Project Title: Therapeutic Potential of Human-Induced Pluripotent Stem Cells (IPSCs) in the Sandhoff Disease Mouse Model of Lysosomal Storage Disorders

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PROGRESS REPORT □ Annual □X Final

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Project Title:	Therapeutic Potential of Human-Induced Pluripotent Stem Cells (IPSCs) in the Sandhoff Disease Mouse Model of Lysosomal Storage Disorders.
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1.Summary of the goals and methods used to pursue our project for which the NTSAD (National Tay-Sachs & Allied Diseases Association) research grant was approved.

EXECUTIVE SUMMARY: Goals and methods

Goals: We investigate the therapeutic potential of human-induced pluripotent stem cells (hIPSCs) for central nervous system (CNS) gene therapy and cell replacement. hIPSCs are reprogrammed from foreskin fibroblasts by transduction with chromatin-remodeling transcription factors. Previously, we showed that human embryonic stem cell (hESC)-derived neural derivatives are equally effective as primary human neural stem cells (NSCs) in a mouse model of a GM2-gangliosidosis (Sandhoff disease) (Lee et al, 2007). However, much debate continues as to whether IPSCs and ESCs are identical cell types or not. In this proposal, we aim to determine whether the two cell types are indeed equally effective, as well as safely therapeutic, in a specific disease model.

Methods: For a head-to-head comparison of hIPSCs with human ESCs and human NSCs *in vivo*, we generated hIPSC-derived NSCs (IPS-NSCs) using currently available protocols for neuralizing hESCs (hESC-NSCs). We transplanted IPS-NSCs into a well-characterized mouse model of Sandhoff disease (SD), whose neurons inexorably degenerate. SD results from a deficiency in both β-hexosaminidase A and B (HexA & HexB).

Multiple mechanisms: neural stem cell action: We previously profiled the multiple mechanisms by which neural progenitors derived from human ESCs, as well as primary CNS-derived NSCs, benefit brains with gangliosidosis using the following methods: **(a)** compensating for Hex A and B deficiency in the SD mouse brain, **(b)** reducing ganglioside storage, **(c)** replacing lost neural elements, and **(d)** diminishing inflammation (Fig.1; Lee et al., 2007).

Preliminary data led to Specific Aims: We followed the impacts of IPS-NSCs after their intracranial transplantation into SD mice brains. Our preliminary data show that, although engrafted IPS-NSCs increase life span, the effects of IPS-NSC were inferior to those obtained with ES-NSC transplantation. The following specific aims have been made in response to these results:

Aim #1): Investigate the mechanism underlying the lower impact of IPS-NSCs in SD mice.

Aim #2): Optimize the use of IPS-NSCs to achieve the therapeutic potential we reported previously for ES-NSCs. Specifically enhance migration of those cells via co-administration with a recently developed synthetic CXCR4 agonist SDF-1α (1~8)-GG-DV1.

2. Specific measurable progress toward your goal (April 1 – June 30, 2012).

A. Specific Aims

Aim #1) Investigate the underlying mechanism of the basis for the lower impact of IPS-NSCs in SD mice:

a). In a side-by-side comparison with ESC–NSCs, verify the migration and distribution pattern of several different sources of IPS-NSCs in an engrafted SD brain. As a control, human fibroblasts taken prior to derivation of iPS cells will be used (Completed).

We successfully completed Aim 1a. (reported in 6 month progress): We transplanted iPS-NSCs into lateral ventricles at birth. One month (30 days) after transplantation, we examined the distribution/migration pattern of engraftment.

We reported that hIPS-NSCs show limited migratory capacity, but SDF-1α(1~8)-GG-DV1 helps disseminate hIPS-NSCs throughout the brain.

