MATERIALS TRANSFER AGREEMENT

U.S. Environmental Protection Agency (EPA)
Office of Research and Development (ORD)
National Center for Computational Toxicology (NCCT)

Recipient Organization's Legal/Official Name:
President and Fellows of Harvard College
Office of Technology Development
1350 Massachusetts Avenue
Holyoke Center 727E
Cambridge, MA 02138

1. EPA agrees to transfer to Recipient's Investigator named below the following Research Material (please check box to select all that apply):

- ☐ A copy of the ToxCast™ chemical library consisting of chemical samples prepared as solutions in dimethyl sulfoxide at a concentraton of 20 millimolar.
- ☐ In vitro assay data derived from the ToxCast™ Program. This data is derived from chemicals analyzed using a variety of high throughput assay techniques. Below, this is referred to as the "ToxCast™ Data".
- ☐ In vivo whole animal toxicology summary data derived from the EPA Toxicology Reference Database (ToxRefDB). Below, this is referred to as the "ToxRefDB Data."
- ☐ Summary descriptions of the individual data sets.
- ☐ Individual subsets of this data will be delivered to the recipient after they have been prepared for use at the EPA and cleared for release to the Recipient.

2. EPA's Research Material may not be used in human subjects. The Research Material will be used only for research purposes by Recipient's investigator in his/her laboratory, for the research project described below, under suitable containment conditions. Recipient agrees to comply with all Federal rules and regulations applicable to the Research Project and the handling of the Research Material.

3. The EPA Research Material does not include specimens or data derived or collected from human subjects.

A12509

4. The EPA Research Material will be used by Recipient's investigator solely in connection with the following research projects described with specificity as follows *(use space below or an attachment page if necessary)*: See attachment A

A12 509

5. In all oral presentations or written publications concerning the Research Project, Recipient will acknowledge EPA's contribution of this Research Material, if used, unless requested otherwise. To the extent permitted by law, Recipient agrees to treat as confidential, for a period of three (3) years, any of EPA's written information about this Research Material that is stamped "CONFIDENTIAL" ("Confidential Information"). The foregoing shall not apply to (a) information that is or becomes publicly available or (b) which is disclosed to Recipient without a confidentiality obligation; (c) was known to Recipient at the time of disclosure, as shown by written documentation; or (d) as of the date of disclosure or thereafter was or is independently developed by Recipient without use of or reference to Confidential Information. Any oral disclosures from EPA to Recipient which EPA wishes to be treated as confidential shall be identified as being Confidential at the time of the disclosure and by written notice and marked "Confidential" delivered to Recipient within thirty (30) days after the date of the oral disclosure. Recipient may publish or otherwise publicly disclose the results of the Research Project, but if EPA has given Confidential Information to Recipient, such public disclosure may be made only after EPA has had thirty (30) days to review the proposed disclosure to determine if it includes any Confidential Information, except when the shortened time period is pursuant to a court order or to the extent such review period is permitted by law.

6. The Recipient will provide to the EPA all Testing Results obtained by the Recipient using the Research Material. EPA shall keep such Testing Results in confidence until publication by Recipient unless prohibited by law . EPA acknowledges that Recipient owns all Testing Results. Following publication by Recipient, EPA will have the right to make such Testing Results freely available to the public through its database or otherwise upon review and approval by the Recipient.

7. Are Testing Results being provided back to EPA that include specimens or data derived or collected from human subjects?

_____ Yes – Go to item #7(a).
_____ No – Skip to item #8.

7(a). Do theseTesting Results include specimens or data derived or collected from fetuses, children, pregnant women, or nursing women?

_____ Yes
_____ No

7(b). Were these Testing Results obtained under a protocol that was reviewed and approved by an Institutional Review Board (IRB) that operated in accordance with the requirements of EPA Regulation 40 CFR 26, HHS Regulation 45 CFR 46, or any other Federal Regulation for the protection of human research subjects?

A12509

_____Yes (Please indicate the applicable Regulation here and provide copies of the protocol and IRB approval documents.)
_____ No (Please provide explanation with documentary support as appropriate.)

7(c). Can the Provider of the Testing Results identify the subjects directly or through identifiers (codes) linked to the subjects?
_____ Yes – EPA's use of the Research Material may be human subjects research subject to 40 CFR 26. Go to item #7(d).
_____ No – EPA's use of the Research Material is not human subjects research subject to 40 CFR 26. Skip to item #8.

7(d). Is the Provider of the Testing Results prohibited by this agreement from releasing information to the EPA that might allow the identification of any of the subjects, including but not limited to the key to any existing code?
_____ Yes – EPA's use of the Research Material is not human subjects research subject to 40 CFR 26. Skip to item #8.
_____ No – EPA's use of the Research Material may be human subjects research subject to 40 CFR 26. Go to item #7(e).

7(e). Is the Research Material publicly available?
_____ Yes – EPA's use of the Research Material is human subjects research that is exempt from 40 CFR 26.
_____ No – EPA's use of the Research Material is human subjects research that may be subject to 40 CFR 26 and must be further evaluated accordingly by the EPA Human Subjects Review Official.

