function in the gene of interest harboring a premature termination codon. DISCUSSION/SIGNIFICANCE OF IMPACT: The significance of this research lies in its potential to identify novel genes capable of triggering transcriptional adaptation in humans. Understanding this mechanism could pave the way for new therapeutic strategies, particularly for CHCHD10-associated ALS.

398

Targeting oxidative stress via NAD+ to mitigate microvascular disease

Anita Kaw and Laura Hansen
Emory University

OBJECTIVES/GOALS: The primary objective of this study is to test whether NAD+ modulation lowers mitochondrial oxidative stress to restore endothelial nitric-oxide signaling and limit microvascular remodeling in diabetes in murine and 3D tissue-engineered models. The goal is to define NAD-responsive pathways and candidate blood biomarkers of microvascular injury. METHODS/STUDY POPULATION: Use db/db diabetic mice to measure effects of NAD+ modulation on capillary density, perfusion, endothelial nitric oxide (NO) signaling, and mitochondrial reactive oxygen species (ROS) by histology, immunohistochemistry (IHC), qPCR/immunoblot, and functional assays. Build a 3D microfluidic model of diabetic retinal capillary disease from human endothelial/pericyte co-cultures with a retina-on-a-chip model; induce diabetic stress (hyperglycemia + inflammatory cytokines) and apply an NAD+ modulation to map dose–responses for barrier integrity, ROS, NO, and morphology. Analyze publicly available transcriptomic datasets from people with and without diabetic microvascular disease to identify oxidative-stress signatures and candidate circulating biomarkers. RESULTS/ANTICIPATED RESULTS: Expect increasing NAD+ availability to reduce mitochondrial ROS and inflammatory signaling, increase SIRT3/SOD2 activity, improve NO bioavailability, and prevent capillary rarefaction and leak in db/db mice. In the microfluidic model, anticipate a rescue of barrier function, lumen stability, and branching with NAD+, defining an effective dose window. Cross-platform analyses should converge on NAD+-responsive pathways (redox, mitochondrial metabolism, endothelial junctions) and nominate blood biomarkers that correlate with microvascular status. Together, results will provide actionable preclinical evidence for NAD+ modulation as a therapeutic strategy. DISCUSSION/SIGNIFICANCE OF IMPACT: By targeting a shared driver, mitochondrial oxidative stress, this work could deliver a mechanism-based therapy and practical biomarkers for diabetic microvascular disease, accelerating translation from bench models to patient-focused trials and improving cardiovascular outcomes.

400

Modeling osteosarcoma progression and therapeutic response using engineered induced pluripotent stem cell-derived 3D organoids

Sophia Wenthe[1], Rebecca A. Johnson[2,4,5], Kelsie L. Becklin[6], Nicholas J. Slipek[2,4,5], Erin M. Stelljes[2,4,5], Prateek P. Thenge[2,4,5], Anthony P. DeFeo[2,4,5], Lauren J. Mills[2,5], Jaime F. Modiano[6,7], Branden S. Moriarity[2,3,4,5] and Beau R. Webber[2,3,4,5]
[1]University of Minnesota CTSI; [2] Department of Pediatrics; [3] Comparative and Molecular Biosciences; [4] Center for Genome Engineering; [5] Masonic Cancer Center; [6] Department of Veterinary and Biomedical Sciences and [7] Department of Microbiology, Immunology and Cancer Biology, University of Minnesota, Minneapolis, MN 55455, USA

