16th Annual Spotlight on Early Career Investigators—
A Cancer Research Mini-Symposium

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UC Davis Comprehensive Cancer Center

ABSTRACTS

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KEYNOTE ABSTRACT

MY PATH IN CANCER EPIDEMIOLOGY FORGED BY MENTORSHIP, OPPORTUNITY, AND SERENDIPITY

Shehnaz K Hussain, PhD, Public Health Sciences, UC Davis School of Medicine

Dr. Hussain will discuss her research focused on the intersection of infections and cancer. She will contextualize how her research and career trajectory have been influenced by supportive mentors, seizing unusual or unexpected opportunities, and sometimes just being the right person at the right place at the right time.

Shehnaz K Hussain, PhD is Professor in Public Health Sciences at the UC Davis School of Medicine and Associate Director for Population Sciences at the UC Davis Comprehensive Cancer Center. Dr. Hussain earned an M.S. in Epidemiology from Johns Hopkins University and Ph.D. in Epidemiology from the University of Washington. She completed a first postdoctoral fellowship in Genetic Epidemiology at the Karolinska Institute in Sweden, and a second fellowship in Cancer Prevention and Control at UCLA. Dr. Hussain’s research program stems from a long-standing interest in the etiology, prevention, and early detection of infection-associated cancers. She has over a decade of experience in the design, implementation, conduct, analysis, and reporting of multi-center longitudinal cohort studies, case-control studies, and clinical trials. She has developed a particular interest in studying biomarkers that relate to, or modulate, the immune response including serum immune markers, intestinal microbiome, and immunogenic microbial components and metabolites. Dr. Hussain’s current research program is largely focused on the disease continuum from non-alcoholic fatty liver disease (NAFLD) to hepatocellular carcinoma (HCC). Key components of this research program include racial/ethnic disparities, the interplay of diet, microbiome, and metabolome in HCC etiology, primary and secondary HCC prevention with statins, diet modification in NAFLD, and HCC early detection with imaging biomarkers. Additionally, aligned with her long-standing interests in infectious causes of cancer, she is also actively conducting research on EBV- and HPV-associated cancer etiology and prevention, particularly in the setting of severe immunosuppression (chronic HIV infection and solid organ transplantation).
ORAL PRESENTATION ABSTRACTS

1. ESTABLISHING AND CHARACTERIZING PATIENT-DERIVED MODELS FROM RACIAL/ETHNIC MINORITY GASTRIC CANCER PATIENTS TO ADVANCE CANCER PRECISION HEALTH EQUITY

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Gastric cancer (GC) is the second leading cause of cancer-related deaths worldwide. While overall incidence and mortality rates have dropped in recent decades, GC remains a significant cause of health disparities for all federally defined racial and ethnic minority groups in the US, who are all at least twice as likely to be diagnosed with and die from GC compared to non-Hispanic whites (NHW). Despite such high minority cancer burden, few FDA-approved targeted therapies are available for GC. This can be partially explained by limited availability of cancer genome data and patient-derived models from racial/ethnic minority populations, hampering gene target identification and drug efficacy studies. Our group has spearheaded the development of the University of California Minority Patient-Derived Xenograft Development and Trial Center (UCaMP) with the goal of addressing these critical issues for GC patient care. Thus far, we have characterized over 30 GC samples from racial/ethnic minorities (mGC), establishing patient-derived organoid and mouse xenograft lines for most. Genomic analyses have revealed a significantly lower prevalence of chromosomal instability and higher prevalence of genomically stable GC tumors compared to TCGA, which consists predominantly of NHW patients. Our analyses have also identified high prevalence of alterations within the cell cycle regulation/cyclin-dependent kinase (CDK) and PI3K/AKT/mTOR (PI3K) pathways, both of which are therapeutically targetable by inhibitors already FDA-approved for other cancer types. At the individual gene level, these mGC patients demonstrate distinct patterns of somatic mutations, with fewer PIK3CA activating mutations, which is the most mutated gene in the PI3K pathway among TCGA, and TP53 deleterious mutations while demonstrating significantly more deleterious mutations in tumor suppressor genes such as CDH1 and PTEN. Indeed, when we treat our models with PI3K and CDK inhibitors, we observe significant responses both in vitro and in vivo. We are now generating genome-edited organoid lines from normal gastric tissue to model specific alterations observed in our mGC cohort for functional characterization within GC pathogenesis and drug response validation. Our findings highlight an important molecular distinction of GC development in racial/ethnic US minorities, providing a rationale for alternative treatments to address GC health disparities.

2. FACTORS ASSOCIATED WITH UNPLANNED READMISSIONS IN PEDIATRIC SURGICAL ONCOLOGY PATIENTS

Christina M. Theodorou MD, UC Davis Department of Surgery, Julianne J. P. Cooley, UC Davis Health, Theresa H. Keegan, PhD, MS, UC Davis Comprehensive Cancer Center, Erin G. Brown, MD, UC Davis Department of Pediatric Surgery

Purpose: Pediatric oncology patients are at increased risk of unplanned readmissions, but patient and hospital factors associated with readmissions are unknown. To identify targets for risk reduction, we aimed to identify patients at increased risk for readmission as well as characterize the full extent of unplanned readmissions for this vulnerable population.

Methods: Patients <20 years old with a diagnosis of a primary solid organ cancer (central nervous system (CNS), germ cell, hepatic, bone, renal, soft tissue/extraosseous tumors) who underwent surgical intervention from 2005-2017 were identified in the California Cancer Registry linked to statewide hospitalization data via Office of Statewide Health Planning and Development (OSHPD). Unplanned readmissions were defined as admissions for acute medical or surgical problems within 30 days of
discharge from the hospitalization during which the index surgical procedure was performed. Multivariable logistic regression was utilized to identify demographic and clinical factors associated with first unplanned readmission within 30 days of surgery.

Results: A total of 2,507 patients were identified with a median age of 10 years old. Half of patients had a readmission within 30 days (n=1233, 49.2%), and 36.7% (n=452) were unplanned. In multivariable models, those at highest risk of unplanned readmission included children <1 year old (OR 2.72, 95% CI 1.72-4.29) and 1-5 years (OR 1.64, 95% CI 1.20-2.24) vs. ages 13-19, those with metastatic disease (OR 1.6, 95% CI 1.1-2.1) at diagnosis, and those with CNS tumors (OR 2.5, 95% CI 1.6-3.9), hepatic tumors (OR 2.3, 95% CI 1.2-4.2), or soft tissue/extraosseous sarcomas (OR 2.2, 95% CI 1.3-3.9). A longer initial hospitalization was also associated with a higher likelihood of unplanned readmission.

Conclusion: Readmissions after surgical interventions for pediatric oncology patients are prevalent, and many are unplanned. Younger children and those with metastatic disease or CNS tumors are at highest risk of unplanned readmissions.

3. EFFECT OF ENGINEERED PLACENTAL MESENCHYMAL STEM CELLS AND BONE MARROW-DERIVED MESENCHYMAL STEM/STROMAL CELLS ON THE PROLIFERATION OF NEUROBLASTOMA CELLS

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PURPOSE - Neuroblastoma is a common pediatric malignancy with extremely poor survival for children with high-risk disease. Furthermore, most survivors suffer from debilitating long-term side effects. Cellular therapy offers a novel means for targeted treatment as mesenchymal stem cells (MSCs) may act as a vehicle to deliver therapeutics directly to tumor sites. However, MSCs have shown varying effects on tumor growth. We hypothesized that engineered early-gestation placental MSCs (ePMSC) would demonstrate less neuroblastoma cell proliferation compared to adult MSCs such as bone-marrow derived MSCs (BM-MSCs).

METHODS - Three neuroblastoma cell lines (NB1643, CHLA90, SH-Sy5y) were co-cultured with ePMSC (n=3: Donor A; Donor B; Donor C;) and BM-MSC (n=1) cell lines. Neuroblastoma cells cultured alone in media served as controls. Proliferation of neuroblastoma cells were assessed using an MTS assay and normalized to the proliferation of the controls. Studies were performed in triplicate and fold change was analyzed via the Kruskal-Wallis test.

