The Promise and Perils of Compound Discovery Screening with Inducible Pluripotent Cell-Derived Neurons

Elizabeth R. Sharlow,[1,2] Mehmet Murat Koseoglu,[1,2] George S. Bloom,[3–5] and John S. Lazo[1,2,6,7]

[1]Department of Pharmacology, University of Virginia, Charlottesville, Virginia.
[2]Fiske Drug Discovery Laboratory, University of Virginia, Charlottesville, Virginia.
Departments of [3]Biology, [4]Cell Biology, [5]Neuroscience, [6]Chemistry, and [7]Biochemistry and Molecular Genetics, University of Virginia, Charlottesville, Virginia.

ABSTRACT

Neurological diseases comprise more than a thousand ailments that adversely affect the brain and nervous system. When grouped together, these neurological conditions impact an estimated 100 million individuals in the United States and up to a billion people worldwide, making drug discovery efforts imperative. However, recent research and development efforts for these neurological diseases, including Alzheimer's disease and amyotrophic lateral sclerosis, have been exceedingly disappointing and typify the challenges associated with translating in vitro *and cell-based discoveries to successful preclinical models and subsequent human clinical trials. Our viewpoint is that neuronal progenitor cells and neurons derived from inducible pluripotent stem cells afford an innovative translational bridge, with higher pathological relevancy than previous cellular models. We outline some of the opportunities and challenges associated with their evolving usage in drug discovery and development.*

Keywords: pluripotent, inducible, NPC, neuron

INTRODUCTION

Neurological diseases globally cause significant disability and death. Although some neurological conditions are common and well known (*e.g.*, migraine headaches, traumatic brain injury, chronic pain, and stroke), most are rare, with few individuals afflicted (*e.g.*, Alexander Disease, Bobble Head Doll Syndrome) (*Table 1*). Of the more than 7,000 known rare diseases,* about 360 are primarily neurological.[1]

According to a recent study, the incidence of neurological diseases and disorders is expected to increase in the United States from 43 million in 2011 to 84 million in 2050.[1] This may be unsurprising, in some respects, given that the average U.S. life expectancy is approaching 80 years according to the CDC and neurological disorders are more prevalent in older populations.[1] Nonetheless, this represents an unprecedented increase in the number of people affected by neurological maladies. Thus, it is more important than ever to find new treatments that can modify, treat, and/or prevent neurological diseases.

Historically, parental neuroactive chemotypes or substances have been discovered through behavioral phenotyping, often serendipitously, using whole organisms,[2] as has been the case with many neuropsychiatric drugs or substances.[2] However, contemporary drug discovery is predominantly performed on the "microscale/nanoscale" and is propelled by cell-based phenotypic and/or target-based (*e.g.*, *in vitro* biochemical) screening, which precedes *in vivo* evaluation (*Table 2*).

There are strengths and weaknesses to these screening-based approaches for drug discovery. Specifically, while data suggest that more first-in-class drugs are discovered using phenotypic assays, more "follow-on" drugs and/or "best-in-class" drugs are identified using target-based approaches.[3] Accordingly, compounds identified using phenotypic screens will most likely be subject to more target deconvolution steps. Nonetheless, drug discovery and development across all disease modalities are challenging and, unfortunately, the pathophysiology of many neurological disorders is often not well-characterized, making efforts even more complicated.

Recent well-publicized neurological disease drug failures[4,5] highlight the frustrating drug discovery landscape and underscore that current *in vitro* and *in vivo* model systems do not accurately recapitulate the complexities and progression of a neurological disorder/disease in humans.

*(https://report.nih.gov/nihfactsheets)

Table 1. Common and Rare Neurological Diseases

Common neurological disorders	Estimated cases (global)	Rare neurological disorders	Estimated cases (global)
Migraine headache	1.04 billion[42]	Amyotrophic lateral sclerosis	222,801[43]
Traumatic brain injury	69,000,000[44]	Guillain-Barré syndrome	100,000[49]
Epilepsy	65,000,000[45]	Batten disease	14,000[a]
Alzheimer's disease and other dementias	47,000,000[b]	Familial dysautonomia (Riley-Day syndrome)	595[c]
Stroke	15,000,000[d]	Alexander disease	500[e]
Parkinson's disease	6,100,000[46]	Bobble head doll syndrome	58[47]
Multiple sclerosis	2,100,000[48]		
Traumatic spinal cord injury	250,000–500,000[b]		

[a]From BatCure.
[b]From World Health Organization.
[c]From Familial Dysautonomia Foundation, Inc.
[d]www.world-stroke.org
[e]https://ghr.nlm.nih.gov/condition/alexander-disease#statistics

However, efforts to promote brain research, such as the National Institutes of Health's Brain Research through Advancing Innovative Neurotechnologies (BRAIN) Initiative, the Human Brain Project, the Cure Alzheimer's Fund, and the Allen Institute for Brain Science, are accelerating our understanding of the human brain and neurological disorders.

