigated pre-malignant prostate.

Methods: To determine how T cell infiltration influences the onset of prostate cancer, Cd3+, Cd4+ and Cd8+ T cell expression levels were measured in benign prostate biopsies derived from a sample of 558 case-control pairs (White = 302, African American = 256) matched on date, age, and race, utilizing an automated multi-image processing platform. The association between the risk of prostate cancer and the marker expression was assessed using conditional logistic regression adjusting for covariates.

Results: T cell expression levels were significantly lower in African American (AA) compared to white men. In multivariable logistic regression models, Cd4+ (Odds Ratio (OR) = 0.80; 95%

confidence interval (CI) = 0.66 -0.98) and Cd8+ (OR=0.77; 95% CI = 0.57- 1.04) T cell levels were inversely associated with prostate cancer risk in African American men. PSA levels were weakly correlated with T cell expression and significant interactions between PSA levels and both Cd3+ and Cd8+ expression levels were observed. Specifically, as PSA levels increased T cell expression became more inversely associated with prostate cancer. For instance, the prostate cancer OR estimate for Cd3+ T cells ranged from 1.11 to 0.74 as PSA levels ranged from 1 ng/ml to 20 ng/ml. The similar OR for Cd3+ T cells ranged from 0.75 to 0.43. Race-specific analysis revealed that this PSA effect on the prostate cancer risk associated with T cell expression was only present in White men.

Conclusion: Given the strong association between higher PSA levels, inflammation, and prostate cancer risk, in early prostate carcinogenesis T cell infiltration may be acting as a cancer defense mechanism.

Reasons for and Predictors of Cancelled PET-CT Appointments Among Lung Cancer Screening Patients (2022-2023)

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Abstract - Background / Hypothesis: Canceled positron emission tomography - computed tomography (PET-CT) scans can delay cancer diagnosis and treatment and be costly for health systems. The aim of our study was to determine reasons for and predictors of canceled PET-CT appointments among patients with abnormal lung screenings. We hypothesized the most common reason for cancellation would be high glucose levels at appointments and cancellations more frequently among diabetics than non-diabetics.

Methods: Patients with an abnormal lung screening that had an order for a lung PET-CT between 2022-2023 were evaluated. Medical records were abstracted for provider/resource and patient PET-CT appointment cancellations. Demographics and medical history of each patient were also collected. During analysis, patients that had at least one cancellation (patient request or No Show) were compared with patients that had none. T-tests, chi-square tests, and a multivariable logistic regression model were used to evaluate evidence of association between patient cancellation status and possible predictors. These predictors included: age, sex, race, insurance type, marital status, diabetes status, obesity status, and any mental health condition.

Results: Out of 308 PET-CT appointments, 23.1% were canceled. 87.3% were patient cancellations and 12.7% were provider/resource cancellations. Reasons for patient cancellations were insurance/transportation (17.7%), patient preparation (16.1%), and illness/health status (8.1%). Other reasons included personal/family/reschedule (38.7%) and No Show (19.4%). Out of 244 patients, 16.8% had a PET-CT cancellation. No significant differences were found in patient cancellation status between diabetics, pre-diabetics, and non-diabetics (p-value = 0.974). When

adjusting for sex, marital status, and any mental health condition, odds of patient cancellation were 2.43 times greater in patients with a medical history of obesity than patients without this medical history (p-value = 0.05).

Conclusion: Odds of patient cancellation of PET-CT were higher in individuals that had a recorded medical history of obesity. These results will be used to guide the healthcare team in helping patients prepare for follow-up tests after an abnormal lung screening.

Lessons From a Multi-Stakeholder Summit Using Design-Thinking and Implementation Science Principles to Address Disparities in Cancer Care

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Abstract - BACKGROUND: Social determinants of health (SDOH) cause racial and ethnic minority populations to experience disproportionate rates of health disparities, resulting in adverse health outcomes; including populations faced with a cancer diagnosis. A pillar of the Henry Ford Innovations (HFI) mission is to improve the health of Michigan's diverse communities through the development of innovative approaches to healthcare. Core to this approach is cross-functional collaboration—both among various clinical teams as well as with community-based and community-serving organizations and life sciences companies.

METHODS: In 2023, HFI with Premier Applied Sciences and local community organizations convened our second health equity summit, aimed at reducing inequities in cancer care. More than 80 stakeholders participated, across multiple disciplines, including researchers, clinicians, patient advocates, community service organizations, and life science companies. The event was preceded by a series of exploratory meetings to align groups of around shared aims and access to industry funding. Prior to the meeting a national expert in implementation science methodology mediated discussions with the working groups to identify patient and provider gaps in care during the pre-diagnosis and diagnosis stage. They then brainstormed iterative interventions at the summit which leveraged the health system's strengths and were specific, actionable, and feasible.

RESULTS: 6 teams were created in the areas of prostate cancer, lung cancer, multiple myeloma, with focus on access to care barriers, inequity in molecular testing, barriers to preventative screening.

CONCLUSIONS: We continued to iterate on our process to bring diverse stakeholders together to address health inequities. Participants learned how improvement science and design-thinking are used to map the patient journey, identify and prioritize barriers, create high-level patient-centered and community-engaged prototypes, and ultimately create solutions that will have a higher chance of impact. Incorporating this methodology increased engagement with non-HFH stakeholders. We also were able to highlight that inclusion of unique voices and perspectives is a cornerstone to creating far-reaching and multi-faceted solutions to advance health equity.

Health Disparities in Gynecologic Cancers: A Focus on Ovarian, Cervical, and Endometrial Cancer Patients

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Abstract - Background: This study examines gynecologic cancers, including the three most common types—ovarian, cervical, and endometrial. It provides an updated analysis of disparities in cancer type distribution, age at diagnosis, and first-course surgery and chemotherapy among various race-ethnicity groups.

Methods: A retrospective study examined patients aged 18 and older with initial tumors diagnosed between 1/1/2016 and 10/31/2023 using a gynecologic cancer database derived from the Henry Ford Health cancer registry. Descriptive analyses reported frequency counts and percentages for cancer type, diagnosis age group, and first-course treatment and means and standard deviations for diagnosis age overall and by race-ethnicity. Univariable chi-squared tests for categorical variables and Kruskal-Wallis tests for continuous variables assessed racial group differences, followed by post hoc pairwise comparisons using chi-squared and Wilcoxon tests.

Results: This study included 4,437 women revealing that Hispanic patients had higher cervical cancer diagnosis than other race-ethnicity groups (63% vs. ≤48.5%), Asian patients led in ovarian cancer (12.5% vs. ≤11.4%), and White, Black/African American (AA), and Asian patients had higher proportions of endometrial cancer diagnosis than Hispanic patients (≥38.5% vs. 21.2%). Additionally, White and AA patients were oldest at diagnosis, while Hispanic patients were youngest (53.3 and 52.3 vs. 44.4 years; $p<0.05$). Among ovarian cancer patients, 47.7% received combined surgery and chemotherapy, compared to ≤24.3% in other treatment groups. AA ovarian cancer patients less frequently received this combined treatment than White patients (40% vs. 49.1%, $p=0.2$) and more often received only chemotherapy (22.7% vs. 9.5%, $p=0.003$). Cervical and endometrial cancer patients typically underwent surgery alone (81.5% and 54.6%). Hispanic and AA cervical cancer patients had higher proportions of no treatment (surgery/chemotherapy) compared to White patients (17.2% and 18.2% vs. 8.8%, $p<0.01$).

Conclusion: Preliminary analysis indicates racial disparities in age at diagnosis and first-course treatments among gynecologic cancer patients. Further analysis is needed to explore potential reasons for these disparities, including patient health status—specifically staging and histology grade—and social determinants of health.

Developing a Rule-Based Algorithm to Identify Recurrent Non-Hodgkin Lymphoma in Electronic Health Data

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Abstract - Background: Recurrent cancers are not captured in a standardized way by US tumor registries. We aim to develop a rule-based algorithm to identify recurrent cases of two common subtypes of non-Hodgkin lymphoma, diffuse large B-cell lymphoma (DLBCL) and follicular lymphoma (FL) in EHR data. Our goal is to create a tool with a PPV greater than 75% to identify recurrent cases for population-based research.

Methods: DLBCL and FL cases (2000-2018) were identified in tumor registry data from the Virtual Data Warehouse (VDW), housed at two study sites. We compiled a comprehensive list of pharmacy and procedure codes to indicate when each patient started first-line treatment. We defined recurrent cases as those who restarted treatment ≥6 months after completing first-line treatment. The algorithm was built using data from Site A and tested at Site B. Results were validated by chart review. The algorithm was then revised to improve performance and will be applied to EHR data at a third site.

Results: Site A identified 225 patients (137 DLBCL and 88 FL). Twenty-three DLBCL and 19 FL patients met criteria for recurrent disease, with a mean of 3.4 years from diagnosis to first recurrence. At site B, 392 patients were identified (246 DLBCL and 146 FL). Forty-nine DLBCL and 48 FL patients met criteria for recurrent disease. Chart review determined a sensitivity of 94%, specificity of 45%, PPV of 52% and NPV of 92% for the algorithm. Following revisions to reduce the false positive rate, the algorithm had reduced sensitivity (66%) and NPV (75%) but higher specificity (88%) and PPV (82%).

Conclusions: The number of recurrent cases identified by the algorithm are in line with clinical expectations. We have developed two algorithms that may be applied to EHR data for population-based research into recurrence of two subtypes of NHL. Our revised algorithm met our a priori goal for PPV and may be a better choice to apply to large population-based databases.

Association Between Acculturation and Breast Cancer Risk Among Polish Immigrant Women in the U.S.

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Abstract - Introduction: Although acculturation has been identified as a risk factor for breast cancer (BC), few studies have investigated whether acculturation is associated with BC risk in immigrant European populations. Polish immigrant women to the U.S. face tripling in BC mortality in their generation. We investigated the association between the level of acculturation and BC risk in Polish immigrant women.

Methods: Data are from a population-based case-control study of BC in Polish immigrant women residing in Illinois and Michigan. Acculturation was assessed through multiple domains including language preference, neighborhood/community preference, and duration of stay in US through in-person interviews. Language preference included 3 domains: language spoken at home, spoken with friends/work, and used to read or think. Community preference and duration of living in the US (years) were also individually categorized into three levels. We created a 9-point scale to describe language acculturation, by summing individual scores for each language domain, and a 15-point scale for the overall acculturation level by summing scores from all domains. Mean scores were used as the cutoff-point to group women into high vs lower acculturation categories. Odds ratios (OR) and 95% confidence intervals (CI) were estimated by multivariable logistic regression.

