

forms of peripheral neuropathy. **DISCUSSION/SIGNIFICANCE:** The significance of this project is that characterization of the relationship of clinical symptoms, non-invasive imaging and expression of NfL will lead to better diagnostic and prognostic algorithms in the treatment of compressive and traumatic peripheral neuropathies.

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### Digital Spatial Profiling of Allograft Loss in Kidney Biopsies with Chronic Allograft Dysfunction

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**OBJECTIVES/GOALS:** Assess molecular and cellular mechanisms of allograft loss in kidney biopsies using digital spatial profiling and clinical outcomes data. **METHODS/STUDY POPULATION:** Patients with chronic allograft dysfunction (CGD), enrolled in the Deterioration of Kidney Allograft Function (DeKAF) study, with or without eventual allograft loss, were included. CGD was defined as a >25% increase in creatinine over 3 months relative to a baseline. Kidney biopsy tissue was assessed by Nanostring GeoMX digital spatial profiling (DSP) after staining with anti-pan-cytokeratin, anti-CD45, anti-CD68, Syto-13, to identify specific cell populations, and Nanostring's Whole Transcriptome Atlas (WTA), to quantify the distribution of transcripts across the biopsy. Up to 14 regions of interest (ROIs) were selected, with or without glomerulus. CIBERSORT was used to perform cell deconvolution. Clinical and outcomes data were from the DeKAF study and United States Renal Data System. **RESULTS/ANTICIPATED RESULTS:** Macrophage (M1) cell population abundance was significantly different in ROIs with glomerulus between graft loss and no graft loss. Principle component analysis of differentially expressed genes resulted in transcriptomes in ROIs that cluster together by clinical outcome of graft loss or no graft loss. There were 203 DEGs in ROIs with glomerulus that were different by graft loss or no graft loss. By pathway analysis, these 203 DEGS were enriched in the T-cell activation, integrin signaling and inflammation pathways. **DISCUSSION/SIGNIFICANCE:** DSP of kidney allograft biopsies allows for the identification and quantification of specific cell types, such as macrophages and molecular transcripts as potential drug targets. This data can be used to understand mechanisms of kidney allograft loss and may lead to improved immune suppression in kidney transplant recipients.

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### From discovery to the clinical laboratory: a methodological appraisal of untargeted metabolomics platforms to characterize inborn errors of metabolism.

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**OBJECTIVES/GOALS:** Untargeted metabolomics platforms are powerful biomarker discovery tools. However, the absence of uniform study design, data analysis, and reporting standards limits

translation of this research into the clinical lab. The goal was to critically appraise existing untargeted metabolomics platforms that analyzed inborn errors of metabolism. **METHODS/STUDY POPULATION:** A search strategy was conducted in MEDLINE via PubMed from January 16, 2013, to January 16, 2023. The search strategy was limited to primary literature articles written in English that evaluated human subjects with inborn errors of metabolism (IEMs). Articles that performed targeted metabolomic analysis or analyzed non-human samples were excluded. Information on patient cohorts analyzed, sample types, and method design were extracted using a template. Categorical data are summarized as frequencies and percentages. **RESULTS/ANTICIPATED RESULTS:** A total of 96 distinct IEMs were evaluated by the different untargeted metabolomics methods included in this review. However, most IEMs (55/96, 57%) were evaluated by a single platform, in a single study, with a limited cohort size. Only one study validated their results using a separate, validation cohort. There was considerable diversity in the separation techniques and mass spectrometry instrumentation used by the studies to create their untargeted metabolomics methods. Slightly over half (59%) of the studies identified at least some of the metabolites detected in their samples with the highest level of confidence. Importantly, most of the included studies reported adherence to quality metrics, including use of quality control material (65%) and internal standards in their analysis (61%). **DISCUSSION/SIGNIFICANCE:** Future studies analyzing IEM patient samples with untargeted metabolomics platforms should progress beyond single-subject studies and evaluate the reproducibility of the research using a prospective, or validation cohort as well as confirm metabolite annotations with reference metabolites standards to generate clinically useful data.

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### Examining the clinical utility of dance to support social connection - Explorations at the level of the brain, heart, and body

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**OBJECTIVES/GOALS:** Social isolation/loneliness is a public health crisis and one that is unlikely to be solved through pharmacology. Nonpharmacological approaches, such as dance, are needed. The objective of this study is to investigate the physiological correlates of dance-induced improvements in social connection. **METHODS/STUDY POPULATION:** Participants were randomly assigned to participate for 4 weeks (2 times per week, 90-minute sessions) in either 1) improvisational dance training (experimental group; n=7); or a 2) dance movie watching experience (control group; n=7). Before and after the intervention, using mobile brain-body imaging techniques, participants and their instructor had their brain (via electroencephalography) and body physiology (via photoplethysmography) recorded during a series of verbal and nonverbal interactive experiences. Participants were also video recorded via 4 surrounding cameras for later motion capture analysis. Neuropsychological assessments were also conducted before and after the intervention. **RESULTS/ANTICIPATED RESULTS:** We found that dance significantly increased social skills including empathy, interpersonal skills, emotional regulation, mindfulness, and attention. Additionally, we found that dance significantly increased interbrain synchrony during nonverbal experiences including theta (4-8 Hz), beta (12-35 Hz), and gamma

(35–45 Hz) frequencies in the occipital lobe. Increases in interbrain synchrony were also positively correlated with increases in empathy. Additionally, intercardiac synchrony between the participant and instructor showed a significant correlation at post-intervention only. Future investigations will focus on the relationship between interbrain, intercardiac, and movement synchrony. **DISCUSSION/SIGNIFICANCE:** Our findings support the idea that dance increases interpersonal synchrony at the level of the brain, heart, and behavior. Understanding the neural and somatic mechanisms of social behaviors will help promote understanding and development of interventions for the critical problem of social isolation and loneliness.