Multiple resources are accessible to researchers through these programs and include grants, opportunities for collaboration, searchable databases for data mining, and training. Key to progress will be the integration of research across scientific disciplines (*e.g.*, imaging, nanoscience, stem cell technologies, bioinformatics, pharmacology, neuroscience, immunology, and technology development), underscoring the need for team-based science and the development of more physiologically representative neuronal cell-based assay systems.

USE OF INDUCIBLE PLURIPOTENT STEM CELLS IN DISCOVERY EFFORTS

Historically, the heavy reliance on cancer cell lines for the discovery of neurologically targeted chemical probe and drugs has created, perhaps, an unavoidable "disconnect" between the model system and physiological relevancy. Conversely, primary rodent neuronal cultures, while physiologically relevant, have limited translatability to human systems and are not necessarily amenable to the conversion to large-scale compound or genetic screening assay formats. These challenges can now largely be circumvented by the use of neural progenitor cells (NPCs) derived from inducible pluripotent stem cells (iPSCs) for the creation of novel, neurologically relevant cell-based screening assays.

Recent studies have supported the critical inroads that inducible pluripotent stem (iPS) NPCs and neurons have had on screening efforts. For example, human iPS-derived NPCs were foundational in a high-content screening (HCS) assay used to identify drug candidates that not only inhibit Zika virus infection but also rescue Zika virus-induced growth and differentiation defects in NPCs.[6] Moreover, modulators of neurite outgrowth, critical for neural network generation and nerve regeneration, were identified using an iPS neuron-based HCS assay.[7]

For Alzheimer's disease (AD), a nonautomated, three-dimensional (3D) human neuron cell culture model has recently been described that mimics *in vivo* amyloid β (Aβ) and tau pathology.[8] In this study, human neural iPSCs were engineered to overexpress APP and PSEN1 with human familial AD mutations. *In vitro*, these cells expressed extracellular Aβ

Table 2. Benefits and Challenges of Compound Identification Strategies Through Phenotypic and Target-Based Screening

Screening approach	Screening assay components	Disease molecular basis	Benefits	Challenges
Phenotypic (forward pharmacology)	• Whole organism • Cell based	Unknown	• Generally, results in more first-in-class drugs • Identification of cell-permeable compounds	Target deconvolution required
Target-based (reverse pharmacology)	• Cell-based • *In vitro* biochemical	Known	• Often leads to follow-on and/or best-in-class drugs • Target is already identified	May have issues with cell permeability

aggregates and Aβ-induced pathological tau aggregation; however, this model system did not recapitulate the neuroinflammatory environment observed in human AD patients and AD mouse models. To address this deficiency, this same research group then developed a 3D human *in vitro* cell culture model using iPS neurons, astrocytes, and microglia in a 3D microfluidic platform.[9] This model maintained key human AD pathologies (*i.e.*, Aβ aggregation and phospho-tau accumulation) and introduced the AD neuroinflammatory environment (*i.e.*, microglial recruitment, inflammatory cytokine release, axonal cleavage, and nitric oxide release). To date, data from HCS screening with these two AD-iPS neuron relevant assay formats have not been published. Thus, the impact of iPS neurons on high-throughput screening, lead identification and optimization, and drug development has yet to be fully realized. Nonetheless, iPS NPCs and neurons have expanded our ability to interrogate neuronal functional endpoints beyond neuronal toxicity, when damage is too great for therapeutic repair.

