the survival of TP53 mutant cells that can be targeted in TNBC. We have identified Kif11 as one such target and aim to further investigate its function in TP53 mutant TNBC. METHODS/STUDY POPULATION: We conducted a dual in silico/in vivo screen that identified Kif11 inhibition as preferentially inhibiting the growth of TP53 mutant TNBC. We obtained data on TP53 mutational status, KIF11 mRNA expression levels, and clinical characteristics from TCGA, METABRIC, and CCLE datasets. We treated breast cancer cell lines with the KIF11 inhibitor SB-743921. Cell counts were obtained through staining with DAPI or Hoechst and imaging on the ImageXPress PICO. We detected cell death by DRAQ7 staining and flow cytometry analysis following Annexin V-PI staining. To investigate mitotic spindle organization, we performed immunofluorescent staining with an anti-tubulin antibody and DAPI co-staining. Cell cycle analysis was performed through flow cytometry. RESULTS/ANTICIPATED RESULTS: KIF11 is highly expressed in TP53 mutant and TNBC clinical samples. High KIF11 expression is associated with poorer clinical outcomes. Kif11 inhibition suppresses growth of both TP53 mutant and wild-type breast cancer cells, but preferentially induces the death of TP53 mutant cells as detected by DRAQ7 and Annexin V/PI staining. Kif11 inhibition induces a G2-M block and growth inhibition in TP53 wild-type cells. On the other hand, following treatment with the Kif11 inhibitor SB-743921, TP53 mutant cells undergo mitotic spindle dysfunction leading to the formation of multinucleated cells and cell death. DISCUSSION/SIGNIFICANCE: These results demonstrate that Kif11 is a promising therapeutic target in aggressive, TP53 mutant TNBCs. Kif11 inhibitors, including SB-743921, have been tested in human trials, and are well tolerated, but it is unclear which patients would most benefit. Our studies show that Kif11 inhibitors may be most useful in patients with TP53 mutant TNBCs.

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The role of leucine-rich PPR motif-containing protein (LRPPRC) in myelin lipid metabolism*

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OBJECTIVES/GOALS: Leigh Syndrome, French Canadian-Type (LSFC) is a neurometabolic disorder caused by mutation of mitochondria-related gene, LRPPRC. White matter lesions and demyelination in central nervous system are common in LSFC. LRPPRC is enriched in myelinating glial cells, yet its role is not known. Our goal is to elucidate its mechanistic role in myelination. METHODS/STUDY POPULATION: We crossed C57BL/6N mice bearing a LRPPRC-loxP allele with mice bearing a Plp-CreERT2 allele. Mice with the Plp-CreERT2 allele expresses a tamoxifen-inducible Cre under the control of the Plp promoter, which drives expression in oligodendrocytes. Using these strains, we can target the deletion of LRPPRC, via tamoxifen injection, in both newly formed myelin and mature myelin. Plp-CreERT2; LRPPRCL/L (LRPPRC-KO) or control littermate mice will be injected for LRPPRC deletion at developmental and maturation stages of myelin. Immunofluorescence and electron microscopy of isolated brain tissues will be used for myelin integrity analysis. Cognitive functions of the mice will be measured via behavioral tests. Lastly, we will submit tissues for lipidomic analyses to observe any lipid metabolite variation. RESULTS/ANTICIPATED RESULTS: Behavioral and motor defects would be expected in LRPPRC-KO mice performing in cognitive function tasks across myelin maturation stages. Electron microscopy-based structure analysis of optic nerve, corpus callosum, and spinal cord should reveal thin or loss of myelin on the axons of LRPPRC-KO compared to control. Immunofluorescence staining of major myelin structural proteins, including myelin proteolipid protein (PLP), myelin basic protein (MBP), and myelin-associated glycoprotein (MAG) would be expected have lower levels in LRPPRC deficient tissues. Since myelin is a lipid-rich species, we would also expect lipid concentrations to be affected. LRPPRC-KO lipidomic analyses of myelin-related lipids should depict lower levels in comparison to control, which would imply dysfunctional lipid metabolism. DISCUSSION/SIGNIFICANCE: There are limited studies in ameliorating neural deficits caused by LS and LSFC. Successful completion of this project would help elucidate the functions of LRPPRC in myelination and lipid metabolism and potentially provide insights for developing novel therapeutic strategies for alleviating the demyelination and neural deficits in LSFC.

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The Role of the IL-6-IGF-II Axis in Systemic Sclerosis-Associated Lung Fibrosis

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OBJECTIVES/GOALS: Interleukin (IL)-6 is produced in excess in Systemic Sclerosis (SSc). Likewise, microarray analysis of Insulin-like Growth Factor (IGF)-II-treated NL fibroblasts revealed increased expression of the basic helix-loop-helix transcription factor, BHLHB2. Our goal is to delineate the role of BHLHB2 in the fibrotic response to IGF-II and IL-6. METHODS/STUDY POPULATION: Primary lung fibroblasts were cultured from human lung tissues at 37oC and 5% CO2. Cell cultures were stimulated with IL-6. Gene expression was measured using quantitative PCR (qPCR). IGF-II mRNA expression levels after IL-6 stimulation were compared with those of the housekeeping gene PPIB (Peptidylprolyl Isomerase B). Western blot was performed on nuclear and chromatin-bound subcellular fractions from treated lung fibroblasts. BHLHB2 protein levels were assayed in response to IGF-II in comparison to PBS as vehicle control. RESULTS/ANTICIPATED RESULTS: Results: Our results show that IL-6 increases IGF-II levels in fibroblasts. In turn, IGF-II increases BHLHB2 nuclear localization. We further show that IL-6 increases BHLHB2 levels and its nuclear localization in lung fibroblasts. Our findings are novel since the role of the transcription factor BHLHB2 in the IL-6 induced/ IGF-II-mediated fibrotic response in SSc lung disease remains unexplored. DISCUSSION/SIGNIFICANCE: Our findings may provide a rationale for combination therapy to block IL-6 and IGF-II function concomitantly and thus halt the progression of SSc pulmonary fibrosis (PF). Our findings may have wide implications for lung fibrosis associated with various diseases, since SSc-PF, is characterized by the activation of common fibrotic pathways.

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A Pilot Study of Tear Cytokine Profiling in a Patient with Ocular Graft versus Host Disease

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OBJECTIVES/GOALS: Ocular graft versus host disease (oGVHD) affects ~50% of individuals after an allogeneic hematopoietic stem cell transplant for treating blood cancers. OGVHD results in severe

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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-1β, MCP-1, IL2, IL4, IL5, IL-7A, IL9, IL-13, IL-15, IFN-γ, 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.

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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 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.

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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.

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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

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