war veterans with/without post-traumatic stress disorder (PTSD). METHODS/STUDY POPULATION: The peripheral blood mononuclear cells (PBMCs) from war veterans with/without post-traumatic stress disorder (PTSD) were used to monitor transcription profile of the molecular markers of mitochondrial biogenesis and fusion/architecture, as well as the markers of mtDNA copy numbers. The human male immortalized monocytes were exposed *in vitro* to hormonal markers of PTSD in order to monitor the effects of each particular hormonal marker on the molecular markers of mitochondrial biogenesis and fusion/architecture, as well as the markers of mtDNA copy numbers. RQ-PCR analyses were used to define transcriptional profile of above mentioned markers. RESULTS/ANTICIPATED RESULTS: The transcription profiles of above mentioned markers were disturbed, with high individual variability within the groups. A significant increase in the expression of the *PPARGC1A* transcript was observed in a group of subjects with current PTSD, as well as in the subjects with "life-time" PTSD, compared to healthy controls. *PPARGC1B, NRF2 and MFN2* transcripts increased only in PBMCs of "life-time"-PTSD, while the level of transcripts for other investigated genes and the ratio of markers of mtDNA copy numbers showed no significant difference between groups. The *in vitro* results showed parallelism with the results obtained using the PBMCs from the subjects of the PTSD study. DISCUSSION/SIGNIFICANCE OF IMPACT: Although preliminary (the analysis require a larger number of subjects), the results are first findings and a solid base for further extensive multidisciplinary research in order to clarify the molecular mechanisms for the prevention and treatment of trauma-induced pathological conditions.

**4564**

## Nilotinib alters microRNAs that regulate specific autophagy and ubiquitination genes in the cerebrospinal fluid of Parkinson's patients†

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OBJECTIVES/GOALS: Our preclinical data demonstrate that the principal effects of nilotinib, a multi-tyrosine kinase inhibitor, in models of neurodegeneration is clearance of misfolded proteins via autophagy. Here we aimed to evaluate the effects of nilotinib on microRNAs in the cerebrospinal fluid of Parkinson's disease patients. METHODS/STUDY POPULATION: Cerebrospinal fluid (CSF) was collected as part of an open label phase I (NCT02281474) (n = 12, 300 mg nilotinib taken orally once daily for 6 months), and a phase II randomized, double-blind, placebo-controlled study (NCT02954978) (n = 75, randomized 1:1:1 into placebo, 150 mg or 300 mg nilotinib taken orally once daily for 12 months). RNA was isolated from CSF and Indexed sequencing libraries were prepared from total RNA plus miRNA. Next generation whole-genome sequencing (single-end 1x75 bp, 25 million raw reads per sample) was performed to identify miRNAs significantly differentially expressed (fold-change ≥ 2, Benjamini-Hochberg FDR p-value ≤ 0.05 or Empirical Bayes FDR ≤ 0.05) with treatment compared to baseline. RESULTS/ANTICIPATED RESULTS: Next generation whole-genome sequencing of microRNAs in the CSF demonstrated that nilotinib significantly increases microRNAs that specifically regulate expression of autophagy and ubiquitination genes in individuals with Parkinson's disease. In the open label phase I, samples, 28 microRNAs found to regulate autophagy and ubiquitination genes, were significantly altered with treatment (Benjamini-Hochberg FDR p-value ≤ 0.05). In the phase II randomized, double-blind, placebo-controlled study samples, we verified several of those 28 candidate microRNAs had been significantly deferentially expressed with treatment (Empirical Bayes FDR p-value ≤ 0.05). DISCUSSION/SIGNIFICANCE OF IMPACT: Our data provide robust evidence that nilotinib's effects on misfolded protein clearance is via autophagy and CSF miRNA sequencing is a valid biomarker of nilotinib's effects in a definitive phase III study to investigate nilotinib in Parkinson's and other neurodegenerative diseases. CONFLICT OF INTEREST DESCRIPTION: Charbel Moussa is listed as an inventor on several Georgetown University patents for the use of tyrosine kinase inhibitors as a treatment for neurodegenerative diseases

