Neural restricted, FAC-sorted, human neural stem cells to treat traumatic brain injury

Reporting Period: Year 1

In the first year of this Early Translation Award for traumatic brain injury (TBI), our goal was to develop the stem cell lines necessary to begin testing of stem cells in an animal model of TBI in year 2. If we are fortunate to demonstrate that the stem cell products are effective in animal models of TBI, these cells will need to be grown in a way that is acceptable to the FDA for future use in man. Xenofree means that the cells are not exposed to possible animal product contaminants (e.g. serum or blood products) and that every component that the cells were exposed to is chemically defined and can be traced to the original source. First, we obtained three separate embryonic stem (ES) cell lines from Sheffield, UK and imported them to the United States. These lines where then thawed and grown in “xenofree” cell culture conditions. Many labs have had difficulty transitioning human ES cells to xenofree conditions without introducing genetic defects in the cell lines or killing the cells. We were able to work out the correct conditions for all three ES cell lines to be grown xenofree. We were also successful in converting two of the three ES lines into neural stem cells (the subtype of stem cell needed for transplanting into brain tissue). These neural stem cells (NSCs) were further purified by labeling them for a stem cell surface marker present on NSCs (called CD133) and then magnetically sorting out just the CD133 positive cells and continuing to grow them. This approach is thought to enrich the stem cell population for NSCs and eliminate any remaining non-differentiated ES cells (which have an added risk of forming tumors if injected into animals or man). We successfully “sorted” both Shef cell lines and we now have four candidate populations of sorted and unsorted Shef4 and Shef6 cells. We grew these cells in culture and tested whether they differentiated into neuronal precursor or glial precursor cells. Quantification of the type of cells they turn into after 2 weeks showed that the four cell populations were different. These differences were even more apparent when looking at the cells in a microscope. At the end of year one, we have four different populations of neural stem cells which are growing in defined xenofree conditions, are frozen down in master cell banks, and which are genetically normal. There are sufficient quantities of these human neural stem cells (hNSCs) to complete the remaining aims of the ETA grant over the remaining two years. In the first year we also trained staff in the surgical procedures required to produce controlled cortical impact injuries in Athymic nude rats (ATNs), a type of rat that has no immune system. These procedures were necessary because no one has ever used ATN rats to model TBI. Our goal in year two is to transplant hNSCs into rats with TBI. If the rats had a normal immune system, their bodies would detect the foreign human cells and reject them. Also, because no one has ever tested TBI in ATN rats, we needed to find out if ATN rats respond like regular rats to the injury and if they have similar, predictable deficits on the cognitive tasks we plan to use in year 2 to measure whether hNCSs improve the animal’s recovery or not. This training and these pilot tests in ATN rats were completed successfully. Finally, the hypothesis is that by “sorting” the hNSCs to be CD133 positive, we are making the stem cell population safer for transplantation. This will be tested in year 2 using a tumorigenicity assay. We worked out how to conduct these assays in year 1 using a population of ES cells known to cause tumors so that we will have a positive control to compare the hNSCs to in year 2. In summary, we met all of our goals and milestones for year 1 and are poised to make good progress in year 2.

Reporting Period: Year 2

The goal of this project is to take three human embryonic stem cell lines (Shef3, Shef4, and Shef6), transition them to multipotent neural stem cell (hNSC) populations, sort/enrich these hNSC stem/progenitor populations, and then test these cell lines for efficacy in a rat model of controlled cortical impact (CCI) model of traumatic brain injury (TBI). Our strategy is to develop xenofree culture methods for the transition of hESCs to NSCs, use magnetic activated cell sorting (MAC) for the cell surface markers CD133+/CD34- to enrich the hNSC populations for stem/progenitor cells, test these sorted vs unsorted cell lines in tumorigenicity assays, and use the best two non-tumorigenic lines in a CCI model of TBI. Efficacy will be assessed on a battery of cognitive tests, via a reduction in spontaneous seizure, and in histological outcomes. At the Two Year time-point in the grant, we have (A) generated 6 hNSC populations, (B) completed short-term teratoma assays which demonstrate that none of our hNSC populations form teratomas in either of two transplantation sites (sub cutaneous into the leg or intracranially into the brain, (C) established parameters for graded contusion traumatic brain injuries in ATN rats that (D) yield long-term (≥8 weeks) deficits in both learning and memory on the Morris Water Maze. (E) We have also determined that TBI yields an altered response on a conditioned taste aversion task (neophobia) and on the elevated plus maze compared to sham controls. (F) Determined that unsorted hNSCs (both
Shef4 and Shef6) do not survive long-term in uninjured brain and (G) transplanted two large cohorts of TBI injured animals with Shef6 sorted NSCs of high passage. Shef6 sorted hNSCs of low passage, sham animals, and animals with a vehicle control. These two cohorts are too large to run simultaneously, so they are being run in parallel. Animals from both cohorts will complete functional all assessments by the end of June 2013.