RESULTS - Rates of proliferation varied across MSC and neuroblastoma cell lines. Rates of proliferation of NB1643 were not significantly different across all MSC groups or significantly increased compared to controls. Proliferation rates for CHLA90 were increased with all ePMSC lines as compared to BM-MSC (p = 0.0024, 0.0211, 0.0269, respectively); but were only significantly increased compared to controls with one e-PMSC cell line (Donor A, p =0.0175). SH-SY5Y proliferation was significantly increased with all ePMSC lines (p =0.0081, 0.0036, 0.0243; respectively) compared to controls as well as between all ePMSC lines and BM-MSC (p=0.072, 0.0022, 0.0332; respectively).

CONCLUSION - Effects of MSCs on proliferation of neuroblastoma cells are highly variable by both MSC and neuroblastoma cell lines. This may be due to the specific chemokine and cytokine milieu of each neuroblastoma or MSC cell line. Donor characteristic may also play a role in this variability. Further analysis of the secretome of the cell lines in co-culture as well as expanding the number of BM-MSCs, ePMSC and neuroblastoma cell lines studied may elucidate the mechanism of these variable growth effects and identify cell lines most suited for use as drug delivery vehicles.
4. REPRESSION OF PRMT5-MEDIATED DIMETHYLATION STABILIZES VIMENTIN AND PROMOTES METASTASIS IN MTAP-LOSS LUNG CANCER

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The aggressive nature of lung cancer with a poor prognosis has led us to explore the mechanisms driving disease progression. We identified a cancer susceptibility gene, methythioadenosine phosphorylase (MTAP), as a metastasis suppressor by utilizing our invasive cell-based model. Both overexpression and CRISPR/Cas9 knockout studies demonstrated that MTAP not only inhibits cell invasion and colony formation in vitro, but also mitigates metastasis and tumorigenesis in vivo. Patients with low MTAP expression displayed worse overall and progression-free survival. Mechanistically, significant accumulations of the MTAP substrate, methythioadenosine (MTA), in MTAP-deficient cells reduce the level of protein arginine methyltransferase 5 (PRMT5)-mediated symmetric arginine dimethylation on proteins. Methyproteomic screening by LC-MS/MS revealed vimentin, a mesenchymal marker, as a novel dimethyl-protein. The symmetric dimethylarginine (sDMA) modification on vimentin protein trivially affects the structure of vimentin filaments but reduces the protein abundance of vimentin. In MTAP-loss cells, we found that lower sDMA level prevents ubiquitination-mediated vimentin degradation, and thereby stabilizes vimentin, contributing to cell invasion. This inverse association of the MTAP/PRMT5 axis with vimentin proteins was noted in both proteogenomic study of lung cancer and immunohistochemical staining of tissue microarrays. Together, we propose a novel mechanism of vimentin post-translational regulation and provide new insights for metastasis.

5. HIGH LEVELS OF CD47 EXPRESSIN IN THYMOMA AND THYMIC CARCINOMA

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CD47 is a tumor marker that inhibits phagocytosis thereby providing tumor cells with a means of escape from immune surveillance and elimination. Anti-CD47 therapy is a promising new immunotherapy across numerous tumor types but has not been tested in thymic tumors. Thymomas and thymic carcinomas are rare tumors which are difficult to treat, especially with PD-1/PD-L1 checkpoint inhibitors, due to the excessive rates of immune-related adverse effects. This study investigated the levels of CD47 expression in thymic tumors to explore the possibility of anti-CD47 therapies. A total of 67 thymic tumors (64 thymomas and 3 thymic carcinomas) and 14 benign thymus controls, and their clinical data were included. Samples with an average of 3 cores each were stained for CD47 expression (rabbit monoclonal antibody SP279, Abcam, USA) and scored for both intensity and H-score (intensity multiplied by the percentage of tumor involved). Intensity was defined as: 0 = none, 1 = weak, 2 = moderate, and 3 = strong. H-scores ranged from 0 to 300. Samples with an intensity score below 2 or an H-score below 150 were considered CD47low, while the rest were CD47high. Multivariate regression and survival analyses revealed significant correlations. CD47 expression was more frequently present in Thymic Epithelial Tumors (TETs) than in normal thymic tissue. The level of expression was on average 16-fold higher in TETs. Among tumors, higher CD47 expression was correlated with a lower stage and more complete resection. A multivariate analysis taking into account these factors showed that CD47 expression by both H-score and intensity were each highly correlated with WHO histology subtype and paraneoplastic syndromes. CD47 expression did not correlate with overall survival, but tumor samples with relatively higher CD47 expression were associated with a less aggressive histology and stage, but with a higher frequency of paraneoplastic syndromes. This is the first study to explore CD47 expression in thymic cancers, and lends support for ongoing investigation of anti-CD47 macrophage checkpoint inhibitor therapy in these tumors.
6. ALLOGENEIC NATURAL KILLER CELLS AND PALLIATIVE RADIOTHERAPY IN THE TREATMENT OF CANINE CANCER

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Natural killer (NK) are cytotoxic innate cells with a crucial role in anti-tumor responses. Their use in cellular therapy is promising partly because allogeneic sources can be used for off-the-shelf treatment without the risk of graft-versus-host disease. We evaluated the combination of palliative radiotherapy (RT) and allogeneic NK cell transfer in a first-in-dog feasibility trial to speed translation of novel NK modalities in both dogs and people. Our objective was to establish feasibility and describe preliminary data for safety and outcomes in dogs with naturally occurring melanoma treated with allogeneic NK cells following palliative RT. Five dogs with unresectable oral melanoma seen at the UC Davis Veterinary Medical Teaching Hospital (VMTH) were enrolled in this IACUC-approved pilot trial. Allogeneic NK cells were expanded over fourteen days from blood obtained from five healthy donor beagles. Patients underwent weekly RT for four weeks followed by infusion of intravenous allogeneic NK cells (7.5 x 10^6 cells/kg) on the day of the fourth and final RT treatment. Peripheral blood was obtained for biochemical and immune monitoring at baseline and day one, seven, and fourteen days post-treatment. RNA sequencing was performed on patient PBMCs using a 3'-Tag-RNA-Seq protocol for gene profiling. Adverse events related to NK infusion were classified as grade 1 or 2 and included emesis, fever, lymphopenia, metabolic acidosis, and hypoglycemia. Lymphocyte counts decreased one day post NK infusion but increased and peaked on day seven post-infusion. Median survival time was 145 days with maximum and minimum survival times of 445 days and 48 days, respectively. Gene expression profiles generally clustered by individual patient and survival time. This study suggests that allogeneic NK cell infusions are well tolerated when administered to dogs with melanoma. This proof-of-concept trial provides preliminary data validating the canine model for investigating allogeneic adoptive NK cell transfer alone or in combination with other immunotherapies.
1. CANCER VARIATIONS AND DISPARITIES IN THE CENTRAL VALLEY

Naod Kelete, Julie Dang, PhD, MPH, Frederick J. Meyers, MD, MACP, University of California, Davis

There are variations in cancer incidence and mortality rates among counties in the Central Valley of California. These differences may be attributable to social determinants of health (SDOH) such as access to education and healthcare, income level, and food insecurity and behavioral risk factors such as tobacco use. We used secondary data sources (e.g. California Cancer Registry and the California Health Interview Survey) to characterize cancer rates, SDOH and behavioral risk factors among the 19 counties of the University of California, Davis Comprehensive Cancer Center's catchment area. We performed an analysis of two counties, El Dorado and Yuba, that are geographically proximate. From 2014 to 2018, both counties had similar cancer incidence rates. El Dorado county had an overall cancer incidence of 426.2 per 100,000 population and Yuba county had an overall cancer incidence of 442.6 per 100,000 population. Despite both having high cancer incidence rates, El Dorado had a much lower cancer mortality rate (136.4 per 100,000 population) than Yuba county (188.7 per 100,000 population). A closer examination of SDOH and behavioral risk factors for these two counties revealed that El Dorado County had higher levels of education, less of the population that smoked, more individuals per capita with health insurance, and greater percentage of population living above the federal poverty line. We hypothesize that the variation in cancer rates in these two counties are a result of differences in SDOH and behavioral risk factors. A formal statistical analysis is being prepared to test the comparison of cancer rates between the two counties and the impact of the SDOH and behavioral domains. This analysis will guide community collaboration and identification of individuals and neighborhoods at greatest risk and guide targeted community-based interventions.