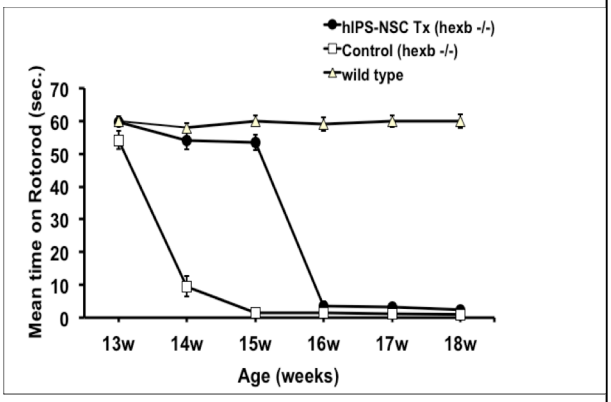

Fig. 1. Transplantation of hIPS-NSCs preserved the rotarod function (n = 22) otherwise lost in nontransplanted *Hexb*$^{-/-}$ mice (n = 14). Motor function test of hIPS-NSC-transplanted *Hexb*$^{-/-}$ mice compared with that of untreated *Hexb*$^{-/-}$ control mice (P < 0.001, t-test). Data represent mean ± s.e.m; n = 12 wild-type mice. Motor function deteriorated at 13 weeks in untreated control *Hexb*$^{-/-}$ mice (n = 14) but was delayed to at least 16 weeks, with a more gradual decline between 14 and 16 weeks, in IPS-NSC-transplanted *Hexb*$^{-/-}$ mice.

b). Test if implanted IPS-NSCs generate electrophysiologically active neurons in a degenerating brain. To analyze the type of neurons generated, lentiviral vector systems will be used to label the IPS-NSCs with GFP, and immunohistochemistry will be used to identify donor-derived neurons. Year 1. (Completed).

We successfully completed Aim 1b. (reported in 9 month progress): We reported that IPS-NSCs engraft widely and generate neurons based on electrophysiological properties using live cortical slices from transplanted adult *Hexb*$^{-/-}$ brains.

We engineered hIPS-NSCs *ex vivo* to express enhanced green fluorescent protein (eGFP) with a lentivirus to visualize the donor cells during patch-clamp. We transplanted them at birth. Under current-clamp conditions, at 2 months of age, we found that injecting depolarizing currents produce a burst of spikes of action potentials from transplanted hIPS-NSCs.

c). Examine the effect of IPS-NSCs on the muscle strength and survival. Muscle strength will be analyzed on a weekly basis beginning 14 weeks post-transplantation using the rotarod test. Year 1. (completed)

Measurable progress during 3 months:
We evaluated motor function by rotarod performance tested twice weekly for 10-week-old-mice until they could no longer walk. We found that transplantation of hIPS-NSCs delayed the motor deterioration

(n = 22) compared with that of nontransplanted control *Hexb*$^{-/-}$ mice (n = 14) (mean ± s.e.m.; P < 0.001, t-test) (Fig. 1) and prolonged their lifespan (data not shown).

Aim #2) Optimize the use of IPS-NSCs to achieve the therapeutic potential for human ES-NSCs we reported previously. Specifically enhance migration of those cells via co-administration with our recently developed synthetic CXCR4 agonist. The SD mouse brains treated with or without SDF-1α (1~8)-GG-DV will be subjected to analyses detailed in Aim #1. Year 1. (completed).

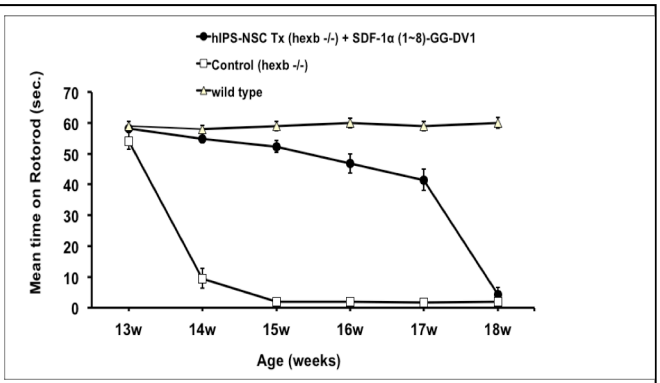

Fig. 2. When co-administered with SDF-1α (1~8)-GG-DV, transplantation of hIPS-NSCs further delayed the onset of motor dysfunction in *Hexb*$^{-/-}$ mice. Motor function test of hIPS-NSC-transplanted *Hexb*$^{-/-}$ mice compared with that of untreated *Hexb*$^{-/-}$ control mice (n = 14, P < 0.001, t-test). Data represent mean ± s.e.m; n = 12 wild-type mice. Motor function deteriorated at 13 weeks in untreated control *Hexb*$^{-/-}$ mice (n = 12) but was delayed to at least 18 weeks, with a more gradual decline between 16 and 18 weeks, in IPS-NSC-transplanted *Hexb*$^{-/-}$ mice when co-administered with SDF-1α (1~8)-GG-DV.

(A). Transplanted IPS-NSCs reduce GM2 ganglioside storage when co-administered along with SDF-1α (1~8)-GG-DV. Completed).