**Reporting Period:** Year 3

Summary: We have very promising preclinical efficacy data in a rodent model of traumatic brain injury (TBI) using stem cells as a potential therapeutic. We have found that intra-cranial transplants of Shef-6 derived human neural stem cells (hNSCs) appear to induce improvement on two different behavioral domains after long-term (>2 months) survival. Importantly, Shef-6 hNSCs did not form tumors when transplanted at high doses into naïve brain. Shef-6 hNSCs are xenofree, GMP compatible, suitable for use in man (the donor and cells were certified to be free of HIV, Hepatitis A, B, C, HTLV, EBV, CMV, and are mycoplasma free). Furthermore, Shef-6 is on the FDA embryonic stem cell registry, enabling future Federal funding of their clinical testing in man if warranted. Specifically, we have demonstrated long-term efficacy in a moderate to severe controlled cortical impact (CCI) model of TBI using Shef-6 derived hNSCs on both a cognitive task (MWM Reversal Learning) and an emotional task (Elevated Plus Maze for anxiety). This dual improvement across cognitive and emotional domains is unique to the field and supports external validity of the model. These behavioral findings need to be correlated with quantification of the total number of surviving human cells and their terminal cell fate (whether the hNSCs differentiated into neurons, oligodendrocytes, or astrocytes) to confirm efficacy. Stereological quantification is currently ongoing and very labor intensive. If the correlation between surviving cells and cognitive improvements holds up after the quantification is complete, these findings will support a future Preclinical Development Award application to CIRM. Additionally, we are the first group to couple kindling and TBI to model the critical complication of post-TBI seizures. Traditional TBI models yield seizures in less than 20% of rodents, making hNSC studies cost prohibitive. Coupling kindling with TBI ensures that all animals start with a hypersensitive neural circuit so hNSCs can be tested in a more relevant environment; we will be ready to begin this important kindling test coupled with hNSCs in the Spring of 2014. These studies have paved new ground for a field with huge economic costs, no treatments, and no GMP qualified ES based solutions on the horizon.