Results: A total of 186 controls and 89 cases were included in current analyses. Compared to lower overall acculturation, high overall acculturation was associated with increased risk of BC (OR = 2.09, 95% CI, 1.15-3.80) after adjustment for site and age at diagnosis. The association was attenuated (OR=1.68, 95% CI, 0.88-3.19), after further adjustment for other confounders such as parity, BMI and age at first full term pregnancy. The association between language acculturation and BC was not statistically significant.

Conclusion: These findings suggest that high overall acculturation potentially impacts BC risk in Polish immigrant women. This is a part of a larger study where we plan to investigate whether acculturation also impacts DNA methylation levels in BC.

Nature-Based Auditory Meditation for Bereaved Cancer Caregivers

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Abstract - Background: Bereavement is the period of grief and mourning following the death of a loved one. Informal cancer caregivers (CGs) experience the emotional, physical, and social toll associated with bereavement but often have limited access to supportive interventions. Limited research has addressed bereavement for cancer CGs despite the increased risk of prolonged grief disorder recognized in the DSM-5.

Methods: This recently funded NIH study provides a nature-based healing meditation (NBHM) designed to support home-based cancer caregivers during early bereavement. The study will include 50 adult CGs who experienced the death of a friend or family cancer patient in home hospice. A single-group, longitudinal, pre-post mixed methods design will evaluate the feasibility and acceptability, and preliminary efficacy. Acceptability and feasibility will be determined via numbers eligible vs. number consented; numbers consented vs. numbers completed; and number of weeks using the intervention. Efficacy data will be collected at baseline (week 0), mid-intervention (week 6), and post-intervention (week 12). Outcome measures include the Prolonged Grief (PG-13-Revised) scale, Attention Function Index (AFI), Quality of Life (QOL; PROMIS-29), and depressive/anxiety symptoms (subscales of PROMIS-29). Finally, semi-structured interviews with CGs will evaluate benefits, satisfaction, and challenges. The NBHM intervention consists of a selection of audio-guided modules to be used weekly. Each 10-minute module combines nature-based imagery with gentle language to direct attention toward QOL, grief recovery, and respite from anxiety/depression through mindfulness practices. For example, the solar system module encourages focused attention to envision the vastness of the universe as they float among the stars and planets in space, helping CGs move beyond the feelings of bereavement and gain a new perspective.

Results: Expected outcomes include high acceptability and feasibility, reduced grief intensity, improved directed attention, and improved QOL and emotional well-being.

Conclusions: This intervention will set the stage for a larger-scale trial to integrate NBHM practices into routine hospice care, ultimately supporting the emotional health of bereaved cancer CGs through nature-based meditative practices.

Development, Implementation, and Evaluation of a National Cross-Generational Strategy to Support Introduction of the Human Papillomavirus Vaccine and Utilization of Cervical Cancer Screening in Nepal

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Abstract - Background: Cervical cancer (CC) is the fourth most common cancer in women globally, with the highest rates of incidence and mortality occurring in low- and middle-income countries. Henry Ford Health's Global Health Initiative collaborates with governments, health facilities and community organizations in several countries, including Nepal, to support Human Papillomavirus (HPV) and CC screening uptake to address this issue. The Nepal government has prioritized free screening and is in the process of introducing the HPV vaccine into the national immunization plan.

Methods: We utilized mixed methods including interviews, focus groups, and surveys of healthcare providers (HCPs) and parent/guardian-daughter dyads for a randomized control trial of interventions for both HPV vaccine and CC screening. Items and scales included knowledge, perceptions, and awareness of HPV and CC, vaccine hesitancy and other factors which affect intentions to uptake the HPV vaccine and use of CC screening. A total of 404 HCP, 483 mothers and 479 girls (age 9 to 14) representing four districts participated in the survey.

Results: HCP were knowledgeable about CC, but only 9% of HCP stated they had received the recommended training for CC screening. A majority of HCP (89.3%) were aware of the link between HPV and CC. However, HCP had limited overall knowledge about HPV and HPV vaccines. Less than one-third of mothers were aware that HPV infection can increase risk for CC. A vast majority of girls had not heard about HPV (87.6%) or HPV vaccines (88.5%). Reported barriers to discussions about HPV and CC were lack of knowledge about risks and embarrassment discussing reproductive health topics.

Conclusion: In Nepal, data was utilized to develop manuals for HCP and health volunteers to support advocacy efforts for HPV vaccine uptake and CC screening. Findings from this study will also inform an ongoing HPV vaccine uptake project in Colombia, as well as similar projects proposed for India and Jordan as part of GHI's efforts to advance health equity globally.

Physician Performance in Referring Patients for Lung Cancer Screening

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Abstract - Background: Lung cancer remains the leading cause of cancer-related deaths worldwide, accounting for 25% of all cancer fatalities. Early detection via screening is essential to improving survival rates, as lung cancer often presents late in its course, leading to a 5-year survival rate of just 19% in the U.S. The U.S. Preventive Services Task Force recommends annual lung cancer screening for high-risk individuals, yet screening uptake remains low, with only 5-20% of eligible individuals undergoing screening. At Henry Ford Health (HFH), 17,545 patients have been screened since the launch of the lung cancer screening program in 2012, resulting in 41,613 screening CTs and 416 lung cancer diagnoses. This study aimed to evaluate physician referral patterns for lung cancer screening at HFH.

Methods: We used outpatient visit data and electronic health records (EHR) to calculate referral rates among eligible patients aged 50 to 77. Two algorithms—Cross-sectional and Longitudinal—were employed to determine patient eligibility based on smoking history. Data from 232,609 patients and 432 physicians (family and internal medicine) over a one-year period were analyzed. We investigated differences in referral rates by physician specialty, gender, age, and health system-region, along with CT completion rates.

Results: Family medicine physicians had a referral rate of 48.7%, while internal medicine physicians referred 65.2% of eligible patients. Our analysis also examined the relationship of physician's sex and patient referral rates by patient's sex. For male physician, the referral rates were not significantly different between male and female patients (p=0.65). However, female physicians were significantly more likely to refer female patients compared to male physicians (p=0.06). No significant association were observed for male physician and patient's referral rate

Conclusions: Our findings highlight variations in physician performance that could guide interventions to improve screening rates. Specifically, addressing the low referral rates among certain providers may enhance overall screening coverage, ultimately improving early lung cancer detection and outcomes.

Cancer Imaging & Early Detection

Investigation of Unsupervised Race-Based Predictive Modeling Strategies to Improve Prediction Power of Tumor Recurrence in Head and Neck Cancer Patients in Diverse Racial Groups

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Abstract - Background: Significant racial disparities currently exist in head and neck cancer outcomes, with 5-year overall-survival (OS) being ~30% lower in African American (AA) versus Caucasian (CC) patients. After adjusting for age, sex, tumor site, and year of diagnosis, AA race has been found to be associated with worse OS and Disease-Specific-Survival (Hazard Ratio=1.67, $p<0.0001$). Epidemiological studies suggest several reasons for the disparity. It is important to examine how imaging biomarkers and clinical factors can contribute to these outcome disparities.

Purpose: This study investigates the predictive value of different race-based models constructed from radiomics and clinical factors, to predict tumor recurrence at three years for AA and Caucasian head and neck cancer patients.

Methods: Two-hundred-two patients (78 AA, and 124 Caucasian), treated with definitive radiation/chemoradiation (70Gy, 35-Fraction) were studied. A five-fold nested-cross-validation (NCV) technique was used to evaluate forty-five unsupervised Kohonen-Self-Organizing Maps (KSOMs, topology: 5×5, five-folds for nine different models corresponding to different combined cohorts and information modalities) constructed from twenty-six one-hot encoded clinical factors and 447 radiomics features extracted from patients' gross-tumor-volume on planning-CT images.

Results: The NCV-based unsupervised predictive accuracy for AA and Caucasian-based models were: 0.712±0.048 and 0.633±0.066 for clinical-factors, 0.736±0.069 and 0.678±0.074, for radiomics, and 0.756±0.088 and 0.693±0.093, for the ensemble model (combined information: radiomics + clinical-factors). The accuracy for the combined cohort ensemble models (combined clinical-factor and Radiomics) were: 0.798±0.093 and 0.734±0.084 for AA and Caucasian patients, respectively.

Conclusions: This study demonstrates the value of different unsupervised modeling strategies for combining different information modalities, their sparsity levels and influence on the model sensitivity according to their class imbalance, and patient cohort sizes to reveal and improve the outcomes prediction power in diverse racial groups. Although radiomics serve as a useful tool for distinguishing differences in the patients' outcome, it is important to note that this study does not offer causal associations. Causality must include consideration of different health care disparities (e.g., access to healthcare, costs-of-care, etc.).

Acute Prediction of Radiation Therapy Induced Tumor Response in an Animal Model from Dynamic Contrast Enhanced MRI Using an Unsupervised Kohonen Self-Organizing Map Technique

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Abstract - Background: Spatial assessment of RT-affected brain regions can play a key role in the optimization of treatment planning in cancer patients, using Dynamic-Contrast-Enhanced (DCE)-MRI and pharmacokinetic models to assess these regions has traditionally been challenging.

Purpose/Hypothesis: Here, we introduce an unsupervised adaptive model for the prediction of the probability of the RT-affected regions in an animal model of cerebral tumors.

Methods: Twenty-four immune-compromised-RNU rats were implanted with human U-251N cancer cells to form an orthotopic glioma. For each rat, 28 days after implantation, two DCE-MRI studies (Dual-Gradient-Echo) were performed 24h apart using a 7T MRI scanner. A single 20Gy-stereotactic radiation exposure was performed before the second MRI (acquired 1-6.5 hours post-RT). DCE-MRI pharmacokinetic analysis was performed using a nested-model-selection (NMS) technique to distinguish three different pathophysiological brain regions. Spatiotemporal signals of the rat brain voxels (two cohorts: 24 pre, and 24 post-RT) were used to build an unsupervised Kohonen self-organizing-map (K-SOM, topology: 10X10 neurons) to quantify RT-induced signal changes on the feature space. The best-matching-units (BMUs) based hit maps were estimated for the two cohorts and the average values of their Silhouette-Coefficients (SCs) and Coefficient-of-Variations (CVs) were calculated to quantify the K-SOM performance. Ultimately, a brain voxel-wise probability map of the RT-induced changes was reconstructed.

Results: Non-leaky and highly permeable brain regions showed less RT-induced effect compared to the peritumoral regions pertaining to leaky tissues with no back-flux to the vasculature. The average values of SCs and CVs were: 0.571/0.587, 0.709/1.081, and 0.594/0.727 for the three NM regions, respectively.

