

that subjects with nonresolving SA-PARDS, defined as intubation and mechanical ventilation, will have a monocyte/macrophage transcriptome characterized by continued hyper-inflammation (M1-like phenotype) that does not transition over time to an anti-inflammatory and pro-repair phenotype (M2-like). Additionally, we expect to see that subjects with non-resolving SA-PARDS will have evidence of continued inflammation driven by hyper-inflammatory neutrophils. Finally, we expect that subjects with non-resolving SA-PARDS will have epithelial cells characterized by continued upregulation of canonical pathways of innate immunity including interferon signaling and the damage associated molecular pattern recognition pathway. **DISCUSSION/SIGNIFICANCE OF IMPACT:** The discovery of immuno-endotypes in SA-PARDS would represent a major step toward developing precision medicine therapies for this group of patients. It would simultaneously provide a strategy to reduce biological heterogeneity and identify novel pathways and targets for therapy.

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### **Effectiveness of the addition of yoga to a behavioral weight loss intervention on measures of glycemic control for adults with overweight or obesity (MOVE for Health Study): A methods description**

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**OBJECTIVES/GOALS:** \* Examination of the acute glucose-lowering response to physical activity within a comprehensive behavioral weight loss intervention in adults without T2DM. \* Explore whether the acute response to yoga differs from the acute response to brisk walking and to examine whether these responses vary across the intervention period. **METHODS/STUDY POPULATION:** Participants in the behavioral weight loss and aerobic exercise group will start with 100 minutes per week of moderate-intensity aerobic activity, increasing every four weeks to 250–300 minutes, spread over five days. Activities will be self-selected, such as walking. Participants in the combined aerobic exercise and yoga group will do aerobic exercise three days a week and yoga two days a week, also progressing from 100 to 250–300 minutes weekly. All participants will follow an energy-restricted diet (1200–1800 kcal/day) and participate in weekly education sessions to learn lifestyle modification skills for successful weight loss. The study will explore differences in acute responses to yoga versus walking and how these vary during the intervention, controlling for initial and changing weight status. **RESULTS/ANTICIPATED RESULTS:** Primary and secondary outcomes from the parent study will include body weight, BMI, body composition (via DXA), cardiorespiratory fitness, energy intake, and physical activity. Glucose and insulin levels will be measured pre- and post-exercise, with HOMA-IR computed. Continuous glucose monitoring (CGM) will be used to track glucose responses during each session, with the area under the curve (AUC) as the primary metric. The study will also explore advanced CGM analytics in collaboration with the KUMC Diabetes Institute that will include in-depth analysis of peak and trough change velocity as well as novel correlations between glucose dynamics and physical activity patterns with an aim to uncover insights that transcend conventional CGM analyses. **DISCUSSION/SIGNIFICANCE OF IMPACT:** This study uses advanced CGM analytics to examine glucose control during physical activity, collaborating with experts to create comprehensive models for glucose fluctuations. It compares acute responses to

walking and yoga, addressing a key gap in research, with potential clinical insights for managing glucose in obesity.

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### **Unraveling the pathogenicity of a novel variant in Diamond Blackfan anemia\***

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**OBJECTIVES/GOALS:** Diamond Blackfan anemia (DBA) is caused by loss of ribosomal proteins leading to death of red blood cell progenitors. We identified a novel heterozygous variant (c.167+769C>T) in RPL30 in a patient with DBA. We hypothesized that this variant, in a gene not previously studied in DBA, would demonstrate DBA phenotype and reveal early drivers of disease. **METHODS/STUDY POPULATION:** To study the role of our novel variant, we developed an induced pluripotent stem cell (iPSC) model, including wild type (WT) and CRISPR-edited RPL30 mutant clones. We differentiated the iPSC into hematopoietic stem cells, identified cell populations with flow cytometry, and applied single-cell RNA sequencing. We identified erythroid clusters for differential gene expression analysis, using R Studio DESeq followed by Gene Ontology (GO) enrichment analysis. We are differentiating cells into red blood cells for further comparison with flow cytometry, bulk RNA sequencing, protein analysis, and hemoglobin staining. Our approach has relied on multidisciplinary expertise in clinical hematology and genetics, basic science study of ribosomes, computational biology, stem cell, and hematopoietic biology. **RESULTS/ANTICIPATED RESULTS:** Compared to WT hematopoietic stem cells, RPL30mutant cells had significantly decreased expression of RPL30. Analysis of top differentially expressed genes revealed downregulation of HSPA1A which encodes heat shock protein 70 (HSP70), chaperone of a critical red blood cell transcription factor. Loss of HSP70 protein has been implicated in RPL-mutated red blood cells previously as a potential modulator of severe DBA phenotype. Upon GO enrichment analysis of downregulated genes, biologic process terms GO:0042254 ribosome biogenesis, GO:1903708 positive regulation of hemopoiesis, and GO:0045646 regulation of erythrocyte differentiation were all highlighted as driver terms. We expect further differentiation to reveal early death of RPL30mutant cells with associated downregulated HSP70. **DISCUSSION/SIGNIFICANCE OF IMPACT:** Our results support our hypothesis that the RPL30 variant downregulates erythropoiesis, with a potential early role of HSP70 protein. Upon completion of our study, we will demonstrate the role of RPL30 in DBA pathogenesis as well as provide understanding of its drivers, which is critical for improved management of this disease.

