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**Quarterly Report
Human Neural Cell-Based Biosensor**

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Summary

Human neural progenitor cells have a strong potential for use as cell-based biosensors for environmental toxins. The overall goal of this project is to develop a human neural cell based biosensor using ArunA's neural progenitor cell line, hNP1™. In this report, we detail the progress in development for the following areas: (1) neural progenitor isolation from induced pluripotent stem cells, (2) directed differentiation of progenitors into dopaminergic neurons, motoneurons and astrocytes using defined medium conditions, (3) cell-based methods to detect botulinum toxin, and (4) fluorescence based assays for proliferation, cell migration, mitochondrial function, reactive oxygen species generation and apoptosis as sensor elements.

(1) Neural progenitor isolation from induced pluripotent stem cells

Our previous studies identified three particular cell surface markers in our hNP1™ neural progenitors that could be used to isolate neural progenitors from mixed populations, such as induced pluripotent stem cells (iPSCs). We also developed lentiviral based methods to generate iPSCs in partnership with Thermo Scientific. We are now working on generating new iPSC-derived neural progenitor lines using the markers we identified.

(2) Directed differentiation using defined media conditions

We are continuing development of methods for directed differentiation of neural progenitors into dopaminergic, motoneurons and astrocytes. Substantial progress has been made with astrocyte differentiation procedure. We are now working to optimize the differentiation protocol and establish robust assays to thoroughly phenotype the differentiated cells. Work continues on optimizing the differentiation protocols for dopaminergic and motor neurons. We have set up a phenotype screening assay for dopaminergic differentiation and are doing the same for motor neurons. We have also developed a dopaminergic progenitor cell line for potential commercial distribution; beta testing on this cell line will begin shortly.

(3) Development of cell based methods to detect botulinum toxin

There has been substantial progress in the development of an hN2™ cell based sensor for botulinum toxin (BoNT). Western blotting experiments firmly establish the expression of SNAP-25, the target for BoNT, in hN2™ cells. Treatment of hN2™ with BoNT-A caused the cleavage of SNAP-25 in a dose-dependent fashion. This cleavage was blocked by co-treatment with inhibitors of BoNT action, indicating the specificity of the BoNT effect. A manuscript of these findings has been written and will be submitted for publication shortly.

(4) Development of fluorescence based assays as sensor elements for neurotoxicity

We continue to make rapid, substantial progress in developing fluorescence based assays as sensor elements in cell-based biosensors.

We have optimized conditions for using both hNP1™ and hN2™ cells with the Alamar Blue assay. This assay measures mitochondrial reductase activity in the non-proliferative hN2™ cells and both mitochondrial health and cell number in the proliferative hNP1™ cells. The next step will be to test a small set of known neurotoxins to complete proof-of-concept studies for this assay. We have also begun development of assays that measure cellular ATP levels in hNP1™ and hN2™ cells. Moreover, development of a reactive oxygen species (ROS) assay using novel dyes continues; current efforts here focus on optimization of the dye chemistry.

In collaboration with Platypus Technologies, we have developed an HTS-amenable cell migration assay using hNP1™ neural progenitors. Proof-of-concept studies show that cytochalasin D, an actin polymerization inhibitor, can block hNP1™ migration while certain individual growth factors stimulate migration. The next step will be to test a small set of known neurotoxins to complete proof-of-concept studies. Our progress to date has been presented in poster format at the American Society for Cell Biology meeting in Philadelphia, PA on December 14th 2010 and at the Society for Toxicology meeting in Washington, DC on March 8th 2011 and will be presented at the Hilton Head Regenerative Medicine Conference in Hilton Head, SC on March 17th 2011. We are also investigating the potential for co-marketing the migration assay with our neural progenitors as a ready screening tool for investigators interested in drug discovery as well as toxicology.