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## Translation: Screening for Novel Therapeutics Using Disease-Relevant Cell Types Derived from Human Stem Cell Models

Stephen J. Haggarty<sup>1,2,3,4,5,6</sup> and Roy H. Perlis<sup>2,3,4</sup>

<sup>1</sup>Chemical Neurobiology Laboratory, Massachusetts General Hospital, Harvard Medical School, 185 Cambridge Street, Boston, MA 02114

<sup>2</sup>Center for Human Genetic Research, Massachusetts General Hospital, Harvard Medical School, 185 Cambridge Street, Boston, MA 02114

<sup>3</sup>Center for Experimental Drugs & Diagnostics, Massachusetts General Hospital, Harvard Medical School, 185 Cambridge Street, Boston, MA 02114

<sup>4</sup>Department of Psychiatry, Massachusetts General Hospital, Harvard Medical School, 185 Cambridge Street, Boston, MA 02114

<sup>5</sup>Department of Neurology, Massachusetts General Hospital, Harvard Medical School, 185 Cambridge Street, Boston, MA 02114

### Abstract

The advent of somatic cell reprogramming technologies, which enables the generation of patient-specific, induced pluripotent stem cell (iPSC) and other trans-differentiated human neuronal cell models, provides new means of gaining insight into the molecular mechanisms and neural substrates of psychiatric disorders. By allowing a more precise understanding of genotype-phenotype relationship in disease-relevant human cell types, the use of reprogramming technologies in tandem with emerging genome engineering approaches provides a previously ‘missing link’ between basic research and translational efforts. In this review, we summarize advances in applying human pluripotent stem cell and reprogramming technologies to generate specific neural subtypes with a focus on the use of these *in vitro* systems for the discovery of small molecule-probes and novel therapeutics. Examples are given where human cell models of psychiatric disorders have begun to reveal new mechanistic insight into pathophysiology and simultaneously have provided the foundation for developing disease-relevant, phenotypic assays suitable for both functional genomic and chemical screens. A number of areas for future research are discussed, including the need to develop robust methodology for the reproducible, large-scale production of disease-relevant, neural cell types in formats compatible with high-throughput screening modalities, including high-content imaging, multidimensional, signature-based

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<sup>6</sup>Correspondence to S.J.H.: haggarty@chgr.mgh.harvard.edu.

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screening, and *in vitro* network using multielectrode arrays. Limitations, including the challenges in recapitulating neurocircuits and non-cell autonomous phenotypes are discussed. While these technologies are still in active development, we conclude that as our understanding of how to efficiently generate and probe the plasticity of patient-specific stem models improves, their utility is likely to advance rapidly.

## Keywords

reprogramming; induced pluripotent stem cells; neural progenitors; disease-relevant cell type; phenotypic assays; neuroplasticity; neuropharmacology; high-throughput screening

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## 1. Introduction

The development of effective treatments for neuropsychiatric disorders presents one of the greatest challenges and areas of unmet medical need in the 21<sup>st</sup> century. Affecting millions of individuals worldwide, they present a tremendous burden to individuals, their families, and society as whole (1, 2). As the causes of severe mental illnesses are likely to be complex and heterogeneous in nature, the application of new approaches and tools to gain insight into the underlying etiology and pathophysiology is critically needed. Recent advances in human genetics have led to an explosion of our understanding of the role that genetic (3), and epigenetic (4), variation plays in determining the susceptibility to a wide range of psychiatric disorders. However, despite these important advances, our increased knowledge has yet to be translated to the discovery and validation of significantly improved, targeted therapeutics (5, 6). Moreover, the polypharmacology of many psychopharmacological agents, along with poor understanding of their precise mechanism of therapeutic action, have limited the development of improved therapeutics that offer either disease-modifying or prophylactic effects, in marked contrast to other disorders, such as rheumatoid arthritis (7).

One of the main challenges to developing next-generation therapeutics for psychiatric disorders is the fact that no common genetic variants of large effect have been identified. Instead, the genetic susceptibility for common disorders, such as bipolar disorder, major depression, schizophrenia, and autism is clearly polygenic in nature (3). This polygenicity renders it impossible to create truly genetically accurate models of human psychiatric disorders in animal models that additionally often lack neuroanatomical regions of the brain thought to contribute to such pathologies (8). Moreover, recent comparisons of mouse and human model systems provide a cautionary tale about the challenges in extrapolating from one to the other(9).