It seems likely, however, that next-generation HCS assays will capitalize on these complex co-culture systems as well as other innovative human iPSC-based technologies such as 3D neural or cerebral organoids (*i.e.*, "mini-brains"), which mimic brain development, cell-cell and cell-extracellular matrix interactions, and cellular organization in neurotypical or neuroatypical brain states.[10] These mini-brains may be useful for screening applications, although perhaps not with the throughput previously available with two-dimensional (2D) cell culture platforms.[11] Nonetheless, cerebral organoids can reach sizes of up to a few millimeters and represent a variety of pathologically important brain regions, including dorsal cortex, ventral forebrain, midbrain-hindbrain boundary, choroid plexus, hippocampus, and retina.[12]

These innovative 3D models have been supplemented with microphysiological systems comprising "brains-on-chips" or engineered 3D constructs using multiple cell types, including human iPS neurons, which have the potential to expand pharmacological and toxicological screening strategies as well as studies of the blood–brain barrier.[13,14] It is noted these more physiologically and highly complex model systems have not yet yielded tangible successes in the field of drug discovery and development.

BENEFITS OF iPSC-DERIVED NPCs AND NEURONS FOR ASSAY DEVELOPMENT AND SCREENING

There are multiple benefits to working with iPSC-derived NPCs and neurons, and foremost, these cell populations circumvent any of the ethical issues, as well as the experimental challenges, which exist with the use of human embryonic stem cells.[15] iPSC-derived NPCs can be maintained in culture and scaled up, in a reasonable amount of time, enabling the creation of low passage number cell banks and a reliable source of cells for experimentation, a critical component of HCS. iPS-derived NPCs and neurons can also be engineered, by eukaryotic expression vector transfection or lentiviral transduction, to ectopically express a variety of proteins.[8,9,16] Moreover, there are a variety of reagents available that can be used to track the maturation of neurons (from NPCs), including antibodies to neuronal nuclear antigen (NeuN), microtubule-associated protein 2 (MAP2), and Milli-Mark™ Fluor-pan, a monoclonal antibody blend that binds to multiple neuronal proteins[17–20] (*Fig. 1*).

Critically, differentiation protocols are also available to derive specific neuronal populations, including motor, sensory, interneurons, and neurons in the brain (*i.e.*, dopaminergic, GABAnergic, cholinergic, glutamatergic, and serotonergic neurons), allowing the derivation of complex co-culture systems, which may better mimic the human brain.[21–27] Since human iPS-derived NPCs and neurons can also be used without artificially enforcing protein expression, the proteins and receptors they express are likely to be found at more physiological levels, which could assist the translational fidelity of screening assays using these cells.

Examples also exist of disease-specific iPSC-derived NPCs and neurons, which should enable clarification of the molecular basis of human brain diseases and facilitate the identification of new drug targets,[22,23,28] as well as to provide a screening platforms for small molecules that can reverse or ameliorate the disease phenotype *in vitro* and, ultimately, *in vivo*.[29,30] Moreover, it is conceivable that iPSCs, NPCs, and neurons can be generated from patients at different disease stages, including those before overt symptom onset, providing experimental strategies to identify new disease and disease-stage biomarkers, as well as molecular drug targets.[31]

CHALLENGES ASSOCIATED WITH iPSC-DERIVED NPCs AND NEURONS IN ASSAY DEVELOPMENT AND SCREENING

Despite the significant theoretical and experimental benefits of using iPSC-derived NPCs and neurons for compound screening, there are considerable challenges associated with their wide acceptance. For example, iPS NPCs and neurons are often used to study diseases of old age (*i.e.*, AD and Parkinson's Disease), but there is increasing evidence suggesting that iPSC reprogramming resets the epigenetic state, making the cells more epigenomically similar to embryonic neurons.[32–34] Moreover, while NPCs are more facile