2. EFFECT OF A KETOGENIC DIET ON PANCREATIC CARCINOGENESIS IN MICE

Tarek Bacha, Undergraduate, UC Davis; Natalia Cortez Penso, PhD candidate, UC Davis; Gerardo Mackenzie, PhD, UC Davis

Pancreatic Ductal Adenocarcinoma (PDAC) remains among the most lethal cancers. Although chemotherapy is the primary therapeutic method to treat patients suffering from PDAC, its efficacy is limited. Thus, there is an urgent need for new strategies to combat this disease, and the exploration of dietary interventions is a critical component. A diet that we are exploring in the lab is the ketogenic diet (KD), which has been gaining attention for their anti-tumor anti-inflammatory potential. KDs are characterized by a high fat, moderate protein and very low carbohydrate content (typically ≤50 g/d carbohydrate intake with >70% fat). The extreme restriction of carbohydrates in KDs causes the body to be in a state of nutritional ketosis, using fats as its main energy source, which produces ketone bodies such as β-hydroxybutyrate. We hypothesize that a KD is a useful dietary intervention in pancreatic cancer. In particular, the objective of my work is to evaluate the effect of a KD on pancreatic carcinogenesis in an animal model of pancreatic cancer carrying a pancreas-specific oncogenic KRASG12D mutation [LSL-KRASG12D; P48+/Cre; (KC mice)]. KC mice develop PDAC at 12-15 months of age on average, and we decided to study the effect of diet intervention in the later stages of PDAC development. For this purpose, 6 months-old male and female KC mice were randomized and fed, either a control diet (CD) or a KD for 6 months and then euthanized at 12-months of age for further histological analysis. At 12-months of age, both, male and female KC mice fed a KD showed significant reduction in PDAC incidence rates compared to KC mice fed a CD. Given these promising results, we are now evaluating the cellular mechanisms by which a KD reduces PDAC incidence. In preliminary results, we have showed that consumption of a KD reduces serum insulin levels, prompting us to study the mechanisms related to the insulin signaling pathway. I am currently examining the effect of a KD on the ERK, AKT, and mTOR signaling pathways in KC mice.
3. SALMETEROL XINAFOATE, SELECTIVELY TRIGGERS DEATH ON GLIOMA CELLS

Orli Algranatti, Student, UC Davis School of Veterinary Medicine; James Angelastro, PhD, UC Davis School of Veterinary Medicine

Stress accentuates cancer progression by the release of epinephrine and norepinephrine hormones. Both hormones activate the α and β-adrenergic receptors. Blockage of the receptors inhibits the growth of several cancer types. Epidemiological studies showed that β-adrenergic antagonists (β-blockers) caused a low incidence of several cancer types. Of the cancer types, Glioblastoma (GBM) expresses β-adrenergic receptors (significantly β-2-adrenergic receptors). GBM accounts for 50% of central nervous system cancers, with a survival time of 15 months after Standard of Care therapeutic intervention. The Standard of Care consists of surgical resection, radiation, chemotherapy, or all three. We first hypothesized that interfering with the β-2-adrenergic receptor’s function will destroy GBM cells grown in culture. Our goal will be to develop a translational therapy that will trigger the death of GBM cells while preserving healthy cells.

One of the highest potency drugs was the β-2-adrenergic receptor agonist, salmeterol xinafoate. Salmeterol xinafoate is a biased agonist that activates the G-protein pathway but not the β-arrestin pathway. Biased agonists activate signaling through either the G-protein or the β-arrestin pathway of G-Protein Coupled receptors (i.e., β-adrenergic receptor). In contrast, conventional unbiased agonists trigger both of these pathways. Therefore, efforts were aimed at repurposing this drug to treat GBMs. Our data shows that disrupting β-2-adrenergic receptor signaling by salmeterol xinafoate leads to cell death of glioma cells but spares healthy neural cells when using similar doses. In addition, the survival of glioma cells appears to be regulated by the β-arrestin pathway. By contrast, balanced agonists do interfere with the viability of glioma cells. Finally, antagonists have also been shown to promote the death of glioma cells. Chronic Obstructive Pulmonary Disease is the current use of this drug. Repurposing this FDA-approved drug for GBM could be fast-forwarded into clinical trials because drug safety has previously been determined.

4. DEVELOPMENT OF A SPONTANEOUSLY METASTATIC SOFT-TISSUE SARCOMA MODEL

Maria Munoz; Janai Carr-Ascher, MD, UC Davis Comprehensive Cancer Center

Soft-tissue sarcoma (STS) is a rare connective tissue cancer that encompasses over 50 subtypes that are each histologically and genetically different. These subtypes include liposarcoma, undifferentiated pleomorphic sarcoma (UPS) and pleomorphic rhabdomyosarcoma. Despite the heterogeneity within this disease, each subtype can metastasize to the lungs. The mechanisms of metastasis are unknown, and there are poor survival rates for patients with metastatic disease. As such we have developed a robust system to enrich and study spontaneous lung metastasis in STS. This will allow us to identify mechanisms of metastasis to enhance our understanding of the disease and improve patient therapeutics. To do this, we first screened several cell lines to identify metastatic activity in-vivo. This was done by injecting the cells intramuscularly into immunocompromised mice. These models had a high tumor latency and low metastatic burden. From this screen, we identified a liposarcoma model with the most lung penetrance and generated a cell line from the primary tumor. These serially passaged cells were then injected into immunocompromised mice and allowed to spontaneously metastasize. This resulted in a shorter tumor latency, and higher metastatic burden in the lungs. For downstream analysis, we enriched the metastatic cells through magnetic cell depletion and fluorescence-activated cell sorting (FACS). This method enriches the human cell population to ensure we solely analyze the human cells. Downstream analyses of these cells will include RNA, DNA and/or ATAC-sequencing. We hypothesize that comparing the metastatic and primary tumor cell populations will identify metastatic pathways in STS. These pathways can be targeted to improve patient therapies. Overall, this robust system is reflective of the human disease and allows us to study spontaneous lung metastasis to further our understanding of STS.
5. GENOMIC LANDSCAPE OF SMARCA4-DEFICIENT LUNG TUMORS BY CLINICAL RNA SEQUENCING

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Background - SMARCA4-deficient lung cancer is an undifferentiated lung cancer subtype associated with poor prognosis. These tumors are known to be resistant to standard of care surgery, radiation, and chemotherapy and possibly resistant to immunotherapy. An assessment of the genomic and transcriptomic features of SMARCA4-deficient thoracic tumors may identify potential novel targets and treatment strategies.

Methods - We retrospectively analyzed de-identified NGS data from 8,484 thoracic formalin-fixed, paraffin-embedded tumor biopsies from lung cancer patients sequenced using the Tempus|xT solid tumor assay (DNA-seq of 595-648 genes at 500x coverage; whole-exome capture RNA-seq). Tumor-normal match sequencing was performed for all tumors, enabling the detection of incidental germline alterations across 46 genes. SMARCA4-deficiency was defined as tumors with a pathogenic or likely pathogenic SMARCA4 single nucleotide variant, insertion/deletion, or copy number alteration. Statistical significance was determined using Fisher's exact test and Wilcoxon rank-sum tests.

Results - SMARCA4-deficiency was detected in 370 (4.4%) tumors, of which over 80% were stage III or IV. SMARCA4-deficient tumors included more male patients (63% vs 49%, p<0.001) and younger age at diagnosis (median 64 vs 68 years, p <0.001). There were more patients with high tumor mutational burden (TMB-H, ≥10 mutations per megabase) (34% vs 15%, p<0.001), and fewer patients with positive PD-L1 immunohistochemical staining (44% vs 54%, p=0.009) compared to SMARCA4 wild-type tumors. Microsatellite instability status occurred at similar low frequencies across SMARCA4-deficient vs wild-type tumors (0.8% vs 0.5%, p=0.5). SMARCA4-deficient tumors showed enrichment for somatic mutations in TP53 (71% vs 47%, q<0.001), STK11 (22% vs 6.8%, q<0.001), KEAP1 (15% vs 4.2%, q<0.001), and CDKN2A (15% vs 5.9%, q<0.001) compared to wild-type. Tumor normal-match sequencing identified incidental germline mutations in MUTYH (2.2%), ATM (1.1%), ATP7B (0.5%), and MSH6 (0.5%) for SMARCA4-deficient tumors. RNA-sequencing analysis confirmed reduced transcriptional expression of SMARCA4 (p<0.001), CD274 (PD-L1; p<0.001), TNFRSF18 (p<0.001), and TNFRSF4 (p=0.035) in deficient tumors vs wild-type. Furthermore, SMARCA4-deficient tumors revealed reduced infiltration of CD4+ T cells (19% vs 22%, p<0.001).