Given these challenges and limitations to solely using rodent model systems, the ability to use genetically accurate human models to investigate the molecular and cellular mechanisms of disease and to investigate the step-by-step development of pathophysiology would have a major impact on our understanding of psychiatric disease mechanisms. Moreover, by developing human cellular model systems capable of supporting screening for novel targets and lead structures for therapeutic development could help address current bottlenecks in the drug discovery process for psychiatric disorders leading to the next-generation of

therapeutics (6). To date, an obstacle in developing such models has been the inaccessibility of the relevant human tissue in patients, making routine sample collection by biopsy infeasible. Even where tissue is available, terminally-differentiated cells such as neurons cannot be maintained in culture, so experiments cannot be repeated and these resources cannot be scaled up to the extent required to utilize them in chemical screens. Conversely, more accessible tissues such as lymphocytes do not necessarily recapitulate all of the signaling pathways and processes needed to explore pathophysiologic processes in neurons and other brain tissue; this is particularly the case for phenotypes which are not cell-autonomous, but rather require interaction between multiple cell types.

Fortunately, recent advances in the field of human stem cell biology, namely the ability to create patient-specific induced pluripotent stem cells (iPSC) that can be differentiated into a growing number of defined cell types using reprogramming technology (10–12), provides new avenues to investigate the pathophysiological mechanisms of psychiatric disorders. This approach allows more accessible cell types, such as dermal fibroblasts from a skin biopsy or lymphocytes in peripheral blood, to be reprogrammed to pluripotent cells. Using this technique, which relies on the expression of cocktails of transcription factors, such as *OCT4*, *SOX2*, *KLF4*, and *c-MYC* (10, 12), or *OCT4*, *SOX2*, *NANOG*, and *LIN28* (11), there now exist a growing number of human iPSCs models of monogenic psychiatric disorders such as Fragile X syndrome (13–15), Rett syndrome (16–22), along with smaller number of examples of complex, polygenic psychiatric disorders, including schizophrenia (23–26), and bipolar disorder (27).

These new patient-derived cell lines effectively model a human disease genome in a form amenable to *in vitro* investigation. These models allow access to otherwise difficult or impossible to obtain living cells that comprise the human nervous system. As important, they enable repeated experiments and larger-scale investigations, in contrast to tissue obtained from neurosurgery or through post-mortem studies. Overall, disease-specific, human iPSC models provides an emerging, scalable platform from which to build a set of tools and an integrated approach for human chemical neurobiology that will allow: 1) genotype-phenotype correlations to be understood for complex genetic disorders; and 2) to develop phenotypic assays capable of supporting high-throughput screening for novel therapeutic agents that target molecular mechanisms not currently modulated by the existing pharmacopeia used to treat psychiatric disorders (Figure 1). As additional encouraging signs of the potential of this approach, outside of the field of psychiatry large-scale therapeutic screening using iPSC-derived disease models has already been successfully applied in a number of examples (28–30), pointing to the generality of the approach for studying human disease biology.

## 2. Neurons Generated *In Vitro* from Multipotent, Self-Renewing, Neural Progenitor Cells

The isolation of multipotent, self-renewing, neural stem and progenitor cells from tissues derived from the rodent central nervous system (CNS) was first described over two decades ago by Reynolds and Weiss (31). Building on these studies, Carpenter and colleagues described the existence of similar multipotent progenitor cells in the human embryonic

forebrain that could be expanded *in vitro* in the presence of basic neurons, astrocytes, and oligodendrocytes (32). However, these initial studies were limited at the time to working with post-mortem human brain tissues, which, for the reasons described below, limited the full potential of human disease modeling. Ultimately, the ability to expand multipotent neural stem and progenitor cells from human pluripotent stem cells as either non-adherent neurospheres, adherent monolayer cultures, or 3-dimensional structures that can form synaptically active, excitatory and inhibitory neuron subtypes by multiple groups over the past few years has brought this approach to modeling human CNS disorders to the forefront (33–44). Examples of these iPSC-derived neural progenitor cells (NPCs) and neurons that can now be generated and used for functional genomic studies and for chemical neurobiology studies are depicted in Figure 2.