OBJECTIVES/GOALS: Our objective is to develop and validate a human iPSC-derived 3D osteosarcoma (3D-iOSA) model that replicates chromosomal instability (CIN), metastasis, and chemoresistance. The model will serve as a platform to evaluate chemotherapy and immune cell therapeutics, offering improved predictive fidelity compared to 2D and spheroid systems. METHODS/STUDY POPULATION: Human iPSCs were engineered by CRISPR/Cas9-mediated knock out of TP53 and RB1, differentiated to mesenchymal stem cells with overexpression of cMYC ± hRAS. Cells were embedded in hyaluronan and osteogenically differentiated to generate 3D organoids (3D-iOSA), with osteoblastic commitment confirmed by RUNX2 and BGLAP immunohistochemistry (IHC). Tumorigenic and metastatic potential was assessed via subcutaneous implantation into immunodeficient mice, followed by histopathology, IHC, RNA sequencing, and 5X whole genome sequencing (WGS). MAP (methotrexate, doxorubicin, and cisplatin) chemotherapy response and chimeric antigen receptor (CAR)-immune cell cytotoxicity were compared across 2D and 3D systems to evaluate chemoresistance, immune evasion, and translational fidelity of the 3D-iOSA model. RESULTS/ANTICIPATED RESULTS: 3D-iOSA exhibited osteogenic lineage commitment with RUNX2 and BGLAP expression and disorganized, anaplastic morphology correlated with mutational burden. Tumors displayed greater histologic complexity than 2D-derived tumors, with pulmonary metastases in 50% of primary and 70% of secondary recipients. WGS revealed recurrent copy number alterations and subclonal variation, indicating ongoing CIN. MAP half-maximal inhibitory concentration values were 106-260× higher in 3D-iOSA organoids than 2D cultures, increasing with genetic complexity. B7H3 CAR-iPSC NK-cell and B7H3/HER2 dual-CAR γδ T-cell screening showed system-dependent efficacy, with reduced cytotoxicity in 3D-iOSA organoids versus 2D and spheroid cultures, highlighting 3D-iOSA

Downloaded from https://www.cambridge.org/core. UNIVERSIDAD AUTONOMA METROPOLITANA, on 17 Nov 2026 at 05:57:45, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/cts.2026.10558

superior predictive power for immunotherapy evaluation. DISCUSSION/SIGNIFICANCE OF IMPACT: 3D-iOSA is the first human OSA model to replicate CIN-driven evolution, chemoresistance, and metastasis. It distinguishes differential immune therapy responses with greater predictive power than 2D or spheroid models, providing a scalable, physiologically relevant platform to accelerate targeted therapy development.

402

Longitudinal transcriptomic and proteomic analysis of immune dynamics in amyotrophic lateral sclerosis patients treated with FUS antisense oligonucleotide*

Olivia Rifai, Samuel HS Levy, Benjamin N Hoover, Maedot Yidenk, Ryan Talcoff, Anand Goswami, Neil A Shneider and Wassim Elyaman
Department of Neurology, Columbia University Irving Medical Center, Columbia University, New York, NY

OBJECTIVES/GOALS: Lowering of FUS with antisense oligonucleotide (ASO) has shown promise in a subtype of amyotrophic lateral sclerosis (FUS-ALS). As inflammation is associated with ALS progression, we characterized immune trajectories in FUS-ALS patients treated with FUS ASO to understand the relationship inflammation may have with treatment response. METHODS/STUDY POPULATION: Twelve participants (10 with a diagnosis of ALS, 1 symptomatic carrier, and 1 asymptomatic carrier) received monthly intrathecal administrations of FUS ASO (i.e., jacifusen). Motor function was measured using the ALS Functional Rating Scale-Revised. Axonal injury was measured by cerebrospinal fluid (CSF) neurofilament light chain (NfL) concentration. 10x Genomics single-cell RNA sequencing (scRNA-seq) with T- cell receptor (TCR) sequencing was performed on paired peripheral blood mononuclear cells (PBMCs) and CSF cells. Proteomic analysis was conducted using the Olink Explore HT platform. Complementary analyses included spectral flow cytometry validation and immunohistochemical staining of post-mortem lumbar spinal cord. RESULTS/ANTICIPATED RESULTS: We observed a proportional shift starting at 6 months, whereby T-cell proportions decreased, and myeloid cell proportions increased over treatment. Decreases in T-cell proportions over treatment were not accompanied by increased CD8+ T-cell infiltration in the ventral horn of the lumbar spinal cord. Differential expression revealed long-term, chronic activation and lysosomal dysregulation in myeloid cells. While CSF monocyte proportions were positively associated with CSF NfL concentration, positive associations with ALSFRS-R score were observed only in PBMC T-cell proportions, particularly cytotoxic subclusters. Proteomic analysis also highlighted a shift toward long-term innate immune cell activation associated with reduced ALSFRS-R score, accompanied by decreases in CSF lysosomal proteases. DISCUSSION/SIGNIFICANCE OF IMPACT: These findings provide insight into the immune dynamics of FUS-ALS in the context of CNS FUS knockdown, as well as their potential relationship with clinical outcomes. Further, the long-term shift toward monocyte activation and persistent lysosomal dysregulation signature outside the CNS highlights potential avenues for combination therapy.