Conclusions - This study reveals the unique genomic and transcriptional characteristics of SMARCA4-deficient lung tumors. Further studies are needed to assess the impact of immunotherapies and targeted therapies.

6. TARGETING OF SOFT TISSUE SARCOMA CANCER STEM CELLS IMPROVES DOXORUBICIN-SENSITIVITY IN VITRO

Edmond O'Donnell, MD, PhD, Department of Orthopaedic Surgery, UC Davis Medical Center; Maria Munoz, Department of Hematology & Oncology, UC Davis Medical Center; R Lor Randall MD, Department of Orthopaedic Surgery; Janai Carr-Ascher MD PhD, Department of Hematology & Oncology, UC Davis Medical Center

Soft tissue sarcomas (STS) are rare tumors encompassing over 70 distinct histopathological subtypes that are treated similarly with surgical resection, radiation, and chemotherapy. In STS, recurrence and resistance to anthracycline-based chemotherapy are associated with worse outcomes and represent significant barriers to improving patient survival. We are interested in the contribution of STS cancer stem cells (STS-CSCs) to the phenomenon of chemo-resistance to doxorubicin. Specifically, we hypothesized the presence of a common genetic signature across unique STS subtypes involved in CSC-regulation that could be targeted to improve the efficacy of existing treatment regimens. To this end, STS-CSCs were profiled by flow cytometry using the Aldeflour assay. This is a well-established technique to measure the aldehyde dehydrogenase activity of cells which is high in the stem cell population. This fluorescently labels Aldeflour bright and dim cells as CSCs and non-CSCs, respectively. The abundance of the CSC population in several STS cell lines modeling dedifferentiated liposarcoma, leiomyosarcoma, and undifferentiated pleomorphic sarcoma were assessed by Aldeflour assay. In order to gain insight into
the molecular pathways active in STS-CSCs, Aldeflour-bright and -dim populations were isolated by FACs and analyzed by RNA-sequencing. Gene-set enrichment analysis of genes upregulated in STS-CSCs identified a signature for the histone methyltransferase Enhancer of Zeste homolog 2 (EZH2), part of the polycomb repressive complex 2 (PRC2) responsible for H3K27 methylation. As an epigenetic modulator, increased EZH2 expression and activity potentiates decreased activity of genes involved in growth suppression and thereby has oncogenic activity. EZH2 can be inhibited with small molecules such as Tazemetostat, an approved treatment for metastatic and locally advanced epithelioid sarcoma. To test the effects of EZH2 inhibition on STS-CSCs, we first generated doxorubicin resistance STS cell lines by serial selection with increasing concentrations of doxorubicin. We identified a positive correlation between CSC abundance and doxorubicin IC50 in the resistant cell lines. Further, co-treatment of doxorubicin and tazemetostat was not only synergistic in the parent cell lines, but restored chemosensitivity in doxorubicin resistant lines. These data confirm the presence of shared genetic programs across distinct subtypes of STS that are unique to CSCs and amenable to therapeutic targeting.

7. **NUCLEAR RECEPTOR ROR-GAMMA PROMOTES ABERRANT CHOLESTEROL HOMEOSTASIS IN ADVANCED PROSTATE CANCER**

_Nianxin Yang, UC Davis Biochen & Molecular Medicine, Yatian Yang, PhD, UC Davis Biochen & Molecular Medicine, Junjian Wang, PhD, UC Davis Biochen & Molecular Medicine, Hongwu Chen, PhD, UC Davis Biochen & Molecular Medicine_

Advanced prostate cancer (PCa), including metastatic castration resistant prostate cancer (mCRPC), features high intratumoral cholesterol levels, resulting from aberrant regulation of cholesterol homeostasis. Our previous studies have found that retinoid acid receptor-related orphan receptor gamma (RORy) plays an important role in promoting mCRPC tumor growth and that RORy functions as a master regulator of cholesterol biosynthesis in triple negative breast cancer (TNBC). However, whether RORy plays a role in the aberrant cholesterol homeostasis in mCRPC remains unknown. Our current study showed that RORy plays a crucial role in aberrantly elevated cholesterol levels in mCRPC, which promotes mCRPC cell survival and proliferation. Our RNA-seq, qRT-PCR and immunoblotting data showed that RORy inhibition resulted in downregulation of many key cholesterol biosynthesis enzyme gene and protein expression, including those of HMGCS1, HMGCR, and SQLE. Interestingly, our further RNA-seq analysis revealed that RORy inhibition significantly enhanced the expression of cholesterol efflux gene program. Since liver X receptors (LXRs) are the master regulator of cholesterol efflux pathway, we performed LXR siRNA knockdown with RORy inhibition and found that RORy regulates cholesterol efflux via controlling LXR expression. Next, since statins are most commonly used as a cholesterol lowering drug, we tested whether RORy antagonist in combination with statins has any synergistic effect in killing mCRPC cells. Our results showed that RORy antagonist possesses significant synergy with statins in mCRPC growth inhibition and blocked statin induced overexpression of cholesterol biosynthesis gene program. Importantly, RORy antagonist and statins also have similar synergy in mCRPC tumor growth inhibition, indicating that their combination treatment is a potential therapeutic strategy for mCRPC patients. To conclude, our work revealed that RORy functions contribute to aberrant cholesterol homeostasis in mCRPC and offered a potential therapeutic strategy for mCRPC.

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8. **IDENTIFYING THE ROLE OF PDZRN3 HYPERMETHYLATION IN PANCREATIC CANCER METASTASIS**

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Pancreatic ductal adenocarcinoma (PDA) is the third leading cause of cancer related deaths in the United States largely because most patients are diagnosed after the cancer has metastasized. Despite several attempts, no recurrent genetic mutation driving PDA metastasis has been found, suggesting that PDA metastasis is driven by epigenetic rather than genetic factors. One such epigenetic factor that is likely driving PDA metastasis is DNA methylation. When at promoters, methylation silences the associated gene. While promoters are typically unmethylated, aberrant promoter hypermethylation is
characteristic of many cancers, including PDA. However, the specific genes whose DNA methylation-mediated silencing promote PDA metastasis are largely unknown. Our preliminary data shows that the Pdzrn3 promoter is hypermethylated and associated with gene downregulation in metastatic compared to primary tumor samples. This Pdzrn3 downregulation is associated with worse survival outcomes in PDA patients. In addition, Pdzrn3 is a ubiquitin ligase implicated in regulation of the non-canonical Wnt/planar cell polarity (PCP) pathway, which has been shown to promote metastasis when dysregulated in several cancers. Thus, I hypothesize that Pdzrn3 promoter methylation inhibits Pdzrn3 gene expression to promote metastatic characteristics in PDA tumors via Wnt/PCP. To test this hypothesis, I aim to 1) determine the effect of Pdzrn3 expression on PDA metastasis, 2) determine the effect of Pdzrn3 expression on Wnt/PCP-mediated cytoskeletal rearrangement and cell-cell adhesion, and 3) assess the prognostic capability of Pdzrn3 promoter methylation as a cell-free DNA (cfDNA) biomarker. I will carry out these aims using gain- and loss-of-function approaches both in vitro and in vivo. This proposed work will be the first to comprehensively elucidate the role of Pdzrn3 in PDA metastasis and assess the potential use of Pdzrn3 hypermethylation as a cfDNA prognostic marker. Pdzrn3 or other Wnt/PCP components can then be considered in targeted therapeutic strategies for PDA and Pdzrn3 hypermethylation can be considered for non-invasive prognostic biomarker screens.

