but the current research demonstrated that 90% of the sample chose to continue working together across disciplines after EAGER awards. Therefore, future research should dedicate more attention to the nontangible benefits members receive in interdisciplinary teams. Moreover, quality measures revealed higher H-indices for multidisciplinary than unidisciplinary journals and conferences. DISCUSSION/SIGNIFICANCE: Our archival results revealed that NSF EAGER grants are having their intended effect of being a catalyst for 1) continued multidisciplinary (and especially multidirectorate) collaboration) and 2) high-quality multidisciplinary publication and conference output. These results have contributed to NSF policy changes to reinstate the EAGER grant.

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Characterization of the human iridocorneal angle in vivo using a custom design goniolens with OCT gonioscopy

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OBJECTIVES/GOALS: The trabecular meshwork (TM) and Schlemm's canal (SC), located within the iridocorneal angle (ICA), form the main outflow pathway and a major target for glaucoma treatments. We characterized the human ICA in vivo with Optical Coherence Tomography (OCT) imaging using a customized goniolens and a commercial OCT device (Heidelberg Spectralis). METHODS/STUDY POPULATION: Imaging these structures is difficult due to the optical limitations of imaging through the cornea at high angles. Therefore, a clinical gonioscopy lens was modified with a 12mm plano-convex lens placed on its anterior surface to focus light on the ICA structures, and capture returning light. Each subjects' eye was anesthetized with 1 drop of Proparacaine 0.5%. The goniolens was coupled to the eye with gonio-gel and it was held by a 3D adjustable mount. OCT volume scans were acquired on 10 healthy subjects. The linear polarization of the OCT was rotated with a half-waveplate to measure dependence of the ICA landmarks on polarization orientation. RESULTS/ANTICIPATED RESULTS: The TM was seen in 9 of 10 subjects. Polarization rotation modified the brightness of the band of extracanalicular limbal lamina (BELL) and corneoscleral bands due to the birefringent nature of the collagenous structures, increasing the contrast of SC. SC width was 99 ± 20Âµm varying in size over space, including a subject with SC narrowing in the inferior-temporal quadrant. DISCUSSION/SIGNIFICANCE: This clinically suitable gonioscopic OCT approach has successfully been used to image the human ICA in 3D in vivo, providing detailed characterization of the TM and SC as well as enhancing their contrast against their birefringent backgrounds by rotating the polarization of the imaging beam.

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Establishing the efficacy of naturally occurring endocannabinoid-like substance in an in vitro model of Fragile X Tremor/Ataxia Syndrome (FXT/AS)

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OBJECTIVES/GOALS: FXT/AS is a devastating, rare neurological syndrome that negatively impacts movement and cognition and is suspected to induce mitochondria dysfunction. Currently, no effective pharmacological treatments for FXTAS exist. The goal is to restore mitochondrial viability using endocannabinoid-like compounds in a cell culture model of FXTAS. METHODS/STUDY POPULATION: To establish a cell model of mitochondrial dysfunction, fibroblast baby hamster kidney (BHK-21) cell lines were treated with glucose oxidase (GluOx) at varying concentrations and times. Mitochondrial viability was assessed by the colorimetric Janus B Green Assay, which stains the mitochondria and enables assessment of cell numbers and the presence of oxygen in anchorage-dependent cell culture. Upon establishing this model of mitochondrial dysfunction, we next investigated the ability of three novel mitochondrial antioxidants (e.g., macamides) to protect mitochondrial viability. RESULTS/ANTICIPATED RESULTS: GluOx treatment of BHK-21 cells caused a dose- and time-dependent increase in oxidative stress. The data demonstrated significant disruption in the morphology of BHK-21 cells at a high glucose concentration, i.e., 40 nM, between 2 and 24 hours post-exposure. The morphology data were confirmed by the Janus B Green colorimetric assay. In examining the effects of glucose on mitochondrial viability, we demonstrated that at 15, 30, 35, and 40 nM, glucose significantly decreased mitochondria viability compared to the untreated, with 40 nM having the greatest effect. Under these conditions of mitochondrial dysfunction, co-incubation of the cells with the 0.5 uM MAM69 macamide attenuated the GluOx-induced increase in oxidative stress, with 0.5 uM MAM69 alone showing no effect on mitochondria viability. DISCUSSION/SIGNIFICANCE: This study illustrates the efficacy of macamides, natural occurring endocannabinoid like-compound, as novel prognostic and therapeutic candidates in the treatment of mitochondrial dysfunction that is associated with FXTAS. The use of BHK-21 fibroblast cells provides a rapid screening model to test for pharmacological therapeutic efficacy.