404

Ensuring rigor and reproducibility in monitoring aortic stenosis progression with echocardiography

Jacob Fischman, Benjamin Wessler, Manlik Kwong and Ludovic Trinquart
Tufts University

OBJECTIVES/GOALS: Medical therapies to halt progression of aortic stenosis (AS) are now being studied; however, the sensitivity of transthoracic echocardiography (TTE) measures to detect disease progression remain unclear. We will evaluate alternative TTE-derived measures of AS severity as potential markers of disease progression to improve trial efficiency. METHODS/STUDY POPULATION: We will conduct a retrospective cohort study of adults with mild or moderate aortic stenosis who had TTE imaging at Tufts Medical Center from 2012 to 2024. Two cohorts will be examined: 1) a reproducibility cohort of patients with studies less than 2 months apart to assess measurement consistency; and 2) a progression cohort with studies more than 6 months apart to evaluate disease progression over time. Echocardiographic parameters including peak velocity, mean pressure gradient, and aortic valve area will be used. We will use recurrent quantitative measurements to evaluate reproducibility, variability, and signal-to-noise characteristics and to model implications for efficient clinical trial design. Analysis will use RStudio. RESULTS/ANTICIPATED RESULTS: Overall there are 2,668 patients with progressive AS. Median age at first TTE is 76 (68–83) years. 1,120 (42%) are female. The reproducibility cohort includes 991 patients (1,728 paired TTEs, median 10 days (0–16) apart), and the progression cohort includes 323 patients (323 paired TTEs, median 504 days (394–750) apart). Additional demographic and comorbidity data will be obtained. Recurrent measurements will quantify reproducibility, variability, and signal-to-noise characteristics. Anticipated results include 1) identification of the most reliable measures for tracking progression, 2) characterization of measurement variability, and 3) modeling of implications for efficient clinical trial design and evidence-based patient management. DISCUSSION/SIGNIFICANCE OF IMPACT: Interest in therapies to halt aortic stenosis progression is growing. Establishing consensus on the most reproducible and practical imaging endpoints is essential. By quantifying real-world variability, we aim to optimize trial design so that effective treatments can be studied and improve outcomes.

405

Uncovering population-specific signals and hundreds of new loci from a multi-ancestry study of blood pressure traits in over 2.5M individuals

Hannah Seagle[1], Jeewoo Kim[2], Joseph H. Breeyear[3], Catherine A. Greene[4], John B. Buse[5], Lanna Caruth[6], Lindsay Guare[7], Navya S. Josyula[8], Qianqian Liang[9], Chelsea Okeh[10], Hannah Poisner[11], Yash Pershad[12], Serena Houghton[13], Yuk-Lam Anne Ho[14], Xiaofeng Zhu[15], Alexander G. Bick[16], Digna R. Velez Edwards[17], J. Michael Gaziano[18], Alison A. Motsinger-Reif[19], Helen R. Warren[20], Patricia B. Munroe[21], Shefali S. Verma[21], Anne E. Justice[21], Peter

Downloaded from https://www.cambridge.org/core. UNIVERSIDAD AUTONOMA METROPOLITANA, on 17 Nov 2026 at 05:57:45, subject to the Cambridge Core terms of use, available at https://www.cambridge.org/core/terms. https://doi.org/10.1017/cts.2026.10558