9. EPIGENETIC REGULATOR BRD4 INHIBITION DISRUPTS NEURONAL SIGNALING TO SUPPRESS NEPC PROGRESSION

Xiong Zhang, Yatian Yang, and Hong-Wu Chen

Neuroendocrine prostate cancer (NEPC) is a highly aggressive form of prostate cancer with a short survival time (typically < 12 months) from detection, arising either de novo or from anti-androgen receptor (AR) signaling inhibitor (ARSI) therapy treatment of prostate adenocarcinoma (PCa) and hence t-NEPC. However, very few drivers and therapeutic targets of NEPC diseases have been identified. Dysregulation of the epigenome and transcriptional networks are thought to drive progression of both de novo NEPC and t-NEPC. Here, we report that through small-molecule perturbation analysis of major epigenetic regulators, we identified chromatin regulator BRD4 as a strong candidate of therapeutic target in NEPC. BRD4 antagonists/BETi AZD5153 and JQ1 displayed strong activities in inhibition of the growth of NEPC cells and PDX organoids. Tumor growth of NEPC pre-clinical models was also potently inhibited by the antagonists. Our RNA-seq gene expression profiling demonstrated that among gene programs downregulated by the treatments, synaptic transmission and neuronal cell differentiation pathways are significantly enriched. Further analysis revealed that pro-neurogenesis signaling and signaling pathways of neurotransmitter receptors were strongly inhibited. Our BRD4 ChIP-seq analysis indicated that expression of the pro-neurogenesis signaling and the receptors was directly controlled by BRD4. H3K27Ac ChIP-seq and ATAC-seq showed that BRD4 inhibition induced significant alteration of local chromatin structure. Therefore, our study identified a new strategy for treatment of NEPC by targeting BRD4.

10. CHARACTERIZATION OF NATURAL KILLER AND CYTOTOXIC T CELL IMMUNE INFILTRATES IN PANCREATIC DUCTAL ADENOCARCINOMA

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Background: Pancreatic ductal adenocarcinoma (PDAC) is characterized by a poor prognosis and resistance to systemic therapies including immunotherapy. Although PDAC has been linked to low T cell infiltrate, the contribution of natural killer (NK) cells has received less attention. Our objective was to
evaluate the immune parameters PDAC, including markers of NK cells, to determine if NK cells associate with patient outcomes.

Methods: We analyzed tumors from 93 PDAC patients treated from 2012 – 2020. Predictor variables included tumor infiltrating lymphocytes (TILs), T cell markers (CD3+, CD8+, CD45RO+), NK markers (NKp46) and NK inhibitory markers (TIGIT and MHC-I). TILs were scored from 0-3, and immune markers were scored from 0-300. We also evaluated neutrophil and lymphocyte levels in the blood. Primary outcome variables were metastasis-free survival (MFS) and overall survival (OS), analyzed by the Kaplan-Meier method.

Results: Mean age was 70, 55% were female, and the mean tumor size was 3.1±1.1cm. 89% involved the pancreatic head, and 63% were lymph node positive. The majority of patients received adjuvant therapy. With a median follow up of 24 months, median survival was 35 months. Blood lymphocytes levels prior to surgery were positively correlated with TILs (p=0.008 r=0.3). Median TILs infiltration was 1.1, and 50% were 0 or 1. Mean CD3+ score was 20.6±1, and mean NKp46 was 3.1±3.9. Although there was slight positive correlation between T cell and NK cell scores (CD3+/NKp46 p=0.005, r=0.3; CD8+/NKp46 p=0.05, r=0.2), neither T nor NK cell infiltration was associated with MFS or OS. Similarly, there was a tight positive correlation between MHC-I expression and all T cell markers (CD3+, CD8+ and CD45RO+), but not with NKp46 nor with survival outcomes (MFS/OS, P>0.05). TIGIT expression was also low (mean 30.8±21.9).

Conclusion: NK and T cell infiltrates are overall low in PDAC and do not associate with oncologic outcomes. Further characterization of the immune infiltrate in PDAC, including inhibitory signals and suppressive cell types, may yield better biomarkers of prognosis and immune targeting in this refractory disease.

11. PREDICTIVE FACTORS FOR ONCOLOGIC DIAGNOSIS IN PEDIATRIC PATIENTS UNDERGOING OPEN CERVICAL LYMPH NODE BIOPSY

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Background: Optimal management of cervical lymphadenopathy, particularly the decision to biopsy, remains a topic of discussion. The goal is to reduce unnecessary biopsies balanced with providing timely diagnosis, particularly oncologic diagnoses. We aim to elucidate clinical factors that predict oncologic diagnosis from open cervical lymph node biopsy.

Methods: Patients <18 years old who underwent open cervical lymph node biopsy from 1/1/2016 to 12/21/2021 at our tertiary care children’s hospital by pediatric general surgeons were included. Demographic, patient history, imaging and laboratory data were collected by chart review and univariate analysis was used to identify independent predictors for cancer diagnoses.

Results: 36 patients underwent open lymph node biopsy for cervical lymphadenopathy, with 50% of patients having a cancer diagnosis. Patients with oncologic diagnoses were significantly more likely to have symptoms of malignancy on presentation (88.9% vs 55.6%, p=0.03). Out of the signs and symptoms concerning for malignancy, dyspnea (38.9% vs 0%, p=0.008, palpable supraclavicular node on exam (50% vs 5.6%, p=0.0007) and abnormal chest radiograph (70.6% vs 0%, p=0.0007) were predictive of oncologic diagnosis on biopsy. Other predictors of malignancy included a leukocytosis (13.86 vs 7.23 k/mm3, p=0.002), elevated erythrocyte sedimentation rate (ESR) (68.58 vs 19.36 mm/hr, p=0.001), and elevated C-reactive protein (CRP) (5.25 vs 1.04 mg/dL, p=0.001). Of note, inpatient work-up was more frequent in patient with oncologic diagnosis, but did not reach significance (88.9% vs. 55.6%, p=0.06).

Conclusion: Palpable supraclavicular node, dyspnea, abnormal chest x-ray, leukocytosis, elevated ESR and CRP are independent predictors for oncologic diagnosis after open cervical lymph node biopsy. Multivariate regression is planned to further assess relationships among these factors. Elucidating predictors of oncologic diagnoses will aid in decision-making for pediatric patients with cervical lymphadenopathy.
12. INHALED RECOMBINANT HUMAN IL-15 IN DOGS WITH NATURALLY OCCURRING PULMONARY METASTASES FROM OSTEOSARCOMA OR MELANOMA: A PHASE 1 STUDY OF CLINICAL ACTIVITY AND CORRELATES OF RESPONSE


Background: Although recombinant human interleukin-15 (rhIL-15) has generated much excitement as an immunotherapeutic agent for cancer, activity in human clinical trials has been modest to date, in part due to the risks of toxicity with significant dose escalation. Since pulmonary metastases are a major site of distant failure in human and dog cancers, we sought to investigate inhaled IL-15 in dogs with naturally occurring lung metastases from osteosarcoma (OSA) or melanoma. We hypothesized a favorable risk/benefit profile given the concentrated delivery to the lungs with decreased systemic exposure.

Experimental Design: We performed a Phase I trial of inhaled rhIL-15 in dogs with gross pulmonary metastases using a traditional 3+3 cohort design. A starting dose of 10 mg twice daily x 14 days was used based on human, non-human primate, and murine studies. Safety, dose-limiting toxicities (DLT), and maximal tolerated dose (MTD) were the primary objectives, while response rates, progression-free and overall survival, and pharmacokinetic and immune correlative analyses were secondary.

Results: From October 2018 to December 2020, we enrolled 21 dogs with 18 dogs reaching the 28-day response assessment to be evaluable. At dose level 5 (70 mg), we observed 2 DLTs, thereby establishing 50 mg BID x 14 days as the MTD and recommended phase 2 dose. Among 18 evaluable dogs, we observed 1 complete response > 1 year, 1 partial response with resolution of multiple target lesions, and 5 stable disease for an overall clinical benefit rate of 39%. Pharmacokinetic analysis revealed detectable and sustained plasma rhIL-15 levels between 1- and 6-hours post-nebulization. Decreased baseline lymphocyte counts prior to treatment were significantly associated with clinical benefit. Cytotoxicity assays of banked peripheral blood mononuclear cells revealed significant increases in peak cytotoxicity against canine melanoma and OSA targets which correlated with overall survival.