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### **Foundations for prescribing song-based therapies: A quantitative analysis of laryngeal exercises\***

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**OBJECTIVES/GOALS:** Explore and compare the functional mechanisms of song-based exercises compared to speech-language

pathology exercises for dysphagia. The long-term goal is to increase patient outcomes through song-based programs that are accessible, enjoyable, and personalizable. **METHODS/STUDY POPULATION:** We will pilot the use of combined electroencephalography (EEG) and electromyography (EMG) technologies to analyze both central and peripheral contributors to laryngeal control in a cohort of healthy individuals. This approach provides detailed insight into the coordination between neural and muscular activity, which will serve as a baseline for future studies in clinical populations. Song-based vocal exercises will be compared with standard dysphagia exercises prescribed by speech-language pathologists to assess their mechanistic differences. **RESULTS/ANTICIPATED RESULTS:** We anticipate identifying specific song-based tasks, such as variations in pitch, rhythm, and intensity, which differentially impact laryngeal musculature. Additionally, we will localize neural activation hotspots using EEG during these tasks, providing a more comprehensive understanding of how song-based therapy influences both peripheral and central mechanisms. **DISCUSSION/SIGNIFICANCE OF IMPACT:** This project will lay the groundwork for developing evidence-based song-based therapies for dysphagia, providing an alternative to traditional SLP exercises. By creating an engaging therapeutic program, we aim to reduce dysphagia's healthcare burden, including aspiration events, healthcare costs, and related mortality.

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#### **Eight weeks of creatine monohydrate supplementation is feasible and associated with increased brain creatine in patients with AD**

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**OBJECTIVES/GOALS:** The creatine (Cr) system is impaired in Alzheimer's disease (AD). Data show that creatine monohydrate (CrM) supplementation may improve AD symptoms in AD mouse models, but no human studies have been reported. Thus, we investigated whether an eight-week CrM supplementation was feasible and associated with increased brain creatine in patients with AD. **METHODS/STUDY POPULATION:** Twenty participants with probable AD were allocated to an open-label, eight-week intervention of 20 g/day CrM. Fasting blood draws were taken at baseline, 4-, and 8-week visits to measure serum creatine (Quest Diagnostics). 1H magnetic resonance spectroscopy was performed at baseline and 8-week visits to measure brain Cr as a ratio to unsuppressed water. Self-reported compliance (with assistance from study partners) was assessed with daily CrM trackers. The mean compliance percentage across all participants was used to describe overall compliance with the intervention. We used paired t-tests to analyze the mean changes in serum Cr levels from baseline to 4- and 8-week visits and the mean change in brain Cr from baseline to 8-week visits. Statistical significance was set at  $p < 0.05$ . **RESULTS/ANTICIPATED RESULTS:** Participants were 65% male with a mean age of  $73.1 \pm 6.3$  years. All participants completed the study, with 19 out of 20 achieving the dose compliance target of  $\geq 80\%$ . The mean self-reported dose

intake was 90%. Serum Cr levels were significantly increased at 4- and 8-week visits compared to baseline ( $0.6 \pm 0.4$  mg/dL vs.  $14.0 \pm 9.9$  mg/dL and  $15.0 \pm 13.6$  mg/dL, respectively;  $p < 0.001$ ). Brain Cr levels also significantly increased ( $330.5 \pm 36.80$  i.u. vs.  $366.9 \pm 57.52$  i.u.,  $p < 0.001$ ). **DISCUSSION/SIGNIFICANCE OF IMPACT:** We are the first to demonstrate that 20 g/day of CrM for eight weeks is feasible and associated with increased brain Cr in patients with AD. Our findings support further investigation of brain target engagement of CrM and its efficacy in AD. With AD cases expected to rise, CrM could serve as an effective, affordable therapeutic to slow AD progression.

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#### **Engineered multifunctional wound healing patch: an antimicrobial living bandage to improve wound healing**

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**OBJECTIVES/GOALS:** The study focuses on developing a wound patch that employs a biocompatible matrix which incorporates mesenchymal stem cells (MSCs) with wound healing and antimicrobial properties, along with antimicrobial metallic nanoparticles covered with keratinocytes derived from induced pluripotent stem cells to replicate the skin's barrier function. **METHODS/STUDY POPULATION:** In vitro experiments will be conducted to combine bacteria with MSCs and metallic nanoparticles to assess whether bacterial killing is improved by this combination. The MSCs will then be evaluated in the presence of the nanoparticles to confirm that their functionality and phenotype are not altered. To verify the cells' functional integrity, they will undergo trilineage differentiation, surface marker phenotypic testing, and evaluation of their capacity to inhibit lymphocyte proliferation in the presence of the nanoparticles. Subsequently, this living bandage will be created using a biomatrix embedded with induced pluripotent stem cell-derived keratinocytes and tested on a canine wound model to study the impact on healing. The model will assess the rate of healing and cellular response at weekly intervals until healed. **RESULTS/ANTICIPATED RESULTS:** The combination of mesenchymal stem cells and antimicrobial nanoparticles works synergistically to enhance bacterial killing in vitro with *S. aureus*. The presence of the nanoparticles in combination with MSC did not affect the ability of the MSC to undergo trilineage differentiation. We anticipate that the surface phenotype will be similarly unaffected. In addition, we expect that the presence of the nanoparticles should not interfere with the ability of MSC to suppress lymphocyte proliferation. Utilization of the wound patch in the in vivo canine wound model is expected to enhance healing and prevent infection. We expect that we will observe a shift in the cellular composition of the wound with less inflammatory cells and more M2 or wound healing anti-inflammatory monocytes. **DISCUSSION/SIGNIFICANCE OF IMPACT:** The incidence of resistant infections with no pharmacologic therapy available are on the rise. The development of an antimicrobial living bandage that increases the body's ability to fight off infection, while providing a barrier to reinfection would provide a new way to treat infections regardless of their acquired antibacterial resistance.