We successfully completed Aim 2a. (reported in 9 month progress): We reported that SDF-1α (1~8)-GG-DV1 helps disseminate hIPS-NSCs throughout the brain and showed that, at 3 months of age, transplanted IPS-NSCs reduce GM2 ganglioside storage. Specifically, there was no detectable GM2 accumulation in the well-engrafted area of an IPS-NSC transplanted *Hexb*$^{-/-}$ mouse brain, however, substantial amounts of GM2 were noted in the same region of an age-matched, nontransplanted *Hexb*$^{-/-}$ mouse brain.

(B) IPS-NSC transplantation reduced microglial activation when co-administered with SDF-1α (1~8)-GG-DV. (Completed).

We successfully completed Aim 2b. (reported in 9 month progress): Abundant microglial activation and macrophage infiltration is a hallmark of Sandhoff disease pathogenesis. We reported that IPS-NSCs reduced the expression of pro-inflammatory genes including *Iba 1* whose immunoreactivity was abundant in the unengrafted *Hexb*$^{-/-}$ cortex at 3 month. In an age matched, well-engrafted IPS-NSC *Hexb*$^{-/-}$ mouse brain, there was no detectable microglial activation.

c). Examine the effect of co-administration of SDF-1α (1~8)-GG-DV with IPS-NSCs on the muscle strength and survival. Muscle strength will be analyzed on a weekly basis beginning 14 weeks post-transplantation using the rotarod test. Year 1. (completed).

Measurable progress during 3 months:
In this study, we co-administered a synthetic CXCR4 agonist to improve dissemination of stem cells, and found that co-administration further delayed the onset of motor dysfunction in *Hexb*$^{-/-}$ mice (Fig. 2) and extended the life span (data not shown). We successfully completed proposed aims during the past 12 months.

Specific Methods:

Neural stem cell culture. Secondary hNSCs, those derived *in vitro* from IPSCs, are maintained under defined, feeder-free culture conditions, in serum-free medium containing bFGF (20 ng/ml), heparin (8 µg/ml), and leukemia inhibitory factor (LIF) (10 ng/ml). To direct these IPSCs toward hNSCs, we employed documented procedures based on exposure to retinoic acid (10 µM) with minor modifications.

Human IPSC-NSC Transplantation

Guided by transillumination of the newborn mouse head, we gently expelled 2 µl of the NSC suspension (5 $X10^4$ NSCs/µl in PBS) through a glass micropipette into each cerebral ventricle of anesthetized Hexb-/- pups (and unaffected littermate controls). This allowed the NSCs access to the subventricular germinal zone. Immediately after stem cell transplantation, 1 µl of 3.2 mM SDV1a peptide was injected by barely puncturing the membrane surface of the mid-region of the cortex. The drug was injected into two sites, one to each side of the cortex. For neonatal cerebellar transplants, we transilluminated the hindbrain and injected 2 µl of the NSC suspension into the external germinal layer of each hemisphere and the vermis.

Histological and immunocytochemical assessment

For immunocytochemical analysis of 20-µm paraformaldehyde-fixed coronal cryosections, routine procedures were employed. To identify human IPS-NSCs, human-specific antibodies are used. We used immunochemistry to assess GM2 storage (GM2 antibody, Matreya, Inc) in the tissue. We assessed CNS inflammation in part with antibodies to markers of microglia/macrophage activation, *Iba I* and *Mac 2*.

Electrophysiology.

We performed whole-cell patch-clamp recordings on fluorescent donor-derived cells in live cortical slices obtained acutely from the brains of *Hexb*$^{-/-}$ mice 2 months after neonatal transplantation. Briefly, we engineered hIPS-NSCs to express eGFP by transduction *ex vivo* with a replication-defective lentivirus. We obtained living coronal slices (400 µm thick) from decapitated adult mice using a vibrating microtome in chilled, gassed artificial cerebrospinal fluid (ACSF) followed by incubation in gassed ACSF at 30°C. After placing the slices in a submerged chamber at 25°C, we obtained whole-cell recordings in current- or voltage-clamp mode from donor-derived cells identified by their eGFP fluorescence.

Confocal microscopy.

We acquired images using a Bio-Rad MRC 1024 equipped with a single photon Kr/Ar laser and a Bio-Rad Radiance 2100MP equipped with a multiphoton laser. Image pixel resolution was 1024 × 1024, and images were acquired sequentially to negate channel cross-talk. Negative and positive control images were taken with the same settings.

Behavioral testing.

We evaluated motor function by rotarod performance tested twice weekly for 10-week-old-mice until they could no longer walk.