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Delineating the immune landscape of Kaposi Sarcoma using multiplexed immunofluorescence at single-cell resolution

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OBJECTIVES/GOALS: KSHV is well documented to infect and establish latency in endothelial cells; however, infected cell types in the Kaposi Sarcoma (KS) tumor microenvironment are not well defined. Here, we present the first application of the Lunaphore COMETTM multiplexed immunofluorescence platform for spatial proteomic analyses of KS lesions. METHODS/STUDY POPULATION: Custom antibody panels were validated and optimized for characterization of the TME components, as compared to marginal structures. The Ab panels supported the detection, quantification, and comparison of >15 cell types in regions of interest in the KS tissues in a single automated run. For data analysis, we developed a rule-based decision tree for accurate cell classification and used the resulting cell populations to perform a nearest-neighbor analysis. We assessed spatial proximity among cell types located within, adjacent to, or distal from the areas with a large number of cells expressing KSHV LANA. RESULTS/ANTICIPATED RESULTS: The highest KSHV load was detected in CD34+ cells with or without CD31, consistent with an endothelial progenitor phenotype (EPCs). We also defined 12 cell types with KSHV-LANA detected in <1% of each cell type. Among them, tumor-infiltrating CTLs were proximal to or overlapping with infected EPCs, suggesting they have cytolytic activities. In addition, we observed areas with T-cells detected at high frequency near normal vascular endothelial cells (VECs), indicating immune cell trafficking toward the lesion in these marginal areas. While supporting previous findings that T-cells are not infected by KSHV, our data also revealed areas having high viral Ag load and abnormal vasculature, versus surrounding areas with much lower Ag load, normal vasculature, and robust immune infiltration. DISCUSSION/SIGNIFICANCE OF IMPACT: Our approach not only demonstrated its ability to characterize the KS TME at a previously unapproachable level of complexity but has also revealed gaps in lineage assignments in substantial proportions of the tumor, which will be subjected to further characterization.

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Social participation and cognitive functioning after traumatic brain injury

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OBJECTIVES/GOALS: The goal of this project is to integrate human and animal data to explore the relationship between social participation and cognitive functioning after traumatic brain injury (TBI). Further, we aim to determine whether reduced social participation is not only a consequence of TBI but a potential driver of post-injury cognitive deficits. METHODS/STUDY POPULATION: This study uses a translational framework to examine how social participation influences cognitive outcomes after traumatic brain injury (TBI) using complementary human and animal models. Longitudinal TBIMS data will be analyzed to assess associations between social participation (PART-O: productivity, out and about, social relations) and cognition (BTACT: memory, fluency, reasoning, processing speed) using linear regression (potentially a Random Intercept Cross-Lagged Panel Model to capture bidirectional effects over time). Further, mice with mild, moderate, or severe TBI will be housed in isolated, standard, or enriched social environments. Post-injury cognitive performance (NOR, Y-maze) and neuronal proliferation and survival in the prefrontal cortex and hippocampus will be evaluated. RESULTS/ANTICIPATED RESULTS: We anticipate that both humans and animals with greater social participation will demonstrate superior cognitive performance over time. In the animal model, we further expect that mice housed in social or enriched environments will exhibit reduced cell death and enhanced neuronal survival in the prefrontal cortex and hippocampus compared to socially isolated counterparts. DISCUSSION/SIGNIFICANCE OF IMPACT: Together, these findings may inform future rehabilitation strategies that integrate social participation as a core component of cognitive recovery supporting more personalized and multidisciplinary approaches to chronic TBI care.

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Enteric colonization with fluoroquinolone-resistant Enterobacterales is associated with increased Enterobacterales and Bacteroidales in hematopoietic cell transplant recipients prior to transplantation

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OBJECTIVES/GOALS: Our objectives were to determine enteric microbiome characteristics in fluoroquinolone-resistant Enterobacterales (FQRE)-colonized and non-FQRE-colonized hematopoietic cell transplant (HCT recipients, and to understand the enteric microbiome-specific factors associated with the development of FQRE BSI in FQRE-colonized HCT recipients. METHODS/STUDY POPULATION: This was a prospective cohort study of patients undergoing HCT at Weill Cornell Medicine from November 2016 to August 2019. All participants received levofloxacin prophylaxis during post-HCT neutropenia. Stool samples were collected prior to levofloxacin prophylaxis initiation and during the week after HCT. Our overall study population consisted of 26 FQRE-colonized HCT recipients and 69 non-FQRE-colonized HCT

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