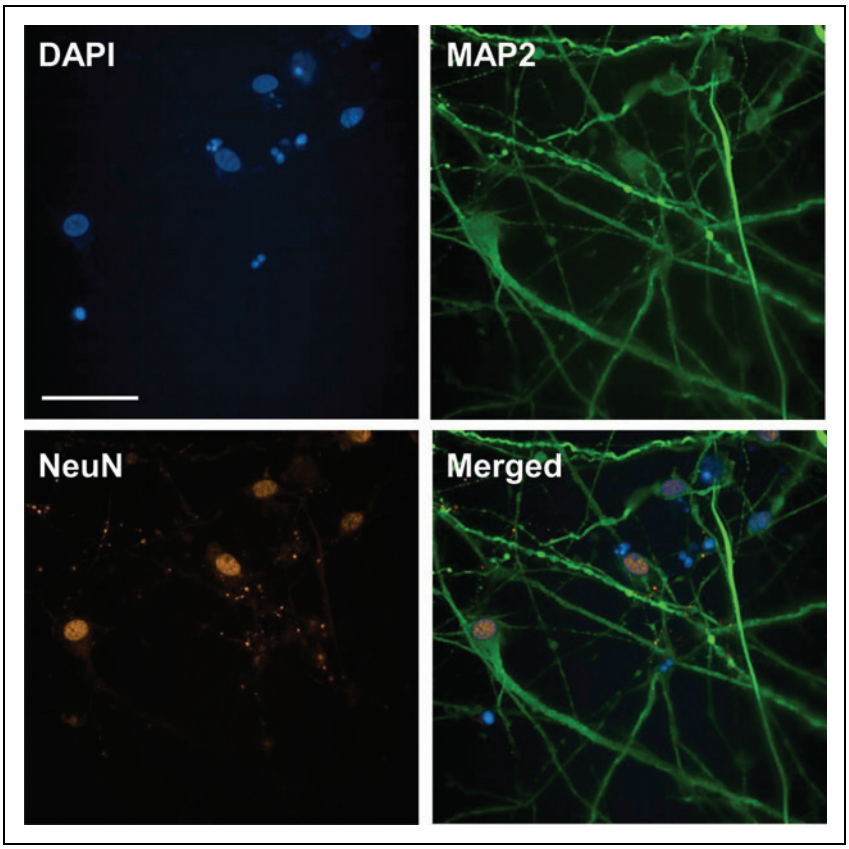

Fig. 1. Inducible pluripotent NPCs differentiated for 6 weeks. NPCs were cultured in neural differentiation medium for 6 weeks in 96-well microtiter plates. Neurons were fixed, permeabilized, and processed for immunofluorescence using antibodies for mature neurons (*i.e.*, NeuN and MAP2) and DAPI to visualize the nucleus. Images were captured by a Perkin Elmer Operetta high-content screening confocal microscope (63 × objective) using Harmony software. Scale bar = 50 μm. MAP2, microtubule-associated protein 2; NeuN, neuronal nuclear antigen; NPC, neural progenitor cell. Color images are available online.

to culturing than the differentiated neurons, they are growth factor dependent, requiring frequent medium changes, and may be subject to selection pressures when passaged for extended time periods or at low confluency. However, there is evidence that suggests extended passaging actually enables the complete reprogramming of iPSCs to neural populations.[35] Nonetheless, NPCs, although critical, have limited application as they have not yet been differentiated into neurons.

iPS NEURON DIFFERENTIATION MEDIUM COMPOSITION

Conversely, iPS neurons offer more challenges with respect to culturing and, subsequently, assay validation and optimization. Overall, the number of protocols for differentiating iPSC-derived NPCs into specific neuronal lineages is limited and they are not standardized, constraining systematic transferability and potential reproducibility.

Neuronal differentiation medium composition is also problematic. It varies among research groups and commercial sources, and there is evidence that the base medium commonly used for iPS neuronal differentiation may not be optimal. The critical nature of the neuronal differentiation medium composition is highlighted in Bardy *et al.*[36] In this study, it was demonstrated that culturing iPS neuronal cells in traditional basal medium (*i.e.*, Dulbecco's modified Eagle medium and Neurobasal) and serum enabled neuron survival and differentiation; however, it also resulted in impaired action potentials, synaptic communication, and basic neuronal function.

Moreover, Neurobasal medium was also independently demonstrated to induce neurotoxicity in primary hippocampal neuronal cultures.[37] Thus, while commonly used base media may provide nutrient environments that support neuronal viability, other facets of neuronal differentiation and function appear to be adversely affected, biasing results. Further complicating, the neuronal differentiation medium composition is the proprietary nature of some medium supplement (*e.g.*, B27) formulations, making medium deconvolution and optimization difficult.