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### **A CTS team approach to Gold Nanorod (GNR) Theranostics in Adoptive Cell Therapy (ACT)**

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**OBJECTIVES/GOALS:** The objective of this study is to use GNR technology to track immune cells infiltrating malignant brain tumors that are delivered as part of a novel immunotherapeutic strategy. We seek to implement this new platform to elucidate the underlying mechanisms of therapeutic benefit from ACT via correlation between biodistribution and efficacy. **METHODS/STUDY POPULATION:** Utilizing the inherent two-photon luminescent signal of GNRs, we will identify uptake and phenotype of lineage negative hematopoietic stem cells (HSCs) in vitro. HSCs will be isolated from the bone marrow of 6-week-old C57bl/6 female mice. Following isolation, HSCs will be co-cultured with varying concentrations of GNRs in DMEM w/o sodium pyruvate for 24 hours, tested for viability, and images to quantify uptake and identify phenotyping. CT contrast of our novel Iodine-capped PEGylated gold nanorods will be confirmed through microCT and biodistribution of HSCs at time points after injection will be identified via CT visualization in vivo. **RESULTS/ANTICIPATED RESULTS:** We expect that increased GNR signaling 24 hours post-transplant in the tumors of glioma-bearing mice will be positively correlated with long term survival following ACT. Published data from our labs have revealed that CCR2+ lineage-negative HSCs significantly accumulate in tumor of glioma-bearing mice<sup>12</sup>. Importantly, CCR2+ lineage-negative HSCs promote differentiation to dendritic cells in the tumor, increase antitumor T cell responses mediated by cross-priming and cross-presentation, and improve efficacy of immune checkpoint inhibition<sup>12</sup>. Given that HSCs are important in mediating immunotherapy efficacy, we seek to correlate the accumulation of GNR signaling within the tumor as a marker of treatment response. **DISCUSSION/SIGNIFICANCE:** Adoptively transferred cells have been imaged using numerous published methods. While promising to the field of immunotherapy, these methods lack significant clinical validation. GNRs have not been used to study hematopoietic stem cells in the context of ACT and brain malignancies. Our research is poised to address this gap.

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### **Clinical and Radiographic Features of Mesenteric Ischemia after Intra-Aortic Balloon Pump Placement**

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**OBJECTIVES/GOALS:** Intra-aortic balloon pumps are commonly used as circulatory support in patients with critically reduced cardiac

function. The goal of this study is to estimate the incidence of mesenteric ischemia as an understudied vascular complication and to describe the clinical and radiographic characteristics of patients experiencing this complication. **METHODS/STUDY POPULATION:** We will be conducting a retrospective analysis of the electronic medical records of all patients who underwent intra-aortic balloon pump (IABP) placement between October 2020 and April 2023 at our academic medical center to identify the incidence of mesenteric ischemia. We will describe the clinical course of these patients and characterize them based on demographic features and risk factors for vascular complications including medical comorbidities. Finally, we will assess available chest x-ray and thoracoabdominal CT imaging for adequacy of balloon tip positioning, concordance between balloon size and aortic dimensions, and compromise of any visceral arteries in patients who experienced mesenteric ischemia vs. those who did not. **RESULTS/ANTICIPATED RESULTS:** We anticipate approximately 150 patients to have received IABPs over this period with at least 4 known cases of mesenteric ischemia. We will describe the clinical presentation of these cases and their often fatal outcomes. We expect several known risk factors will be present in these patients, including history of peripheral vascular disease, diabetes, or smoking history. On chest x-ray, we predict balloon tip positioning to be suboptimal (defined as >5cm below the aortic arch) in many patients, both those with mesenteric ischemia and those without, but a greater discordance on CT imaging between balloon size and aortic dimensions with greater evidence of visceral compromise in patients with mesenteric ischemia compared to those without. **DISCUSSION/SIGNIFICANCE:** Mesenteric ischemia is a serious and poorly studied complication of intra-aortic balloon pumps. Understanding the hospital course, clinical characteristics, and radiographic features present in these patients may guide clinicians in the early recognition and management of this potentially fatal complication.

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### **Pain and falls among persons with multiple sclerosis.**

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**OBJECTIVES/GOALS:** Falls are very common among persons with multiple sclerosis (PwMS) due to the disabling symptoms associated with the disease. The relationship between pain and falls is underexplored. This study investigated the relationship between the facets of pain (intensity and interference) and falls in the context of co-occurring symptoms of MS. **METHODS/STUDY POPULATION:** This is a survey-based study that included 915 adults with MS. Participants provided data on demographics, clinical data, concerns about falling, symptom severity, and occurrence of falls in the past 6 months. Participants also completed the Patient Reported Outcome Measurement Information System (PROMIS) pain interference and pain intensity short forms. Pain interference and pain intensity were separately entered into univariate and multivariable logistic regression models developed to examine the associations between falls incidence and pain. Multivariable models were adjusted for age, sex, years since diagnosis, MS type, Patient Determined Disease Steps, MS status, concerns about falling, fatigue severity, PROMIS depression short form, and PROMIS physical function short form. **RESULTS/ANTICIPATED RESULTS:** Univariate regression analyses indicated that pain interference (OR = 1.05; 95% CI 1.03 to 1.06; p < 0.01) and pain intensity (OR = 1.03; 95% CI 1.02 to 1.04; p < 0.01) were both associated with