As a scalable platform for chemical neurobiology and novel therapeutic discovery, the use of these patient-specific, human iPSC-derived NPCs and differentiated neurons extends previous efforts with rodent stem cell-derived neurons (45–50), as well as efforts to use postmortem, human brain-derived immortalized (51, 52), or non-immortalized (53, 54) NPCs. First, while the rodent and human nervous systems share a number of evolutionary conserved properties there are also radical differences in terms of neurogenesis and neural patterning, most apparent grossly in the lissencephalic nature of rodent brain. Thus, in order to ultimately develop human disease-relevant neuronal cell models, it will be important to be able to routinely access neural cell types found within regions of the human brain, including the cerebral cortex (8, 39, 40, 55). Moreover, many aspects of the epigenetic regulation of the genome, including non-coding RNAs and regulator enhancers are unique to the human genome and cannot be adequately investigated in non-human systems (56–58). Third, since immortalization process itself interferes with the normal mechanisms of cell proliferation, the use of non-immortalized cells allows access to a more physiologically relevant state of self-renewing NPCs (59). Fourth, the use of iPSCs from living patients removes the dependency on post-mortem tissue-derived cells thereby allowing study of neurodevelopmental mechanisms by providing access to less-mature cell types in which terminal differentiation can be manipulated. It also allows application of human iPSC models as biomarkers, for diagnosis or treatment selection in a particular patient (60). Finally, the accessibility of somatic tissues allows efficient development of biobanks large enough to represent most common genetic variation, enabling investigation of particular genetic variants without needing to engineer each variant individually, particularly where they cannot readily be created with existing genome engineering technology, as with some large copy number variants, for example.

## 2. Phenotypic Screens Using Stem Cell-Derived Neural Progenitors and Neurons

As the example of Huntington's disease illustrates, identifying a causal genetic variation is only the first step in understanding the process by which that gene contributes to the pathophysiology of disease (61). To complement the use of genetic approaches, the use of chemical biology provides a means to identify small-molecule probes that can help reveal pathophysiology and identify novel targets for therapeutic intervention. Recent reports in

non-neuropsychiatric patient-derived disease models illustrate the potential for this approach (28–30). Moving beyond the testing of individual drugs described in a number of published studies to date, large-scale, high-throughput screens have only recently begun to be performed with stem-cell derived NPCs and differentiated neurons with potential for screens to be performed at multiple stages of human neurodevelopment (Figure 3).

## 2.1 Identification of glutamate receptor potentiators as cognitive enhancers

One of the first, and still largest-scale phenotypic screens performed using stem cell-derived neurons that has been publicly disclosed is that of McNeish and colleagues that sought to identify novel structural classes of the ionotropic AMPA subtype of glutamate receptors using a combination of mouse embryonic stem cell (mESC) and hESC-derived neurons (62). To perform the primary screen, mESC-derived NPCs obtained through embryoid body differentiation were further differentiated in 384-well plates into a mixed population of neural and glial subtypes (62). Using a Fluorimetric Imaging Plate Reader (FLIPR) assay to measure calcium influx, the screen was performed in the presence of the AMPA receptor potentiator cyclothiazide to enable the identification of potentiators that were dependent upon partial AMPA receptor activation. In total, the high-throughput screen was performed on an impressively large 2.4 million compound library resulting in the identification of 5,911 initial hits, 17 of which were confirmed as positive upon retesting in dose responses, with secondary assays using human NPCs (ArunA Biomedical Inc.) derived from WA09 hESCs (62). These results provide an exciting example of screening a challenging CNS relevant target in stem cell-derived neurons to produce chemical matter that can be further optimized as leads for therapeutic development of cognitive enhancers.

## 2.2 Screens for neuroplasticity regulators using Human iPSC-derived neural progenitors

To date there have been two examples of large-scale screening using human iPSC-derived CNS NPCs, which are illustrative of what is likely to emerge with differentiated human iPSC-derived neurons.

