Concise Review: Drug Discovery in the Age of the Induced Pluripotent Stem Cell

HUAISING C. KO, BRUCE D. GELB

Key Words. Induced pluripotent stem cells • Drug discovery • Drug screening • Preclinical drug evaluation • High-throughput screening methods

ABSTRACT
For decades, the paradigm of drug discovery and development has relied on immortalized cell lines, animal models of human disease, and clinical trials. With the discovery of induced pluripotent stem cell (iPSC) technology in 2007, a new human in vitro drug testing platform has potentially augmented this set of tools by providing additional ways to screen compounds for safety and efficacy. The growing number of human disease models made with patient-specific iPSCs has made it possible to conduct research on a wide range of disorders, including rare diseases and those with multifactorial origin, as well as to simulate drug effects on difficult-to-obtain tissues such as brain and cardiac muscle. Toxicity and teratogenicity assays developed with iPSC-derived cells can also provide an additional layer of safety before advancing drugs to clinical trials. The incorporation of iPSC technology into drug therapy development holds promise as a more powerful and nuanced approach to personalized medicine.

INTRODUCTION
Preclinical studies of therapies for rare genetic diseases have often been undertaken with animal models. Subsequent clinical trials to demonstrate safety and efficacy in humans can have difficulty achieving sufficient statistical power. Additional roadblocks to studying human disease arise in the fact that certain tissues, such as brain and cardiac muscle, are difficult to obtain and/or cannot survive long-term culture in vitro. In 2007, the reprogramming of adult human dermal fibroblasts into a pluripotent, embryonic stem cell-like state introduced a new source of difficult-to-obtain human tissues, a milestone for regenerative medicine and a novel way to model human diseases [1, 2]. The result of this discovery was human induced pluripotent stem cells (hiPSCs), which can be differentiated into many cell types, including neurons, cardiomyocytes, neural crest cells, and hematopoietic progenitor cells. hiPSCs have a pluripotency level comparable to that of human embryonic stem cells (hESCs) and can serve as a tool with a similar function in research while avoiding the ethical restrictions of being derived from fertilized human embryos [3, 4]. Moreover, hiPSCs possess the genetic background of the patient, allowing for the creation of disease- or patient-specific cell lines with which to test potential therapeutics with minimal risk to the patient, enabling advances in drug screening in human in vitro models and personalized medicine.

This review discusses the capacity of hiPSCs to model human disease and their current role in the realm of discovery and development of new drugs. Unpublished material was not included in this work.

HIpscS AS DISEASE MODELS
iPSCs were originally generated by using viral vectors to introduce key reprogramming factors (OCT3/4 and SOX2, with either a combination of KLF4 and C-MYC or a combination of NANOG and LIN28) into skin fibroblasts, or other terminally differentiated cells obtained from the patient of interest. These reprogramming factors induce an embryonic-like state, in which these cells can be passaged in culture indefinitely while retaining their pluripotency or can be differentiated via cytokine signaling into cell types derived from any of the three germ layers (endoderm, mesoderm, and ectoderm). DNA-integrating viruses were used to create the first generation of iPSCs, but as this method introduces genomic alterations, current research protocols achieve reprogramming using nonintegrative methods such as Sendai virus, nonintegrating adeno-associated virus, episomal vectors, microRNAs, or PiggyBac transposons to minimize the genomic perturbations [5–11].

Although the use of murine models for human diseases is standard in biomedical research, interspecies differences make the study of particular diseases difficult to translate to human physiology. For instance, mouse cardiomyocytes display shorter action potentials compared with human ones because of differential expression of ion channels [12], spurring the need for approaches
like humanized mouse models. Transgenic mice expressing amyloid precursor protein, a widely accepted mouse model of Alzheimer disease (AD), show significant differences from the human form of AD in PET imaging [13]. Thus, conventional mouse models, along with nonhuman in vitro and in vivo systems, have inherent limitations in representing human disease pathology or for testing potential therapeutics.