Conclusions: This pilot study shows for the first time that unsupervised analysis combined with an NMS technique can effectively capture RT-induced changes of DCE-MRI information. Results suggest that peritumoral zones corresponding to infiltrative tumor borders with contrast-enhanced rim are highly affected by RT.

An Unsupervised Probabilistic Nested Model Selection Technique in Pharmacokinetic Analysis of Dynamic Contrast Enhanced MRI Data in Animal Model of Brain Tumor

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Abstract - Purpose/Hypothesis: Dynamic contrast enhanced (DCE)-MRI nested-model-selection (NMS) theory assumes the measured time trace of contrast-agent (CA) concentration in a voxel, traces a single physiologically nested model. In reality, combinations of different models may exist within a voxel's CA time-trace. This study introduces an unsupervised feature engineering method, Kohonen-Self-Organizing Map (KSOM), to estimate the probability of each model in a specific voxel, generating a more accurate estimation of permeability parameters.

Methods: Thirty-two immune compromised RNU rats were implanted with human U-251N cancer cells, and DCE-MRI (7T Dual-Gradient-Echo) were acquired from all rat brains. The time-trace of change in the longitudinal-relaxation-time (ΔR1) in all the voxels of the animal's brain were calculated. DCE-MRI pharmacokinetic (PK) analysis was carried out using conventional NMS and an extended-Patlak graphical method. 143,057 ΔR1 profiles were extracted from the brain voxels and used to build the KSOM (topology-size: 10X10, 250 epochs, competitive learning algorithm along with Best-Matching-Unit strategy). The NMS results (at a confidence-level of 0.95%) were used to label the feature space and probability maps for each model.

Results: The KSOM probabilistic model choice maps (at 50% threshold) for the leaky tumor regions were strongly similar (Dice Similarity Coefficient, DSC= 0.914±0.043, and 0.936±0.038 for Models 2 and 3, respectively) to their respective NMS maps (CL=0.95%). The KSOM-NMS technique was able to capture the voxel-wise probability and model uncertainties (mainly at the borders of the model regions) according to their ΔR1 profiles' similarities and dissimilarities. The KSOM-NMS also produced more stable maps of vascular parameters and nested-model regions that were less impacted by the arterial input function dispersion effect.

Conclusions: This work introduces for the first time an unsupervised model averaging technique to estimate the contribution of different models in PK analysis and provides a more accurate estimation of vasculature parameters from DCE-MRI studies.

Noninvasive Classification of Central Nervous System Tumors Using cfDNA Methylation Profiling: Development and Validation of a Multiclass Random Forest Model

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Abstract - Background: Accurate classification of central nervous system (CNS) tumors using tissue samples has been well-established. However, a noninvasive approach utilizing liquid biopsy samples offers significant advantages for patient diagnosis and monitoring. This study aims to develop a multiclass classification model for CNS tumors based on circulating cell-free DNA (cfDNA) methylation profiles obtained from blood samples.

Methods: We performed genome-wide methylation profiling (EPIC array v1.0) on 227 serum liquid biopsy specimens from patients with CNS tumors, including glioma (n=109), meningioma (n=81), ependymoma (n=23), and pituitary tumors (n=14). A random forest classifier was trained on 155 samples, while 72 samples were reserved for independent testing (held-out set). Publicly available methylome data from CNS tumor tissue samples were used to further validate the tumor-specific signatures derived from the blood samples.

Results: Our random forest model demonstrated a classification accuracy of 91% on the held-out test set, confirming the presence of tumor-specific methylation signatures in blood samples from patients with CNS tumors. Tumor-specific methylation signatures detected in liquid biopsy samples were able to discriminate between related tumors in an independent set of tissue samples.

Conclusions: These findings suggest that liquid biopsy-derived cfDNA methylation signatures are a promising noninvasive tool for the classification and surveillance of CNS tumors, offering an alternative or complement to tissue-based and imaging methods.

Targeted Biodegradable Near-Infrared Fluorescent Silica Nanoparticles (FSNs) for Colorectal Cancer Imaging

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Abstract - Colorectal cancer (CRC) is a particularly dangerous disease and is the third leading cause of cancer related death in the United States. While early detection is key to effective treatment, current methods of detection are unable to adequately detect small or flat lesions or

define the tumor boundaries to allow for precision treatment. Fluorescence endoscopy is a promising imaging technology for detection of CRC due to its wide field of view and high sensitivity and temporal resolution. Use of new, targeted diagnostic agents will allow us to take advantage of fluorescence endoscopy for effective early detection of CRC. We developed near-infrared (NIR) fluorescent silica nanoparticles (FSNs) of size 50-200 nm conjugated with PEG and carcinoembryonic antigen (CEA) antibodies. These particles were then characterized, and targeting was validated through high uptake of CEA-FSNs in CEA expressing HT29 cells and low uptake in CEA negative HCT116 cells. Smaller (50 nm) FSN were internalized better than larger particles. In xenografted mice, CEA-FSN injected intravenously were taken up better by HT29 tumors than HCT116 tumors validating the specific targeting of these particles. In F344-PIRC rats, lesions were detected by white light endoscopy, and after ex vivo topical treatment with CEA-FSN, fluorescence was detected in the excised intestinal tissue. Immunofluorescence imaging taken of slides of the excised tissue show excellent colocalization of CEA, CEA-FSNs, and the CRC marker β-catenin which indicates that CEA-FSNs are a promising molecular imaging tool for the early and effective detection of CRC.

LDCT Screening in a Mid-Western Community Hospital Network: A Retrospective Cohort Study

Authors - Jason Law MS MD, Alisha Kennedy BS, Niket Shah MD, Ling Wang MS PhD, Borys Hrinczenko MD PhD

Abstract - Context: Lung cancer is the number one cause of cancer deaths in the United States, resulting in approximately 235,760 deaths per year. In accordance with USPSTF guidelines, adults ages 50-80 with an equivalent 20-pack-year smoking history are recommended for annual lung cancer screening with low-dose computed tomography (LDCT). This applies to current smokers and adults with a significant history of smoking that have quit within the past 15 years. The NLST and NELSON trials showed significant mortality benefit in smoking populations undergoing LDCT through early detection, informing the USPSTF recommendation. However, these high-powered studies still faced limitations in the diversity of their patient populations.

Objective: Identify population characteristics and risk factors that will inform screening recommendations in addition to USPSTF guidelines. Our exploratory objective is to quantify the association of these risk factors with the stage of diagnosis to inform future studies.

Results: A total of 2638 subjects, 49.91% females, 50.09% males, mean BMI 29.50 ± 7.17 (SD), pack-years 44.52 ±18.29, Medicaid 6.66%, Medicare 32.01%, Private Insurance 57.62%, Self-pay /unknown 3.71%. Current smoker 65.49%, Former smoker 34.51%, other CT findings 51.99%, pre-COVD 46.08%, during COVD pandemic 53.92%. Lung-RADS® category 0 (0.04%), 1 (35.33%), 2, (50.61%), 3 (6.56%), 4a (4.85%), 4b (1.55%), 4x (1.06%). A statistically significant association with a Lung-RADS® category ≥3 was found with higher BMI, OR=0.980 (95% CI, 0.969-0.991), p=0.0005, and female gender, OR=1.232 (95% CI, 1.039-1.462), p=0.0166.

Future Direction: Publication is in progress; we plan on using this data to improve lung cancer screening in this hospital network and will follow-up with data representative of the 2021 change in USPSTF recommendation. This study may help identify the extent to which risk factors, in addition to smoking, contribute to lung cancer progression

Development of Disease-Specific Biomarkers Using Extracellular Vesicles for Early Detection of Osteosarcoma in Children

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Abstract - Background: Osteosarcoma (OS) is a malignant bone tumor predominantly affecting child, with a high mortality rate of approximately 50%. Although surgical excision and chemotherapy can be effective if OS is detected early, delays in diagnosis often lead to poorer outcomes and reduced survival rates. Developing disease-specific biomarker for early diagnosis is crucial; however, significant biomarkers for OS in children have not yet been identified.

Hypothesis: This study provides novel insights into the identification of OS-related biomarkers for early diagnosis that can improve patient outcomes and survival rate

Methods: Extracellular vesicles (EVs) were isolated from the serum of patients with OS (n = 5) and healthy individuals (n = 5, control group) and characterized based on our defined protocol. After filter aided sample preparation (FASP) digestion for protein quantification using the bicinchoninic

acid (BCA) assay, desalting was performed for further analyses. Liquid chromatography–mass spectrometry (LC–MS) was conducted as per defined protocols, and bioinformatic analyses, including functional annotation, pathway analysis, network visualization, were performed to compare the expression patterns of proteins identified through proteomics. Protein markers were examined and validated via enzyme-linked immunosorbent assay (ELISA) using additional blood samples from both groups (n = 10 each).

Results: EVs were characterized completely via NTA, FACS, TEM. Scatter plots and heatmaps of protein data from LC-MS analysis revealed upregulated levels of CFHR1, VWF, LYZ, FGB, FBRN2, PRG4, and ZSWIM9 in the OS group compared with those in control group. Regarding protein–protein interaction, bioinformatic analyses showed that some of the proteins with altered levels were potentially related to OS biomarker. Finally, ELISA demonstrated that LYZ and CFHR1 were significantly expressed in patients with OS.

Conclusions: The EV proteins LYZ and CFHR1 are promising biomarkers for the early diagnosis of OS, which is paramount for improving treatment outcomes and survival rates. Identifying these biomarkers is essential to facilitate rapid diagnosis and proactive intervention.

