progression, showing remarkable early biomarker potential. These findings lay the groundwork for early detection and innovative therapies to halt DKD and improve patient outcomes.

38

Biomarkers and neurocognitive impairment in traumatic brain injury patients

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OBJECTIVES/GOALS: This study aims to explore the relationship between plasma biomarkers (GFAP, NF-L, and IL-1β) and cognitive impairment in moderate to severe TBI patients. We will assess biomarker levels and their link to neurocognitive outcomes at acute and chronic stages of injury. METHODS/STUDY POPULATION: We will recruit 100 patients aged 21 years and older with moderate to severe TBI (Glasgow Coma Score 3–12) from a trauma hospital. Blood samples will be collected at 24–72 hours post-injury and again at 3 and 6 months. Plasma levels of GFAP, NF-L, and IL-1β will be measured using multiplex ELISA. Neurocognitive tests will be administered at 3 and 6 months to assess cognitive function. Correlations will be made between biomarker levels, neurocognitive performance, and disability scores (Disability Rating Scale and Glasgow Outcome Scale). Exosome isolation from plasma will allow for detailed analysis of astrocyte-derived biomarkers and their association with long-term cognitive impairment and recovery. RESULTS/ANTICIPATED RESULTS: We anticipate that plasma levels of GFAP, NF-L, and IL-1β will be elevated in the acute phase of moderate to severe TBI and will correlate with injury severity. At 3 and 6 months, higher levels of IL-1β, in particular, are expected to be strongly associated with cognitive deficits. We also anticipate that biomarkers in astrocyte-derived exosomes will provide more specific insights into long-term neuroinflammation and its impact on cognitive function. These findings could pave the way for targeted, personalized interventions to improve recovery in TBI patients. DISCUSSION/SIGNIFICANCE OF IMPACT: This research focuses on inflammation's role in cognitive impairment and disability in TBI patients. We propose using multiple biomarkers – GFAP, IL-1β, NF-L – paired with advanced techniques like exosomes and multiplex analyses to identify novel therapeutic targets, aiming for personalized treatment strategies, as well as prognosis.

39

Nanoscale imaging of pT217-tau in aged rhesus macaque entorhinal and dorsolateral prefrontal cortex: Evidence of interneuronal trafficking and early-stage† neurodegeneration

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OBJECTIVES/GOALS: pT217-tau is a novel fluid biomarker that predicts onset of Alzheimer's disease (AD) symptoms, but little is known about how pT217-tau arises in brain, as soluble pT217-tau is dephosphorylated postmortem in the humans. Aging macaques naturally develop tau pathology with the same qualitative pattern and sequence as humans, including cortical pathology. METHODS/STUDY POPULATION: The etiology of pT217-tau in aging brains can be probed in rhesus macaques, where perfusion fixation allows capture of phosphorylated proteins in their native state. We utilized multi-label immunofluorescence and immunoperoxidase and immunogold immunoelectron microscopy to examine the subcellular localization of early-stage pT217-tau in entorhinal cortex (ERC) and dorsolateral prefrontal cortex (dlPFC) of aged rhesus macaques with naturally occurring tau pathology and assayed pT217-tau levels in blood plasma using an ultrasensitive nanoneedle approach. RESULTS/ANTICIPATED RESULTS: pT217-tau labeling is primarily observed in postsynaptic compartments, accumulating in: 1) dendritic spines on the calcium-storing smooth endoplasmic reticulum spine apparatus near asymmetric glutamatergic-like synapses and 2) in dendritic shafts, where it aggregated on microtubules, often "trapping" endosomes associated with Aβ42. The dendrites expressing pT217-tau were associated with autophagic vacuoles and dysmorphic mitochondria, indicative of early neurite degeneration. We observed trans-synaptic pT217-tau trafficking between neurons within omega-shaped bodies and endosomes, specifically near excitatory, but not inhibitory synapses. We also examined pT217-tau in blood plasma in macaques across age-span and observed a statistically significant age-related increase in pT217-tau. DISCUSSION/SIGNIFICANCE OF IMPACT: We provide direct evidence of pT217-tau trafficking between neurons near synapses to "seed" tau pathology in higher brain circuits, interfacing with the extracellular space to become accessible to CSF and blood. The expression of pT217-tau in dendrites with early signs of degeneration may help to explain why this tau species can herald future diseases.

40

Impact of feminizing hormone therapy on rectal mucosal HIV target cells in Thai TGWSM†

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OBJECTIVES/GOALS: Transgender women who have sex with men (TGWSM) have higher HIV risk. The rectal mucosal (RM) immune environment of TGWSM who choose feminizing hormone therapy (FHT) has been shown to be distinct from the RM of cisgender men who have sex with men (MSM). We studied the impact of FHT on the adaptive immune cellular composition of the RM. METHODS/STUDY POPULATION: We sampled cross-sectional and longitudinal cohorts of TGWSM and cisgender MSM from The Silom Clinic in Bangkok, Thailand from December 2020 to December 2023. We included participants aged >18 years, all cisgender MSM and TGWSM with FHT levels in the therapeutic range for cisgender women. We performed RM biopsies and analyzed the adaptive immune cell characteristics via flow cytometry. We will perform binary linear regression to assess the association between systemic FHT levels and the percentage of CD4+ T cells expressing key biomarkers. Primary outcomes include the percentage of CD4+ T cells that express CCR5, with a secondary outcome of the percentage of CD4+ T cells that express Ki67. RESULTS/ANTICIPATED RESULTS: The cross-sectional cohort included 100 TGWSM on FHT and 50 cisgender MSM. The longitudinal cohort included 25

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TGWSM who were initiating FHT. Similar primary and secondary outcomes are to be elucidated in both cohorts. We anticipate the RM environment of TGWSM using FHT in both cohorts compared to the RM environment of cisgender MSM in the cross-sectional cohort will be associated with greater percentages of activation/co-receptor expression of CD4+ T cells that express biomarkers of interest. In the longitudinal cohort, we similarly anticipate increased percentages and activation/co-receptor expression of CD4+ T cells expressing biomarkers of interest in TGWSM after in comparison to before initiating FHT. DISCUSSION/SIGNIFICANCE OF IMPACT: This is the largest study of its kind to compare HIV target cells in RM of TGSWM, which challenges prevailing perspectives suggesting to group cisgender MSM with TGWSM. Anticipated results will inform HIV prevention strategies and future vaccine studies in this high-risk population.

