optogenetics in treating such severe arrhythmias. RESULTS/ANTICIPATED RESULTS: Immunostaining shows 87.339% of iPSC-CMs, treated with All-trans retinoic acid (RA) (1 uM) on days 7 and 12 [RA 7,12], and 23.84% of those, treated on days 3 and 5 [RA 3,5], expressed MLC-2V ($p<0.001$). Calcium reuptake (τ) is 0.5914 s in RA 7, 12 while 0.2247s in RA 3, 5 ($p<0.001$). APD90 and APD50 of RA 7, 12 are 2- and 5-fold higher than RA 3, 5, showing distinct ventricular and atrial phenotypes. Protein expression of βII-spectrin and ankyrin-2 and their co-localizations were reduced in the ANK2 phenotype compared to the healthy phenotype. We found prolongation of Ca2+ waves and τ with blue light on iPSC-CMs, expressing ChR2. We anticipate that such prolongation of calcium transients would prevent aberrant calcium spikes, rescue Ca2+/calpain-induced βII-spectrin loss and provide electrical stability. DISCUSSION/SIGNIFICANCE: Animal models cannot accurately recapitulate human cardiac electrophysiology. The proposed human iPSC-CM-based ANK2 model would provide better mechanistic insights of severe ventricular arrhythmias. Also, the proposed optogenetic cardioversion has the potential to provide safe, targeted and painless cardioversion to manage arrhythmias.

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An Investigation of Novel Urinary Cell mRNA Profiles for Noninvasive Diagnosis of Acute Rejection in Kidney Transplant Recipients

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OBJECTIVES/GOALS: RNA-seq of urine and kidney allograft biopsies (bx) found that urinary cell immune landscape reflects intragraft molecular events and we discovered a shared set of 127 mRNAs in urine matched to T cell mediated and antibody mediated rejection bx. We prioritized ITM2A, SLAMF6 and IKZF3 mRNAs and herein investigate if these accurately predict rejection. METHODS/STUDY POPULATION: We collected urine samples from adult kidney allograft (KA) recipients at the time of KA bx. KA bx were classified by pathologists by Banff criteria. Total RNA was isolated from KA bx-matched urine samples. Absolute copy numbers of ITM2A, SLAMF6, and IKZF3 mRNAs and 18S rRNA were measured using our customized RT-qPCR assays. Logistic regression used to derive an equation for a combined signature score of 18S-normalized urinary cell mRNA levels of ITM2A, IKZF3, and SLAMF6 that best predicts Acute Rejection (AR= both T cell mediated rejection and antibody mediated rejection). Area under the ROC curve (AUC) was calculated to discriminate between AR and No Rejection (NR) biopsies for 18S-normalized urinary cell levels of ITM2A, IKZF3 and SLAMF6 and the composite signature score. AUCs were compared by DeLong Method. RESULTS/ANTICIPATED RESULTS: Urinary cell 18S-normalized levels of ITM2A, IKZF3, and SLAMF6 mRNAs in urine discriminated KA recipients with AR biopsies (n=95) from those with NR biopsies (n=160) (All P values <0.05, Mann-Whitney test) and the AUC was 0.69 (95% CI, 0.62 to 0.76) for ITM2A, 0.61 (95%CI, 0.53 to 0.68) for IKZF3, and 0.60 (95%CI, 0.53 to 0.68) for SLAMF6. The derived combination signature score of urinary cell 18S-normalized levels of ITM2A, IKZF3, and SLAMF6 mRNA discriminated KA recipients with AR from those with NR ($P<0.0001$, Mann Whitney test) and the combined signature score AUC was 0.72 (95%CI, 0.65 to 0.79). The combination signature score discriminated AR vs NR better than IKZF3 and SLAMF6 alone, but was not significantly different than ITM2A alone (DeLong method). (Additional results/figures to be included in poster) DISCUSSION/SIGNIFICANCE: Our RNA-seq offered a unique opportunity to diagnose AR by measuring the mRNAs in urine. We now found that urinary cell mRNA levels of ITM2A, IKZF3, SLAMF6 and the combined signature are diagnostic of AR, a major and serious post-transplant complication. This allows for much-needed KA molecular surveillance and personalization of immunosuppression.