Exosome-Coated Gadolinium-Doped Prussian Blue Nanoparticles as a Multi-Theranostic Agent for Glioblastoma Treatment

Authors - Maggie Lee, MS, Michigan State University; Meghan Hill, PhD, Michigan State University; Praveen Kumar, PhD, Michigan State University; Kay Hadrick, PhD Candidate, Michigan State University; Taeho Kim, PhD, Michigan State University

Abstract - Glioblastoma is one of the most common and aggressive types of brain cancer, with no known cure and a survival of less than 15 months. FDA approved, Prussian Blue nanoparticles (PBNPs) are being explored as a new class of near-infrared (NIR) laser-driven, photothermal ablation/photoacoustic imaging agents. Laser interstitial thermal therapy (LITT) is an emerging, minimally invasive, magnetic resonance imaging (MRI)-guided procedure used for cytoreductive treatment of glioblastoma. This study introduces a novel multi-theranostic agent for glioblastoma treatment: exosome-coated gadolinium-doped Prussian Blue nanoparticles (Exo:GdPB). These nanoparticles combine the photothermal ablation capabilities of Prussian Blue, the MRI contrast enhancement of gadolinium, and the brain-targeting ability of glioblastoma-derived exosomes. We synthesized uniformly sized GdPBNPs, using a co-precipitation method between gadolinium(III) chloride and potassium(III) ferricyanide in the presence of citric acid and polyvinylpyrrolidone. These NPs with near-infrared absorbance, coated with exosomes, were characterized and demonstrated their efficacy in vitro. PBNPs (210.4 nm, PDI: 0.053) and GdPBNPs (256.4 nm, PDI: 0.028, Gd content: 1.28 μg/mL) showed absorption peaks at 750 nm and 800-900 nm, respectively, allowing the particle to be used as a photothermal ablation agent with an 808 nm laser and clinically used 980 nm lasers in the Visualase apparatus. Size analysis and uranyl acetate staining confirmed successful exosome coating. MRI analysis showed that the

particle has a T1 relaxivity between that of water and Dotarem. The Exo:GdPBNPs showed similar uptake patterns between bare exosomes and Exo:GdPBs, and enhanced photothermal ablation effects when exposed to an 808 nm laser with a significant reduction in cell viability at low doses of nanoparticles (< 55% at 0.03 mg/mL of NPs) after treatment of the cells with Exo:GdPB and 808 nm laser (1.5 W/cm2 for 1 min). This nanohybrid system has the potential to improve the precision and effectiveness of laser interstitial thermal therapy (LITT) for glioblastoma treatment, particularly near sensitive brain regions, by allowing better visualization and more efficient tumor cell targeting.

The Advanced Molecular Imaging Facility: A Preclinical Imaging Core Facility for MSU

Authors - Christiane Mallett, Jeremy Hix, Erik Shapiro

Abstract - Department of Radiology and Institute for Quantitative Health Science Engineering, Michigan State University The Advanced Molecular Imaging Facility (AMIF) supports the small animal imaging needs of MSU researchers and off-campus partners. We can image mice, rats and rabbits using a wide range of techniques.

PET/MRI: The 7T MRI is designed for rodent preclinical MRI and has a PET insert for high resolution simultaneous PET imaging. We have specialized RF coils for proton imaging of mouse brain and body, rat brain and body, and larger animals up to rabbit size. FDG for PET imaging is acquired locally; other isotopes can be received from facilities around the country.

Photo-Acoustic Imaging: This is a hybrid optoacoustic/ultrasound imaging system. Whole body, co-registered tomographic ultrasound and photoacoustic images are produced. Contrast can be endogenous (deoxy-hemoglobin vs oxy-hemoglobin) or from exogenous agents such as indocyanine green or nanoparticles.

Optical Imaging: We have systems for bioluminescence and low and high wavelength fluorophores. This is ideal for non-invasive longitudinal monitoring of disease progression, cell trafficking and gene expression patterns in living animals.

µCT: The system is suitable for all analytical µCT applications and features cardiac gated imaging and an adjustable bore size that enables imaging of a wide range of species up to rabbits in size. Flexible scan lengths and resolutions ensure low radiation dose exposure for serial studies.

3D Histology: The 3D histology system can be used to automatically section and image samples ranging from organs to whole animals. Samples are embedded in OCT and then placed in the chamber of the Xerra for sectioning. Images are then reconstructed into a 3D file for further analysis. Ideal for specimens with fluorescent tracers or particles, or genetically-encoded expression of fluorescence in structures of interest.

Magnetic Particle Imaging (MPI): This system sensitively and specifically detects superparamagnetic iron oxide particles in mice and can be applied to labeled cells or particles, e.g., tracking immune cells, cancer metastasis, and targeted nanoparticles. 2D or 3D images can be acquired and are easily co-registered with μCT.

Experimental and Clinical Therapeutics

Validation of Platelet-derived Growth Factor Receptor Alpha (PDGFRα) as a Therapeutic Target in Glioblastoma

Authors - Artem Berezovsky, PhD, HF, Neurosurgery, Detroit MI; Susan Irtenkauf, BS, HF, Neurosurgery, Detroit, MI; Laura Hasselbach, BS, HF, Neurosurgery, Detroit, MI; Andrea Transou, MS, HF, Neurosurgery, Detroit, MI; Katherine Gurdziel, PhD, Wayne State University, Genomic Sciences Core, Detroit, MI; Indrani Datta, MS, HF, Neurosurgery, Detroit, MI; Ruicong She, MS, HF, Public Health Sciences, Detroit, MI; James Snyder, DO, HF, Neurosurgery, Detroit MI; Laila Poisson, PhD, Public Health Sciences, Detroit, MI; Ana C. deCarvalho, PhD, Neurosurgery, Detroit, MI

Abstract - Platelet-derived growth factor receptor alpha (PDGFRα), a mitogen receptor tyrosine kinase in oligodendrocyte precursor cells (OPC), is a promising therapeutic target in glioblastoma (GBM) due to its frequent oncogenic activation in these tumors. To validate PDGFRα as a driver of GBM tumor maintenance, we employed a patient-derived model (HF3253) harboring an extrachromosomal (ecDNA) PDGFRA amplification, driving high copy number and heterogeneity, and an in-frame deletion leading to constitutive activation. Tumorigenicity of cell subpopulations containing different proportions of PDGFRA ecDNA(+) and (-) cells were tested in mouse orthotopic-xenografts. The higher the frequency of PDGFRA ecDNA(+) cells implanted, the shorter the symptom-free survival, due to a strong selection for PDGFRA ecDNA(+) cells during tumor growth, based on histology and TaqMan DNA Copy-Number assay. Four clones derived from single PDGFRA ecDNA(-) cells, expressing very low levels of PDGFRα, formed xenograft tumors at a much slower growth rate relative to parental ecDNA(+) (Kaplan-Meier survival-curve comparison, Log-rank $p< 0.0001$; $n\geq4$/group). In contrast to parental HF3253, ecDNA(-) tumors demonstrated diffuse tumor morphology and weak PDGFRα activation, with no re-emergence of PDGFRA amplification. Comparing ecDNA(+) and (-) tissue and cells using bulk and single-cell RNAseq, PDGFRA expression was associated with a biphasic injury response/developmental transcriptional signature typical of CNS fibroblasts and OPCs, respectively. The ecDNA(-) tissue and cells were enriched in neurodevelopment pathways, including ion channels. The ectopic expression of PDGFRA in ecDNA(-) clones partially rescued the defective tumor growth, decreasing median survival by 50% in orthotopic xenograft relative to original ecDNA(-) tumors (Log-rank $p=0.0028$, $n=5$/group). Avapritinib, a brain-penetrant PDGFRα/KIT-selective kinase inhibitor, or vehicle control, was administered to HF3253 xenografts at daily oral doses (40 mg/kg) for 5 weeks. Avapritinib treatment was highly effective in slowing tumor growth, relative to control (Log-rank $p=0.0047$; $n\geq9$/group). Non-invasive bioluminescence imaging revealed stalled

tumor growth during treatment, which resumed upon withdrawal. This work strongly supports PDGFRα as a major driver and therapeutic target in GBMs and the clinical potential of avapritinib.

Validation of DNA-PK as a Therapeutic Target for Combination Treatment of Glioblastoma

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Abstract - Non-homologous end joining (NHEJ) is responsible for the repair of most of DNA double strand breaks (DSB) in somatic cells. DNA-dependent protein kinase catalytic subunit (DNA-PKcs) has an essential role in NHEJ, a role in tumor cell resistance to DSB agents. The standard of care for glioblastoma (GBM) consists of DNA damaging: radiation treatment (RT) and Temozolomide, being a need for agents that potentiate these treatments.

The goal of this study was to evaluate DNA-PKcs as a therapeutic strategy for GBMs.

We tested the sensitivity of 14 GBM patient-derived cancer stem cells (CSCs) to treatment with two compounds, selective inhibitors of DNA-PKs, M3814 and AZD7648. IC50 concentrations and area above the curve (AAC)) were calculated from dose-response curves of cell viability measurements (CellTiterGlo), after 7-day treatment with inhibitor or control (n=5). AACs varied from 0.004 (resistant) to 0.748 (sensitive) and did not correlate with sensitivity of these cells to radiation treatment, or with p53 status. Combination of M3814 (IC30 concentrations) and RT (0, 2 and 4 Gy) had synergistic effect in 7 out 13 CSCs, and additive effect in the remaining cell. AZD7648 was less effective.

To complement the pharmacological studies, we have knocked down (KD) DNA-PK catalytic subunit (PRKDC) in CSCs, using PRKDC-shRNAs and non-silencing control-shRNA. Knockdown (KD) was verified by expression of total DNA-PKs in the transduced cells by RT-PCR and Western blot. Surprisingly, in all 4 CSC lines tested, PRKDC KD, did not sensitize cells to RT or other DNA-damaging treatment in vitro but, PRKDC-KO did sensitize a resistant CSC to RT ad TMZ in mouse orthotopic xenografts (Kaplan-Meier curve comparison p-values <0.02).

These results prove the potential for DNA-PK inhibitors in combination treatments for GBM. The pharmacological inhibition of DNA-PK in vitro sensitized different GBM CSCs to radiation treatment, PRKDC knockdown sensitized resistant GBM PDXs to radiation, to Temozolomide and CDK4/6 inhibitors, pointing to incorporation of DNA-PK inhibition in therapy of GBM patients.

First Use of Cesium-131 Embedded Collagen Tiles for Brachytherapy in the Spine: Combined Surgical Resection and Brachytherapy for Recurrent Rectosigmoid Adenocarcinoma Metastasis to the Sacrum

Authors - Anisse N. Chaker, MD[1]; Heegook Yeo, MD[1]; Jessie Huang-Vredevoogd, PhD[2]; Anthony Doemer, MS[2]; Lisa Scarpace, BS[1]; Alexandra Moceri, BFA[3]; Ali Al Asadi, MD[2]; Mira Shah, MD[2]; Ian Lee, MD[1]; Steven Kalkanis, MD[1]; Salim Siddiqui, MD, PhD[2]; Adam Robin, MD[1]

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Abstract - Background: GammaTile (GT Medical TechnologiesTM) is a Cesium-131 embedded collagen tile used for high-dose rate brachytherapy. Advantages of Cesium-131 include less tissue penetration thus lowering the risk of damage to nearby organs and neural elements. Utilization of brachytherapy allows for a surgically targeted radiation source that takes effect immediately upon intraoperative implantation. Cesium-131 embedded collagen tiles (Cs131CT) have so far only been implanted intracranially. We aim to present the first utilization of Cs131CT brachytherapy in the spine.