Conclusions: In this first-in-dog clinical trial of inhaled rhIL-15 in dogs with advanced metastatic disease, we observed promising clinical activity when administered as monotherapy for only 14 days. These data have significant clinical and biological implications for both dogs and humans with refractory lung metastases and support exploration of combinatorial therapies using inhaled rhIL-15.

13. A FIRST-IN-HUMAN PHASE I OPEN-LABEL STUDY OF A NOVEL CANCER VACCINE LABVAX 3(22)-23 AND ADJUVANT GM-CSF IN PATIENTS WITH ADVANCED STAGE ADENOCARCINOMAS

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Background: Adenocarcinoma is the most common histologic type of solid tumors that may occur almost anywhere in the body. Labyrinthin is a novel tumor-specific protein expressed on the cell surface of the majority of adenocarcinomas of various cancer types. We hypothesize that vaccination against labyrinthin can elicit strong immune responses against the labyrinthin-positive adenocarcinomas in cancer patients. LabVax 3(22)-23 is a novel anti-tumor vaccine that contains 4 synthetic labyrinthin-based peptides designed to elicit both B-cell and T-cell responses. Preclinical studies showed that LabVax 3(22)-23 significantly inhibited tumor growth that was augmented by GM-CSF sargramostim without any significant toxicity in C57/BL6 transgenic mice expressing human PD-1/PD-L1 implanted with the murine colon adenocarcinoma cell line MC-38-huPD-L1. This first-in-human, phase I trial (UCDCC#296) evaluates LabVax 3(22)-23 and adjuvant sargramostim in patients with labyrinthin-positive metastatic or recurrent adenocarcinoma of any primary tumor site after all standard-of-care therapies.
Methods: The primary endpoint is dose limiting toxicity (DLT), which is defined as grade ≥ 2 allergic and autoimmune reaction, grade ≥ 3 injection site reaction, any grade 3 toxicities lasting >1 week, or any grade ≥ 4 toxicities by NCI CTCAE V5.0. With a sample size of 10 patients, there is 89-97% of chance to observe ≥1 DLT if the true event rate is 20-30%. A secondary endpoint is the preliminary assessment of tumor response rate by Response Evaluation Criteria in Solid Tumors (RECIST) v1.1. Exploratory objectives measure the effect of LabVax 3(22)-23 on various immune responses (cytokines, anti-labyrinthin antibody production) and the correlation between the level of labyrinthin expression and the efficacy of LabVax 3(22)-23. Eligible patients are required to have labyrinthin expression on their tumor cells by immunohistochemistry (IHC) and adequate organ function. Patients receive sargramostim subcutaneously and LabVax 3(22)-23 intradermally on weeks 1, 2, 4, 8, and 12 in the absence of disease progression or unacceptable toxicity. After completion of study treatment, patients are followed every 3 months for 1 year.

Results: Three patients are enrolled at the time of submission. All tumors expressed labyrinthin by IHC. First two patients tolerated the treatment well without any SAE, and first patient had stable disease at 3 months.

14. CHARACTERIZATION OF THE TUMOR IMMUNE MICROENVIRONMENT IN SOFT TISSUE SARCOMA PATIENTS UNDERGOING SURGERY

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Background - Tumor infiltrating lymphocytes (TILs) have been shown to predict survival in soft tissue sarcomas (STS), but the specific contribution of natural killer (NK) and CD8+ T cells to outcomes is undefined. Therefore, we sought to characterize the extent of NK and CD8+ T cell infiltration in STS.

Methods - Prospectively, we evaluated 15 patients using fresh tumor from surgery for flow cytometric analysis. Retrospectively, we evaluated archived tumor tissue from 90 STS patients by immunohistochemistry (IHC) for CD3, CD8, CD45RO, NKp46, TIGIT, MHC-I, and p53. We analyzed metastasis-free survival (MFS) and overall survival (OS) by Kaplan-Meier method and log-rank test.

Results - By flow cytometry, we observed significant variability in CD45+ leukocytes in the STS TME (mean 29±24% of total live cells) with low percentages of tumor-infiltrating CD3-CD56+ NK cells (1.7±1.9% of total live cells and 5.3±3.0% of live CD56+ cells) and CD8+ T cells (1.6±1.6% of total live cells and 29.6±30.5% of live CD8+ cells). By IHC, NK and T cell infiltrates were low (median H score 0, range 0-66.5 and 2.7, range 0-110, respectively). We confirmed a positive correlation between CD8+ T cell infiltration and significantly improved OS (P<0.05) and a trend for improved MFS. We also observed a trend for improved OS among patients with higher NKp46 scores (P=0.07). MHC-I expression positively correlated with both T and NK cell infiltration (P<0.05), whereas TIGIT expression positively correlated with T cell infiltration (P<0.05), but not NK infiltration.

Conclusion - Infiltration of NK and CD8+ T cells is overall low in STS patients undergoing surgery but associated with superior OS. Further characterization of the immune infiltrate in STS may yield better biomarkers of prognosis and immune targeting.
15. LOW LYMPHOCYTE COUNTS ARE ASSOCIATED WITH POOR CLINICAL OUTCOMES IN HOSPITALIZED CANCER PATIENTS RECEIVING IMMUNE CHECKPOINT INHIBITORS

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Immune checkpoint inhibitor (ICI) therapy has improved survival outcomes in a variety of cancers. Due to these promising results and ever expanding accessibility, use of ICI across a spectrum of clinical settings continues to evolve. In this study, we explore outcomes and predictive factors of patients who received ICI therapy while hospitalized at UC Davis. We performed a retrospective chart review of cancer patients who received ICI therapy from 08/2016 to 07/2021. For each patient we reviewed cancer histology, ICI treatment administered, time from treatment to discharge, time from treatment to progression (PFS) or death (OS), and complete blood counts (ALC<800, dNLR ≥4) prior to therapy (time 0). 38 patients were identified who received a total of 47 doses of ICI therapy. The most common cancer type to receive therapy was lung cancer (13, 33.3%), followed by melanoma (8, 20.5%), and lymphoma (6, 15.4%). The majority of patients were men (N=30) and the average age was 54 years. Average hospitalization length in days was 23.5 (95% CI 16.8, 30.2). Most of the patients died during the study period, and only 9 (23.1%) remained alive at the time of study closure. 10 patients (25.6%) died during the same hospitalization in which they received treatment. Of the patients who followed up, 22 (64.7%) died within 90 days of receiving inpatient therapy. The average PFS in months was 2.18 (95% CI 0.587, 3.77). On review of basic pretreatment lab work lymphopenia was observed as a common derangement (n=18). It was found that in patients with an absolute lymphocyte count (ALC) of <800 PFS was notably lower (0.55 months) compared to patients with approximately normal counts (ALC≥800, 3.24 months). Furthermore, patients ALCs <800 were less likely to receive a follow up dose of ICI therapy than their counterparts (28.6% vs 36.4%). Administration of inpatient ICI therapy is associated with poorer clinical prognosis. The majority of patient demonstrated high rates of both inpatient mortality and death within 3 months of discharge. The data collected in our review suggests a possible correlation between ALC and ICI treatment, warranting further investigation into these routinely examined biomarkers.

16. LYMPHOCYTE-SPARING RADIOThERAPY: REDUCING DOSE TO LYMPHOCYTE-RELATED ORGANS AT RISK IN LOCALLY ADVANCED LUNG CANCER

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Purpose: To investigate the dosimetric feasibility of lymphocyte-sparing radiotherapy in locally advanced lung cancer. We hypothesized that dose to lymphocyte-related organs at risk (LOAR) can be reduced while maintaining target coverage and adherence to standard OAR constraints.

Methods: Fifteen consecutive patients with stage III non-small cell lung cancer were selected from a previous prospective clinical trial. LOARs included thoracic active bone marrow (ABM), heart and lungs. Thoracic ABM was defined as subvolume of thoracic vertebrae with a standardized uptake value greater than the mean on the coregistered FDG-PET image. Each patient had two volumetric modulated arc therapy plans: baseline and LOAR-sparing. Baseline plans used conventional dose-volume constraints for the planning target volume (PTV) and standard OARs, while LOAR-sparing plans used the same constraints and the following additional constraints for the LOARs: thoracic ABM V20Gy ≤45%, heart V5Gy ≤48%, and lung V5Gy ≤51% (defined based on published data demonstrating an association with radiation-induced lymphopenia). We compared dose-volume metrics of the PTV, standard OARs, LOARs, and effective dose to circulating immune cells (EDIC) (previously associated with lymphopenia) between the baseline and LOAR-sparing plans using the Wilcoxon signed-rank test.

