enhanced nuclear staining localized to astrocytes in association with the induction of astrogliosis. These findings strongly implicate the STAT3 pathway in astrocytes as a key signaling pathway for astrogliosis.

PS 373 BAC-TRAP Technology in Neurotoxicology: The ALDH1L1 BAC-TRAP Mouse As a Tool to Assess Astrocyte Specific Responses to Neural Injury

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A central problem in neurotoxicology is detecting the selective and unpredictable damage to specific cells produced by toxic agents and mixtures. Evaluating astrogliosis overcomes this problem because reactive astrocytes show the location of toxicant-induced damage occurring anywhere in the CNS. Enhanced expression of GFAP is a hallmark of reactive astrocytes; however, few other astrogliosis biomarkers are known. Thus, determining the specific in vivo transcriptomic profile of astrocytes under control and reactive conditions will allow for the identification of additional astrogliosis biomarkers. Heintz and Greengard (2008) introduced BAC-TRAP (translating ribosome affinity purification) technology as an approach for identification of cell-type-specific responses in vivo. Here, we implemented this approach for the assessment of mRNA translation specific to astrocytes responding to damage caused by known neurotoxicants. ALDH1L1 is an enzyme thought to serve a housekeeping function in astrocytes. Using hippocampal and striatal damage due to TMT and MPTP, respectively, we evaluated the localization and response of ALDH1L1 compared to astrogliosis seen by GFAP immunohistochemistry and ELISA. Staining of ALDH1L1 revealed localization to astrocytes after TMT and MPTP, while immunoblots of ALDH1L1 revealed basal expression of this protein but little enhanced expression after MPTP and TMT, confirming it to be an astrocytic "housekeeping" gene/protein. We then used the ALDH1L1 BAC-TRAP mouse to evaluate astrocytic-specific mRNA with expression analyses by gene array in control conditions. Tissue was subjected to TRAP utilizing an eGFP antibody that only binds to actively translating RNA in astrocytes. This revealed numerous genes in "resting" astrocytes, including genes previously localized to astrocytes (e.g., GFAP), as well as novel genes to this cell type (e.g. PHOX2A). MPTP expression data are reported in accompanying poster. The ALDH1L1 BAC-TRAP mouse represents a promising tool to investigate astrocytic responses to neural injury.

PS 374 BAC-TRAP Technology in Neurotoxicology: Assessing the Astrocyte Response to MPTP-Induced Damage in the ALDH1L1 BAC-TRAP Mouse

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A central problem in neurotoxicology is detecting the selective and unpredictable damage to specific cells produced by toxic agents and mixtures. Evaluating astrogliosis overcomes this problem because reactive astrocytes show the location of toxicant-induced damage occurring anywhere in the CNS. Enhanced expression of GFAP is a hallmark of reactive astrocytes; however, few other astrogliosis biomarkers are known. Heintz & Greengard (2008) introduced BAC-TRAP (translating ribosome affinity purification) technology that allows the characterization of the actively translating transcriptome of a particular cell type. For example, ALDH1L1 is an astrocyte specific enzyme thought to be a housekeeping gene. Thus, ALDH1L1 BAC-TRAP mice can be used to characterize the transcriptome of astrocytes under various conditions. To begin to characterize additional biomarkers of astrogliosis occurring in response to neurotoxic damage ALDH1L1 BAC-TRAP mice were given a single 12.5 mg/kg s.c. dose of MPTP, a well characterized dopaminergic neurotoxicant that induces significant astrogliosis, Striatal tissue was obtained at 12, 24, and 48 hrs following a single s.c. dosage of saline or 12.5 mg/kg MPTP. Striatal tissue was subjected to TRAP utilizing an eGFP antibody that only binds to actively translating RNA in astrocytes. Changes in the actively translating RNA induced by MPTP damage were determined by microarray (Illumina MouseWG-8 v2 Expression BeadChip) and the dataset interrogated using Ingenuity Pathway Analysis (IPA). MPTP induced robust transcriptome changes in genes previously identified as astrocyte specific (e.g., 403, 399, 804 fold increases in TIMP1 at 12, 24, 48 hrs, respectively) as well as others not previously considered astrocyte-specific (e.g., 219, 203, 3.69 fold increases in PHOX2A at 12, 24, 48 hrs). Our data indicate the BAC-TRAP technology can be used to identify additional biomarkers of astrogliosis and will aid in characterizing various astrocyte phenotypes.