8. This Research Material represents a significant investment on the part of EPA and is considered proprietary to EPA. Recipient's investigator therefore agrees to retain control over this Research Material and further agrees not to transfer the Research Material to other people not under his/her direct supervision without advance written approval of EPA. EPA reserves the right to distribute the Research Material to others and to use it for its own purposes. When the Research Project is completed, the Research Material will be returned to the EPA or disposed, if directed by EPA.

9. This Research Material is provided as a service to the research community. It is being supplied to Recipient with no warranties, express or implied, including any warranty of merchantability or fitness for a particular purpose. EPA makes no representations that the use of the Research Material will not infringe any patent or proprietary rights of third parties.

10. Recipient shall retain title to any patent or other intellectual property rights in inventions made by its employees in the course of the Research Project. However, if said inventions

A12509

contain any portion of the Research Material, are derived from the Research Material, or could not have been produced but for the use of the Research Material, Recipient agrees to contact the EPA to determine what ownership interests, if any, the EPA may have, and, where applicable, to negotiate in good faith the terms of a commercial license. Inventorship for a patent application or a commercialized product based on said inventions shall be determined according to United States patent law.

11. Recipient agrees not to claim, infer, or imply endorsement by the Government of the United States of America (hereinafter referred to as "Government") of the Research Project, the institution or personnel conducting the Research Project or any resulting product(s). Recipient agrees to hold the Government harmless against all liabilities, demands, damages, expenses and losses arising out of Recipient's use of the Research Material in the Research Project.

12. When EPA receives Testing Results in accordance with Section 3 and 7, from the partner, the partner will not be liable to EPA for any claims or damages arising from EPA's use of the Testing Results.

13. This Agreement shall begin on the date of its execution and continue for twelve (12) months thereafter, and shall automatically renew for successive year-long periods (a) unless one party notifies the other party no sooner that thirty (30) days prior to such renewal date that it elects not to renew the Agreement, or (b) unless earlier terminated as provided in the next sentence. The EPA shall have the right to terminate this Agreement at any time if Recipient breaches any of the terms of this Agreement. Upon termination, Recipient shall return to the EPA all unused portions of the Research Materials upon written request of the EPA. Recipient may retain one copy of the Confidential Information solely for the purpose of monitoring its obligations under this Agreement.

14. All notices pertaining to or required by this Agreement shall be in writing and shall be signed by an authorized representative and shall be sent by mail or commercial courier addressed as follows:

EPA's Contact Information

Monica R. Linnenbrink
National Center for Computational Toxicology (NCCT)
US EPA (MD-B-205-01)
4930 Old Page Rd.
Research Triangle Park, NC 27711
Ph. 919-541-1522; fax 919-541-1194
Linnenbrink.monica@epa.gov

A12509

Kelly Haston-Rubin Lab
HSCI, Harvard University

March 2010

Attachment A
TOXCAST-EPA Phase I Chemical Assay Proposal

The role of environment in etiology and pathogenesis of neurodegenerative diseases such as Amyotrophic Lateral Sclerosis (ALS) and Parkinson's disease (PD) is largely unknown. These diseases are characterized by progressive and devastating loss of motor neurons (MN) and dopaminergic neurons (DPN), respectively. For both, the majority of cases are sporadic; only 5-10% of cases have an identified genetic basis, which can often display variability in penetrance. Furthermore, most transgenic animal models fail to recapitulate the full pathological scope seen clinically [1-4]. Recent epidemiological work has linked ALS and PD to environmental factors including metals, pesticides, solvents, tobacco, and nutrition [4-6]. However, these studies, while highly suggestive, are often inconclusive, failing to fully parse out environmental versus genetic factors, and rarely providing a cellular or molecular mechanism of action[7].

We hypothesize that environmental contaminants play a causative role in the origin and progression of ALS and PD, both in the presence and absence of known genetic mutations, most likely as effectors within a multi-factorial pathway, leading to the full pathological phenotype of neuronal cell death. To address this hypothesis we propose a collaboration with TOXCAST-EPA to utilize Phase I