TIMING OF NEURONAL DIFFERENTIATION

Additional complications focus on the lack of consistency with respect to the timing of differentiation to yield mature neurons. As a result, the "differentiated neurons" may actually represent an immature stage of neuronal development[38,39] and because they typically do not express a full complement of adult neuronal proteins, they may lack functional activities associated with fully mature neurons. For example, synaptogenesis (*i.e.*, formation of synapses) is generally observable at 6 weeks postdifferentiation *in vitro*,[40] yet there are many instances when iPS neurons are differentiated for shorter or undisclosed time periods, so the maturation state of the neurons is obscured.[31,32]

These knowledge gaps reflect a significant need for further development of standardized neuron subtype-specific differentiation protocols, phenotypic characterization, and functional

correlation with *bona fide* mature iPS neurons. Thus, once model systems have been optimized and validated, they will be an invaluable source for drug discovery (*e.g.*, biomarkers, drug targets, pharmacodynamic endpoints, and screening assays).

LOGISTICS OF iPS NEURON DIFFERENTIATION, ASSAY DEVELOPMENT AND BATCH MODE SCREENING

Since iPS neuronal differentiation may take weeks in culture, a major challenge associated with iPS neurons will be the potential variation that might and most likely will occur during each neuronal differentiation induction. Thus, methodical assay development and optimization will be complex and more assay variation should be expected. As a result, this variation will almost assuredly require any high-throughput or HCS processes to occur in batch mode (*i.e.*, with multiple "batches" of iPS neurons). Batch-mode screening is not uncommon for HCS assays as screening campaigns often take weeks to complete, depending on throughput and compound library size.

Complicating the issue, however, is that iPS neurons cannot be easily scaled up like cancer cell lines as the neurons (and their accompanying neurites) fail to thrive if passaged after neuron morphological differentiation. Thus, NPCs usually are seeded directly into the microtiter plates for screening and then induced (through medium changes) along the differentiation pathway. However, there is some evidence that NPCs can be differentiated *en masse* for up to 7 days before seeding in microtiter plates.[38] Nonetheless, the remaining time during differentiation will include multiple medium changes and edge effects will be a constant concern.

LESSONS LEARNED FROM NEUROLOGICAL DRUG FAILURES

Similar to drug discovery efforts in other disease areas, our failure to identify and develop efficacious neurological disease-focused therapeutics reflects an incomplete understanding of disease etiology and pathogenesis. However, it also reflects a disconnect between when drugs are patient tested versus when the drug would be temporally efficacious (*i.e.*, "right drug-right time[31]"). It also suggests that the assay