Since the description of iPSC technology in 2007, close to 70 hiPSC models of rare and difficult-to-study human diseases have been published, and this number is rapidly growing (Tables 1, 2, 3). hiPSCs can display characteristics of the donor’s clinical phenotype and also can reveal underlying cellular characteristics not clinically evident in the patient, which may be valuable for diseases that show incomplete penetrance. Type 2 long QT syndrome (LQT2) hiPSC-derived cardiomyocytes generated from an asymptomatic mutation carrier with a normal QT interval (437 ms) exhibited a corrected QT interval that was 170% longer than that of the control [14]. By paring the disease down to a given cell type, hiPSCs can provide a window into the disease mechanism and allow the study of tissue-specific influences of the disease mutation in the patient. The mutation responsible for the fetal or neonatal-like phenotype often observed in iPSC-derived target cells is sometimes seen as a limitation to their use in disease modeling [17, 18]. However, this characteristic can make them especially useful to model diseases of development. Rett syndrome is an autism spectrum developmental disorder manifesting at approximately 18 months of age. Rett syndrome hiPSC-derived neurons show many characteristics implicated in the clinical phenotype, including reduced spine density, consistent with the neurons found in the brains of affected patients [19–21].

On the other end of the spectrum, hiPSC-derived models can also recapitulate diseases that manifest later in life, exemplified in hiPSC models of AD, Huntington chorea, and Parkinson disease. Neurons derived from patient-specific familial AD hiPSC lines secrete increased levels of amyloid β42, consistent with previous studies showing similar results in patients’ serum and fibroblasts [22]. Interestingly, hiPSC-derived neurons reprogrammed from patients with trisomy 21 also show increased levels of these amyloid peptide aggregates and hyperphosphorylated tau protein after a prolonged time in culture, consistent with the clinical feature of early-onset AD in patients with Down syndrome [23]. Treatment with a γ-secretase inhibitor reduced production of aberrant amyloid peptides in these neurons, similar to studies on conventional AD hiPSC models. The capacity to represent disorders on the opposite sides of the age range underscores the utility and relevance of hiPSC-derived disease models.

The ability of hiPSCs to retain the entire genomic background of a patient is integral for modeling diseases of multifactorial origin having no single causal genetic mutation, such as schizophrenia. hiPSC-derived neurons from subjects with schizophrenia displayed diminished neuronal connectivity with decreased neurite number,

### Table 1. Drug testing in iPSC-derived models of cardiovascular diseases

| Disease                  | iPSC-derived cell type | Mutation | Drugs tested                                      | Aim                     | Novel | Reference          |
|--------------------------|------------------------|----------|--------------------------------------------------|-------------------------|-------|-------------------|
| CPVT                     | Cardiomyocytes         | RYR2 S406L | Dantrolene | Therapeutic | New to CPVT | Jung et al. [29], 2012 |
| CPVT                     | Cardiomyocytes         | CASQ2 D307H | Isoproterenol | Characterization | No | Novak et al. [57], 2012 |
| Familial dilated cardiomyopathy | Cardiomyocytes         | TNNT2 R173W | Metoprolol | Therapeutic | No | Sun et al. [58], 2012 |
| LQT2                     | Cardiomyocytes         | KCNH2 (HERG) A614V | Isoproterenol hydrochloride, nifedipine, ranolazine dihydrochloride, pinacidil monohydrate, cisapride and E-4031 | Therapeutic | No | Itzhaki et al. [27], 2011 |
| LQT2                     | Cardiomyocytes         | KCNH2 (HERG) G1681A | Isoprenaline, propranolol, PD118057, and nadolol | Therapeutic and Characterization | Yes | Matsa et al. [59], 2011 |
| LQT8                     | Cardiomyocytes         | CACNA1C G1216A | Roscovitine | Therapeutic | No | Yazawa et al. [31], 2011 |
| LQT8                     | Neurons, neural progenitor cells | CACNA1C G1216A | Roscovitine and nimodipine | Therapeutic | Yes | Pasca et al. [60], 2011 |