Results: Compared with the baseline plans, LOAR-sparing plans had a mean absolute reduction of 6.2% (range, -2.8%–21.7%) in the ABM V20Gy (p=0.012), 14.4% (-1.4%–42.5%) in the heart V5Gy (p=0.002), 12.4% (0.5%–32.8%) in the lung V5Gy (p=0.001), and 0.7 Gy (0.0–2.3 Gy) in EDIC (p<0.001), along with reductions in the conventional heart and lung constraints. LOAR-sparing plans had greater dose heterogeneity in the PTV and higher maximum dose to the spinal cord, which were clinically acceptable.
Conclusion: In this first investigation on the feasibility of lymphocyte-sparing radiotherapy, we demonstrated that dose to LOARs can be reduced while maintaining target coverage and adherence to standard OARs, providing evidence to support further investigations.

17. A PHASE IA STUDY OF CERITINIB + TRAMETINIB IN PATIENTS WITH ADVANCED ALK POSITIVE NON-SMALL CELL LUNG CANCER (NSCLC): PRELIMINARY RESULTS

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Background: In NSCLC patients harboring oncogenic ALK alterations, monotherapy with ALK inhibitors has high response rates. However, responses are not durable and patients eventually succumb. In preclinical EML4-ALK NSCLC models, ALK and MEK co-inhibition resulted in increased anti-tumor activity. We sought to evaluate the combination of the ALK inhibitor ceritinib and the MEK inhibitor trametinib in stage IIIB/IV ALK or ROS-1 rearranged NSCLC.

Methods: An investigator-initiated phase Ia trial of ceritinib plus trametinib in ALK- or ROS1-rearranged NSCLC patients who had progressed on prior oncogene-targeted therapy was conducted. Primary endpoints were to determine the safety/tolerability and the recommended phase 2 dose for the combination. We used 3+3 dose escalation starting at dose level 1 (ceritinib 300 mg po QD + trametinib 1.5 mg po QD), with dose escalation cohorts of: (level 2) ceritinib 450 mg po QD + trametinib 1.5 mg po QD and (level 3) ceritinib 450 mg po QD + trametinib 2.0 mg po QD. Dose limiting toxicity (DLT) was defined as any attributable grade 3 or 4 non-hematologic toxicity; grade 4 neutropenia or thrombocytopenia lasting > 7 days; or febrile neutropenia.

Results: Nine patients were enrolled and completed at least 1 cycle. There were six ALK+ patients in dose level 1 and two ALK+/one ROS1+ patients in dose level 2. The median number of prior lines of therapy was five. Most common adverse events (AE, all grades) were rash (n=6; 67%), diarrhea (n=5; 55%), and elevated AST/ALT (n=4; 44%). The most common attributable grade 3 or higher AE was elevated AST/ALT (n=3; 33%). One DLT (grade 3 rash) occurred at dose level 1. Of nine evaluable patients, two (22%) had partial response (PR) (both in dose level 1), three (33%) had stable disease (SD) (all in dose level 1), and four (44%) had progression (1 in dose level 1 and 3 in dose level 2). The sole ROS1+ patient had PD. Preliminary overall response rate (ORR) was 22%; disease control rate was 56%. One ALK+ responder with four prior lines of therapy experienced an 88% tumor size reduction.

Conclusions: The combination of ceritinib and trametinib appears safe and tolerable thus far with no unexpected toxicities. The ORR of 22% in a pre-treated patient population suggests that the approach of targeting both ALK and MEK may be an effective strategy for a subset of patients who have progressed on ALK-targeted monotherapies. Further evaluation of biomarkers of response and resistance as well as treatment of molecularly-defined expansion cohorts is planned.

18. IDENTIFYING LATE-STAGE CERVICAL CANCER PRESENTATION IN CALIFORNIA: A MACHINE LEARNING APPROACH

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Background: Screening can detect cervical precancer and early-stage disease (stage I). However, approximately half of cervical cancer diagnoses in California are late stage (stages II-IV). Identifying subgroups of women that could benefit from additional screening is crucial to reduce the morbidity and mortality of this highly preventable disease in California.

Purpose: To characterize combinations of patient sociodemographic characteristics associated with high risk of late-stage cervical cancer (LSCC) diagnosis.

Methods: Using California Cancer Registry data, we identified 12,587 women ≥21 diagnosed with a first primary cervical cancer of known stage from 2010-2019. The machine learning model classification and regression tree (CART) was used to identify combinations of sociodemographic characteristics
associated with homogenous risks of LSCC. Each combination was categorized as low, medium, or high risk of LSCC diagnosis. Descriptive statistics (proportion of LSCC, confidence intervals) were used to describe the risk of LSCC within each combination.

Results: We identified 11 combinations and categorized them as low, medium, and high risk. The combination with the lowest proportion of LSCC (p=0.32; CI=0.30, 0.33) included women <50 with private health insurance. The combination with the highest proportion of LSCC (p=0.79; CI=0.73, 0.86) included women <50 with public or unknown health insurance and ≥2 comorbidities. Women at median risk (p=0.56; CI=0.53, 0.59) included those 50-64 with 0, 1, or unknown comorbidities, private or unknown health insurance, and known tobacco status. Of variables included, the most important was age, followed by health insurance type, comorbidity index, tobacco use, and marital status. The model’s positive predictive value (PPV) and negative predictive value were 71% and 57% respectively.

Conclusions: Despite early-detection screening methods, women in California continue to present with LSCC. The CART algorithm had a high PPV and was good at identifying homogenous sociodemographic subgroups of women diagnosed with LSCC. Our findings identified the most vulnerable populations, including women <50 with public or unknown health insurance and ≥2 comorbidities, to target for additional screening in order to decrease morbidity and mortality from cervical cancer.

19. COMPARATIVE ANALYSIS OF BIOTIN LIGASES AIRID, ULTRAID, AND TURBOID FOR MECHANO-TRANSDUCTION

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Significant knowledge gaps exist in the molecular details of force-initiated signaling (i.e., mechano-transduction) and its role in embryogenesis, tissue homeostasis, and cancer metastasis. Additionally, mechano-transduction plays a major role in the regulation of cell behavior and as such may play an even greater role in cancer tumor formation, tumorigenesis, and metastasis. In order to begin filling in these knowledge gaps, we developed an experiment using proximal biotinylation to identify force-dependent protein interactions in live cells. Based on our discovery that cten, a focal adhesion protein, is recruited to cytokeratin fibers in the presence of applied forces, our goal is to identify proteins surrounding cten under force-bearing conditions so that we can potentially determine the function of cten as well as its protein network’s role in cellular mechano-transduction. Because new biotin ligases with improved functions have been reported, as the first step in this investigation, I have tested the comparative biotinylation efficiencies of AirID, UltraID, and TurboID and determined which biotin ligase is best suited for this application. Thus far, my initial findings indicate that TurboID has the greatest biotinylation efficiency, followed by AirID and then UltraID, which is significant considering that both AirID and UltraID were engineered to improve upon TurboID biotinylation function. Moving forward in this experiment, I will be tagging the full-length cten with TurboID to test the force-sensitive interactions surrounding cten. This approach will allow us to gain a better understanding of mechano-transduction within cells and eventually its role in cancer.