**2.2.1 Modulators of the Wnt/GSK3-mediated neuroplasticity pathways**—In order to identify novel small-molecule probes of a pathway critical to neurogenesis and neuroplasticity, Zhao and colleagues recently reported on a large-scale screen of the Wnt/GSK3 signaling pathway using human iPSC-derived CNS NPCs (63). Dysregulation of Wnt/GSK pathway has been implicated in the pathophysiology of a number of neuropsychiatric disorders and in the response to a wide variety of clinically used drugs, including the mood stabilizers lithium and valproate, antidepressants, and antipsychotics (64–66). The putative psychosis risk gene *DISC1* has been shown to be a direct inhibitor of GSK3 kinase activity, such that its absence leads to inhibition of Wnt signaling and alteration of adult neurogenesis as well as behavior (67). Similarly, loss of FMRP in Fragile X syndrome leads to the dysregulation of Wnt/GSK3 signaling (68–70). These findings are consistent with the observed adult neurogenesis phenotype seen in *Fmr1*-KO mice (71), as well as the neurodevelopmental defects observed in the Fragile X syndrome human iPSC model (14).

In light of these data, the identification of novel compounds that selectively modulate Wnt/GSK3 signaling in human NPCs and neurons may prove therapeutically useful for multiple psychiatric disorders. To advance testing of this notion, Zhao and colleagues introduced a TCF/LEF-responsive luciferase reporter into human iPSC-derived NPCs and validated the assay using Wnt3a conditioned media, lithium, and the GSK3 inhibitor, CHIR-99021 (63). To perform a pilot screen, a collection of ~1,500 compounds from a library of F.D.A.-approved drugs and known bioactive compounds was screened with cells subject to media alone, or the addition of an EC<sub>25</sub> amount of Wnt3a-conditioned media or 10 mM lithium. With follow-up dose responses, this pilot screen identified a number of known bioactive molecules that could potentiate Wnt or lithium signaling (or both). This included potent mGluR1 antagonists and riluzole (Rilutek), an F.D.A.-approved drug, which was also identified in previous screens using immortalized mouse hippocampal progenitors (HT-22) (72), providing confidence in the validity of the iPSC-derived NPCs for characterizing Wnt pathway modulation.

**2.2.2 Enhancers of human neural progenitor proliferation/viability**—With the goal of identifying novel pro-neurogenic compounds, McLaren and colleagues recently reported on a screen of 1,000 compounds for their ability to enhance proliferation and/or viability of human iPSC-derived NPCs using a Cell Titer-Glo ATP bioluminescence-based, end-point assay (50). After two rounds of screening and testing, 5 compounds were validated as increasing the proliferation or viability of the cells, which may ultimately prove to be useful tools for enhancing the ability to expand NPCs on a large scale and potentially as therapeutic agents for modulating neurogenesis (50).

### 3. Future of Phenotype-Based Screens Using Neuropsychiatric Disorders iPSC Models

A number of new tools, technologies and screening approaches are likely to be applied in the near future to impact the discovery of new targets and leads for therapeutic development. These developments will enable screens to be performed at multiple stages of human neurodevelopment, including defined NPCs and neuron subtypes (Figure 3).

#### 3.1 Prospective isolation of defined neural progenitors using cell surface markers

While a number of approaches exist for the patterning of iPSCs into early neuroectodermal cells that can subsequently be patterned and differentiated into neurons, the derivation of neural rosette-derived progenitors has emerged as a particularly useful method for generating cells expandable into telencephalic NPCs that can subsequently be differentiated into a range of neuron subtypes (33–36, 73). Directly isolated neural rosette-derived progenitor cell cultures, however, contain a mixture of cell types with the capability to form cells of both the CNS and as well as neural crest-derived cells that form the peripheral nervous system. To enable purification of more homogenous CNS iPSC-derived NPCs from heterogenous populations of cells, recent studies performed by Yuan and colleagues sought to identify cell-surface markers on hESC and human iPSCs subject to neural induction that could be used for prospective isolation of defined subpopulations of progenitor cells (74). Using fluorescence activated cell sorting (FACS), these studies

identified a cell-surface signature that enables the isolation of progenitors that could be expanded for multiple passages and retained the ability to differentiate into electrophysiologically active neurons (74). Importantly, since this method selects against CD271+ cells, its use enables the isolation of an enriched population of progenitors that yield CNS rather than peripheral nervous system subtypes of neurons. Given the importance of being able to work with distinct neuron subtypes for disease modeling and therapeutic screening, a critical need for the field moving forward will be to develop additional markers that allow, ideally prospectively, the isolation of different types of NPCs. These tools will also enable the standardization of methods and results between laboratories—a key issue that if not addressed will limit progress as investigators struggle to replicate each others' work.