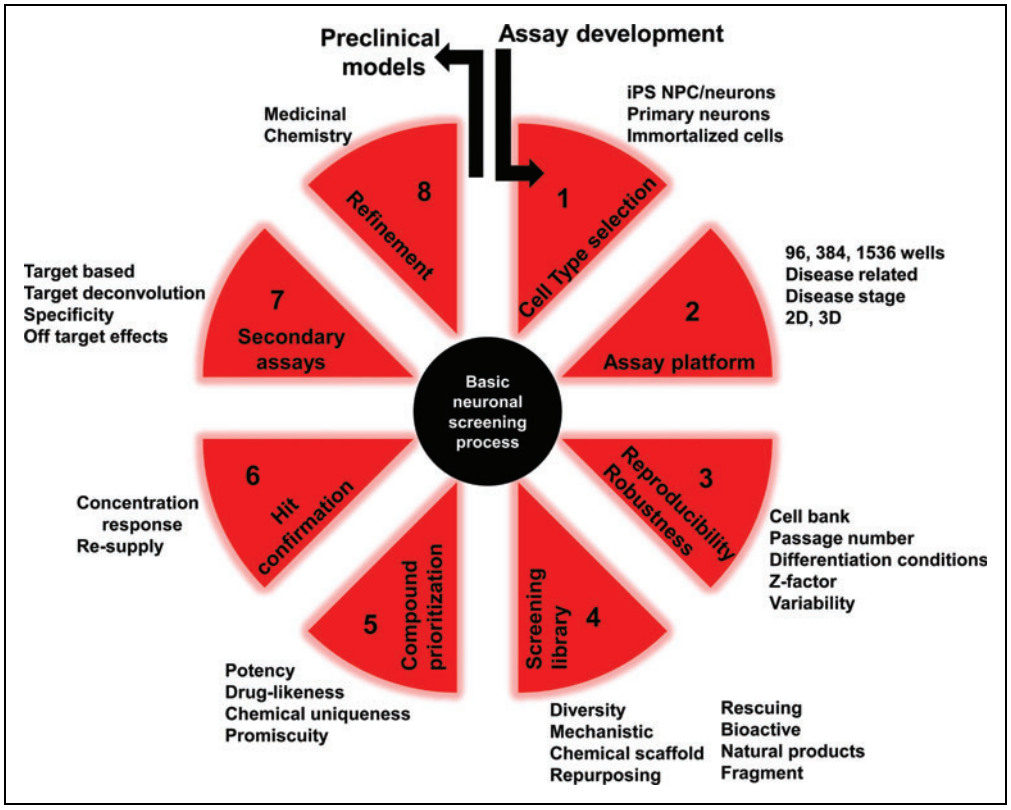

Fig. 2. Basic neuronal screening processes. iPS NPCs and neurons are foundational to the basic neuronal screening process and lead to the first decision point–assay platform or readout. To enhance the chance of identifying meaningful chemical probes or drugs, assays should be disease and disease stage specific. 2D, two dimensional; three dimensional; iPS, inducible pluripotent stem. Color images are available online.

systems we use to identify and validate molecular targets as well as to screen for drugs or compounds may not be physiologically relevant since they do not represent a physiologically relevant disease state for therapeutic discovery. Moreover, we now understand that our assay systems need to not only reflect relevant disease stages or progression but also measure target engagement, especially if efficacious therapeutic intervention is needed before disease presentation.[31]

We therefore posit to build upon this "right time-right drug"[31] reasoning to "right cell type-right assay format," which will lay the foundational rigor and reproducibility needed to move potential drug leads and candidates through the developmental pipeline (*Fig. 2*). We understand that neurological diseases are highly complex and are influenced by genetics, brain injury, and inflammation and that therapeutic options may need to be combined with life style changes.[31] However, if we build assay systems that better recapitulate the neurological disease pathogenesis, which can be used to demonstrate target engagement, we should be able to accelerate the identification of useful chemical probes and drugs. iPSC-derived NPCs and neurons, which can be derived from patients, may be able to help bridge the translation gap between preclinical and clinical drug development by offering more predictive cell-based model systems for screening.[31]

iPSC-derived NPCs and neurons enable the development of more physiologically relevant and complex 2D and 3D assay systems. These high physiocontextual[41] assay systems, which yield rich data sets, may necessitate an evolution in screening strategies from larger to smaller compound library-based screens to accommodate the fundamental complexity of the brain (as well as data analysis and interpretation). Nonetheless, iPS NPCs and neurons are a valuable resource, but they are essentially "undiscovered country" with refinements in methodologies occurring almost simultaneously with implementation limiting the realization of their full potential. Thus, it is critical that investigators using iPSC-derived NPCs and neurons carefully codify their model development, optimization, and standardization processes to ensure robust, reproducible, and transferable results.