Hypothesis : Utilization of Cs131CT brachytherapy in the spine can circumvent aggressive resection thus preserving patients' neurological function and quality of life.

Methods: We present a 55-year-old male with symptomatic lumbosacral compressive polyradiculopathy from locally advanced and recurrent rectosigmoid adenocarcinoma involving the sacrum and lumbosacral nerve roots. Multidisciplinary discussion amongst the Henry Ford Spine Tumor Board determined conventional radiation was not an option due to reaching the radiation dosing limit to his colon. Options included disease progression, sacrectomy, or off-label compassionate Cs131CT brachytherapy with tumor debulking and partial sacrectomy. Patient declined sacrectomy given significant morbidity including potential need for bowel and bladder diversion. A shared-decision was made with the patient to undergo L5-S3 decompression and partial sacrectomy with tumor debulking and placement of Cs131CT brachytherapy.

Results: A 3D model of the patient's spine was created to simulate the operation in a lab setting prior to surgery. This allowed for estimation of the local radiation dose delivered and develop a strategy to avoid damage to the cauda equina. Surgery was then performed with successful debulking of the tumor and circumferential decompression. Cs131CT were then placed with adequate distance from the neural elements.

Conclusions: We present the first ever use of Cs131CT brachytherapy in the spine. Utilization of a pre-surgical model to determine feasibility and safety, as well as establishment of a multi-disciplinary team, were critical in providing an alternative for a patient faced with either disease progression or a highly morbid life-altering surgery.

PTEN Function Restoration by MN-anti-miR10b Nanodrug Suppress Glioblastoma Growth in vitro and in Xenografts

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Abstract - Glioblastoma is the most frequent and malignant glioma in adults, stands as one of the most prevalent and aggressive forms of brain cancer, holding the unfortunate distinction of the highest mortality rate among all brain malignancies. Within the intricate landscape of tumorigenesis, the role of microRNAs (miRNAs) has come to the forefront. These non-coding RNA molecules wield significant influence over a wide array of biological processes. Recent scientific investigations have particularly illuminated the prominence of a specific miRNA - miR-10b - which emerges with pronounced expression levels in high-grade glioblastoma while remaining conspicuously absent in the normal neuroglial cells of the brain. Accumulating evidence indicates that inhibition of miR-10b in glioblastoma cells leads to deregulation of multiple pathways in tumorigenesis, resulting in repression of tumor growth and increased apoptosis. In our study, we investigated the anti-proliferative effects of phosphatase and tensin homolog (PTEN) restoration by iron oxide nanoparticles with miR-10b inhibitors (termed MN-anti-miR10b) on U251 and LN229 glioblastoma cells in vitro and in intracranial xenografts. The iron oxide nanoparticles serve as delivery vehicles for the antagomirs as well as imaging reporters guiding the delivery in in vivo studies. Restoration of PTEN in glioblastoma cells markedly down-regulates the phosphorylation level of AKT, inhibits cell proliferation and promotes cell apoptosis. PTEN restoration by miR-10b downregulation is also able to efficiently inhibit the growth of human U251 glioblastoma xenografts in nude mice. In addition, we found that the mice survival rates were significantly extended in U251 cells by PTEN restoration, suggesting that antitumor effects may also be partly attributed to PTEN restoration. Our results demonstrate that PTEN functions play specific roles in tumorigenesis, and that treatment of PTEN restoration by MN-anti-miR10b is a promising gene therapy strategy for GBM.

High Incidence of Thoracic Lesions with Characteristic Mutational Signature in Mouse Strains Commonly Used in Pre-Clinical Studies After Treatment with a DNA-Alkylating Agent

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Abstract - Introduction: Temozolomide (TMZ) is an oral DNA-alkylating agent used to treat glioblastoma (GBM) and lower grade gliomas. We have previously determined that GBM patient-derived mouse orthotopic xenografts (PDX) present variable sensitivity to TMZ treatment, depending on the expression of DNA repair enzyme O6- methylguanine-DNA methyltransferase (MGMT). Furthermore, frequent symptomatic secondary thoracic lesions in TMZ-treated PDX mice were observed.

Method: We prospectively measured the incidence and characterized thoracic lesions after TMZ-treatment of 3 mouse strains. Immunocompromised NCRNU-F (NCr, athymic nude) and CBSCBG-F (SCID) mice, naïve or bearing GBM tumors, were randomized into 3 treatment groups (n=5-10 mice/group): 1 or 2 cycles of 5 x 40 mg/kg daily oral doses of TMZ, or vehicle control; immunocompetent (C57BL) mice received control or 2-cycle TMZ treatment. Mice bearing GBMs were sacrificed when symptoms associated with GBM or thoracic lesions developed (<150 days). Untreated GBM naïve mice did not develop symptoms within the 300-day observational period.

Result: Nude mice treated with 1 or 2 cycles of TMZ developed respiratory distress and lung tumors at 100% incidence, SCID mice treated with 2 cycles TMZ developed lung and thymus tumors at 66.7% and 58.3% incidence rate. TMZ-treated C57 BL mice developed thymus tumors at 43.8% incidence rate, and lung tumors at 12.5%. Lung and thymus tumors were positive for lineage-specific markers TTF1 and CD5, respectively. Genomic DNA samples isolated from SCID thymus tumors (n=4), nude lung tumors (n=3) and non-tumor lung tissue were analyzed by whole exome sequencing.

Conclusion: The predominant class of somatic mutations in the mouse thoracic tumors were C to T transitions, matching COSMIC Mutational Signature SBS11 related to exposure of DNA alkylating agents. TMZ treatment has efficacy for GBM patients, but also affects host tissues, resulting in a high incidence of leukopenia and to a lesser extent secondary leukemia. We show tumorigenic potential of systemically delivered TMZ, particularly in immunocompromised mouse strains, a confounding factor in pre-clinical studies, and show TMZ-mutational signature in mouse tissue.

Kynurenine Stimulates Growth and has Differential Effects on AhR Activity in Breast Cancer Cell Lines: Implications for Obesity-Driven Tumor Progression

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Abstract - Breast cancer (BC) remains a leading cause of mortality, with distinct subtypes such as triple-negative breast cancer (TNBC) and estrogen receptor (ER)-positive (+) BC presenting unique therapeutic challenges. TNBC has few treatment options and ER+ BC can develop resistance to anti-endocrine therapies. Obesity increases the risk of TNBC and promotes resistance to anti-endocrine therapy leading to tumor reoccurrence. Understanding the mechanism by which TNBC and endocrine-therapy resistant BC progress independently of estrogen will lead to novel therapeutic approaches. Our previously published studies demonstrate that adipose tissue-derived and serum-derived kynurenine are significantly elevated in pre-clinical models of obesity. This corresponds to clinical data demonstrating kynurenine pathway activation in patients with obesity. Kynurenine is a metabolite derived from tryptophan and an endogenous ligand of the aryl hydrocarbon receptor (AhR). We published that high AhR gene expression is associated with worse BC outcomes for all subtypes. Therefore, we hypothesize that kynurenine promotes proliferation through AhR activation in mammary epithelial pre-cancer and cancer cell lines. To test this hypothesis, we treated MCF-10A (ER- pre-cancer), MCF7 (ER+ BC) and tamoxifen-resistant MCF7 (ER+ BC, TAMR7) cells with kynurenine and measured AhR target genes and 3D growth. We found that kynurenine concentration-dependently stimulated 3D growth. Colony count was measured in MCF-10A cells as a surrogate marker of malignant transformation and colony size was measured in MCF7 and TAMR7 as a surrogate marker of cancer progression. Kynurenine-induced 3D growth was dependent on AhR activity as determined using AhR knockout cells and a pharmacological AhR antagonist. Surprisingly, AhR activity was reduced in TAMR7 cells compared to MCF7 cells and this activity was further reduced by kynurenine treatment. These opposing effects of kynurenine on AhR activity warrants further investigation and suggests that AhR activity may have distinct roles in tumor progression depending on the ER status, cancer stage and/or resistant phenotype. While kynurenine stimulated 3D growth in each cell line, understanding differences in AhR responses will be important for developing personalized treatments.

Wide Representation of the Diverse Population of Glioblastoma Patients in the HBTC Live Biobank

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Abstract - The Hermelin Brain Tumor Center (HBTC) at Henry Ford Health is one of the leading biorepositories of brain tumors in the US, and a major contributor of glioma samples to The Cancer Genome Atlas (TCGA). We have added to our tumor biorepository the routine culturing of fresh surgical brain tumor specimens in a selective media to create a comprehensive collection of cryopreserved renewable source of cancer stem cells for pre-clinical studies. Because glioblastomas, the most frequent and malignant primary brain tumor in adults, present high level of genomic diversity. To assure for a wide representation of patient-derived models in pre-clinical studies, we aimed to evaluate the success rate of obtaining long term self-renewing neurosphere cultures (NS(+)) from glioblastoma tumors over a 17-year period, and to identify factors influencing the establishment of success versus failure to obtain neurosphere cultures (NS(-)). Resected tumor samples were enzymatically dissociated and cultured in serum-free neurosphere media. To enrich for long-term self-renewing stem cells, primary neurospheres were serially passaged for a minimum duration of two months. We observed an overall rate of NS(+) of 41.4%, with no difference between newly diagnosed or recurrent tumors (odds ratio = 1.003; Fisher's exact test, $p > 0.999$). Neither sex nor race influenced the proportion of NS(+) tumors in newly diagnosed or recurrent cases. Focusing on a cohort of 119 sequentially resected newly diagnosed glioblastomas, we observed that neurosphere formation was significantly correlated with a shorter time to first progression (TTP1) (log-rank $p = 0.0096$) and shorter progression-free survival (log-rank $p = 0.0327$), but not with overall survival (OS) ($p = 0.202$). Furthermore, we have previously shown that the most significant somatic genomic abnormalities and all three transcriptional subclasses are represented in the glioblastoma models. Thus, the only clinical or molecular characteristics affecting representation of glioblastomas in the HBTC live biobank collection was a shorter TTP1, validating the wide representation and value of this panel for pre-clinical work

Effective Delivery of Therapeutic RNA Oligos Conjugated to Magnetic Iron Oxide-Dextran Nanoparticles to Glioblastoma Patient - Derived Mouse Orthotopic Xenografts

