

nocturnal limb movement cluster. As we incorporate more EMR variables, we will select a final set of OSA sub-types. We anticipate patients in different clusters to have different risks of various adverse OSA-associated outcomes that are tracked in our EMR data. Notable outcomes with sufficient incidence rates (>3%) after OSA diagnosis include essential hypertension (43.4%), hyperlipidemia (28.8%), type 2 diabetes (21.9%), anxiety disorder (19.2%), coronary atherosclerosis (14.9%), cerebrovascular disease (7.7%), and pulmonary heart disease (5.9%). **DISCUSSION/SIGNIFICANCE:** If our results match anticipations, we will show how EMR data can be used to define OSA sub-phenotypes and predict patient risks of various OSA-associated outcomes. This analysis enables work in personalized risk and treatment predictions for OSA patients. By better understanding these risks, providers can better tailor treatments to patients.

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Decoding the role of polyamine metabolism on anti-tumor immunity in head and neck cancer

Richard Alexander Harbison¹, William Andrews², Michael Mikula¹, Aleksandra Ogurtsova¹, Liz Engle¹, Ogechi Nwankwoala¹, Reagan Willis¹, Pritam Sadhukhan¹, Mark Burns³, Drew Pardoll¹, Tanguy Seiwert¹, Carole Fakhry¹, Robert A. Casero¹, Elana Fertig¹, Erika Pearce¹

¹Johns Hopkins University ²University of Maryland ³Aminex Therapeutics

OBJECTIVES/GOALS: The effect of immunosuppressive metabolites on anti-tumor immunity in human papillomavirus (HPV)-associated vs carcinogen-driven head and neck cancer is unknown. The objective of this study is to define the extent to which metabolites impair this response and identify novel metabolic targets for enhancing anti-tumor immunity. **METHODS/STUDY POPULATION:** HPV-associated and carcinogen-driven head and neck squamous cell carcinoma specimens were frozen following surgical excision, and tumor sections were cut onto glass slides. Slides were coated in alpha-cyano-4-hydroxy-cinnamic acid (CHCA) matrix and subjected to mass spectrometry imaging using matrix-assisted laser desorption ionization (MALDI) on a Bruker Solarix XR 12T Hybrid QqFT-ICR mass spectrometer run in positive mode. Slides were then stained for immunohistochemistry (IHC) using markers of CD8 T cells, macrophages (CD163), B cells (CD20), and tumor cells (panCK). Mass spectrometry imaging and IHC spatially resolved data will be co-registered and metabolite intensity in regions of interest (cell types) quantified. **RESULTS/ANTICIPATED RESULTS:** A total of seven HPV-associated (three metastatic lymph nodes and four primary tumors) and six carcinogen-driven (primary tumors) HNSC specimens were subjected to MALDI and IHC. Metabolites significantly enriched in HPV-associated HNSC relative to carcinogen-driven HNSC include 2,3-diphosphoglyceric acid, xanthine, 2,3,5-Trichloromaleylacetate, and indole-3-carboxyaldehyde. Metabolites significantly enriched in carcinogen-driven HNSC relative to HPV-associated HNSC include hesperetin 3'-O-sulfate, hypoxanthine, phosphorylcholine, and L-homocysteine sulfonic acid. In ongoing analyses, we anticipate identifying a relationship between CD8+ T cell enriched vs depleted regions and immunosuppressive metabolites (e.g., kynurenine, adenosine monophosphate). **DISCUSSION/SIGNIFICANCE:** Defining the extent to which CD8+ T cells interact with the metabolic milieu of the microenvironment will provide a foundation for metabolic Precision Medicine. Strategically targeting metabolic pathways to enhance

the anti-tumor immune response will be leveraged for the design and implementation of immune modulatory metabolic therapy.