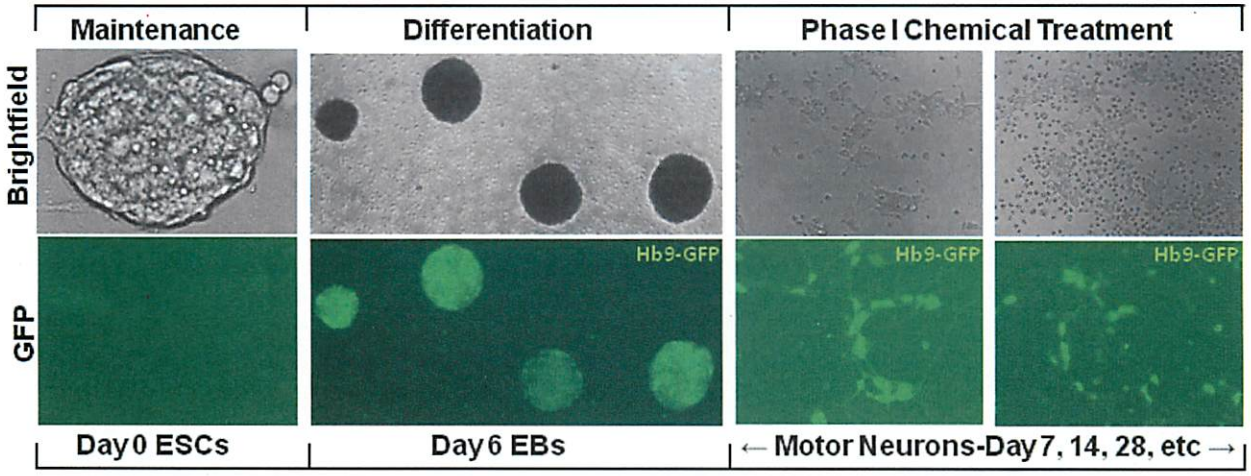

Figure 1: Timeline of motor neuron differentiation and chemical treatment from embryonic stem cells (ESCs). Cells will be differentiated towards the neuronal lineage and assessed for characteristic markers such as the post-mitotic motor neuron maker Hb9-GFP (shown above). Hb9-GFP is not expressed in undifferentiated ESC; however it is present in a subset of cells within day 6 embryoid bodies (EBs) and in post-day 6 dissociation and replated motor neurons. Timepoint and concentrations of Phase I chemical treatment of post-mitotic MNs will be determined experimentally prior to highthrough put screening.

chemicals in a high throughput screen to identify chemical entities that perturb development of embryonic stem cell (ESC) and disease-specific induced pluripotent cell (iPSC) derived DPN and MNs (Figure 1 describes MN example).

We will utilize well established protocols to differentiate ESC/iPSC to MN (Figure 1) and DPN [7-8]. Cells will be exposed to Phase I chemicals at specific time points of differentiation to be determined experimentally. The chemicals will be applied at multiple concentrations, also to be determined during pilot experiments. The first wave of analysis will be image-based to determine divergence in neuron number from controls. Chemicals that promote loss of MN or DPN characteristics, formation of protein

aggregates, or cell death will be investigated at greater depth to determine molecular mechanism of action. We will also utilize them in a combinatorial assay to determine the multi-factorial nature of ALS or PD origin and progression.

The ultimate goal of this project is to uniquely combine human pluripotent stem cell biology with a high throughput environmental screening modality to develop human *in vitro* models of genetic and non-genetic based neurodegenerative diseases that recapitulate cellular level pathology. Successful completion of this project will provide a platform for investigating environmental impact and mechanisms underlying neurodegenerative disease, and importantly will also provide an efficient method for testing preventative measures and treatments, ultimately improving medical science's ability to predict, treat, and one day prevent these diseases.

References

1) Pasinelli P, Brown RH. Molecular biology of amyotrophic lateral sclerosis: insights from genetics. In: Nat Rev Neurosci; 2006:710-23.

2) Migliore L, Coppedè F. Genetics, environmental factors and the emerging role of epigenetics in neurodegenerative diseases. In: Mutat Res; 2009:82-97.

3) Ravits JM, La Spada AR. ALS motor phenotype heterogeneity, focality, and spread: deconstructing motor neuron degeneration. In: Neurology; 2009:805-11.

4) Shaw CA, Höglinger GU. Neurodegenerative diseases: neurotoxins as sufficient etiologic agents? In: Neuromolecular Med; 2008:1-9.

5) Brown RC, Lockwood AH, Sonawane BR. Neurodegenerative diseases: an overview of environmental risk factors. In: Environ Health Perspect; 2005:1250-6.

6) Bronstein J, Carvey P, Chen H, et al. Meeting report: consensus statement-Parkinson's disease and the environment: collaborative on health and the environment and Parkinson's Action Network (CHE PAN) conference 26-28 June 2007. In: Environ Health Perspect; 2009:117-21.

7) Cho MS, Lee YE, Kim JY, et al. Highly efficient and large-scale generation of functional dopamine neurons from human embryonic stem cells. In: Proc Natl Acad Sci USA; 2008:3392-7.

8) Wichterle H, Lieberam I, Porter JA, Jessell TM. Directed differentiation of embryonic stem cells into motor neurons. In: Cell, 2002: 385-397.

9)depts.washington.edu/terisweb/teris;

10)**Error! Hyperlink reference not valid.**;

11)ec.gc.ca/inrp-npri;

12) epa.gov/ncct/toxcast/index.html;

13) Martin MT, Judson RS, Reif DM, Kavlock RJ, Dix DJ. Profiling chemicals based on chronic toxicity results from the U.S. EPA ToxRef database. In: Environ Health Perspect; 2009: 392-399.

14) Park I, Arora N, Huo H, Maherali N, et al., Disease-specific induced pluripotent stem cells. In: Cell, 2008:877-886.

15) Dimos JT, Rodolfa KT, Niakan KK, et al. Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons. In: Science; 2008:1218-21.