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Progression of silica-induced pulmonary fibrosis is arrested after selective ablation of Col1a1+ fibroblasts

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OBJECTIVES/GOALS: Silicosis is a highly fatal progressive fibrotic disease of the lungs characterized by accumulation and persistence of fibroblasts that excessively deposit Collagen1a1. We sought to eliminate Collagen1a1-expressing fibroblasts through a targeted genetic ablation strategy and hypothesized that this would arrest the progression of Silicosis. METHODS/STUDY POPULATION: Silicosis was induced with a single intratracheal (i.t.) instillation of silica particles (RESULTS/ANTICIPATED RESULTS: Targeted ablation of Col1a1 + fibroblast in established Silicosis resulted in a decrease in: 1) Col1a1+ fibroblasts by flow cytometry and within fibrotic nodules by immunofluorescent staining, 2) total lung collagen content by histology and hydroxyproline assay, 3) tissue-associated disease by microCT and an increase in arterial oxygen saturation by pulse oximetry. Cessation of targeted Col1a1+ fibroblast ablation resulted in a rebound effect in Silicosis disease progression. Following ablation, Col1a1+ fibroblasts expanded by proliferation (Ki67+) and total lung collagen levels returned to pre-ablation levels. DISCUSSION/

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SIGNIFICANCE: Silicosis is a often fatal disease with no FDA approved therapies. These results suggest that targeted loss of Col1a1+ fibroblasts in Silicosis is sufficient to arrest disease progression. Thus, it is essential to understand how targeted loss of pro-fibrotic fibroblasts can alter disease progression as a tool to develop novel therapeutic strategies.

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Interactions between tumor, age, and chemotherapy in cognitive impairments and neuroinflammation*

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OBJECTIVES/GOALS: We will use a novel syngeneic model of prostate cancer to examine impairments and uncover potential changes in inflammatory signaling in the brains of animals with and without tumors. We will then investigate the interaction between peripheral tumor, age, and chemotherapy on cognitive impairments and any accompanying neuroinflammation METHODS/STUDY POPULATION: Male Copenhagen rats (aged 3 or 10 months) were subjected to tumor fragment implantation (Dunning R2237G cells) or sham surgery. Once tumors were palpable, animals received either docetaxel (4.5mg/kg, intraperitoneal) or it's vehicle once every other day for 5 days (3 injections total) followed by a two-week recovery period. During this time, TNFa and IL-6 was quantified in plasma samples obtained once per week for two weeks. Hippocampal-mediated visuospatial and working memories were assessed using the novel object task and percent alternation in a y-maze, respectively. Afterwards, trunk blood and hippocampal tissue were isolated. TNFa and IL-6 protein was quantified in plasma. Hippocampal tissue was probed for markers of neuroinflammation, including increases in TNFa, IL-6, and reactive microglia RESULTS/ANTICIPATED RESULTS: The presence of a tumor alone produces deficits in hippocampal-mediated visuospatial memory and working memory regardless of treatment and persistent elevations in TNFa and IL-6 in plasma. Docetaxel administration also produces impairments in hippocampal-mediated visuospatial memory, but not in working memory. We anticipate these cognitive impairments will be accompanied by hippocampal neuroinflammation. We expect age and docetaxel chemotherapy to exacerbate working memory deficits and markers in hippocampal neuroinflammation, including increases in TNFa, IL-6 and reactive microglia DISCUSSION/SIGNIFICANCE: This study will provide insight into the interaction between tumor, age, and chemotherapy in impairments in visuospatial memories. This model provides a substrate upon which interventions can be tested to ensure the efficacy of the cancer treatment is maintained when treating these cognitive impairments.