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Genetic risk factors for drug-induced long QT syndrome: Findings from a large real-world clinical cohort.

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OBJECTIVES/GOALS: The objective of this research was to determine the associations of candidate genetic variants withdrug-induced long QT syndrome (diLQTS) risk, an adverse effect of over 150 FDA-approved drugsthat can lead to cardiac arrhythmias and sudden cardiac death. METHODS/STUDY POPULATION: This was a retrospective observational study of the genomic biobank at the University of Michigan Health System. Patients treated with a high-risk QT-prolonging drug and ECG measurements were included. The primary outcome was exaggerated prolongation of the QTc interval (i.e., >60 ms change from baseline and/or >500 ms absolute value) corrected using Bazett. We analyzed 3 genetic variants: KCNE1-D85N (rs1805128), SCN5A-G615E (rs12720452) and KCNE2-I57T (rs7415448) in the dominant genetic model. A Bonferroni-corrected p-value of 0.017 was considered statistically significant using logistic regression adjusted for clinical covariates. RESULTS/ANTICIPATED RESULTS: In total 6,083 self-reported white patients were included (12% event rate). The adjusted odd ratio for KCNE1-D85N was 2.24 (95%CI: 1.35-3.57; p=0.0011). The adjusted odds ratio forKCNE2-I57T was 1.40 (95%CI: 0.26-5.78, p=0.662). Only 4 total patients carried the SCN5A-G615E variant, and none of the carriers had prolonged QTc. DISCUSSION/SIGNIFICANCE: This is the largest study of candidate genetic variants in cardiac ion channels associated with the diLQTS risk. KCNE1-D85N was associated with diLQTS risk, while KCNE2-I57T was suggestive of a potential association. KCNE1-D85N should be considered in clinical guidelines as a risk factor of diLQTS.

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Evaluating the therapeutic efficacy of combination IL-12 and trabectedin for the treatment of triple negative breast cancer

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OBJECTIVES/GOALS: IL-12 has potent immune effects but the presence of myeloid-derived suppressor cells (MDSC) can inhibit IL-12-induced NK cell cytotoxicity. Thus, we hypothesized that combining IL-12 with trabectedin, an immunosuppressive myeloid cell

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depleting agent, would improve its therapeutic efficacy in triple negative breast cancer (TNBC). METHODS/STUDY POPULATION: Combination IL-12 and trabectedin was tested in the 4T1 mouse model of TNBC. 4T1 cells were injected into the mammary fat pad of female BALB/cj mice. When tumors reached 50 mm3, mice were randomly divided into 4 groups and treated with PBS, IL-12 (0.5 μg/mouse 3x/wk), 0.15 mg/kg trabectedin weekly or the combination. Tumor volumes were measured by calipers. Mass cytometry was performed on spleens and tumors using a 35-antibody panel. Plasma IFN-γ levels were measured by ELISA. The role of NK cells was evaluated via depletion with anti-asialo-GM1. The Luminex Discovery Assay was used to measure plasma cytokines and immunohistochemistry was performed for CD4 and CD8a. Linear/nonlinear mixed effects modeling was used for in vivo data analysis and applicable t- or ANOVA tests were used for in vitrodata analysis. RESULTS/ANTICIPATED RESULTS: Combination IL-12 and trabectedin led to a significant reduction in tumor burden compared to single-agent IL-12, trabectedin and control treatments (all $p<0.001$), as well as higher levels of IFN-γ (all $p<0.04$). One combination treated mouse had complete tumor regression. Splenic MDSC were significantly decreased in combination treated mice. NK depletion abrogated the effects of combination therapy. Compared to mice receiving a control antibody, NK depletion of combination treated mice led to lower levels of CCL5 ($p<0.01$) and CXCL10 ($p<0.001$) and significantly higher tumor burden ($p=0.001$). CD8+T cell levels were significantly higher in combination treated mice compared to those receiving IL-12 ($p<0.01$), and these levels were decreased when mice were depleted of NK cells ($p=0.01$). DISCUSSION/SIGNIFICANCE: TNBC represents 15% of all breast cancer diagnoses and is associated with a worse prognosis compared to other subtypes. Black women are twice as likely to be diagnosed with TNBC and more likely to die from disease than White women. Thus, there is an increasing need to develop additional therapeutic options for this disease.