**4306**

## Periodontal disease and the oral microbiome in antiretroviral-treated patients with HIV

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OBJECTIVES/GOALS: People living with HIV, despite antiretroviral therapy (ART), have increased burden of inflammatory and aging-related comorbidities such as periodontitis. Oral microbiota have been linked to periodontitis, but not in the context of HIV. We aim to compare relationships between the oral microbiome and periodontal disease in HIV+ vs healthy controls. METHODS/STUDY POPULATION: In an ongoing cohort study we have been recruiting pre- and post-menopausal women with HIV+ on ART for ≥6 months and HIV- controls matched by menopausal status (target n = 30 per arm; currently HIV+: n = 30 post- and 9 pre-M; HIV-: n = 15 post- and 6 pre-M). Patients age <18 or on antibiotics within 3 mos., except prophylaxis, are excluded. Patients provide saliva, then subgingival plaque collection during a dental examination through scaling from six index teeth. Standard CDC/AAP classifications of periodontitis are used. We will perform 16S rRNA and ITS sequencing to profile bacterial and fungal communities in saliva and plaque. Linear mixed effect regression and differential abundance analyses will be used to identify microbial and mycobial oral signatures of periodontal disease severity in HIV+ and HIV- populations. RESULTS/ANTICIPATED RESULTS: We found a markedly high prevalence of severe periodontal disease in HIV+ women despite ART (59%, compared to 11% in HIV- controls). In post-menopausal women with HIV, saliva bacterial α- and β-diversity in the saliva differed significantly with periodontal disease severity. Fungal α-diversity was also significantly lower in plaque from teeth with severe loss of tissue attachment (CAL ≥4 mm). We identified bacterial and fungal taxa significantly enriched in post-menopausal HIV+ women with severe compared to no or mild periodontitis. We hypothesize, similarly, associations between the oral microbiome and periodontitis in HIV- controls. However, we expect overall diversity metrics to be significantly altered in HIV+ compared to HIV- patients, indicating long-term dysbiosis despite treatment with ART. DISCUSSION/SIGNIFICANCE OF IMPACT: Contrasting associations between the oral microbiome and periodontal disease with respect to HIV will provide evidence for the role of microbiota in accelerated aging phenotype caused by HIV. Our results would also provide rationale for interventions

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targeting co-morbidities in people living with HIV to account for both inflammation and dysbiosis.

4472

## Plasma Neurofilament Light as a Biomarker for Pediatric Patients with Huntington's Disease

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OBJECTIVES/GOALS: The goal of this study is to compare plasma neurofilament light (NfL) concentrations in asymptomatic children and young adults that carry the gene expansion (GE group) that causes Huntington's Disease to similar subjects that do not carry this genetic mutation (GNE group). METHODS/STUDY POPULATION: Subjects from the Kids-HD study in the GE group were divided into groups based on their estimated years to motor onset. Each subgroup was compared to the subjects from the GNE group. Additionally, a group of participants with juvenile HD were compared to the GNE group. These comparisons were made by utilizing linear mixed effects regression models that included a random effect per subject and family and also included the covariates of age and parental socioeconomic status. A post-hoc analysis of subjects in the GE group who were within 20 years from their predicted motor onset was conducted to assess the relationship between striatal volume and plasma NfL concentrations. RESULTS/ANTICIPATED RESULTS: GE participants more than 20 years from their predicted motor onset did not have elevated plasma NfL concentrations relative to the GNE group. However, participants who were 15-20 years from their predicted motor onset had a mean NfL concentration of 1.61 pg/uL compared to 1.31 pg/uL in the GNE group ($p = 0.036$). Participants who were within 15 years from their predicted motor onset had a mean NfL concentration of 2.08 pg/uL, which was also significantly elevated relative to the GNE group ($t = 3.03$, $p = 0.003$). Additionally, the participants with juvenile HD had a mean NfL level of 3.22 pg/uL, which was significantly elevated compared to the GNE group ($p<0.0001$). NfL concentrations were significantly correlated with striatal volume amongst participants who were within 20 years of onset ($p = 0.017$). DISCUSSION/SIGNIFICANCE OF IMPACT: The huntingtin protein is essential to neurodevelopment but current gene therapies for HD focus on blocking production of this gene. These results will provide guidance on the optimal timing of administration of gene therapies by identifying neurodegeneration decades prior to motor onset of HD.

