

dry eye disease and decreased vision. Quantifying specific cytokine changes in tears may reveal biomarkers and future treatment targets for patients with oGVHD. **METHODS/STUDY POPULATION:** The goal of this study is to determine if cytokines can be measured in tears with the Isoplexis platform. This pilot validation study evaluates the tears of a patient with oGVHD utilizing the Isoplexis platform. The Isoplexis has specific advantages for tear samples including high-throughput analysis, and small sample requirements, but has yet to be validated in tears. A sample from normal and oGVHD patient tears were collected for comparison. Samples were analyzed on two separate backgrounds—standard Bovine Serum Albumin (BSA) background and artificial tears (ATs). The negative control was ATs and positive control was a concentrated cytokine solution. Analysis of 22 cytokines was performed. **RESULTS/ANTICIPATED RESULTS:** Analysis of 22 cytokines was performed. As expected, the cytokine levels of the ATs alone were below the limit of detection (LOD). The oGVHD patient tears showed elevated TNF-alpha, TNF-beta, perforin, MIP-1a, MIP-1b, MCP-1, IL2, IL4, IL5, IL-7A, IL9, IL-13, IL-15, IFN-gamma, granzyme B, and GM-CSF with ATS background, but no cytokines above the LOD in the BSA background plate. The control tears had elevated IP-10. The elevated cytokines for the oGVHD patient corresponded to symptom severity and clinical findings. **DISCUSSION/SIGNIFICANCE:** These results suggest that using ATs as the background with the Isoplexis platform improves the sensitivity to detect tear cytokines. Findings of elevated IL-7A and GM-CSF in tears parallels literature findings for oGVHD. Further evaluation of samples will continue to validate the Isoplexis multiplex assay for tear cytokine analyses.

384

Alterations in the fungal microbiome in ulcerative colitis

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OBJECTIVES/GOALS: Although gut fungi have been implicated in the immunopathogenesis of inflammatory bowel disease, the fungal microbiome has not been deeply explored across endo-histologic activity and treatment-exposure in ulcerative colitis. **METHODS/STUDY POPULATION:** Our retrospective cohort was derived from the Study of a Prospective Adult Research Cohort with Inflammatory Bowel Disease. We evaluated the fungal composition of fecal samples from 98 ulcerative colitis patients across endoscopic activity (n=43), endo-histologic activity (n=41), and biologic-exposure (n=98). Across all subgroups, we assessed fungal diversity and differential abundance of specific taxonomic groups. **RESULTS/ANTICIPATED RESULTS:** We identified 504 unique fungal amplicon sequence variants across the cohort of 98 patients, dominated by phylum Ascomycota. Compared to endoscopic remission, patients with endoscopic activity had an increased global fungus load (p < 0.001). **DISCUSSION/SIGNIFICANCE:** Endoscopic inflammation in ulcerative colitis is associated with altered fungal diversity driven by expansion of *Saccharomyces* and *Candida* compared to remission. The role of these fungal taxa as potential biomarkers and targets for personalized approaches to therapeutics in ulcerative colitis should be evaluated.

385

Combining Cannabidiol with Prolonged Exposure Therapy for PTSD: Design and Methodology of a Pilot Randomized Clinical Trial

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OBJECTIVES/GOALS: There is increasing evidence that cannabidiol (CBD) has promising potential to treat PTSD. However, more research is warranted to fully understand the benefits of CBD for PTSD. This poster will describe the design and methodology of one of the first ever pilot RCTs examining CBD (vs. placebo) combined with prolonged exposure therapy for PTSD. **METHODS/STUDY POPULATION:** This study is an early Phase II double-blind, pilot RCT. Participants are 24 individuals 18-65 years old who meet DSM-5 criteria for PTSD on the CAPS-5 and were recruited from local hospitals and the community. Individuals complete a standardized baseline assessment with an independent evaluator to assess study eligibility. Participants who meet study inclusion are randomized to 18 days of CBD 250mg (BID) or placebo delivered in combination with 10-sessions Prolonged Exposure (PE) psychotherapy over 2 weeks. Individuals begin medication 3 days prior to beginning PE to ensure steady state. Participants complete self-report and biomarker outcomes at select timepoints during study participation, and are also asked to complete a 1-month follow-up assessment following treatment. **RESULTS/ANTICIPATED RESULTS:** This aims of this study are to: 1) examine the safety, feasibility, and PTSD symptom reductions associated with the combined intervention; 2) evaluate biomarkers associated with the endocannabinoid system and stress response; 3) determine the association between changes in biomarkers and PTSD symptoms following treatment. It is expected that CBD+PE will be safe and feasible, and that there will be a detectable signal of CBD vs. placebo in the reduction of PTSD symptoms. It is also anticipated that CBD will have higher levels of endocannabinoids and lower stress response levels compared to placebo. Lastly, we expect that greater changes in biomarkers will be associated with lower levels of PTSD severity following treatment. **DISCUSSION/SIGNIFICANCE:** Although there is growing interest in cannabinoids for psychiatric conditions, such as PTSD, controlled trials are limited and have yet to examine the proposed intervention for PTSD. If successful, this study will enhance the feasibility of a larger, adequately powered RCT to address immediate and long-term improvements for PTSD treatments.

