

endometrial cancer (EC) are well documented, but differences in distress have not been previously explored. Here we characterize the association between race/ethnicity, distress scores, and stressors reported by patients with EC. **METHODS/STUDY POPULATION:** Patients presenting to a single academic outpatient gynecologic oncology practice for initial evaluation of known EC from January 2013-May 2020 were included. The electronic health record was used to abstract demographics, National Comprehensive Cancer Network Distress Thermometer and Problem List (NCCN DT) scores and stressor categories (physical, emotional, spiritual, practical, and family) from the initial encounter. Referral to support services occurs at NCCN DT score ≥ 4 . We excluded women who received prior cancer-directed therapy and those without an initial NCCN DT score. Summary statistics were tabulated for demographics. Mann-Whitney U tests were used for inter-group difference on continuous variables and 2-sample tests for equality of proportions were used for binary variables. **RESULTS/ANTICIPATED RESULTS:** 412 non-Hispanic White (NHW, mean age 63) and 149 non-Hispanic Black (NHB, mean age 65) women were included in our analysis. More NHB women presented with high-grade EC (53.7%) vs NHW women (21.9%) and fewer NHB women were privately insured (32% vs 52%). Median distress scores were higher in NHW women compared to their NHB counterparts (4 vs. 2, $p < 0.001$) and NHB women were more likely to report a distress score of 0 compared to their NHW counterparts (32% vs 19%, $p = 0.001$). 50.5% NHW women had a score ≥ 4 and thus qualified for referral to services compared to 20.7% of NHB women ($p = 0.02$). Of those referred, NHB and NHW women declined referral to support services at similar rates (35.1% vs 34.5%; NS). There was a significant difference in the median number of stressors reported by NHW and NHB women, (4 vs 3 stressors; $p = 0.02$). **DISCUSSION/SIGNIFICANCE OF FINDINGS:** The NCCN DT, a widely used tool in cancer clinics, may fail to adequately measure distress in NHB women presenting with a diagnosis of EC, despite $> 30\%$ more high-risk histology cancers in this cohort. This difference leads to disparities in referral to additional support services, which may affect quality of care and quality of life.

Mechanistic Basic to Clinical

Basic Science

10061

Assessing immunogenicity of an Ebola vaccine in humans using a systems biology approach*

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ABSTRACT IMPACT: Understanding gene expression changes after viral vaccination and booster may help predict vaccine efficacy. **OBJECTIVES/GOALS:** Utilize a systems biology approach to identify gene expression changes after administration of Zaire Ebola virus glycoprotein expressed in a Chimp Adeno3 vector (ChAd3-EBOZ) and either boosted ~7 weeks later with modified vaccinia Ankara MVA expressing Zaire and Marburg GPs plus Tai forest NP (MVA-BN[®]Filo) or given saline (placebo). **METHODS/STUDY POPULATION:** As part of the phase 1b, open-label vaccination trial

of ChAd3-EBO-Z in Mali, West Africa, peripheral blood mononuclear cells were isolated from eight volunteers for whole genome transcriptomics analysis. Four subjects received the MVA-BN[®]Filo booster and four received saline. Samples were taken prior to receipt of the booster or placebo, as well as 1, 7, and 14 days afterwards. Significant differentially expressed genes were identified using RNA-seq between baseline and post-MVA-BN[®]Filo. Functional enrichment analysis against the GO Ontology Database and the Immune Signatures C7 collection of MSigDB (ImmuneSigDB) was performed. These differentially expressed genes were also examined for associations with Ebola antibody titers and cell-mediated immune responses. **RESULTS/ANTICIPATED RESULTS:** The majority of gene expression changes occurred on day 1 post-MVA-BN[®]Filo administration. 870 genes had significantly different expression when day 1 samples were compared to pre-booster baseline (791 upregulated/79 downregulated). Those upregulated genes are mainly involved type I interferon and regulation of viral life cycle pathways. The downregulated genes are involved in regulation of cellular defense response, lymphocyte mediated immunity. Comparing to the C7 Immune Signatures collection datasets, we identified more than 100 upregulated genes from 6 studies of yellow fever vaccination that were also significantly upregulated in our study. The top enriched ontological pathway of those genes is cellular response to type I Interferon. **DISCUSSION/SIGNIFICANCE OF FINDINGS:** The use of a systems biology approach to compare gene expression changes among vaccine studies utilizing whole genome transcriptomics data allows the identification of genes involved in the immune response to vaccination and might aid in predicting vaccine efficacy.

24435

Pathogen-specific metabolic pathways and innate immune responses associated with Chlamydia trachomatis infection and other STIs

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ABSTRACT IMPACT: This project seeks to identify unique host responses that are biomarkers for specific urethral pathogens, and which can be used in the development of point-of-care (POC) STI diagnostics. **OBJECTIVES/GOALS:** How Chlamydia trachomatis (CT) and other common STIs, e.g. Neisseria gonorrhoeae, evade immunity and elicit pathology in the male urethra is poorly understood. Our objective is to determine how STI-infected urethral epithelial cells, as well as the uninfected 'bystander' cells with which infected cells communicate, respond to CT and other STIs. **METHODS/STUDY POPULATION:** We evaluated how immortalized urethral cell lines - including transduced human urethral epithelial cells (THUECs) - respond to increasing doses of CT infectious particles using in vitro one-step progeny assays performed in the presence or absence of cycloheximide, a drug that inhibits eukaryotic protein synthesis. We will perform concurrent single-cell RNA sequencing (scRNA-seq) and multiplex cytokine analyses to determine how different CT doses impact the transcriptomes of infected and bystander urethral epithelial cells and modulate cytokine production of the overall monolayer. Results of these experiments will inform the feasibility of performing similar analyses in situ using urethral swabs from men with clinically diagnosed urethritis. **RESULTS/ANTICIPATED RESULTS:** Our results demonstrate that immune-competent urethral cell monolayers strongly resist CT infection, unless most of the cells are simultaneously infected.

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.

30580

A TL1 Approach to Assessing Peripheral Immune Changes in PTSD

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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-naïve 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.

48218

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

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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. 1.5×10^4 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.