PS 375 Human iPSC Neurons: *In Vitro* Models to Predict Clinical CNS Toxicity

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A major cause of drug attrition from clinical trials is neurotoxicity, a problem stemming from the lack of a relevant model to predict human CNS safety. Current neurotoxicity assessment depends on in vivo animal models, which are time-consuming and expensive. In vitro models consist of immortalized cell lines and primary rodent cultures, which are often a poor representation of human physiology. There is need for high throughput, human-based assays that can narrow down drug candidate panels for in vivo animal studies, and augment those studies with human data before initiation of clinical trials. We chose to establish such assays with human induced pluripotent stem cells (iPSCs) that have been differentiated into mature neurons. These iPSC neurons offer a renewable source of mature cells, relevant to human drug safety, and free from ethical concerns. CNS toxicity results from disruption of complex neural networks, which is why our test panel addresses neuronal activity, in addition to neurite outgrowth, cytotoxicity and mitochondrial stress. Few studies tackle neuronal activity in a high throughput system, and fewer still integrate changes in activity with other functional toxicity endpoints. Our novel platform will combine a semi-high throughput multi-electrode array (MEA) with high throughput neurotoxicity assays for potential screening of drug candidates. By using a panel of test compounds known to cause CNS toxicity in vivo we put forward a panel of in vitro human physiological-based assays to evaluate the translatability of drug safety.

PS 376 Live Cell-Based Assay Using GFP+ Human Stem Cell-Derived Neurons

J. N. Le. *ArunA Biomedical Inc, Athens, GA.* Sponsor: S. Stice.

Adverse outcome pathways (AOP) are conceptual frameworks that portray existing knowledge concerning the linkage between a molecular initiating event and an adverse outcome. High throughput screening (HTS) and high content screening (HCS) approaches seek to gain a greater perspective of the AOP, leading to more efficient and reliable identification of adverse outcomes of compounds and chemicals. However, HCS assays often utilize primary or stem cell sources which are not amenable to large scale screening and can require extensive cell culture, fixation and permeation, and immunocyotochemistry prior to imaging. Thus, major costs are associated with 96-well based high content neurotoxin screening. In addition, preserving or fixative steps can alter cell architecture and a new plate of cells is required for each time point, thus introducing variability to data. This new human cell-based assay provides a scalable solution to the previously labor intensive, single-end-point, and hence limited nature of neurotox assays, while preserving the breadth and quality of data. The assay utilizes clonal, embryonic stem cell derived, human neural cells that have been genetically modified with a non-viral vector encoding a Green Fluorescent Protein (GFP) reporter gene driven by a ubiquitous promoter. Previously, hN2™ human neuronal cells faithfully reproduced early brain development, providing a cell model > 95% positive for neuronal markers while demonstrating higher sensitivity to neural toxins than mouse cortical neurons, and our new GFP+ neurons are equally sensitive. Additionally, previous studies using rodent primary cells examined mixed and unknown ratios of neural cells, often at various stages of proliferation and differentiation. These new GFP+ neurons are differentiated from a clonal GFP+ neural progenitor population, thus removing variability from cultures while maintaining an adherent monolayer amenable to high content imaging. By eliminating the need for fixing and staining cells, these GFP+ human neurons provide a scalable means to analyze neurite outgrowth in live cells spanning the course of hours to days following exposure to test compounds.

PS 377 A Functional Phenotypic Screen for Synapse Formation in Human iPS-Derived Neurons

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Exposure to low levels of neuronal toxicants may affect neuronal structure subtly, leading to developmental and cognitive impairment. Synaptic transmission is the fundamental unit of communication between healthy neurons. We used human induced pluripotent stem (iPS) neurons grown in low volume 384 well dishes for 1-3 weeks to measure synapse formation. Work done in other laboratories has demonstrated that these cells are capable of electrical transmission. Therefore, we used antibodies to measure expression levels of the pre- and post-synaptic proteins, Synapsin I and post-synaptic protein 95 (PSD95), respectively. Furthermore, using advanced image analysis algorithms we masked neurite regions demarked by

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