41

Elucidating the altered metabolism of NAD+ in the selective targeting of glioblastoma with the NQO1-activated drug β-lapachone*†

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OBJECTIVES/GOALS: NAD+ synthesis is enhanced in glioblastoma (GBM) allowing GBM to resist chemotherapy. NQO1 is upregulated in GBM and may be targeted by β-lapachone (β-lap) to induce NAD-depletion and cell death. This project investigates NQO1 as a selective target for GBM and the contributions of glucose and uridine to NAD+ synthesis. METHODS/STUDY POPULATION: Survival Studies and NQO1 expression. RNA-seq and survival data from TCGA of glioma patients (n = 667) was obtained using the UCSC Xena platform. Western blots were utilized to determine expression levels of NQO1 and NAMPT in normal human astrocytes, U87 cells, and patient-derived GBM cell lines. Immunocytochemistry: γ-H2AX staining was used to evaluate β-lap induced DNA damage. NQO1-dependence was evaluated with the NQO1-inhibitor dicoumarol. Cytotoxicity measurements. Cells were exposed to β-lap and other inhibitors, and cell survival was determined by trypan-blue exclusion assay. Co-culturing experiments were performed with fluorescently labeled U87 cells and unlabeled astrocytes. NAD+ quantification. Intracellular NAD+ was acid extracted and quantified by an enzyme-cycling reaction. RESULTS/ANTICIPATED RESULTS: NQO1 overexpression is linked to decreased survival in glioma patients. In glioma patients, high NQO1 expression was associated with a decreased overall survival and high-grade tumors. β-lap induces selective NAD+ depletion and cell death in NQO1-expressing GBM cells. Western blots demonstrate NQO1 expression to be elevated in GBM cell lines compared to normal human astrocytes. β-lap induces NQO1-dependent NAD+ depletion and cell death in GBM compared to astrocytes in mono- and co-culture experiments. Glucose and uridine facilitate NAD+ regeneration in GBM. We demonstrate extracellular glucose and uridine facilitate NAD+ regeneration and cell survival in β-lap exposed GBM cells. Utilizing inhibitors, we determined that glucose and uridine facilitate NAD+ regeneration through the NAD+ salvage pathway. DISCUSSION/SIGNIFICANCE OF IMPACT: GBM is the most common primary adult CNS tumor with a median survival of 14 months. Despite significant research in therapeutic strategies, treatment has not improved in 2 decades. There is a significant need to discover new targets that may improve GBM treatment. We demonstrate here that targeting NQO1 with β-lap induces selective GBM toxicity.

43

Calcium channel blockers for treatment of triple-negative breast cancer*

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OBJECTIVES/GOALS: Triple-negative breast cancer (TNBC) is a highly aggressive form of breast cancer (BC) with limited treatment options. Mortality rate is especially high in African American (AA) women of reproductive age. High levels of intracellular calcium (Ca2+) have been shown in TNBC cells. This study is to investigate Ca2+ channel blockers (CCBs) as therapy for TNBC. METHODS/STUDY POPULATION: Two human TNBC cell lines obtained from ATCC – HCC1806, and MDA-MB-453 are treated with CCBs, Cilnidipine (Cil), and Mibefradil (Mib), in a concentration- and time-dependent manner. Cell proliferation assays are performed by the MTS cell viability assay. Intracellular Ca2+ levels are measured using the fluorescent dye: Fluro 4-AM. Apoptosis is determined by flow cytometry using Annexin V staining and mitochondrial permeability will be assessed by the Mito JC-1 assay. Expression of Ca2+ signaling genes will be quantitated by real-time polymerase chain reaction (RT-PCR). Potential pathways of CCB efficacy will be identified by ingenuity pathway analysis (IPA). RESULTS/ANTICIPATED RESULTS: Our findings show both CCBs decrease cell proliferation in a concentration- and time-dependent manner to a maximum of 80% vs. control in both TNBC cells. Flow cytometry findings on both TNBC cells treated with both drugs at 20 μM for 24 hours depicts late apoptosis. Interestingly, Mib did not change the intracellular Ca2+ level in HCC1806 cells yet decreased in MDA-MB-453 cells by fivefold, while Cil increased the intracellular Ca2+ level in both cells almost twofold. It is anticipated that Mito JC-1 assay depict decreased mitochondrial potential in both cells. For reverse transcription polymerase chain reaction, it is anticipated that CCB treatment will increase transient receptor potential Ca2+ channels and decrease voltage-gated Ca2+ channels in both cells. IPA analysis is expected to show apoptotic pathways are involved in TNBC via CCB treatment. DISCUSSION/SIGNIFICANCE OF IMPACT: TNBC lacks the estrogen receptor, progesterone receptor, and human epidermal growth factor receptor 2. Treatment options for TNBC remain severely limited. Our findings that both Cil and Mib can inhibit proliferation of human breast cancer cell lines indicate repurposing CCBs as treatment for TNBC warrants further investigation.

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