### 3.2 Multidimensional signature-based neuronal screening

As an alternative to selecting individual genes or molecular events such as phosphorylation for performing high-throughput screens, an alternative strategy is the selection of a multigene mRNA expression signature that can serve as a surrogate for complex cellular states (75). Once defined, a high-throughput, inexpensive, multiplexed mRNA profiling assay, such as that using Luminex-bead platform can be performed (76). Recent development of this technology have led to the ability to measure up to 1000 distinct transcripts that have been selected on their basis to allow through a computational inference model to allow assessment of over 80% of the variation that has been observed in the human transcriptome in the form the L1000 assay (T. Golub, A. Subramanian, *manuscript in preparation*). The L1000 assay is well-suited for the characterization of complex disease states where multiple gene expression changes often occur and where it may be desirable to screen for therapeutic agents that can reverse many of the changes observed. As part of the NIH-sponsored LINCS project (<http://www.linccscloud.org>), the Haggarty laboratory in collaboration with members of the Broad Institute's Connectivity Map project (CMap) has recently completed a successful pilot study of this strategy for signature-based screening using human iPSC-derived NPCs and neurons treated with a collection of CNS-active drugs and probe compounds, at multiple time points, with multiple replicates, and in multiple states (e.g. +/- KCl-induced depolarization) (W. Zhao, A. Subramanian, T. Golub, S. Haggarty, *manuscript in preparation*). Extension of this multidimensional signature-based screening approach to include specific disease models, a larger collection of compounds, alteration of cell states, and different cell types derived from iPSC models are in the works. Overall, the expansion of this "Neuro-CMap" to include diverse human iPSC-based disease models and perturbations could prove a valuable resource for the psychiatric disease research community.

### 3.3 Neuronal circuit-based and information processing assays

While not yet adapted to the point of being able to perform large-scale, high-throughput screens, yet another area of great potential utility for the generation of functional networks of pluripotent stem cell-derived neurons with the capacity for information processing applies multielectrode array technology (77–80). Using mESC-derived neurons, Ban and colleagues demonstrated the feasibility of generating functional networks *in vitro* that exhibit spontaneous bursting activity and evoked action potentials leading to a propagation of the

electrical activity throughout the culture (77). In the case of hESC-derived neurons, Heikkila and colleagues have demonstrated that the spontaneous network electrical activity could be blocked by antagonizing sodium ion channels and multiple glutamate receptor subtypes (79). Importantly, these assays with pharmacological agents demonstrate the existence of functional synapses that corroborate immunocytochemical studies and receptor expression. Multiplexed versions of multielectrode arrays with neural circuits formed from defined neuronal subtypes rather than the heterogeneous cultures may reveal subtle phenotypes that would otherwise be missed by other functional assays.

#### 4. Alternatives to Human iPSC Models for Chemical Neurobiology Studies

The ability to directly reprogram fibroblasts into so-called induced neurons (iNs) has been now demonstrated by multiple groups (81–85). Variations of this iN approach with different transcription factors have yielded defined neuronal subtypes, such as dopaminergic neurons through the expression of *Nr4a1*, *Lmx1a*, and *Ascl1* (85). For further details of this approach, see other articles in this issue. By eliminating the need to generate iPSCs, iNs may substantially shorten the time between sample acquisition and establishment of a cellular model. A further advantage, while largely hypothetical, is the possibility that eliminating this intermediate step would reduce the disruption in epigenetic marks associated with iPSC generation. However, a key current limitation of this approach as it applies to high-throughput screening is the relatively small number of neurons produced: the methodology in its current form is highly inefficient, with only a small % of fibroblasts transdifferentiating to iNs, which would be limiting for large-scale screens. One potential solution to limited number of neurons is the creation of induced neural progenitor cells (iNPCs), rather than iNs, along with approaches using olfactory biopsies, which we discuss in more detail in the accompanying supplemental information.

#### 5. Important Considerations and Remaining Challenges

A summary of the considerations for high-throughput screening with patient-derived stem cell models is provided in Table 1. Besides these consideration and the complementary approaches described above, more general technical issues emerge that require focused attention before the promise of human stem cell-based therapeutic screening can be fully realized. First, despite rapid progress in identifying the developmental trajectory of specific types of neurons and glia (86, 87) reducing these techniques to practice remains challenging. At present, while remarkable progress has been made (40, 55), it is still difficult to generate a specific cell type reliably and in adequate numbers, as might be required for a large-scale chemical screen, with the exception of a few cell types which have been the focus of intense study over the past decade (30, 88). On the other hand, while the mixed cell populations that result from standard differentiation protocols are generally framed as a limitation, they may also represent an opportunity: for diseases where the cell type of interest is unknown, the opportunity to interrogate multiple cell types simultaneously could be advantageous with emerging approaches for single cell analysis by imaging and transcriptomic approaches.

