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

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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

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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 algin 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

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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

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