386

A novel truncating variant of EBF2 disrupts human adipocyte differentiation in lipodystrophy syndromes: an example of a discovery from a clinical translational pipeline

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OBJECTIVES/GOALS: Aiming to better understand the molecular pathogenesis of familial partial lipodystrophy (PL), we initiated whole-exome sequencing for our patients with PL syndromes. A novel variant of early B cell factor 2 (EBF2) was identified. Here

we report the biological impact of a novel truncating EBF2 variant. **METHODS/STUDY POPULATION:** Using 3T3-L1 and human primary subcutaneous preadipocytes, we performed loss-of-function and gene rescue experiments. All cells were cultured in DMEM with 10% bovine calf serum (Invitrogen) at 5% CO₂. After lentivirus transfection, cells were grown to confluence and then exposed to adipogenesis induction media containing dexamethasone (0.25 ÅµM), insulin (1 Åµg/ml) and isobutyl methylxanthine (0.5 mM). Total RNA was extracted using RNeasy Mini Kit (Qiagen) and cDNA was synthesized using IScript (Bio-Rad). Real-time qPCR was performed using TaqMan probes for Pparg and Fabp4, two key adipogenesis markers. **RESULTS/ANTICIPATED RESULTS:** Patient was found to carry a heterozygous nonsense mutation in exon 6 of EBF2, causing the premature termination of the protein at amino acid position 165. Adipogenesis was significantly suppressed in 3T3L1 cells when endogenous Ebf2 was suppressed with siRNA and lentiviral shRNA. Adipocytes with suppressed Ebf2 expression showed marked reduction of intracellular lipid content and Pparg and Fabp4 expression (>80% reduction). With lentiviral gene transfer, EBF2 fully rescued adipogenic potential, whereas the truncated variant EBF2 did not. Of note, 3T3-L1 cells transfected with the EBF2 variant displayed impaired adipogenesis, suggesting a dominant-negative effect of the EBF2 variant on adipogenesis. We confirmed the dominant effect of the EBF2 variant in human adipocyte differentiation. **DISCUSSION/SIGNIFICANCE:** Our data suggest that EBF2 is indispensable for adipogenesis. The loss of function and dominant-negative effect of the truncating variant of EBF2 likely plays a pathogenic role in PL. Whole exome sequencing of PL patients and ex-vivo functional analysis help identify novel gene variants and better understand the molecular pathogenesis of PL.

388

Early Neurorehabilitation of Disorders of Consciousness after Acute Hemorrhagic Stroke

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OBJECTIVES/GOALS: Accurate classification of disorders of consciousness (DoC) is key in developing rehabilitation plans following brain injury. The Coma Recovery Scale-Revised (CRS-R) is a sensitive measure of consciousness. We explore feasibility, safety and impact of CRS-R guided rehab in hemorrhagic stroke patients with DoC and evaluate predictors of recovery. **METHODS/STUDY POPULATION:** Consecutive patients with non-traumatic hemorrhagic stroke, defined as subarachnoid hemorrhage (SAH) or intracerebral hemorrhage (ICH), receiving serial CRS-R assessments during their ICU stay at University of Maryland Medical Center from 2017-2021 were retrospectively identified. Outcomes of interest included the association with CRS-R and discharge disposition, therapy-based function and mobility and occurrence of safety events during CRS assessment. We also examined the association between CRS-R and physiological and anatomical injury pattern on electroencephalography (EEG) and magnetic resonance imaging (MRI), respectively. **RESULTS/ANTICIPATED RESULTS:** 76

patients with ≥2 CRS-R assessments were identified (22 SAH, 54 ICH, median age = 59, 50% female). Median CRS-R completed was 3 with no SAEs identified during sessions. We identified 4 patterns: persistent VS/UWS (49%), persistent MCS or better (13%), emergence from VS/UWS to MCS or better (27%) and regression from MCS or better to VS/UWS (11%). Persistent low CRS-R correlated with older age in SAH (p=0.01), female gender in ICH (p=0.04), and history of diabetes (p=0.01). 2% of patients with final CRS-R **DISCUSSION/SIGNIFICANCE:** Early neurorehabilitation guided by CRS-R appears to be feasible and safe acutely following hemorrhagic stroke complicated by prolonged DoC and may enhance access to inpatient rehabilitation with a lasting benefit on recovery. Further characterization of DoC patterns and their correlation to clinical markers, including EEG and MRI is needed.

389

Insights into the complex immune environment during pregnancy and association with the developing human connectome

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OBJECTIVES/GOALS: Maternal health and exposures during pregnancy play a major role in shaping the neurodevelopment of our offspring—one influence is maternal immune activation (MIA). Here we explore the association of MIA during pregnancy and the developing human connectome through analysis of 46 markers of activation. **METHODS/STUDY POPULATION:** 74 healthy women with singleton pregnancies underwent blood draws between 34-37 weeks gestation. 46 markers of maternal immune activation, both adaptive (e.g., IgG) and innate (e.g., cytokines and acute phase reactants), were collected. In addition, for preliminary analyses of MIA in relation to the newborn brain, we utilized 30 participants with MRIs between the ages of 0-6 months. **RESULTS/ANTICIPATED RESULTS:** Principal component analysis (PCA) identified the first 5 PCs explains ~68% of the variance and the first 10 explains ~83% (top PC is 42.1%). Using the top PC each edge in the connectome was correlated with the immune profiles. Several regions trended towards significance—one survived correction and included 359 edges, showing. The highest number of edges was observed in the inferior parietal lobe of the left hemisphere—a region associated with functions from basic attention to social cognition, suggesting that deviations in fetal exposure to MIA can longitudinally impact offspring behavior in areas essential for human interaction. **DISCUSSION/SIGNIFICANCE:** This is the first study in understanding how interruptions (i.e., MIA) influence later development. Identification of alterations, and long-term outcomes could lead to the development of mechanism-based health-care, facilitate timely referral for appropriate interventions and provide family support.