DISCLOSURE STATEMENT

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REFERENCES

1. Gooch CL, Pracht E, Borenstein AR: The burden of neurological disease in the United States: a summary report and call to action. *Ann Neurol* 2017;81:479–484.
2. Bruni G, Lakhani P, Kokel D. Discovering novel neuroactive drugs through high-throughput behavior-based chemical screening in the zebrafish. *Front Pharmacol* 2014;5:153.
3. Swinney DC, Anthony J. How were new medicines discovered? *Nat Rev Drug Discov* 2011;10:507–519.
4. Mullard A: BACE failures lower AD expectations, again. *Nat Rev Drug Discov* 2018;17:385.
5. Petrov D, Mansfield C, Moussy A, Hermine O: ALS Clinical Trials Review: 20 years of failure. Are we any closer to registering a new treatment? *Front Aging Neurosci* 2017;9:68.
6. Zhou T, Tan L, Cederquist GY, *et al.*: High-content screening in hPSC-neural progenitors identifies drug candidates that inhibit zika virus infection in fetal-like organoids and adult brain. *Cell Stem Cell* 2017;21:274–283.e275.
7. Sherman SP, Bang AG: High-throughput screen for compounds that modulate neurite growth of human induced pluripotent stem cell-derived neurons. *Dis Model Mech* 2018;11:pii: dmm031906.
8. Choi SH, Kim YH, Hebisch M, *et al.*: A three-dimensional human neural cell culture model of Alzheimer's disease. *Nature* 2014;515:274–278.
9. Park J, Wetzel I, Marriott I, *et al.*: A 3D human triculture system modeling neurodegeneration and neuroinflammation in Alzheimer's disease. *Nat Neurosci* 2018;21:941–951.
10. Camp JG, Treutlein B: Human development: advances in mini-brain technology. *Nature* 2017;545:39–40.
11. Sachs N, Clevers H: Organoid cultures for the analysis of cancer phenotypes. *Curr Opin Genet Dev* 2014;24:68–73.
12. Clevers H: Modeling development and disease with organoids. *Cell* 2016;165:1586–1597.
13. Fan Y, Nguyen DT, Akay Y, Xu F, Akay M: Engineering a brain cancer chip for high-throughput drug screening. *Sci Rep* 2016;6:25062.
14. Wevers NR, Kasi DG, Gray T, *et al.*: A perfused human blood-brain barrier on-a-chip for high-throughput assessment of barrier function and antibody transport. *Fluids Barriers CNS* 2018;15:23.
15. King NM, Perrin J: Ethical issues in stem cell research and therapy. *Stem Cell Res Ther* 2014;5:85.
16. Frega M, van Gestel SH, Linda K, *et al.*: Rapid neuronal differentiation of induced pluripotent stem cells for measuring network activity on micro-electrode arrays. *J Vis Exp* 2017 [Epub ahead of print]; DOI: 10.3791/54900.
17. Mullen RJ, Buck CR, Smith AM: NeuN, a neuronal specific nuclear protein in vertebrates. *Development* 1992;116:201–211.
18. Wolf HK, Buslei R, Schmidt-Kastner R, *et al.*: NeuN: a useful neuronal marker for diagnostic histopathology. *J Histochem Cytochem* 1996;44:1167–1171.
19. Hayashi N, Oohira A, Miyata S: Synaptic localization of receptor-type protein tyrosine phosphatase zeta/beta in the cerebral and hippocampal neurons of adult rats. *Brain Res* 2005;1050:163–169.
20. Huat TJ, Khan AA, Pati S, Mustafa Z, Abdullah JM, Jaafar H: IGF-1 enhances cell proliferation and survival during early differentiation of mesenchymal stem cells to neural progenitor-like cells. *BMC Neurosci* 2014;15:91.
21. Suzuki S, Akamatsu W, Kisa F, *et al.*: Efficient induction of dopaminergic neuron differentiation from induced pluripotent stem cells reveals impaired mitophagy in PARK2 neurons. *Biochem Biophys Res Commun* 2017;483:88–93.