**Reporting Period:** Year 4/NCE

Traumatic Brain Injuries (TBI) are the leading cause of death and disability in the young population. Falls resulting in injury to the brain are also a major problem in the elderly. The rate of TBI is greater than the number of people diagnosed with brain, breast, colon, lung, and prostate cancers combined, yet nationally the US invests 95% more research dollars on cancer compared to TBI. 1.7 million new cases of TBI occur each year, at an economic cost of $60 billion. Extrapolating to California (12% of US population), there are ~210,000 new cases of TBI a year in our state, with a yearly cost that exceeds $7 billion. TBI results in permanent long-term deficits, including memory impairments and emotional disfunction, that affect both the patient and their families. There are no treatments to alleviate the long-term consequences of TBI. Yet a small reduction in damage, restoration of just some nerve fibers to their targets beyond the injury, or moderate improvement in learning, memory, or emotional outcomes could have significant implications for an individual’s quality of life. Our hypothesis was that human neural stem cells (hNSCs) might alleviate some impairments associated with TBI in a new animal model of neurotrauma. Our first goal was to grow hNSCs under cell culture conditions free from contamination of non-human products (referred to as “xenofree”), and then sort these cells based on cell surface markers known to be present in high concentrations on migratory neural stem cells (and not other byproducts of the culture conditions). Our second goal was to develop an animal model of TBI with long-lasting cognitive and emotional deficits; this animal model had to be “immuno-deficient”, or lacking a functional immune system, so that “foreign” human cells would not be rejected. Long-lasting deficits were need so that there would be a sufficient time window of dysfunction to allow the hNSCs to divide, migrate through the brain, and possibly restore function. If animals recover function too quickly on their own (as happens in some models of neurotrauma), then there would not be a large enough difference between control animals and injured animals to detect an effect of the hNSCs or not. Goal three was to test the therapeutic effects of hNSCs in this model. Finally, because a large number of people with TBI also experience seizures long after the initial injury, our forth goal was to combine “kindling” with TBI and ask whether hNSCs could alter kindling. Kindling involves implanting an electrode in the brain and very gently stimulating the brain every day until seizures occur. One can then measure how strong the seizure are and their duration (called after-discharge). As the result of receiving CIRM Early Translation funding, we successfully generated two “xenofree” human neural stem cell lines (hNSCs) which are suitable for future therapeutic use in a variety of human neurological conditions (Goal 1). We also developed an athymic nude rat (ATN) model of controlled cortical impact TBI which exhibits sustained (2-months or longer) cognitive and emotional deficits. ATN rats lack T-cells, and thus have a sufficiently impaired immune system that they do not completely reject transplanted human cells. These ATN rats show deficits on novel place recognition (NPR), acquisition and memory of location on
the Morris Water Maze, and disturbances on an Elevated Plus Maze (EPM) task in comparison to sham controls (Goal 2). We also found that sorted hNSCs survive and are not rejected in this model and that performance on the NPR task, learning on the Morris Water Maze and exploration on the EPM are all improved in the hNSC treated group compared to sham controls (Goal 3). Finally, when we repeated a therapeutic transplantation test of sorted hNSCs, but in seizure/kindled animals with TBI we found three interesting results (Goal 4). First, we replicated our earlier finding that hNSCs are efficacious in restoring memory function on the NPR task prior to kindling. Second, we found that after kindling, the improvement found with hNSCs was lost. And finally, we found that hNSCs reduce the number of After Discharge events in TBI+Kindled animals in comparison to TBI+Kindled animals that received a vehicle control injection. In summary, we have successfully met all of our goals: (1) we generated a new human neural stem cell line suitable for future clinical trials in humans. (2) We developed an immunodeficient animal model of traumatic brain injury with sustained behavioral deficits. (3) We found very promising preclinical efficacy of our hNSCs in TBI. And (4), we have shown that hNSCs may play a role in reducing the number or severity of seizures following TBI, but if seizure activity is severe, that activity may interfere with hNSC mediated improvements on memory. With additional funding, we hope to complete the full range of preclinical studies required to translate these positive findings into an FDA approved human trial.

Neural restricted, FAC-sorted, human neural stem cells to treat traumatic brain injury

Grant Type: Early Translational II
Grant Number: TR2-01767
Project Objective: using Athymic rodents testing the development candidate feasibility of cell therapy to improve some of the symptoms caused by TBI (e.g. seizures).
Investigator:

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Disease Focus: Neurological Disorders, Trauma
Collaborative Funder: Maryland
Human Stem Cell Use: Embryonic Stem Cell
Award Value: $1,517,767
Status: Closed
Application Title: Neural restricted, FAC-sorted, human neural stem cells to treat traumatic brain injury
Public Abstract:

Traumatic brain injury (TBI) affects 1.4 million Americans a year; 175,000 in California. When the brain is injured, nerve cells near the site of injury die due to the initial trauma and interruption of blood flow. Secondary damage occurs as neighboring tissue is injured by the inflammatory response to the initial injury, leading to a larger area of damage. This damage happens to both neurons, the electrically active cells, and oligodendrocytes, the cell which makes the myelin insulation. A TBI patient typically loses cognitive function in one or more domains associated with the damage (e.g. attention deficits with frontal damage, or learning and memory deficits associated with temporal lobe/hippocampal damage); post-traumatic seizures are also common. Currently, no treatments have been shown to be beneficial in alleviating the cognitive problems following even a mild TBI.