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Abstract - One of the main challenges in the treatment of glioblastoma, the most frequent and malignant primary brain tumor, is the delivery of therapeutic agents through the blood-brain barrier (BBB). Here we test magnetic nanoparticles consisting of an iron oxide core covered by dextran and conjugated to Cy5.5 near infrared optical dye to deliver anti-sense oligo targeting oncogenic microRNA10b (MN-anti-miR10b) to glioblastoma patient-derived mouse xenografts. Cancer-stem like cells from two newly diagnosed glioblastoma patients expressing high levels of miR10b and constitutively expressing firefly luciferase were each implanted intracranially into athymic nude mice (n=30). Mice were randomized into 3 treatment groups: MN-anti-miR10b, MN-Scr-mirR or untreated controls. Treatments started 2 weeks after the nude mice were implanted and continued for up to 7 weeks. Mice received one 20 mg/kg iron dose i.v. once per week until significant tumor associated symptoms developed. Tumor growth was monitored via weekly bioluminescence imaging (BLI) and nanodrug tumoral accumulation was assessed via Cy5.5 fluorescence imaging (FLI) performed 24h after weekly i.v. MN-anti-miR10b injections. Our results showed that the nanoparticles carrying anti-sense oligo were able to successfully cross the BBB and reach the brain tumor in both xenograft lines, and that the NM-anti-miR10b treatment led to a decrease in miR10b expression in the xenografted tumor. Similarly, our in vitro results demonstrated that MN-anti-miR10b treatment significantly downregulated miR10b in glioblastoma cancer stem cells. While in vitro treatment led to significant cellular toxicity, the in vivo results suggested that downregulation of miR10b alone was not sufficient to impair tumor growth to a significant extent as shown by BLI tumor imaging and Kaplan-Meier survival curve comparison. This agrees with a consensus in the field that single agent targeted therapy is not expected to achieve a threshold of efficacy in treating these highly aggressive tumors in clinic. Higher accumulation of MN-anti-miR10 and combination treatments may be necessary to produce toxicity in tumors in vivo to improve survival outcomes.

Precision Nanotherapeutic Targeting MicroRNA-10b Shows Preclinical Efficacy in a Glioblastoma Murine Model

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Abstract - Glioblastoma (GBM) is the most prevalent primary brain tumor and has a 5-year survival rate of just 5%. This highly aggressive glioma is characterized by molecular heterogeneity, which makes the development of therapeutics against GBM difficult. Furthermore, the blood-brain barrier, while protective in normal physiology, poses as another roadblock to the successful treatment of GBM by restricting the movement of therapeutics into the interstitium of the brain. Recent studies have shown that microRNA-10b is upregulated in most cases of GBM and is implicated in the tumorigenesis of GBM. Importantly, inhibition of miR-10b in GBM decreases cell invasion and migration and is critical for GBM cell viability. To address the clinical need for improved GBM therapy, we have developed an iron oxide nanoparticle, termed MN-anti-miR10b, that can cross the blood-brain barrier, accumulate in the tumor, and suppress miR-10b with an antisense oligo. The MN-anti-miR10b construct is about 25 nm in size and consists of an iron oxide nanoparticle core that is functionalized by an aminated, crosslinked dextran coating, which allows for conjugation of miR-10b antisense oligo and Cy5.5 moieties. In addition to the optical imaging capabilities conferred by Cy5.5, the superparamagnetic properties of ultra-small iron oxide nanoparticles make this construct a potent T2 magnetic resonance imaging contrast agent. In these studies, human U251 glioblastoma cells were orthotopically implanted in nude, athymic mice and administered respective treatments (PBS, MN-Scramble, MN-anti-miR10b) weekly by tailed vein injection beginning on day 7 post-tumor implantation until meeting a humane endpoint. We showed that miR-10b expression is significantly downregulated after multiple weekly doses of MN-anti-miR10b. In addition, we validated the accumulation of MN-anti-miR10b in the tumor region via ex vivo fluorescence imaging, fluorescence microscopy, and laser-ablation inductively coupled plasma mass spectrometry. Most significantly, we have shown that monotherapy with MN-anti-miR10b significantly extends median survival in our U251 murine model. Taken together, these studies show that MN-anti-miR10b has potential as a therapeutic for glioblastoma.

Co-Targeting Telomere Integrity and Repair of Telomere Damage for CRPC Therapy

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Abstract - The critical role of the androgen receptor (AR) in prostate cancer (PCa) cell proliferation and survival is the enduring basis for treating advanced PCa with AR inhibition drugs (ARi). However, a relentless challenge is the development of drug-resistant castration-resistant prostate cancer (CRPC).

Telomeres are DNA-protein structures that cap the ends of chromosomes. Damaged telomeres elicit a DNA damage response (DDR) that leads to damaged telomere repairs. Repair thereby

promotes cell viability. Conversely, blocking repair promotes cell death. We have discovered a role of AR in PCa telomere stability; by targeting both telomere integrity with ARi and the telomere DDR with an ATMi to block repair, we propose a novel approach to treat CRPC.

However, in CRPC cells, like AR, AKT is also essential for proliferation and survival. Because of reports that ARi leads to activation of PI3K/AKT, and PI3K/AKTi leads to activation of AR signaling, both ARi and AKTi may be more effective than either alone. Notably, we found that AKTi induces substantial telomere DNA damage in PCa cells. Therefore, our hypothesis is that dual inhibition of AR and AKT will maximize telomere DNA damage, so that DDR inhibitors can induce maximal cell death of CRPC.

We evaluated the effect of drugs that maximally induce telomere DNA damage and inhibit telomere DDR on CRPC tumor growth in vitro and in vivo. We observed that treatment with AR and/or AKT inhibitors in combination with DDR inhibitor (ATM, Chk1 or wee1) accentuated death of both AKTi and ARi-treated CRPC LNCaP/AR and 22Rv1 cells. In addition, combined treatment of CRPC LNCaP/AR tumor-bearing mice with AKTi +ARi +DDRi significantly inhibited tumor growth compared to the other single or double treatments. Thus, treatment with AR and AKT inhibitors in combination with DDR inhibitor may offer an effective strategy for deeper and/or more durable remission of metastatic CRPC.

Artificial Intelligence Aided Drug Discovery for the Treatment of Idiopathic Pulmonary Fibrosis

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Abstract - Background: Idiopathic Pulmonary Fibrosis (IPF) is a pathological condition resulting from injury to the lungs and an ensuing fibrotic response with unknown etiology. Median survival for IPF patients after diagnosis is approximately 3 years. The FDA approved Pirfenidone and Nintedanib for the treatment of IPF can only slow disease progression, as neither drug reverses lung fibrosis. Thus, there is a need for new effective therapeutics for IPF. Therefore, we propose a systems-based approach to identify drugs that can reverse the global expression of cell-type-specific transcriptional features of IPF lungs.

Methods: We used a single cell-based drug repurposing method for IPF treatment. One recent study conducted scRNA-seq on lung tissues from individuals with IPF. A comparison between diseased samples and controls revealed changes in the transcriptional features of all major cell

types. We compiled the expression signatures derived from this analysis, as well as additional signatures obtained by comparing pathological cell types in IPF patients. Each signature was inputted into our drug repurposing pipeline, OCTAD, to predict drugs capable of reversing the specific signature. Signatures were then inputted into GPS, a deep learning-based drug discovery system for screening of large novel compound libraries. We were able to identify novel compounds that were in top predicted across multiple signatures, these compounds were selected for experimental validation.

Results: To evaluate the efficacy of the drugs, we assessed their ability to inhibit profibrotic markers in precision cut lung slice (PCLS) obtained from IPF lungs. Out of the 20 drugs, 2 significantly decreased the profibrotic protein expression of collagen (Col1A1) and fibronectin. These two drugs are predicted to target MUC5B+ cells and myofibroblasts. We also conducted bulk RNAseq on PCLS and using deconvolution were able to identify cell types changing under treatment.

Conclusion: This study suggests that targeting specific cell types by reversing the disease gene signature could lead to the inhibition of pulmonary fibrosis. We performed validation of novel compounds targeting these cell types.

Development of Prostate Specific Membrane Antigen-Targeted Liposomal Zinc for the Treatment of Prostate Cancer

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Abstract - Introduction: Early-stage prostate cancer is treated with watchful waiting, surgery, or radiation, whereas advanced cases rely on hormone therapy followed by chemo/radiotherapy, despite side effects. More effective, low-side-effect treatments are needed, especially for advanced cases. Unlike normal prostate cells, cancerous ones have reduced zinc (Zn) levels due to ZIP1 zinc transporter downregulation, which keeps m-aconitase active, making prostate cancer cells energy efficient and grow. High levels of Zn are needed to inhibit m-aconitase, the enzyme responsible for the first reaction of Krebs cycle. We aim to induce energy starvation and cell death of prostate cancer cells by inhibiting m-aconitase using the prostate-specific membrane antigen (PSMA)-targeting liposomal zinc.

Methods: Zn-loaded Liposomes were prepared using DOPC, Cholesterol and DSPE-mPEG(2000) lipids. PSMA-expressing (PC3-PIP) and PSMA-null (PC3-FLU) human prostate cancer cells were used. Cellular uptake of PSMA-targeting Zn-loaded liposomes (Zn-TL) was performed with or without pre-incubation of PSMA-specific peptide. Zn-TL cytotoxicity was performed in PC3-PIP

cells using MTS assay. Mouse carrying contralateral subcutaneous tumor of PC3-PIP and PC3-FLU was used to evaluate Zn-TL biodistribution.

Results: PSMA-specific peptide and control peptide (ASP3) were conjugated with lipid and used them to prepare Zn-TL and control liposomes (Zn-CL), respectively. The hydrodynamic diameter of Zn-TL was 114 nm, and zeta potential was ~ -34 mV. Encapsulation efficiency and loading capacity of Zn+2 were 1.1% and 4.6%, respectively. Compared to Zn-CL, Zn-TL accumulated more in PSMA-expressing PC3-PIP cells, which was reduced upon pre-treatment with PSMA-specific peptide. Whereas, both liposomes showed low uptake in PC3-FLU. Further, cytotoxicity studies showed that Zn-TL induces cell death in the PSMA-expressing cells. Finally, biodistribution study showed delivery of Zn-loaded liposome in tumor tissue at appreciable amount (30% for Zn-TL Vs 31% Zn-CL, ID/gm tissue).

Conclusions: Overall, we developed Zn-TL liposomes that selectively target and kill PSMA-expressing prostate cancer cells and accumulate in tumor tissue. Future work will test Zn-TL's impact on m-aconitase activity and its in vivo efficacy in prostate cancer models.