22. Cao SY, Hu Y, Chen C, *et al.*: Enhanced derivation of human pluripotent stem cell-derived cortical glutamatergic neurons by a small molecule. *Sci Rep* 2017;7:3282.
23. Ochalek A, Szczesna K, Petazzi P, Kobolak J, Dinnyes A: Generation of cholinergic and dopaminergic interneurons from human pluripotent stem cells as a relevant tool for in vitro modeling of neurological disorders pathology and therapy. *Stem Cells Int* 2016;2016:5838934.
24. Lu J, Zhong X, Liu H, *et al.*: Generation of serotonin neurons from human pluripotent stem cells. *Nat Biotechnol* 2016;34:89–94.
25. Kang S, Chen X, Gong S, *et al.*: Characteristic analyses of a neural differentiation model from iPSC-derived neuron according to morphology, physiology, and global gene expression pattern. *Sci Rep* 2017;7:12233.
26. Hu BY, Weick JP, Yu J, *et al.*: Neural differentiation of human induced pluripotent stem cells follows developmental principles but with variable potency. *Proc Natl Acad Sci U S A* 2010;107:4335–4340.
27. Lee KS, Zhou W, Scott-McKean JJ, *et al.* Human sensory neurons derived from induced pluripotent stem cells support varicella-zoster virus infection. *PLoS One* 2012;7:e53010.
28. Frati G, Luciani M, Meneghini V, *et al.* Human iPSC-based models highlight defective glial and neuronal differentiation from neural progenitor cells in metachromatic leukodystrophy. *Cell Death Dis* 2018;9:698.
29. Egawa N, Kitaoka S, Tsukita K, *et al.*: Drug screening for ALS using patient-specific induced pluripotent stem cells. *Sci Transl Med* 2012;4:145ra104.
30. Kondo T, Imamura K, Funayama M, *et al.*: iPSC-based compound screening and in vitro trials identify a synergistic anti-amyloid beta combination for Alzheimer's disease. *Cell Rep* 2017;21:2304–2312.
31. Golde TE, DeKosky ST, Galasko D: Alzheimer's disease: the right drug, the right time. *Science* 2018;362:1250–1251.
32. Lapasset L, Milhavet O, Prieur A, *et al.*: Rejuvenating senescent and centenarian human cells by reprogramming through the pluripotent state. *Genes Dev* 2011; 25:2248–2253.
33. Maherali N, Sridharan R, Xie W, *et al.*: Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution. *Cell Stem Cell* 2007;1:55–70.
34. Meissner A, Mikkelsen TS, Gu H, *et al.*: Genome-scale DNA methylation maps of pluripotent and differentiated cells. *Nature* 2008;454:766–770.
35. Koehler KR, Tropel P, Theile JW, *et al.*: Extended passaging increases the efficiency of neural differentiation from induced pluripotent stem cells. *BMC Neurosci* 2011;12:82.
36. Bardy C, van den Hurk M, Eames T, *et al.*: Neuronal medium that supports basic synaptic functions and activity of human neurons in vitro. *Proc Natl Acad Sci U S A* 2015;112:E2725–E2734.
37. Hogins J, Crawford DC, Zorumski CF, Mennerick S: Excitotoxicity triggered by Neurobasal culture medium. *PLoS One* 2011;6:e25633.
38. Xu X, Lei Y, Luo J, *et al.*: Prevention of beta-amyloid induced toxicity in human iPS cell-derived neurons by inhibition of Cyclin-dependent kinases and associated cell cycle events. *Stem Cell Res* 2013;10:213–227.
39. Nieweg K, Andreyeva A, van Stegen B, Tanriover G, Gottmann K: Alzheimer's disease-related amyloid-beta induces synaptotoxicity in human iPS cell-derived neurons. *Cell Death Dis* 2015;6:e1709.
40. Kim JE, O'Sullivan ML, Sanchez CA, *et al.*: Investigating synapse formation and function using human pluripotent stem cell-derived neurons. *Proc Natl Acad Sci U S A* 2011;108:3005–3010.
41. Sharlow ER: Benefits of strategic small-scale targeted screening. *Assay Drug Dev Technol* 2016;14:329–332.
42. Collaborators GBDH: Global, regional, and national burden of migraine and tension-type headache, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol* 2018;17:954–976.
43. Arthur KC, Calvo A, Price TR, Geiger JT, Chio A, Traynor BJ: Projected increase in amyotrophic lateral sclerosis from 2015 to 2040. *Nat Commun* 2016;7: 12408.
44. Dewan MC, Rattani A, Gupta S, *et al.*: Estimating the global incidence of traumatic brain injury. *J Neurosurg* 2018 [Epub ahead of print]; DOI: 10.3171/ 2017.10.JNS17352.
45. England MJ, Liverman CT, Schultz AM, Strawbridge LM: Epilepsy across the spectrum: promoting health and understanding. A summary of the Institute of Medicine report. *Epilepsy Behav* 2012;25:266–276.
46. Dorsey ER, Elbaz A, Nichols E, *et al.*: Global, regional, and national burden of Parkinson's disease, 1990–2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol* 2018;17:939–953.
47. Reddy OJ, Gafoor JA, Suresh B, Prasad PO: Bobble head doll syndrome: a rare case report. *J Pediatr Neurosci* 2014;9:175–177.
48. Browne P, Chandraratna D, Angood C, *et al.*: Atlas of Multiple Sclerosis 2013: a growing global problem with widespread inequity. *Neurology* 2014;83:1022–1024.
49. Willison HJ, Jacobs BC, van Doorn PA. Guillain-Barré syndrome. *The Lancet* 2016;388:717–727.

Address correspondence to:
Elizabeth R. Sharlow, PhD
Department of Pharmacology
University of Virginia
P.O. Box 800735
Charlottesville, VA 22908-0793

E-mail: ers7g@virginia.edu

Abbreviations Used

2D = two dimensional
3D = three dimensional
AD = Alzheimer's disease
Aβ = amyloid β
HCS = high-content screening
iPS = inducible pluripotent stem
iPSCs = inducible pluripotent stem cells
MAP2 = microtubule-associated protein 2
NeuN = neuronal nuclear antigen
NPCs = neural progenitor cells