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Magnetic Resonance Biomarkers of Metabolic Dysfunction-Associated Steatotic Liver Disease

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OBJECTIVES/GOALS: Metabolic dysfunction-associated steatotic liver disease (MASLD) is a major public health concern due to its increasing prevalence and association with type 2 diabetes mellitus. Non-invasive magnetic resonance-based biomarkers can aid in the monitoring of disease progression and identification of patients at risk for chronic liver disease. METHODS/STUDY POPULATION: Over 600 subjects will be recruited from the San Antonio Mexican American Family Study and from a second study, which consists of (i) T2DM patients diagnosed with either MASLD or metabolic dysfunction-associated steatohepatitis (MASH) or (ii) metabolically healthy controls. Hydrogen-1 MRS and diffusion-weighted MRI (DW-MRI) will be used to measure liver fat fraction and liver stiffness biomarkers, respectively. Several potential biomarkers of liver stiffness will be evaluated in vivo using the intravoxel incoherent motion (IVIM) model for DW-MRI. To further improve the diagnostic accuracy of patients with liver fibrosis, we will integrate MRI/MRS data with relevant clinical indicators of hepatic metabolism. Results will be compared to biopsy samples to evaluate the model's diagnostic accuracy. RESULTS/ANTICIPATED RESULTS: Based on preliminary data, we predict that IVIM will be able to accurately diagnose hepatic fibrosis in patients with MASLD, allowing it to be implemented in clinics with high-field MRI units easily. Previous studies have shown correlations between IVIM estimates and fibrosis stages, however, none included additional clinical indicators of liver disease in their models. We have already found significant differences in metabolic measurements such as fasting plasma glucose and HbA1c levels. Additionally, the use of machine learning in developing these models has shown improvements in the ability to extract features from the data. The aim is to achieve high accuracy and robustness in the staging of liver fibrosis. DISCUSSION/SIGNIFICANCE: Over 100 million people in the US are affected by MASLD. Without treatment, it progresses from hepatic steatosis to MASH, fibrosis (liver stiffening), and ultimately to hepatic cirrhosis and hepatocellular carcinoma (HCC). Continued research efforts and clinical implementation of MRI and MRS are vital in combating the growing burden of MASLD.

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Neuropsychiatric Symptom Clusters in behavioral variant frontotemporal dementia: The Role of Early Anxiety/Depression on Functional Progression

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OBJECTIVES/GOALS: To identify empiric neuropsychiatric symptom (NPS) clusters in behavioral variant frontotemporal dementia and to determine the role of early anxiety/depression on functional progression. METHODS/STUDY POPULATION: Analyses were conducted using data from the ARTFL-LEFFTDS Longitudinal Frontotemporal Lobar Degeneration (ALLFTD) study, an established consortium with an ongoing cohort study of FTD patients across 18 clinical sites which includes comprehensive cognitive, neuropsychiatric, and structural neuroimaging data. A polychoric cluster analysis was performed on subjects from the ALLFTD cohort [applewebdata%3A//044E463E-34DA-4677-9EDC-B8309D14C337#_msocom_1] with early-stage disease (N=145, male 61%, median age 62 years) in order to identify empiric NPS clusters. Cox proportional hazard regression was then used to examine the association between early affective symptoms in bvFTD and subsequent functional disabilities adjusted for age, sex, level of education, and FTLD CDR global score. RESULTS/ANTICIPATED RESULTS: We identified a four-factor model as the best fit for the data: (1) an affective cluster with prominent depression, anxiety, agitation, and irritability, (2) a disinhibited symptom cluster with prominent elation and disinhibition, (3) an obsessive symptom cluster with prominent obsessive/ritualistic behavior and hyperorality, and (4) a psychotic symptom cluster with prominent delusions and hallucinations. The hazard of developing impairments in transactions, language, self-care, meal preparation, and incontinence was significantly elevated in those with early affective symptoms (depression/anxiety). DISCUSSION/SIGNIFICANCE: In this study we show that, NPS cluster into four discrete groups: (1) affective symptoms, (2) disinhibited symptoms, (3) obsessive symptoms, and (4) psychotic symptoms. Anxiety and depression are prominent within the affective symptom cluster and are associated with accelerated functional decline in a number of domains.

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