This suggests that uninfected bystander cells sense CT-infected cells and secrete soluble factors that may act to limit CT proliferation in infected cells and to inform remaining uninfected cells that a potential pathogen is present. We anticipate that our scRNA-seq and cytokine analyses will identify both specific effector pathways that protect against CT and intracellular signals that modulate them. We speculate that these pathways and signals may differ during infection with CT and other STIs. Importantly, we anticipate that our in vitro model of CT infection will be highly representative of in situ immune responses observed in urethras of infected men. DISCUSSION/SIGNIFICANCE OF FINDINGS: In men, common STIs including CT are usually managed syndromically due to a lack of POC diagnostics. By determining how STIs elicit urethral inflammation and identifying countermeasures that STIs use to evade urethral immunity, we can identify host responses that serve as biomarkers for urethritis, generally, and for specific urethral pathogens.

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A TL1 Approach to Assessing Peripheral Immune Changes in PTSD

P. M. Mackie, C. Wilkinson, A. Gopinath, L. Knackstedt and H. Khoshbouei
*indicates equal contribution

ABSTRACT IMPACT: We present preliminary data and an outlined approach to assess peripheral immune changes associated with PTSD in a clinical setting and in a pre-clinical rat model of PTSD. OBJECTIVES/GOALS: We report our methodology and findings indicating a relationship between CNS dopamine signaling and peripheral immune cell populations and propose to extend this methodology to a PTSD patient population to elucidate immune-brain connections in this disorder. METHODS/STUDY POPULATION: Using an IRB-approved protocol in collaboration with a board-certified psychiatrist, we will recruit PTSD patients undergoing treatment, newly diagnosed drug-naiive PTSD patients, and age-matched healthy controls. Flow cytometry will be used for immunophenotyping on blood samples from each group. To complement this data, we will also measure serum cytokine levels in each group. In order to elucidate the connection between the observed immunophenotypes in the PTSD population and CNS neurotransmitters levels, we will employ a rodent model of PTSD and high-pressure liquid chromatography to measure dopamine levels in tandem with peripheral immune changes. RESULTS/ANTICIPATED RESULTS: In both humans and rodents with low CNS dopamine, an expansion of monocyte-derived suppressor cells was observed via flow cytometry. We anticipate that human PTSD patients will exhibit a similar expansion in suppressive immune cells" in agreement with existing literature suggesting a chronic inflammatory state in PTSD. Moreover, in an animal model of PTSD we anticipate an inverse correlation between the CNS dopamine levels and the size of the immune suppressor cell population. DISCUSSION/SIGNIFICANCE OF FINDINGS: Our findings will indicate whether altered dopamine neurotransmission underlies peripheral immune system changes in the context of PTSD models and human patients. Thus, these findings will provide an alternative avenue for future investigations on the role of the immune system in PTSD.

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Preclinical modeling of BRAF(V600E)/PTEN-/- melanoma leptomeningeal disease (LMD) to assess intrathecal checkpoint blockade

Renato A. Guerrieri, Grant M. Fischer, Barbara G. Knighton, Courtney W. Hudgens, Debora A. Ledesma, Michael A. Davies and Sherise D. Ferguson
MD Anderson Cancer Center

ABSTRACT IMPACT: Melanoma leptomeningeal disease (LMD) is a devastating subtype of central nervous system (CNS) metastatic disease that is associated with limited treatment options and an extremely poor prognosis, thus requiring the development of pre-clinical models of LMD for therapeutic development. OBJECTIVES/GOALS:

1. Develop an immunocompetent murine model of melanoma LMD with tumors bearing genetic mutations commonly found in patients, specifically BRAF(V600E)/PTEN-/-
2. Assess the safety of intrathecal (IT) immunotherapy, specifically anti-PD1 antibody (aPD1)
3. Evaluate the therapeutic efficacy of IT aPD1 checkpoint blockade in murine melanoma LMD METHODS/STUDY POPULATION: To develop BRAF(V600E)/PTEN-/- LMD models, we acquired BP, D4M, and D4M-UV2 (irradiated) murine melanoma cell lines and luciferase-tagged them. 1.5x10^4 cells were suspended in 10 uL serum-free media and injected into the cisterna magna of female C57BL/6 mice. Brain and spinal cord were harvested for histologic assessment once mice were moribund. To assess safety of IT aPD1, we injected IT control IgG or IT aPD1 (13 ug, 26 ug, 39 ug) and monitored weights or harvested at days 7 or 14 for IHC staining of inflammation markers. To evaluate therapeutic efficacy of IT aPD1, BP cells were directly injected as above. After 3 days, mice underwent imaging to confirm tumor uptake and randomization to receive 13 ug IT control IgG or aPD1 once + 200 ug systemic (Sys) control IgG or aPD1 (days 0, 3, and 5), and then monitored for survival. RESULTS/ANTICIPATED RESULTS: For LMD development, all mice survived cisternal injection of BP, D4M, and D4M-UV2 cells and median survival was 17, 19, and 30 days, respectively. Presence of leptomeningeal deposits was confirmed for all tumor-bearing mice by IHC for MART1. For safety of IT aPD1, all mice survived the procedure and no mice displayed morbidity or >10% weight loss over 14 days of observation. IHC assessment of brain and spinal cord samples from mice treated with 13 ug aPD1 revealed focal ischemia related to injection site and no other signs of neurological damage or inflammation. IT aPD1 treatment of mice with BP leptomeningeal tumors demonstrated no significant survival advantage, although both IT aPD1 +/- Sys aPD1 had mice live up to days 29 and 26, respectively, compared to both IT control IgG +/- Sys aPD1, for which all mice died by day 22. DISCUSSION/SIGNIFICANCE OF FINDINGS: We demonstrate that cisternal injection of murine BRAF(V600E)/PTEN-/- melanoma cell lines yield LMD with reproducible survival and that treatment with IT aPD1 in this model is feasible and safe. Together these findings establish a new model to facilitate the development of more effective immunotherapy strategies for melanoma patients with LMD.

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