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Defining the single-cell transcriptomes of splenic adaptive Natural Killer cells in donors with latent human cytomegalovirus infection

Mohamed Khalil, Scott Terhune, Subramaniam Malarkannan
Medical College of Wisconsin

OBJECTIVES/GOALS: The primary objective of this study was to define the transcriptomes and transcriptional regulatory network required for the development and function of adaptive Natural Killer (NK) cells in donors with latent human cytomegalovirus (HCMV) infection. **METHODS/STUDY POPULATION:** Eight healthy adult human spleens were obtained from four HCMV seropositive and four HCMV seronegative donors. Spleens were provided by the Versiti Organ Donor Center of Wisconsin and were processed to a single cell suspension. CD7+ CD3E- CD14- CD19- CD20- NK cells were isolated, using the BD FACSAria sorter. Following cell sorting, single-cell RNA sequencing (scRNA-seq) was performed, and cDNA libraries were constructed and sequenced via NextSeq 550. Cell Ranger was then used to align the cDNA reads and the Seurat R package was used to analyze the transcriptional data. Cells were filtered and clustered based on the number of uniquely expressed genes. The monocle software was used for single cell trajectory analysis and the SCENIC software was used to decipher gene regulatory networks. **RESULTS/ANTICIPATED RESULTS:** Eight healthy spleens from four HCMV seropositive and four HCMV seronegative donors were obtained and their NK cells were sorted and captured for scRNA-seq. Donor median age was 59 [IQR 48.5-56.5], 50% (n=4) were female and all donors were not experiencing any acute or chronic symptoms. Using scRNA-seq, we observed elevated numbers of NKG2C+ adaptive NK cells in HCMV seropositive individuals when compared to HCMV seronegative individuals. In addition, we identify a set of transcription markers and regulators that are responsible for the development and function of adaptive NKG2C+ NK cells. Finally, our trajectory analysis of adaptive NKG2C+ NK cells revealed a unique developmental pathway. **DISCUSSION/SIGNIFICANCE:** Here, we demonstrate that HCMV infection can induce the formation of adaptive NKG2C+ NK cells that display a unique transcriptional and developmental profile. These findings have the potential to influence the future application of adaptive NK cells in cellular immunotherapies.

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DEGAS: Deep transfer learning reveals cancer-like transcriptional signatures in histologically normal prostate tissue and adjacent-normal tissues in pancreatic cancer

Justin Couetil¹, Ziyu Liu², Jie Zhang¹, Kun Huang³, Travis Johnson³
¹USM, Medical and Molecular Genetics ²Purdue University, Statistics ³USM, Biostatistics and Health Data Sciences

OBJECTIVES/GOALS: Single-cell and spatial transcriptomics have revealed high heterogeneity in the tumor and microenvironment. Identifying populations of cells that impact a patient's prognosis is an important research goal, so researchers can generate hypotheses and clinicians can provide targeted treatment. **METHODS/STUDY POPULATION:** DEGAS uses deep-transfer-learning to identify

patterns between patient tumor RNA-seq and clinical outcomes and map these associations on to higher-resolution data like spatial and single-cell transcriptomics. We apply DEGAS to prostate and pancreatic cancer spatial transcriptomics samples, as well as one normal sample of prostate tissue. We used the TCGA prostate cancer cohort to with the accompanying survival information and publicly accessible prostate cancer ST data from 10X Genomics to predict survival associations in the ST slides derived from the TCGA patients. Based on these survival associations, we identify higher risk subsections of ST slides which can be further studied. RESULTS/ANTICIPATED RESULTS: We were able to validate our method by comparing it to Scissor and were able to show that the number of high-risk regions in prostate cancer slides increased with the stage of disease. Furthermore, we identify transcriptomic signatures enriched for ontology terms associated with growth regulation and apoptosis, inflammation, immune signaling, and autophagy in histologically normal prostate tissues and adjacent normal pancreatic cancer tissues that were identified as high-risk by DEGAS. The regions highlighted by DEGAS could reflect transcriptional precursors to intraepithelial neoplasia—a well-recognized premalignant morphological change in glandular epithelium. DISCUSSION/SIGNIFICANCE: Identifying biomarkers of tissue stress that precede morphologic diagnosis of high-grade pre-malignant lesions by a pathologist may help triage patients at high risk for future development of cancer, or aid in better understanding whether histologically normal pre-malignant tissues at tumor margins contribute to recurrence.