20. Y-90 PET IMAGING POST LIVER RADIOEMBOLIZATION: SYSTEM MODELING WITH MONTE CARLO SIMULATIONS

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Yttrium-90 (Y-90) PET images are inherently noisy due to the extremely low positron (e+) emission rate of Y-90 (32 ppm). However, other factors such as dead-time and scatter modeling can also contribute to the high noise and non-uniformities observed in Y-90 PET images. One plausible additional source is the high bremsstrahlung emission rate from the beta- (β−) emission (99.98% of Y-90 nuclear decays) that occurs with therapeutic activities (typically around 2 GBq) much higher than typical diagnostic activities. As mentioned in early Y-90 PET studies (Lhommel et al. 2009, Pasciak et al. 2014), system saturation through dead-time effects could induce a detection plateau at high count-rates and prevent the system from detecting the rare annihilation photons. To the best of our knowledge, no published work attempted to study this hypothesis. In this work, we use Monte Carlo simulations (GATE/Geant4) of a total-body PET scanner (uEXPLORER) to investigate the effect of system saturation on Y-90 PET imaging by introducing dead-time into the PET detector response model.
An analytical model of the NEMA NU 2 image quality phantom with six fillable spheres and a background activity was used to generate Y-90 counts representative of a therapeutic dose. This preliminary study focused on studying the various count rates and did not include image reconstruction. Positron (e+) and bremsstrahlung emissions were evaluated individually and combined. Six different dead-time values ranging from 6 ns to 60 µs were simulated.

As expected, a 6 ns dead-time decreased both the e+ and the bremsstrahlung detection rates. However, a dead-time longer than 60 ns resulted in a much faster decrease of the bremsstrahlung detection rate than the e+ reaching, at 600 ns, 9% of the detection rate of simulations without dead-time, while the e+ kept a high detection rate of 83%, indicating a possible system saturation. In future work, scatter effects will be evaluated and the actual dead-time will be calculated using an experimental characterization of the PET scanner and used to conduct accurate simulations aiming at the validation of the model and allowing its use on different positron emitter radionuclides.

21. DECIPHERING FORCE-INDUCED INTERACTIONS OF KERATIN AND CTEN USING IN VITRO MICRONEEDLE STRETCH

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Epithelial tissues serve as an important protective structure and account for greater than 80 percent of all human cancer cases. Our current project focuses on keratin 8 (k8) and keratin 18 (k18) since these proteins are the primary intermediate filament proteins in simple epithelia. The keratin network provides mechanical integrity of epithelial tissues and may also serve as the force-sensing element in normal and cancer cells. Our lab has shown that cten, a protein known to act as both tumor suppressor and promoter, accumulates around force-bearing keratin fibers in vivo. Yet, the precise molecular interaction between keratin and cten remains unclear. To understand the mechano-biology of keratin and its implication in cancer, our goal is to define the force-induced protein-protein interactions surrounding the keratin network in vitro. The recombinant keratin 8 and keratin 18 were assembled into filaments, and the efficiency of keratin assembly was verified using ultra centrifugation. These filaments were deposited onto a glass coverslip and visualized using fluorescently labeled antibodies. To visualize stretch-dependent interactions, a microneedle was gently placed onto the keratin filaments and moved across the coverslip, thus pulling surface bound keratin fibers and straining them. Finally, purified cten proteins were added to the stretched keratin filaments. Interestingly, cten accumulated along both unstretched and stretched keratin filaments but more intensely along stretched keratin filaments, suggesting that cten binds keratin filaments directly in a force-dependent manner. Currently, we are improving cten purification and fluorescent labeling of cten proteins to minimize fluorrescent background in the experiments. In addition, we are studying the function of cten force-induced interaction by making stable cten knockout cells using CRISPR Cas9. Using the knockout and rescue approach, my goal is to test cancer cell lines using the cell-stretch device and cell adhesion assays to understand the force-sensitive function of cten in the context of cancer.

22. MODELING OF CATHETER MICROSHERE INJECTION FOR PATIENT SPECIFIC Y-90 RADIOEMBOLIZATION

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Treating cancer patients diagnosed with hepatocellular carcinoma with transarterial radioembolization is increasingly used due to its minimally invasive procedure and sparing of adjacent healthy tissues from radiation exposure. The use of complex physics-based modeling techniques with patient-specific clinical data shows much promise to support pre- and post-treatment strategies for improved tumor targeting through high-precision dosimetry. However, radioembolization requires quick clinical decision-making at the time of the Y-90 microsphere injection, leading to challenges in implementing accurate but computationally expensive pre-treatment models. Unfortunately these models suffer from uncertainty from multiple sources if assumptions are made to speed up the computation. We have developed a
modeling framework, CFDose, that incorporates clinical patient cone-beam Computed Tomography (CBCT) images and then applies physics-based techniques to predict microsphere transport in the patient liver vasculature using computational fluid dynamics (CFD). Radiation dosimetry is then performed from the predicted microsphere transport. We have demonstrated a proof-of-concept and using post-treatment Positron Emission Tomography (PET) imaging of the yttrium-90 microspheres to compare the assess the accuracy of the predicted radiation dose distribution.

The core concept of CFDose is to use patient-specific modeling based on parameters that can be obtained from clinical data (e.g. images, blood flow, and pressure). Challenges resulting from this approach and more broadly physics-based modeling approaches stem from adequate calibration, measurement of the required parameters, and validation. In the proof-of-concept CFDose used some population-based parameters; making it more patient-specific through patient-specific input data and evaluating the key parameters is the next step.

In this work, we focus on improving the accuracy of the CFD modeling by parsing out the various sources of uncertainty in intra-patient geometry and microsphere transport model fidelity. Specifically, the microsphere transport currently implemented through locally evolving massless particles that instantaneously travel with the blood flow in the absence of a catheter model could be improved by i) modeling finite-sized microspheres injected at the catheter site and ii) modeling the injection parameters (e.g. speed, direction). These improvements to the model accuracy could help reduce the uncertainty in patient-specific predictions of the microsphere distribution between liver segments.

23. DEFINING THE MINIMUM FORCE SENSITIVE SEQUENCE OF TENSIN 3

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Mechanical force plays an important role in cell proliferation and differentiation. Tensins are a family of proteins that have significant roles in cell focal adhesion. Other research has also suggested that tensins have a role in cancer cell migration, and possibly metastasis. Previously, our lab has shown that cten (tensin 4) accumulates along tensed keratin fibers. Recently, we found that tensin 3 is also force sensitive and accumulates along keratin fibers. However, the physiological role of this force sensitivity remains ambiguous. The first step to understanding the physiological role is to define the minimum force sensitive sequence of tensin 3, then identifying its molecular relationship with keratin fibers. To achieve this goal, I created truncated tensin 3 with a GFP tag. The areas of truncation were decided through sequence alignment with the corresponding cten minimal force sensitive sequence. MDCK epithelial cells were transfected with the truncated tensin 3 and full length tensin 3 as a positive control. These cells were visualized using live-cell confocal microscopy, and stretched via microneedle to see the effects of mechanical forces on protein localization. After experimenting on multiple truncated sequences of tensin 3, images collected from live microscopy were analyzed to quantify the fiber accumulation in each cell. From this, it was concluded that the force sensitive sequence of tensin 3 resides within amino acid 595-1171. Currently, we are testing whether protein interactions between tensin 3 and keratin fibers are direct using in vitro reconstitution. This molecular understanding will help in resolving the physiological role of force sensitive interactions between tensin 3 and keratin fibers in cells and further explain tensin roles in cancer cells.

24. CRITICAL EVALUATION OF MASS SPECTROMETRY AND RAMAN SPECTROSCOPY FOR LIQUID BIOPSY CANCER DIAGNOSTICS

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To improve patient outcomes, there remains a critical need to develop faster, less invasive platforms capable of identifying biomarkers. Multi-omics approaches, while promising, are high in cost and complexity, low throughput, slow, and require large sample volumes, making them impractical for many stages of clinical care. These limitations are especially prohibitive for large scale routine cancer
screening, thus there are huge advantages to moving towards diagnostic platforms that do not rely on mass spectrometry. Raman spectroscopy (RS) addresses many of these needs: it requires little to no sample preparation, is non-destructive, does not need exogenous dyes or labelling agents, and can be performed directly in aqueous solutions. In this study we carried out comprehensive RS measurements on a 58-person cohort of blood and saliva from head and neck cancer (HNC) patients and benign controls. Using chemical standards of metabolites, we validated that the biomarkers driving discrimination of cancer vs control in RS are the same for MS and we rigorously determined optimal pre-analytical variables (e.g., wet vs dry biofluid) leading to model performance. The results of this study indicate an exciting step in validating Raman as a robust diagnostic tool as well as proof that a more holistic view of an individual’s sample (in this case, a combination of their plasma and saliva) can provide a greater level of information indicative of specific disease states. We achieved diagnostic results in line with the metabolomic gold standards while reducing sample destruction, prep time, and uncertainty.