Abbreviations: CPVT, catecholaminergic polymorphic ventricular tachycardia; iPSC, induced pluripotent stem cell; LQT2, long QT syndrome type II; LQT8, Timothy syndrome.
**Table 2. Drug testing in iPSC-derived models of neurological and neuropsychiatric diseases**

| Disease                          | iPSC-derived cell type | Mutation/characterization | Drugs tested | Aim           | Novel | Reference |
|----------------------------------|------------------------|---------------------------|--------------|---------------|-------|-----------|
| AD                               | Neurons                | PS1 A246E, PS2 N141I      | Compound E, compound W | Therapeutic | No    | Yagi et al. [22], 2011 |
| AD                               | Neurons                | Familial: APP E693del, APP V717L, Sporadic: AD3E211, AD8X213 | DHA, dibenzoylmethane (DBM14-26), NSC23766 | Therapeutic | No    | Kondo et al. [61], 2013 |
| ALS                              | Motor neurons          | SOD1 L144F, TDP-43 M337V | Kenpaullone, dexamfetamine, oleosine | Therapeutic | Yes   | Yang et al. [62], 2013 |
| ALS                              | Motor neurons          | TDP-43 Q343R, TDP-43 M337V, and TDP-43 G298S | Trichostatin A, spliceostatin A, anacardic acid and garcinol | Therapeutic | Yes   | Egawa et al. [32], 2012 |
| Early AD in Down syndrome        | Neurons                | Trisomy 21                | DAPT         | Therapeutic   | Yes   | Shi et al. [23], 2012 |
| FD                               | Neural crest precursors | IKKAPB 2507+ 6T > C       | Kinetin      | Therapeutic   | No    | Lee et al. [63], 2009 |
| FD                               | Neural crest precursors | IKKAPB 2507+ 6T > C       | Tested 6,912 small-molecule compounds for rescued expression of IKKAP | Therapeutic | Yes   | Lee et al. [36], 2012 |
| Rett syndrome                    | Neuronal progenitor cells, glutamatergic neurons | MeCP2 T158M, MeCP2 Q244X, MeCP2 1155del32, MeCP2 R306C | Tetrodotoxin, CNQX, APV, gabazine, IGF1, gentamicin | Characterization: tetrodotoxin, CNQX, APV, gabazine. Therapeutic: IGF1, gentamicin | Not standard therapy | Marchetto et al. [64], 2010 |
| Schizophrenia                    | Neurons                | Complex origin            | Loxapine     | Therapeutic   | No    | Brennand et al. [24], 2011 |
| Schizophrenia                    | Neuronal progenitor cells, neurons | Complex origin | FoF1-ATP synthase inhibitor, oligomycin, FCCP, VPA | Characterization: FoF1-ATP synthase inhibitor, oligomycin, FCCP. Therapeutic: VPA | No | Paulsen et al. [65], 2012 |
| Spinal muscular atrophy          | Motor neurons          | SMN1 SMNdel7              | VPA, tobramycin | Therapeutic   | New to SMA | Ebert et al. [66], 2008 |

Abbreviations: AD, Alzheimer dementia; ALS, amyotrophic lateral sclerosis; DAPT, N-[N-(3,5-difluorophenacetyl)-L-alanyl]-S-phenylglycine t-butyl ester; DHA, docosahexaenoic acid; FCCP, carbonyl cyanide p-trifluoromethoxyphenylhydrazone; FD, familial dysautonomia; iPSC, induced pluripotent stem cell; VPA, valproic acid.

PSD95 protein levels, and glutamate receptor expression compared with wild-type [24]. Even more encouraging, exposure of these neurons to the clinically approved antipsychotic loxapine ameliorated some of the abnormalities.

Epigenetic information from donor cells can also be retained in iPSCs. The characteristic methylation patterns of Angelman and Prader-Willi syndrome—classic DNA methylation disorders—are preserved in hiPSC lines and hiPSC-derived neurons [25, 26]. This shows that using hiPSCs to model diseases of imprinting is feasible even after the “global erasure” of epigenetic markings associated with reprogramming.