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Development of a Predictive Nomogram for Circumferential Resection Margin in Rectal Cancer Surgery

Megan Schroder, Molly M Ford, Fei Ye, Zhiguo Zhao, Aimal Khan, Shannon McChesney, M. Benjamin Hopkins, Alexander T. Hawkins
Vanderbilt University Medical Center

OBJECTIVES/GOALS: A negative circumferential resection margin (CRM) after surgical resection of rectal cancer decreases local recurrence and increases overall survival. While MRI is used to predict this risk, there is no predictive model that incorporates clinical factors to predict the risk of CRM positivity. METHODS/STUDY POPULATION: Utilizing the National Cancer Database from 2010-2014, we performed a retrospective study evaluating factors predictive for positive CRM after surgical resection of rectal cancer. The primary outcome was positive CRM (tumor \leq 1 mm from the surgical margin). Our population included patients with clinical stage I-III rectal cancer who underwent total mesorectal excision. For the primary outcome, multivariable logistic models were used to estimate the probability of a positive CRM. Model performance was evaluated by using the area under the receiver operating characteristic curve (AUC). Model calibration was assessed by examining the calibration plot. Bootstrapping method (300-iteration) was used to internally validate and estimate optimism-adjusted measures of discrimination and overall model fit. RESULTS/ANTICIPATED RESULTS: There were 28,790 patients included. 2,245 (7.8%) had positive CRM. Older age, race, larger tumor size, higher tumor grade, mucinous and signet tumor histology, APR, open operative approach, facility location, higher T stage, lymphovascular invasion, lack of neoadjuvant chemotherapy/radiation, and perineural invasion were all significantly associated with positive CRM (p < 0.05). DISCUSSION/SIGNIFICANCE: An objective model that predicts

positive CRM and associated poor clinical outcomes is possible to be used in conjunction with MRI. Positive CRM is associated with specific patient demographics, tumor characteristics, and operative approach. These factors can be used to predict CRM positivity in the preoperative period and plan accordingly.

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Diagnosis and Detection of Thrombosis in PCOS

Mariel Miller, Caitlin Raymond, Bharathi Kavuri, Christopher Zahner
University of Texas Medical Branch

OBJECTIVES/GOALS: Identify and stratify clinical presentations of thrombotic pathology in PCOS patients. This will be accomplished by 1) evaluating clinical assays for the detection of hypofibrinolysis, and 2) analyzing clinical symptomology of thrombosis in PCOS via Symptom-Disease Pair Analysis of Diagnostic Error (SPADE). METHODS/STUDY POPULATION: Preliminary study populations include n=3 for each of the following groups PCOS with thrombotic complications, PCOS without thrombotic complications, healthy controls, and healthy control samples treated ex vivo with PAI-1. Coagulation assays include ACL coagulation panel, antiphospholipid antibody assays, viscoelastic testing with TEG and Quantra devices, and global fibrinolytic capacity. Coagulation assays will be performed on three samples taken at 4-week intervals. The SPADE techniques will be used to evaluate symptomology of thrombosis in the study period and patient electronic medical history. Molecular testing will be performed for pro-thrombotic polymorphisms (PAI-1 and Apo E) and mutations (Factor V Leiden and Prothrombin). RESULTS/ANTICIPATED RESULTS: We anticipate PCOS and ex vivo PAI-1 samples to show signs of hypofibrinolysis on TEG and Quantra devices outside of reference ranges and with statistical significance. We also anticipate seeing a statistical significance with ACL coagulation panels, however, these results are expected to still be within the reference ranges, as seen in previous studies. We believe the SPADE method will identify clinical presentations of hypofibrinolysis in PCOS patients coinciding with patterns in laboratory tests that were misdiagnosed due to being within reference ranges. We hope to stratify clinical presentations predictive of thrombosis in PCOS patients with standard clinical assays and with increased precision using viscoelastic assays. DISCUSSION/SIGNIFICANCE: The incidence rate of thrombosis is 40x higher in PCOS compared to healthy populations. However, the mechanisms of thrombosis in PCOS are unknown and are undetectable with current clinical assays. We hypothesize that chronic cellular stress in PCOS disrupts the regulation of interconnected immune pathways, causing hypofibrinolysis.

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Differential expression of two Plasmodium falciparum variant surface antigen families in Malian children with cerebral malaria compared to mild malaria*

Jonathan G. Lawton¹, Albert E. Zhou¹, Drissa Coulibaly², Emily M. Stucke¹, Antoine Dara^{1,2}, Matthew B. Laurens¹, Joana C. Silva¹, Mahamadou A. Thera², Mark A. Travassos¹

¹University of Maryland School of Medicine, Baltimore, MD, USA

²University of Sciences, Techniques and Technologies, Bamako, Mali

OBJECTIVES/GOALS: Recent in vitro evidence suggests that diverse parasite protein families called RIFINs and STEVORs are displayed