A second limitation is apparent in most published work to date using patient-derived cellular models, which assumes that cell-autonomous models of disease will suffice – in other



words, that disease-relevant phenotypes can be observed in individual cultured cells regardless of their milieu. While this is a reasonable initial step, and preliminary work suggests it may be possible to identify such phenotypes, it neglects two key aspects of the developing brain. Specifically, at present it is difficult to study cell-cell interactions with these cultured cells, either in terms of differentiation or synapse formation and maintenance. Whether the synapses observed *in vitro*, which can show evidence of being intact and functional, adequately model those *in vivo* where multiple cell types act in concert, remains to be investigated. Understanding diseases that arise at the network, rather than the single cell, level may require other approaches to reconstruct complex connectivity amongst neurons, for example using brain slices from animal models or in some cases discarded human tissue (89, 90). On the other hand, just because a disease is most apparent at the network level, it does not necessarily follow that no deficit can be observed in single cells.

## 6. Summary and Future Directions

Assuming many of the current methodological challenges can be overcome, one can imagine having access to cohorts of iPSCs consisting of multiple clones per patient with defined genotypes (at the level of the whole genome) and rich phenotypic profiles, including knowledge of illness features and course and response to a range of therapeutics. Additionally there would be ready availability of engineered iPSC lines with specific gain- or loss-of-function genetic variants, on multiple genetic backgrounds, introduced using emerging technologies such as TALE nucleases (TALENs) (91–93) and the CRISPR/Cas tools (94, 95).

As part of efforts to identify phenotype in disease relevant cell types that can be used for high-throughput screening, it will be critical that studies rigorously address variation (both genetic and otherwise) between individual iPSC clones of the same patient and between patients with the same disorder. While substantial progress has been made in moving towards non-integrating factors for this purpose, it cannot be forgotten that reprogramming likely entails massive change in cellular states, in particular the epigenome and transcriptome. In certain cases, pooling of data between patients with the same disorder may be appropriate, but careful inspection of the individual iPSC clone performance and its reproducibility over time will be critical if one is to develop robust, reproducible, and scalable assays that can support high-throughput screening. Exactly the number of clones and patients required to rigorously assess genotype-phenotype will depend on the phenotype being investigated and the contribution of background genetic variation to its expressivity of the phenotype. Here, for single gene phenotypes, the use of genome-engineering technologies will be instrumental to establish causality (91–93), and in other cases (e.g. large deletions and duplications), where such engineering technologies are less developed, the consideration of other appropriate genetic controls will be required.

In closing, a general theme emerging from investigation of the human nervous system is the remarkable degree of plasticity it can exhibit (4). Nowhere is this plasticity more evident than the studies emerging from the field of human stem cell biology and the *in vitro* generation of patient-specific iPSC-derived NPCs and differentiated neuronal subtypes. Understanding how to control and direct this neuroplasticity for the purpose of developing