**iPSC-BASED DRUG TESTING**

iPSC technology provides a valuable tool to study disease pathways with well-controlled in vitro human models. It is crucial to demonstrate the validity of the hiPSC model by comparing these cells to the physiological equivalent in primary tissues or animal models, by characterizing their response to known chemical compounds. iPSC-derived cardiomyocytes with LQTS mutations were treated with a panel of ion channel blockers to establish the similarity of their responses to the electrophysiological signature of LQTS [27]. Treating LQTS hiPSC-derived cardiomyocytes with potassium channel blockers exacerbated the LQTS phenotype, whereas a calcium channel blocker had ameliorating effects, correlating with the clinical effects of these drugs on LQTS patients. Proteasome inhibitor treatment of hiPSC-derived hepatocytes from a-1-antitrypsin deficiency exacerbated the disease phenotype, revealing a disease-specific accumulation of a-1-antitrypsin [28]. These drug treatments aimed to characterize the hiPSC-based model, highlighting and quantifying the disease phenotype in vitro, with two goals: allowing the comparison between hiPSC-derived cells and primary tissues, and setting the stage for a biochemical study of a drug’s impact on intracellular pathways.

The existing system for drug development consists of animal models, in vitro screening, and multiphase clinical trials in humans. Although pharmacokinetics and pharmacodynamics can still only be examined with an animal model or clinical trial, hiPSC technology can augment phase II and phase III of a clinical trial as an in vitro arm to each (Fig. 1). In phase II trials, hiPSC lines in...
a case-control pairing can support evidence of drug efficacy. In phase III clinical trials, a wide range of genetic backgrounds can be represented by a library of hiPSCs generated from a diverse pool of individuals, aiding in the demonstration of safety and efficacy in a larger population. As an additional in vitro arm to clinical trials, hiPSC-derived cells can serve as a surrogate “patient” to anticipate adverse side effects and calibrate optimal dosing. The ability to test drugs in human cells, model rare diseases, and scale up to high-volume drug screening make hiPSCs poised for work as a parallel division of the preclinical phase of drug discovery.

**USE OF iPSC-BASED ASSAYS IN TESTING NEW DRUGS FOR EFFICACY**

iPSC-derived cells can serve as a platform for high- and low-throughput drug testing. A low-throughput approach incorporates...
the results from previous studies to increase the likelihood of finding a successful candidate drug.

hiPSCs have served as a basis for drug discovery in various cardiovascular disease models (Table 1). hiPSC-derived cardiomyocytes from patients with catecholaminergic polymorphic ventricular tachycardia (CPVT) were characterized by their calcium handling in response to catecholamines [29]. Dantrolene, successful in rescuing the CPVT phenotype in the knock-in mouse model [30], was also found to alleviate the abnormal delayed after depolarizations seen in CPVT hiPSC-derived cardiomyocytes, reinforcing its possible therapeutic use for CPVT. Using a similar approach, an hiPSC model for Timothy syndrome (LQT8), caused by a mutation in the main L-type calcium channel, was created to characterize the disease in cardiomyocytes and test potential therapeutics [31].

Targeted drug discovery in neurological disorders has also grown in recent years (Table 2). hiPSC-derived motor neurons from patients with amyotrophic lateral sclerosis [32] were tested with drugs to augment the limited treatment options. Four compounds known to act on RNA metabolism or histone acetylation were

