indicating improvements from baseline to end-of-treatment in: AN symptomatology (d=1.37), anxiety ratings about weight gain (d=1.22), anxious beliefs about weight gain (d =1.06), and tolerance of weight gain (d=1.40). Pearson's correlations exploring whether changes in weight gain anxiety were associated with AN symptom improvement met our benchmark (r≥0.5) for anxiety ratings (r=0.55) and anxious beliefs (r=0.65), but not tolerance of weight gain (r=0.18). DISCUSSION/SIGNIFICANCE OF IMPACT: Results support Exp-AN's acceptability and preliminary efficacy. Reductions in weight gain anxiety correlated with symptom improvement, suggesting target engagement. By targeting specific mechanisms, this work advances precision medicine and treatment optimization. Findings warrant a fully powered randomized controlled trial.

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Exploiting global viral immunity to develop a novel viro-immunotherapy using oncolytic viruses and CAR T-cells for hepatocellular carcinoma

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OBJECTIVES/GOALS: Hepatocellular carcinoma (HCC) is a therapeutic challenge due to poor tumor immunogenicity, low endogenous T cell response, and a complex tumor microenvironment (TME). We propose an immunotherapeutic strategy leveraging widespread antiviral immunity – particularly memory T cells against SARS-CoV-2 spike (SPIKE) – to redirect T cells into tumors. METHODS/STUDY POPULATION: By engineering oncolytic viruses (OV) to express SPIKE, the approach aims to recruit virus-specific T cells and re-activate them through viral boosting, enhancing tumor clearance. This has been successfully shown in a B-16 melanoma model, another solid tumor system. We will also develop a dual-specific CAR T cell platform against SPIKE and GPC3, the most common HCC tumor-associated antigen (TAA), enabling antitumor targeting which can also benefit from OV readministration. The experimental plan includes purification and titration of a single-cycle adenovirus expressing SPIKE (SC-Ad-SPIKE), infection of murine and human HCC cell lines to validate SPIKE expression and replication, and *in vivo* treatment of subcutaneous or orthotopic murine tumors in pre-immunized or naïve mice using OV. RESULTS/ANTICIPATED RESULTS: This strategy offers a translatable platform to re-focus existing antiviral memory toward otherwise weakly immunogenic tumors like HCC, potentially enhancing clinical efficacy through oncolytic virotherapy and CAR T cell engineering. We should see better average tumor clearance and survival in the groups treated with the OV and CAR T against SPIKE than controls with only GPC3 given the lack of reliance on tumor-GPC3 expression. DISCUSSION/SIGNIFICANCE OF IMPACT: Our approach could be crucial for patients given how varied current responses are to available therapies. The combination of a generalized OV therapy and a more targeted CAR T provides a promising new solution to the current unmet need of many HCC patients globally.

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Arousability from sleep as a multidimensional construct: Cross-sectional analysis and machine-learning based feature selection*

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OBJECTIVES/GOALS: To develop and assess a multidimensional measure of arousability by integrating respiratory, autonomic, cortical, and movement-related events and to identify sex-specific arousal phenotypes using clustering analyses and LASSO-based machine learning for feature selection and model optimization. METHODS/STUDY POPULATION: We will conduct a retrospective cross-sectional analysis using sleep and clinical data from >10,000 adults in three different cohorts. Arousability will be defined by event-level metrics across respiratory, autonomic, cortical, and movement domains. Variable clustering will be used to construct a multidimensional composite index of arousability based on those domains. Generalized linear models will test sex differences in individual metrics composite indices. LASSO regression will be applied to select arousability features most predictive of sex, mortality, and cardiovascular disease (CVD). RESULTS/ANTICIPATED RESULTS: We anticipate identifying distinct arousability dimensions and sex-specific arousal profiles. Women are expected to show greater non-respiratory arousals, while men will show higher respiratory-related arousals. The resulting multidimensional index will capture arousal burden more comprehensively than traditional metrics like the apnea–hypopnea index. This will aid in understanding sex-based difference of sleep fragmentation, as well as understanding the sleep mechanisms most associated with mortality and CVD. DISCUSSION/SIGNIFICANCE OF IMPACT: This study will advance precision sleep medicine by developing a multidimensional, data-driven arousability index and identifying sex-specific

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phenotypes. These findings may improve diagnostic accuracy, influence tailored interventions, and inform risk stratification beyond traditional metrics.