disease-relevant cell types and phenotypic assays promises to shed light on fundamental questions of pathogenesis of psychiatric disorders and enable new approaches to discover novel treatment targets.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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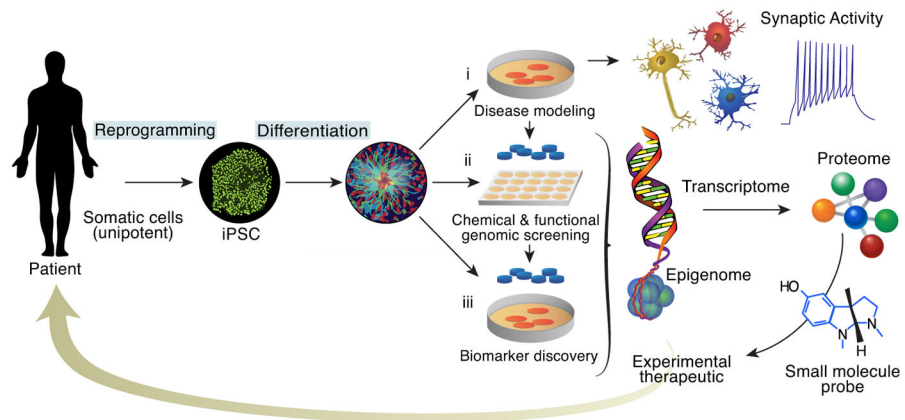
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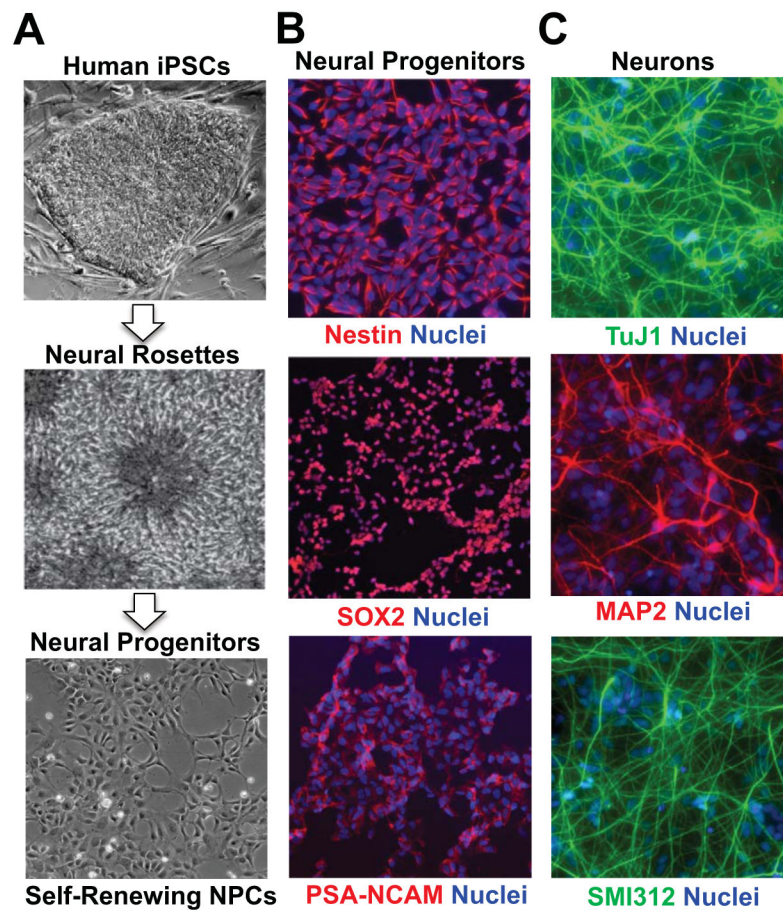
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**Figure 1.** Overview of an Integrated Platform for Biological and Therapeutic Discovery Using Patient-Specific iPSC Models and Chemical Neurobiology.

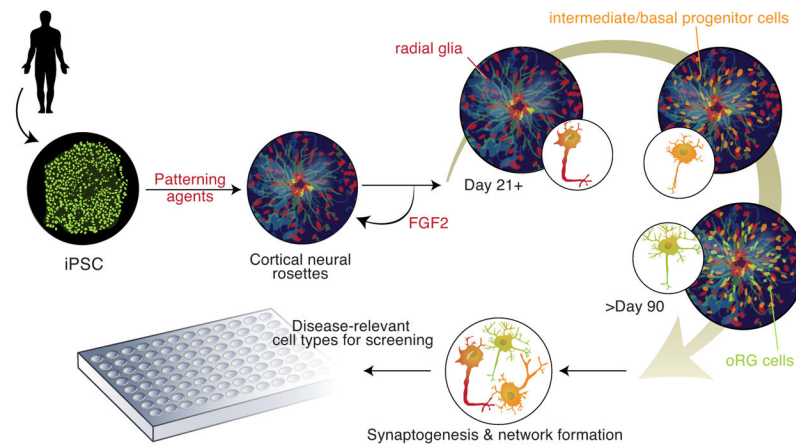




**Figure 2. Generation of Long-Term, Self-Renewing Human iPSC-Derived Neural Progenitors for Use in Chemical Neurobiology Studies and Novel Therapeutic Screening**

(A) Human iPSC-derived NPCs can be derived from the manual isolation of neural rosette structures from iPSC colonies subject to neuroepithelial patterning by a variety of methods (33–44), including “dual SMAD” inhibition, growth factor removal, and from spontaneous formation. Isolated NPCs can be expanded in neural progenitor selective conditions on poly-ornithine/laminin coated surfaces in the presence of the mitogens EGF and FGF2 (bFGF).