Targeting Tribbles 2 with Daclatasvir Reverses Anti-androgen Resistance in Prostate Cancer

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Abstract - Background: FDA-approved enzalutamide is commonly prescribed for advanced prostate cancer. However, enzalutamide-resistant prostate cancer (ERPC) invariably develops, which leads to aggressive, lethal disease. Recently, we found that the Tribbles 2 (TRIB2) is overexpressed in ERPC cells and confers resistance to enzalutamide by promoting lineage plasticity to neuroendocrine differentiation. Though TRIB2 emerged as an excellent molecular target for ERPC, suitable inhibitors are not available for effective targeting.

Methods: Compounds were tested using a luciferase-tagged TRIB2 fusion protein-based assay system. Binding of drugs with TRIB2 protein was analyzed by thermal shift assays and by advanced computer-based homology modeling. Degradation of TRIB2 protein was measured by Western blot. Drug effects on re-sensitization of ERPC cells and synergy with enzalutamide were determined through cell viability and apoptosis assays. To gauge the in vivo effects of Daclatasvir (DCV), ERPC tumor xenograft-bearing mice were treated with varying doses of DCV via oral gavage. Tumor growth was calculated by measuring volumes and molecular markers in tumors were analyzed by immunohistochemistry.

Results: By designing a luciferase-tagged TRIB2 fusion protein-based assay system, we screened a library of about 1,600 FDA-approved compounds and found that DCV, effectively inhibits the TRIB2-luciferase activity but does not inhibit the activity of free luciferase. Notably, DCV directly binds to pure TRIB2 protein, leading to its destabilization and a reduction in the half-maximal melting temperature. Interestingly, we found that DCV degrades TRIB2 proteins via activation of proteasomes and re-sensitizes ERPC cells to enzalutamide. DCV downregulates the master neuronal transcription factor, BRN2, and the stemness factor, SOX2, and synergizes with enzalutamide to decrease the viability of prostate cancer cells. Finally, DCV was found to effectively inhibit the growth of ERPC tumors and decrease the protein level of TRIB2 in tumor xenograft model.

Conclusion: These findings indicate that DCV effectively downregulates TRIB2 both in vitro and in vivo and suggest that further testing of DCV may help design an innovative therapeutic approach for management of ERPC.

A Preclinical Model of Recurrent Glioblastoma Following Laser Ablation of the Primary Tumor Exhibits Increased Genetic Signatures of Cell Cycle and Cell Motility

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Abstract - Image-guided laser interstitial thermal therapy (LITT) is a minimally invasive tumor cytoreductive treatment for recurrent gliomas and tumors in eloquent loci. We have adapted this technique to develop an image-guided glioblastoma (GBM) ablation model, its recurrence and have tested the efficacy of imaging biomarkers in evaluating tumor ablation and recurrence. The cytopathology and molecular signatures of the primary and recurrent tumors were compared. Immune-compromised female rats were implanted with U251N tumor cells in one brain hemisphere (n=20). Tumor growth was monitored using magnetic resonance imaging (MRI). When tumors reached about 4 mm in diameter, they were ablated using a clinical LITT system (Visualase®), under MRI guidance. Five other rats implanted with U251N tumors were used as unablated controls. MRI data were acquired at 24 h post-LITT, and at 2 and 4 weeks. Rats were

sacrificed for histopathology at 2 and 4 weeks and brain sections stained for hematoxylin and eosin, human major histocompatibility complex, Ki67, a cell proliferation marker and sex determining region Y)-box 2 (SOX2), a stem cell transcription factor. An additional cohort of rats with primary (n=4) and post-LITT recurrent (n=4) U251N tumors were used for analysis of their molecular composition using RNA-Seq approach. In the treated groups, MRI showed little tumor tissue at 24 h, evidence of recurrence at 2 weeks and significant tumor tissue at 4 weeks. Tumor DCE-MRI parameters showed elevated intra-tumoral vascular permeability (i.e., Ktrans) values at pre-LITT imaging, that shifted to peri-ablation periphery at 24 h. A trend of progressive decrease in Ktrans was seen until 1-week post-ablation. Increasing Ktrans values at 2 weeks and after 4 weeks coincided with tumor recurrence. RNA-Seq data showed that cell cycle, cellular movement and inflammatory disease genes were the most differentially expressed genes in the recurrent tumors suggesting increased infiltration may primarily underlie treatment resistance.

Enhancing Radiotherapy Treatment Planning with Large Language Model Agents

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Abstract - Background: Radiotherapy treatment planning involves balancing multiple clinical objectives to ensure optimal tumor control while minimizing risks to organs at risk (OARs). Traditional optimization methods rely heavily on manual adjustments, which are time-consuming, resource-intensive, and subject to variability. Large language models (LLMs) offer the potential to automate and accelerate this process, enhancing both consistency and efficiency in treatment planning.

Hypothesis: We hypothesized that LLM-driven iterative optimization could enhance radiotherapy plans by efficiently managing dose constraints, improving plan quality and consistency, and offering time savings over manual methods.

Methods: A retrospective analysis was performed on 20 prostate cancer patients treated between 2015 and 2023 with a prescription dose of 6000 cGy in 20 fractions. The Llama3.1-70B-Instruct LLM was integrated with the Varian Eclipse system to optimize treatment plans over 20 iterations per patient. The LLM adjusted clinical priorities for the planning target volume (PTV) and OARs, including the rectum, bladder, femoral heads, and penile bulb. An LLM temperature of 0.8 and top_k of 2 were used.

Results: LLM-optimized plans showed trade-offs between PTV coverage and OAR sparing. PTV coverage (V58.5Gy) decreased by 6.5% (±7.9%), while the bladder (V60Gy) experienced a dose reduction of 2.4% (±6.0%). Femoral head dose reductions were 1.3 Gy (±8.1 Gy) on the left and

0.3 Gy (±9.3 Gy) on the right. The rectum (V40Gy) saw a decrease of 2.8% (±9.7%), and the penile bulb (V22Gy) a reduction of 2.3% (±11.8%).

Conclusions: Integrating an LLM into radiotherapy planning resulted in OAR dose reductions, particularly for the bladder, rectum, and femur heads. The trade-off in PTV coverage (6.5% reduction) highlights the need for further tuning to avoid compromising tumor coverage. The automation provided by LLMs can significantly reduce planning time and resource demands, suggesting a promising pathway for more efficient and consistent treatment planning.

Nanoparticle-Coated Cisplatin Probe Radiation Enhancement in Triple Negative Breast Cancer Model

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Abstract - Enhancing chemotherapy drug mechanisms can advance cancer treatment and patient outcomes. We aim to use nanoparticle coating to improve the efficiency of Cisplatin damage to triple-negative breast cancer (TNBC) 4T1 cells through combined radio-sensitization and cytotoxicity.

Hydrophobic platinum was synthesized by treating anhydrous cis-$[PtCl_2(NH_3)_2]$ with excess H_2O_2 (30% w/v), forming cis,trans,cis-$[PtCl_2(OH)_2(NH_3)_2]$, which was reacted with hexanoic anhydride in DMSO to afford hydrophobic platinum. Simultaneously, a polymer (PGLA-PEG-COOH) was coupled with the trifluoracetic acid salt of fluorescein hexylamine, yielding a fluoresceinated polymer. The polymer was then nanoprecipitated with the hydrophobic platinum to synthesize fluoresceinated Pt(IV)- nanoparticles (Pt(IV)-NPs). In Vitro, studies were conducted in 4T1 cell line to test the cellular uptake and radiosensitization via clonogenic assay.

We calculated that nanoparticle-coated Cisplatin probe contained 40% of active Cisplatin drug. Fluorescent microscope images show cellular uptake indicated by FITC-labeled probe found within vesicular compartment in 4T1 cells. Clonogenic assay results show radiosensitization whit 1 uM of the probe, or 0.4 uM Cisplatin, compared to Cisplatin alone. Dose of 10 uM probe, or 4 uM active Cisplatin, showed drastic cell death compared to control untreated cells and cells treated with Cisplatin alone.

Concomitant chemoradiotherapy (CCRT) is the standard treatment for many cancers. CCRT trials have demonstrated decreased incidence of distant metastases compared with RT alone. Cisplatin-mediated radio-sensitization mechanisms are proposed to be partially responsible for the improvements seen after CCRT and the benefits are coupled with significant dose-limiting

systemic toxicities. However, using FDA-approved Cisplatin i.v. therapeutic dose, relatively low drug concentration reaches the tumor resulting in a weak radio-sensitization effect. Nanoparticle coated Cisplatin can be targeted to tumor tissues, ensuring adequate Pt-Th intratumor concentrations are needed for radiosensitization. Here, we showed that encapsulated Cisplatin successfully entered 4T1 cells and exhibited radio-sensitizing effect. Our next step will involve the addition of targeting moiety to the current nanoparticle design that will be tested in vivo. The findings will advance the implications of nanoparticles for enhancing CCRT.

Implications of Glutaminolysis in Diet-Induced Ovarian Cancer Chemoresistance

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Abstract - Ovarian cancer is the most lethal gynecologic cancer with approximately 80% of patients developing resistance to chemotherapy. Obesity is associated with poorer survival among patients receiving platinum-based drugs however, the role of obesity in chemoresistance is unclear. We have shown that high-fat diet (HFD)-induced obesity promotes ovarian cancer progression in tumor-bearing mice and altered metabolic balance in glutaminolysis activity when treated with carboplatin. Therefore, we hypothesize that factors secreted from adipose tissue metabolically rewire ovarian cancer and this is associated with glutaminolysis-driven proliferation and chemoresistance. To test this hypothesis, we developed a novel model whereby factors secreted from human omental adipose tissue (OmFTF) stimulate the anchorage-independent 3D growth in soft agar (colony formation) of ovarian cancer cell lines. Our results show that OmFTF induces a significant increase in colony size, demonstrating that culture with adipocyte-secreted factors stimulates proliferation of OVCAR3 and OVCAR8 cells. Interestingly, these aggressively growing clones have a higher IC50 when exposed to cisplatin, and this is associated with elevated glutamate pyruvate transaminase 2 (GPT2), which serves as a pivot between glycolysis and glutaminolysis. To further explore the implications of the adipocyte-secreted factors ex vivo, we exposed OVCAR3 cells to ascites derived from HFD-fed tumor-bearing mice. We demonstrated that exposure to these HFD-derived ascites (but not in regular diet-derived ascites) results in acquired chemoresistance and upregulation of GPT2. These data suggest that adipose tissue

stimulates ovarian cancer chemoresistance through metabolic adaption to the microenvironment. To determine if GPT2 was associated with chemoresistance-independent of OmFTF exposure, we characterized GPT2 gene expression in OVCAR3 and OVCAR8 sensitive (CP0) and resistant (CP5) cells lines. GPT2 was only upregulated in resistant cells and knocking out GPT2 in resistant OVCAR8-CP5 resulted in a significant decrease in 3D growth. These studies implicate GPT2 as a novel metabolic link between excess adiposity and glutaminolysis-driven chemoresistance in ovarian cancer.