Other

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Rapid SARS-CoV-2 testing with duplexed recombinase polymerase amplification and a bacteriophage internal control

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OBJECTIVES/GOALS: Current COVID-19 rapid molecular tests require cartridge-reader detection, expensive circuitry, and complex microfluidics making the most accurate tests unavailable to the masses. Here we present a rapid molecular diagnostic leveraging isothermal amplification and paper-based microfluidics for a low-cost ultra-sensitive COVID-19 assay. METHODS/STUDY POPULATION: We designed a reverse transcription recombinase polymerase amplification (RT-RPA) assay for the detection of SARS-CoV-2 and bacteriophage MS2 RNA. RT-RPA is a sequence specific, ultrasensitive, rapid isothermal DNA amplification technique that is well suited to home based testing due to its rapid assay time, robustness, ease of use, and readout options. RT-RPA reagents are added to a tube and incubated at 39Â°C in a fluorometer. Realtime fluorometer data gives results in under 15 minutes. This assay also provides visual detection via lateral flow readout with results in 23 minutes. RESULTS/ANTICIPATED RESULTS: We have developed a rapid multiplexed nucleic acid amplification assay with an internal process control for SARS-CoV-2 using single-pot RT-RPA. We screened 21 primer combinations to select primers that demonstrated excellent performance and target specificity against common respiratory viruses. We demonstrate the ability to multiplex SARS-CoV-2 and MS2 detection, utilizing MS2 as an internal process control for lysis, reverse transcription, amplification, and readout. We show duplexed detection using both fluorescence readout and visual readout using lateral flow strips. Duplexed fluorescence detection shows a limit of detection of 25 copies per reaction. Duplexed lateral flow readout shows a limit of detection of 50 copies per reaction DISCUSSION/SIGNIFICANCE: We developed a duplexed RT-RPA assay for SARS-CoV-2 with fluorescence or lateral flow readout. Our assay does not re-quire expensive reader, circuity, or fluid handling. The low material cost, temperature, and robustness make it ideal for a more accurate home-based COVID-19 diagnostic.

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A Human 3D Model of Duchenne Muscular Dystrophy Cardiomyopathy to Investigate Calcium Regulation and Mitochondrial Dysfunction

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OBJECTIVES/GOALS: We will use control- and DMD-engineered heart tissues to better model and investigate DMD cardiomyopathy. We will primarily assess cardiac calcium handling, mitochondrial function, and mitochondrial calcium handling, as calcium regulation and mitochondrial function are known to be affected in DMD. METHODS/STUDY POPULATION: We will use patient-derived stem cells, differentiated into cardiomyocytes in bioprinted 3D heart tissue muscle chambers to better model DMD cardiomyopathy. We will look at calcium handling and general mitochondrial function, as well as mitochondrial calcium handling, using a novel multifunctional genetic probe I previously developed allowing for simultaneous observation of cytosolic and mitochondrial calcium in real time. Optical mapping will also be used for tissue-level analysis. We will establish the functional differences at baseline, and then progress heart failure in the tissues to see how the abnormalities seen in the DMD tissues may get worse. Finally, we will investigate the effects of early restoration of dystrophin function on the effects of DMD cardiomyopathy development. RESULTS/ANTICIPATED RESULTS: We anticipate that DMD tissues will show more irregular/abnormal calcium handling, as seen in 2D hiPSC-CMs, as well as disruptions to mitochondrial function and ultrastructural development, as well as a decreased synchronization between cytosolic and mitochondrial calcium dynamics. We anticipate that these

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