4346

## Potential Sudden Unexpected Death in Epilepsy (SUDEP) Biomarkers in Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes with DEPDC5 Loss-of-Function

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OBJECTIVES/GOALS: Sudden Unexpected Death in Epilepsy (SUDEP) is a leading cause of death in epilepsy patients. This study aims to determine whether cardiac mechanisms contribute to SUDEP in epilepsy patients with variants in *DEPDC5*, a gene encoding a member of the mTOR GATOR complex, to identify SUDEP biomarkers. METHODS/STUDY POPULATION: SUDEP has been reported in 10% of epilepsy patients with *DEPDC5* loss-of-function variants. We used human induced pluripotent stem cell-derived cardiomyocytes (iPSC-CMs) to measure changes in cellular excitability that are known to be substrates for cardiac arrhythmias. CRISPR-derived isogenic *DEPDC5* iPSC-CMs and *DEPDC5* patient-derived iPSC-CMs were used in this study. Whole-cell patch-clamp was used to measure voltage-gated sodium current ($I_{Na}$) and calcium current ($I>_{Ca}$) in single iPSC-CMs in voltage-clamp mode; and to measure action potentials (APs) in 3-dimentional iPSC-CM-derived micro-tissues in current-clamp mode. RESULTS/ANTICIPATED RESULTS: CRISPR generated heterozygous deletion of 1 base-pair in the first coding exon of *DEPDC5* gene, resulting in a premature stop codon, simulated the variants identified in *DEPDC5* epilepsy patients. In CRISPR generated heterozygous*DEPDC5* iPSC-CMs, whole-cell voltage-clamp recordings revealed that $I_{Na}$ was increased and $I_{Ca}$ was reduced compared with isogenic control iPSC-CMs. Whole-cell current-clamp recordings revealed that AP duration at 80% and 90% of repolarization, $APD_{80}$ and $APD_{90}$, respectively, were prolonged compared to isogenic control iPSC-CMs. Similar measurements will be performed for iPSC-CMs derived from *DEPDC5* patients. DISCUSSION/SIGNIFICANCE OF IMPACT: This study shows that epilepsy patients with non-ion channel gene variants in *DEPDC5* have altered CM excitability, which may serve as a substrate for cardiac arrhythmias in *DEPDC5* patients. Importantly, this work may allow us to identify biomarkers for SUDEP risk in these patients in the future. CONFLICT OF INTEREST DESCRIPTION: L.L.I. is the recipient of a collaborative research grant from Stoke Therapeutics.

4291

## Quantifying the art of surgical decision-making in total knee arthroplasty

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OBJECTIVES/GOALS: To quantify clinical exam in total knee arthroplasty by answering the following questions: (1) What are the magnitudes of forces applied by surgeons during the varus-valgus exam? (2) Is the choice of tibial insert thickness related to the magnitude of the applied forces? (3) How accurately does a surgeon estimate the gaps in the varus-valgus exam? METHODS/STUDY POPULATION: Three cadaveric knees were implanted with standard TKA trial implants. Four pliable force sensors were wrapped around the foot and ankle of each cadaver to measure the push-pull forces applied during the varus-valgus exam. Six surgeons with varying experience independently conducted a varus-valgus exam in extension and flexion and reported the gaps that they observed. Motion capture was used to measure the gaps between femur and tibia by placing cluster of reflective markers on femur and tibia. Subsequently, each surgeon chose the tibial insert that they thought best fit each knee. The measured peak applied forces were related to the insert thickness and the measured gaps were compared to the observed gaps by surgeons. Since insert thickness was in 1 mm increments, 1 mm gap error was considered a meaningful difference. RESULTS/ANTICIPATED RESULTS: The peak forces varied among surgeons for each cadaver. In cadaver one, the peak forces in varus and valgus in extension were 48±20 and 20±12 N, and in flexion were 27±14 and 8±11 N. Peak forces in cadavers two and three were similar; in varus and valgus in extension, 24±14 and 35 ±10 N, and in flexion, 23±12 and 20±10 N, respectively. It was observed that the larger the valgus force in extension, the thinner

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