Integration of Transcriptional Signatures and Response of Glioblastoma Cancer Stem Cells to MDM2 Antagonist Treatment Identify New Pathways Associated with Resistance

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Abstract - In glioblastoma, wildtype p53 is inhibited by MDM2 and MDM4 amplification, or deletion of CDKN2A (coding for MDM2 inhibitor p14ARF). MDM2 pharmacological antagonists (MDM2a) are currently in clinical trials for wildtype p53 GBM. Here we integrate glioblastoma response to MDM2a with genomic and transcriptional signatures to identify predictive markers and mechanisms of resistance. We employed a panel of 7 wildtype p53 patient-derived cancer stem cells (CSCs), to test sensitivity to MDM2a in clinical trials: RG7112 and AMG232. IC50 concentrations and area above the curve (AAC) were calculated from dose-response curves obtained from cell viability measurements after 4 and 7-day MDM2a treatment or DMSO control, in quintuplicates. These CSCs were then treated with one of the MDM2a at IC50 concentrations or DMSO control for 24h (n=4). RNA was isolated for Illumina Truseq stranded mRNA libraries sequenced at 30M depth. Quantified raw counts were processed using NOISeq R package to determine differentially expressed genes (DEG) between control and treated samples, and enrichment analysis was performed using Metascape. A wide range of sensitivity was observed, with AACs ranging from 0.2 to 0.8, from a scale of 0 (complete resistance) to 1. We found no correlation between sensitivity and genomic features, but using transcriptome we identified that baseline activation of MYC, RNA and DNA metabolism were markers of sensitivity to MDM2a treatment. Transcriptional changes in response to 24h MDM2a treatment led to the expected enrichment in p53 signaling, including upregulation of 212 validated direct p53 targets in at least 2 of the CSC lines. Additionally, we identified 1637 genes upregulated in multiple CSCs which are not validated p53 transcriptional targets, highly enriched in extracellular matrix (ECM) components, calcium binding and NFkB signaling, all pathways involved in cell survival and resistance to treatment. We conclude that MDM2a treatment has potential to treat a subset of

glioblastomas with high MYC activity, and unveiled novel transcriptional programs responsive to MDM2a that are likely associated with resistance.

TPPP3: A New Target to Fight Ovarian Cancer

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Abstract - Ovarian cancer is a highly lethal gynecological malignancy that is characterized by low rates of early detection and a high frequency of recurrence. Approximately 80% of patients develop resistance to the current chemotherapeutic standard of care, cisplatin and paclitaxel. Therefore, there is a pressing need for new targets to be discovered for the development of new treatments to improve patient survival. Recent findings have shown the gene Tubulin Polymerization Promoting Protein 3 (TPPP3) is involved in tumor growth in several cancers. TPPP3 encodes a microtubule-associated protein that promotes microtubule bundling, stabilization, and polymerization. However, its role in ovarian cancer is not well-known. To investigate this, I designed a mouse xenograft model utilizing ovarian cancer cells with high expression of TPPP3 and a TPPP3 knockout. These cells were implanted subcutaneously into the flanks of female BALB/c mice. A significant reduction in tumor growth was seen in the mice implanted with the TPPP3 knockout cells compared to the high TPPP3 expressing cells, which was validated through ex-vivo quantitative PCR analysis. Our findings show the significant decrease in tumor growth is due to the knockout of TPPP3, suggesting a role for TPPP3 in ovarian cancer progression. Thus, this gene is a promising target to develop new treatments that can reduce the growth of ovarian cancer.

VPS72 is a Novel Oncogenic Driver Crucial for Tumor Growth and Survival

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Abstract - Epigenetic chromatin remodeling plays important roles in cancer progression and drug resistance. Vacuolar protein sorting-associated protein 72 homolog (VPS72) is a histone chaperone that exchanges canonical histone H2A with histone variant H2A.Z, causing

transcriptional activation. VPS72 is mapped to chromosome 1q21.3, a region where amplification is trackable for recurrence in breast cancer, an increased risk of drug resistance, disease progression, and death in multiple myeloma and hepatocellular carcinoma (HCC) and is considered a novel epigenetic marker for racial disparities in prostate cancer. Our prior data demonstrates VPS72 is crucial for Treg stability and immune suppressive phenotype; however, little is known regarding VPS72 molecular functions in tumor cells.

To evaluate VPS72 expression, we perform meta-analysis on tumor samples collected from The Cancer Genome Atlas (TCGA), tumor cell lines from the Cancer Cell Line Encyclopedia (CCLE), and single-cell analysis using the Tumor Immune Single-cell Hub 2 (TISCH2) database. Loss of VPS72 function in tumor cells was interrogated using small interference RNA (siRNA) to deplete VPS72 mRNA. We collect cells for VPS72 depletion on cell growth and survival phenotypes.

The analysis of TCGA samples showed significant upregulation of VPS72 mRNA in 14 tumor types and protein upregulation in 6, compared to normal tissue. High VPS72 expression was associated with copy number amplification ($p < 0.001$) in tumors and malignant cells and was linked to genomic alterations in TP53, MUC16, CSMD3, and PIK3CA. Single-cell analysis revealed high VPS72 expression in malignant cells, (CD4+ T, CD8+ T, Tregs, proliferating T cells), and myeloid cells (dendritic cells, monocytes/macrophages). High VPS72 expression also correlated with shorter survival. DepMap CRISPR gene dependency data indicated tumor cells were dependent on VPS72 for survival (Gene Effect Score: -1 Chronos). We validated these results in cholangiocarcinoma cells. VPS72 depletion via siRNA reduced cell growth and Ki67-positive cells.

Our data indicates VPS72 is upregulated in cancer. Silencing VPS72 abrogates tumor cell growth signaling, making it a potential driver of malignant growth.

Role of Alpha-Ketoglutarate, the Gate Keeper of TCA-Glutamine Anaplerosis in Regulating Immunosuppressive CD11b+Gr1+ Myeloid Cell Function in Epithelial Ovarian Cancer

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Abstract - Immunosuppressive myeloid cells (CD11b+Gr1+) are a major constituent of the epithelial ovarian cancer (EOC) microenvironment that confer immunosuppression and promote tumor growth. We recently showed that the EOC microenvironment can induce metabolic reprogramming in CD11b+Gr1+ cells by increasing oxidative phosphorylation via TCA cycle, resulting in increased immunosuppressive ability. Increased TCA cycle activity was due to glutamine anaplerosis, which was facilitated by the upregulation of DLST (dihydrolipoamide succinyl transferase), the E2 transferase subunit of α-KGDC (alpha-ketoglutarate dehydrogenase complex). We selected CPI-613 (Devimistat), an orphan metabolic drug, being actively tested in clinical trials to target the a-KGDC complex. We utilized the syngeneic ID8p53-/- model and FVB/N mice model, a high grade serous ovarian cancer, to determine if CPI-613 may regulate CD11b+Gr1+ cell metabolism and immunosuppressive function and restore an antitumor immune response that inhibits EOC. One week after inducing tumors, mice were treated with CPI-613 (2.5mg/kg body weight) thrice a week by IP injections or with vehicle (corn oil, control). CPI-613 treated mice showed significantly improved overall survival (median survival 75 days versus 48 days in control in ID8p53-/-, 71 days vs 55 days in control in FVB/N mice), decreased tumor progression and burden. CPI-613 decreased the number of CD11b+Gr1+ myeloid cells in ascites, and more importantly, reduced the intracellular immunosuppressive markers arginase 1 and IL-1β, indicating that the immunosuppressive potential was diminished, which was also validated by reversal of T cell suppression by CPI-613 in both mice models. The alleviation of immunosuppression was complemented by remarkable increase in CD8+ T effector cells. Targeted metabolomics of CPI-613 treated CD11b+Gr1+ cells showed a profound decrease in TCA metabolite α-ketoglutarate (αKG). Among all TCA metabolites, only αKG supplementation reversed the OXPHOS inhibition and T cell immunosuppression by CPI-613 and increased immunosuppressive markers in ex-vivo conditions. In vivo treatment of αKG alone replicated the ex-vivo effect and enhanced CD11b+Gr1+ myeloid cell OXPHOS and immunosuppressive ability but had no effect on tumor or survival. However, in combination with CPI-613, αKG was unable to reverse the inhibitory effect of CPI-613 on immunosuppression and OXPHOS. Intriguingly, the combination of αKG and CPI-613 inhibited tumor growth. Though, its role in EOC or CD11b+Gr1+ myeloid cells has never been studied. Our study shows that αKG generated by TCA- glutamine anaplerosis is crucial for supporting an immunosuppressive phenotype of CD11b+Gr1+ myeloid cells in EOC, yet with right combination may have the potential to improve tumor immune response and outcome.

Brd4::Nutm1 Fusion Gene Initiates NUT Carcinoma In Vivo

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Abstract - Background: NUT carcinoma (NC) is an aggressive cancer with no effective treatment. About 70% of NC cases are associated with chromosomal translocations that form a BRD4::NUTM1 fusion gene. Because the BRD4::NUTM1 gene is unequivocally cytotoxic when ectopically expressed in cell lines, questions remain on whether the fusion gene can initiate NC.

Hypothesis: We hypothesized that the Brd4::Nutm1 fusion gene can directly induce NC in vivo by driving tumorigenesis in a genetically engineered mouse model.

Methods: We generated the first genetically engineered mouse model for NC, designed to mimic the human t(15;19) chromosomal translocation that forms the BRD4::NUTM1 fusion gene. Using Cre-Lox technology, we induced a syntenic t(2;17) chromosome translocation in mice, resulting in the formation of the Brd4::Nutm1 fusion gene. Mice were monitored using bioluminescent imaging for tumor development, and histopathological and molecular analyses were conducted to assess the characteristics of the resulting tumors.

Results: The Brd4::Nutm1 fusion gene successfully induced aggressive carcinomas in mice. Tumors exhibited histopathological and molecular features similar to human NC, with enrichment of undifferentiated cells. Moreover, similar to the reports of human NC incidence, Brd4::Nutm1 can induce NC from a broad range of tissues with a strong phenotypical variability. The consistent induction of poorly differentiated carcinoma demonstrated a strong reprogramming activity of BRD4::NUTM1.

Conclusions: We developed a critical preclinical mouse model of NC that faithfully recapitulates key features of the human disease. This model offers valuable insights into the initiation and progression of NC and will lead to better understanding and therapy development for NC.