(B) Example of immunocytochemical analyses of the neural progenitor markers (Nestin, SOX2 and PSA-NCAM) and (C) neuronal markers (TuJ1<sup>+</sup>, MAP2<sup>+</sup>, SMI312<sup>+</sup>) after differentiation for 7 days that is initiated by removal of EGF and FGF2 (bFGF) mitogens. Images adapted from (63).



### Figure 3. Developing Disease-Relevant Cell-Based Assays Through Directed Differentiation of Human iPSCs to

Example of human corticogenesis based upon recent studies of human pluripotent stem cells. Directed differentiation of iPSCs to cortical neurons through rosette-derived neural progenitor cells. The use of morphogens and patterning agents, including inhibitors of SMAD (e.g. Noggin, dorsomorphin, and SB431542) that attenuate bone morphogenetic protein (BMP) and transforming growth factor beta (TGF- $\beta$ ) signal transduction pathways, and/or retinoic acid (vitamin A), leads to the formation within neural rosettes of self-renewing, cortical progenitor cells expressing the transcription factors PAX6+, FOXG1+, OTX1/2+, and TBR2+ (40, 55). Removal of the mitogen FGF2 initiates neurogenesis with the concomitant production of intermediate/basal progenitor cells and then outer radial glia (oRG) cells that produce a diverse range of upper and lower layer cortical, glutamatergic projection neuron subtypes that follows the temporal course of neuronal production observed *in vivo* in the human brain over a 21–90 day period (40, 55). By addition of other patterning agents the fate of the neural progenitors can be altered. For example, addition of the hedgehog signaling agonist puromorphine can ventralize neural rosettes to generate GABAergic (GAD67+) interneurons (40). Scaling these procedures and optimizing protocols for robustness and reproducibility will be critical milestones to achieve in order to support high-throughput screening using patient-derived neuronal subtypes that are relevant to particular psychiatric disorders.

**Table 1**

Considerations for High-throughput Screening with Patient-Derived Stem Cell Models.

Requirement	Current State of Field	Challenges
<b>Characterization of multiple iPSC clones from well clinically phenotyped patients and from multiple patients for each disorder</b>	<ul style="list-style-type: none"> <li>Limited number of examples of intra-clonal variation described; growing number of patients characterized for major disorders (Rett syndrome, Fragile X syndrome, schizophrenia)</li> </ul>	<ul style="list-style-type: none"> <li>Patient samples may have limited clinical phenotype data</li> <li>Increased work load and costs</li> <li>Existence of somatic genetic variation</li> </ul>
<b>Delineation of disease-relevant cell types</b>	<ul style="list-style-type: none"> <li>Unknown in many cases</li> <li>Emerging methods for directed differentiation and patterning (genetically and with small molecules)</li> </ul>	<ul style="list-style-type: none"> <li>Requires hypothesis testing</li> <li>Need for integration with post-mortem studies and other approaches (e.g. neuroimaging)</li> </ul>
<b>Ability to efficiently generate large numbers of cells</b>	<ul style="list-style-type: none"> <li>Possibility of generating expandable neural progenitors</li> </ul>	<ul style="list-style-type: none"> <li>Need for improved methods to prospectively isolate additional type of progenitors</li> <li>Need for methods to enable maintenance of full developmental plasticity</li> </ul>
<b>Disease-relevant readouts</b>	<ul style="list-style-type: none"> <li>Unknown in many cases</li> <li>Can be targeted to specific genes and pathways (e.g. Wnt signaling and neurogenesis)</li> </ul>	<ul style="list-style-type: none"> <li>Extended period for synaptogenesis in human stem cell-derived neurons</li> </ul>
<b>Well-defined and robust assay readout</b>	<ul style="list-style-type: none"> <li>Examples of pathway-selective reporter genes</li> <li>Examples of high- content imaging</li> </ul>	<ul style="list-style-type: none"> <li>Difficulty of long-term (&gt;2 weeks) culture of iPSC-derived neurons in 96- and 384-well plate formats</li> </ul>