Neural stem cells (NSCs) provide a cell population that is promising as a therapeutic for neurotrauma. One idea is that transplanting NSCs into an injury would provide “cell replacement”; the stem cells would differentiate into new neurons and new oligodendrocytes and fill in for lost host cells. We have successfully used “sorted” human NSCs in rodent models of spinal cord injury, showing that hNSCs migrate, proliferate, differentiate into oligodendrocytes and neurons, integrate with the host, and restore locomotor function. Killing the NSCs abolishes functional improvements, showing that integration of hNSCs mediates recovery. Two Phase I FDA trials support the potential of using sorted hNSCs for brain therapy and were partially supported by studies in my lab. NSCs may also improve outcome by helping the host tissue repair itself, or by providing trophic support for newly born neurons following injury. Recently, transplantation of rodent-derived NSCs into a model of TBI showed limited, but significant improvements in some outcome measures. These results argue for the need to develop human-derived NSCs that can be used for TBI.

We will establish and characterize multiple “sorted” and “non-sorted” human NSC lines starting from 3 human ES lines. We will determine their neural potential in cell culture, and use the best 2 lines in an animal model of TBI, measuring learning, memory and seizure activity following TBI; then correlating these outcomes to tissue modifying effects. Ultimately, the proposed work may generate one or more human NSC lines suitable to use for TBI and/or other CNS injuries or disorders. A small reduction in the size of the injury or restoration of just some nerve fibers to their targets beyond the injury could have significant implications for a patient’s quality of life and considerable economic impact to the people of California. If successful over the 3-year grant, additional funding of this approach may enable a clinical trial within the next five years given success in the Phase I FDA approved trials of sorted hNSCs for other nervous system disorders.
Statement of Benefit to California:

The Centers for Disease Control and Prevention estimate that traumatic brain injury (TBI) affects 1.4 million Americans every year. This equates to ~175,000 Californian’s suffering a TBI each year. Additionally, at least 5.3 million Americans currently have a long-term or a lifelong need for help to perform activities of daily living as a result of suffering a TBI previously. Forty percent of patients who are hospitalized with a TBI had at least one unmet need for services one year after their injury. One example is a need to improve their memory and problem solving skills. TBI can also cause epilepsy and increases the risk for conditions such as Alzheimer’s disease, Parkinson’s disease, and other brain disorders that become more prevalent with age. The combined direct medical costs and indirect costs such as lost productivity due to TBI totaled an estimated $60 billion in the United States in 2000 (when the most recent data was available). This translates to ~$7.5 billion in costs each year just to Californians.

The proposed research seeks to generate several human neural-restricted stem cell lines from ES cells. These “sorted” neural-restricted stem cell lines should have greatly reduced or no tumor forming capability, making them ideally suited for clinical use. After verifying that these lines are multipotent (e.g. they can make neurons, astrocytes and oligodendrocytes), we will test their efficacy to improve outcomes in TBI on a number of measures, including learning and memory, seizure activity, tissue sparing, preservation of host neurons, and improvements in white matter pathology. Of benefit to California is that these same outcome measures in a rodent model of TBI can also be assessed in humans with TBI, potentially speeding the translational from laboratory to clinical application.

A small reduction in the size of the injury, or restoration of just some nerve fibers to their targets beyond the injury, or moderate improvement in learning and memory post-TBI, or a reduction in the number or severity of seizures could have significant implications for a patient’s quality of life and considerable economic impact to the people of California. Additionally, the cell lines we have chosen to work with are unencumbered with IP issues that would prevent us, or others, from using these cell lines to test in other central nervous system disorders. Two of the cell lines have already been manufactured to “GMP” standards, which would speed up the translation of this work from the laboratory to the clinic. Finally, if successful, these lines would be potentially useful for treating a variety of central nervous system disorders in addition to TBI, including Alzheimer’s disease, Parkinson’s disease, stroke, autism, spinal cord injury, and/or multiple sclerosis.