

SPECIFIC AIMS – PROJECT 2 – PRECLINICAL MODEL FOR ANTIEPILEPTOGENIC THERAPY SCREENING IN POST-TRAUMATIC EPILEPSY

There is no clinically validated anti-epileptogenic (AEG) treatment to prevent posttraumatic epilepsy (PTE). The scientific premise of this project is: targets and biomarkers for treatment implementation, in combination with the biomarkers of PTE epileptogenesis (PTEgenesis) identified in *Project 1*, will guide rigorous randomized preclinical trials of potential AEG interventions to prevent PTE in a standardized animal model of traumatic brain injury (TBI) / PTE. Animal studies implicate inflammatory pathways, hippocampal hemorrhages, neurodegenerative pathologies, and changes in neuronal ion channel expression and function in epileptogenesis. Small human cohorts show increased susceptibility to either hemorrhagic injury or PTE in persons with polymorphisms of the IL-1 receptor antagonist (IL-1ra) gene⁴² or of the IL-1 β gene respectively²⁵. Biomarkers of epileptogenesis and response to treatment can significantly optimize efforts to identify novel AEG treatments, select and monitor the target population, inform decisions on the type, dose, and timing of treatments and also predict outcomes^{27, 113}. Bragin et al have evidence for pathologic high frequency oscillations (pHFOs) and repetitive HFO and Spikes (rHFOs) as early biomarkers of PTEgenesis in the lateral fluid percussion injury (LFPI) model, present during the 1st week post-TBI¹⁵. We have formed a collaborative, multicenter network with the overall goal to implement rigorous, multicenter preclinical studies to identify AEG therapies for PTE. We will use a blinded, randomized, vehicle-controlled study design, using common data elements (CDEs), harmonized methods for data collection, outcome assessment, and unbiased data analysis, across the four collaborating centers (Einstein, Melbourne, UCLA, UEF) and a Data Safety Monitoring Board (DSMB) to test novel candidate AEG therapies in PTE. Pharmacokinetic (PK) modeling for dose optimization will be done by Dr. Cloyd. We will screen four drugs that target different key processes implicated in acquired epileptogenesis: hyperphosphorylated tau (h-tau) (sodium selenate)⁹³, the IL-1 β pathway (IL-1ra and VX765)^{7, 14, 43, 68, 71, 88, 91}, iron deposition (deferiprone⁹) and low threshold calcium channels (Z944)¹⁷. All have been tested for safety in early stage clinical trials, so a positive result can be rapidly translated into clinical AEG trials. We will compare the effects of these drugs on biomarkers with an antiepileptic drug commonly used in patients post-TBI, levetiracetam^{13, 16, 31, 46, 60, 85, 89, 97, 100, 112, 116}. We will select the lead treatment protocol with the best target relevance and PTEgenesis biomarker modification profile to advance in a multicenter preclinical AEG trial. The DSMB with input by the *Public Engagement Core* will advise on future transition of the lead compound to a clinical AEG trial by *Project 3*. **Overarching hypothesis:** anti-PTEgenesis can be (a) effected by targeting early stage (1st post-TBI week) epileptogenic pathologies and (b) predicted by the treatment response of early stage MRI/EEG/plasma biomarkers of PTEgenesis identified in *Project 1*.

Specific Aim 1: Apply a multi-modality early stage post-TBI screening protocol for selecting early onset candidate AEG treatments in the adult rat LFPI model, using the following criteria:

Specific Aim 1A: define target relevance and treatment window for the tested drugs;

Specific Aim 1B: evidence for modification of early stage candidate epileptogenic targets by the tested drugs;

Specific Aim 1C: prevention of early post-TBI seizures and EEG biomarkers of PTEgenesis (pHFOs, rHFOs, spikes), as a function of dose and treatment exposure;

Specific Aim 1D: normalization of early plasma biomarkers of PTEgenesis, as a function treatment exposure.

Hypothesis 1: A multi-modality early stage post-TBI screening platform for target relevance and persisting modification of early stage post-TBI seizures and EEG/plasma biomarkers beyond treatment exposure will help select optimal treatment protocols for candidate AEG treatments for PTE.

Specific Aim 2: Determine whether an optimized targeted treatment selected by the early stage post-TBI multi-modality screening process can:

Specific Aim 2A: have AEG effects in adult rats with LFPI when given during defined therapeutic windows;

Specific Aim 2B: modify the MRI/ EEG/plasma biomarkers of PTEgenesis identified in *Project 1*, in a manner that can predict its AEG effect.

Hypothesis 2: Targeted early stage treatments that have lasting modifying effects on relevant targets and MRI/EEG/plasma TBI biomarkers also have lasting AEG mitigating effects against PTEgenesis.

Specific Aim 3: Create a Rodent Biospecimen Repository (BioBank)

Hypothesis 3: A Rodent Biospecimen Repository will serve as a resource for future research to identify targets and biomarkers that will support future AEG trials.

Deliverables: (1) Validate a rapid multi-modality screening platform for optimization and selection of lead AEG treatments for PTE. (2) Identify early stage MRI/EEG/plasma biomarkers predicting AEG treatment response. (3) Identify at least one effective AEG treatment in the animal model of PTE that is suitable for clinical trials. (4) Create a Rodent Biospecimen Repository (BioBank) for future AEG target/biomarker discovery in PTE.