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Nutrition education camp intervention reduces dietary variation and improves metabolic control in a large cohort of females with PKU

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OBJECTIVES/GOALS: Dietary variation can compromise metabolic control in phenylketonuria (PKU), yet the impact of improved management on within-subject dietary variation has not been explored. We examined the effect of a 5-day nutrition education camp on dietary variation and metabolic control in females with PKU. METHODS/STUDY POPULATION: Females with PKU (n=103; ages 12–59 years, median=18) who attended the Emory Metabolic Camp from 2014 to 2025 were included in the analysis. Pre- and post-camp 3-day diet records (3DDR) and blood samples (camp day 1 and day 5) were collected. Within-subject coefficients of variation (CVw) in energy, total protein, medical food protein, intact protein, and Phe were calculated from pre- and post-camp 3DDR. Wilcoxon signed-rank tests were used to assess pre- and post-camp dietary variation, and linear mixed models adjusted for age and energy intake were used to examine the relationship between changes in dietary variation and blood Phe concentrations. RESULTS/ANTICIPATED RESULTS: Median (IQR) pre- to post-camp energy intake increased from 1475 (1207–1832) to 1733 (1459–2054) kcal, medical food protein intake increased from 36 (13–54) to 40 (18–54) g, intact protein intake decreased from 17 (10–29) to 12 (7–26) g, and Phe intake decreased from 641 (409–1152) to 527 (332–1015) mg. Blood Phe decreased from 529 (290–895) to 385 (196–721) μmol/L. CVw significantly decreased from pre- to post-camp for energy ($p<0.001$), intact protein ($p<0.05$), and Phe ($p<0.05$), and a reduction in energy CVw was associated with lower blood Phe ($p<0.05$). Notably, greater dietary variation in energy, total protein, and intact protein intake was associated with higher blood Phe at pre-camp but not at post-camp, suggesting improved metabolic stability after intervention. DISCUSSION/SIGNIFICANCE OF IMPACT: These findings demonstrate that a 5-day nutrition education intervention in a large PKU cohort optimized dietary stability and resulted in improved metabolic control, providing strong evidence to support recent FDA guidance on optimizing and standardizing diet prior to and throughout inherited metabolic disorder clinical trials.

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A systematic review of real-world evidence on the clinical relevance, characterization, and utility of CYP2D6 biomarker testing

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OBJECTIVES/GOALS: Limited systematic analysis of real-world evidence (RWE) on pharmacogenomic (PGx) testing utility, which can help provider decision-making and avoid serious adverse effects, exists. This study explores clinical-behavioral outcomes and implementation considerations, for PGx testing in real-world clinical settings for PGx biomarker CYP2D6. METHODS/STUDY POPULATION: Our systematic review investigated drug–gene pairs with strong evidence (Level A, Final classification by the Clinical Pharmacogenomics Implementation Consortium [CPIC]) for CYP2D6. Search strings were deployed across Google Scholar, PubMed, Scopus, and Semantic Scholar. Papers were included only if they presented, measured, and/or described real-world clinical or patient and/or provider behavioral outcomes based on EHR or claims data assessment following PGx testing for the CYP2D6 biomarker, included PGx biomarker(s)–drug pairs with CPIC Level A, Final Evidence designations, and published in English. Study quality and bias risks were assessed using the Newcastle–Ottawa scale or A comprehenSive tool to Support rEporting and critical appraiSal…of reSearch outcomes (ASSESS) tools, where applicable. RESULTS/ANTICIPATED RESULTS: Of the 218 articles identified, 25 met our inclusion criteria and explored 9 CPIC Level A, Final CYP2D6–drug pairs. Overarching qualitative themes were 1) variation in CYP2D6 biomarker testing and interpretation and 2) PGx test implementation and data considerations. CYP2D6–drug pairs were reported across four therapeutic areas (analgesia [n = 21], psychiatry [n = 17], oncology [n = 7], and gastroenterology [n = 6]) with the two most researched drugs being codeine (n = 21) and tramadol (n = 18). Six (6) articles reported PGx clinical outcomes, considered to be a "measurable change in symptoms, overall health, ability to function, quality of life, or survival outcomes" in relation to PGx testing. DISCUSSION/SIGNIFICANCE OF IMPACT: Our findings indicate a need to address both inconsistent phenotype categorization and racial, ethnic, and genetic ancestry classifications and to improve EHR interoperability with PGx test results. Future RWE-PGx studies should associate dosage and regimen changes to both discrete provider choices and patient clinical-behavioral outcomes.

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A CTS team approach to investigating sEV production in magnetic-responsive scaffolds following Salmonella infection‡

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OBJECTIVES/GOALS: This collaboration explores magnetic-responsive scaffolds toward modeling Salmonella infection via the small extracellular vesicle (sEV) route. We aim to elucidate the mechanism by which sEVs reflect host–pathogen interactions to inform new Salmonella therapeutics. METHODS/STUDY POPULATION: Human intestinal epithelial cells (INT-407) are being cultured on magnetic-responsive scaffolds under controlled mechanical stimulation and infected with Salmonella Typhimurium 12023 WT. Kinetic assays are assessed via confocal microscopy, flow cytometry, and qPCR. sEV isolation is being

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