| Drug testing in wild-type iPSC-derived cells |
|--------------------------------------------|
| **Cell type**                  | **Drugs tested**                                                                 | **Objective**                                                                 | **Output measurement**                                                                 | **Reference** |
| Cardiomyocytes                | Ouabain, aconitine, quinidine, dofetilide, flecainide, erythromycin, terfenadine, thioridazine, RO5657, sotalol, cisapride, E-4031, astemizole, ranolazine, alfuzosin, moxifloxacin, nifedipine, amiodarone, verapamil, captopril, amoxicillin, fluoxetine, rofecoxib, aspirin | To create an index to quantify proarrhythmic risk of a given drug | Changes in the beat rate and the amplitude of the impedance measurement | Guo et al. [38], 2011 |
| Cardiomyocytes                | Amlodipine, carbachol, DMSO, E-4031, isoproterenol, mibebradil, zatebradine, T-type calcium-channel blockers Ref1, Ref2, Ref3 | To assess for arrhythmia-like activity in iPSC-CMs in a medium-throughput screening | Changes in the beat rate and the amplitude of the impedance measurement | Jonsson et al. [73], 2011 |
| Cardiomyocytes                | Sorafenib, sunitinib, dasatinib, imatinib, lapatinib, nilotinib | To characterize mitochondrial function and gene and protein expression in the presence of different arrhythogenic kinase inhibitors | (a) Energy consumption via mitochondrial respiration and glycolysis; (b) qPCR for 96 genes; (c) protein expression for OXPHOS human antibodies, COX4, COX6A, SOD1, and BCL2L1 | Rana et al. [74], 2012 |
| Cardiomyocytes                | Quinidine, isoproterenol, verapamil, E-4031 | To assess QT prolongation and characterize various arrhythogenic compounds | Electric field potential of iPSC-CMs in the presence of various compounds | Tanaka et al. [75], 2009 |
| Chondrocyte progenitors       | Ab235, NB61, GDF5 | To create a high-throughput system to screen for enhanced chondrogenesis | (a) qPCR of pluripotent, mesodermal, and chondrocyte genes; (b) immunofluorescence for Col II; (c) mRNA expression of Col II. 4) Luciferase reporter assay after insertion of chondrocyte-specific expression cassette | Yang et al. [76], 2012 |
| Neural progenitor cells       | Ethanol, thalidomide, all-trans retinoic acid, lithium chloride, caffeine | To examine and characterize teratogenic effects on human neuronal development | (a) DNA microarrays; (b) immunochemistry for α-fetoprotein, NCAM1, HOX1A, SOX17 | Mayshar et al. [42], 2010 |
| Neural progenitors            | Preliminary testing: Wnt3a, GSK3 inhibitor, lithium; screened 1,500 compounds from a library of FDA-approved drugs and known bioactives | To create and use a high-throughput screen to find and validate targets for neuropsychiatric disorders | CignalT cell factor/lymphoid enhancer factor luciferase reporter for Wnt/β-catenin signaling | Zhao et al. [35], 2012 |
| Neurons                       | β-Secretase inhibitor, γ-secretase inhibitor sulindac sulfide | To evaluate β-amyloid production after drug treatment as a surrogate for Alzheimer dementia phenotype | β-Amyloid production | Yahata et al. [77], 2011 |

*Ref1–Ref3, reference compounds from Sigma-Aldrich or AstraZeneca.

Abbreviations: CM, cardiomyocytes; Col, collagen; FDA, U.S. Food and Drug Administration; iPSC, induced pluripotent stem cell; qPCR, quantitative polymerase chain reaction.
screened on this platform, including anacardic acid, which was able to prevent motor neuron death and lower the production of mutant protein. These efforts have now opened the possibility of anacardic acid as a treatment for this incurable neurological disease.

Although the targeted approach to selecting drug candidates may seem more efficient, high-throughput drug screening has the advantage of not imposing bias in the search for new drugs. Disease-specific hiPSCs and appropriate control lines can be differentiated into the target cell type(s), grown in multiwell microtiter plates, and exposed to a different compound in each well. The therapeutic response can be approximated by measurements in protein production, enzyme activity, cell proliferation, gene expression, or any quantifiable cell characteristic. In this manner, a high volume of compounds can be tested rapidly by comparing the outcomes simultaneously [33]. It is important to recognize, however, that the current set of hiPSC disease models is limited to a subset of all existing cell types. Some cell types are not easily differentiated from iPSCs, scaled up to grow in large quantities, or maintained long term in culture, which curtails their use for high-throughput drug screening. With the continued development of robust and reliable differentiation protocols and improving techniques in iPSC culture, the range of hiPSC disease models is likely to extend to a broader range of human cell types.

High-throughput methods can also play a role in the development of a disease-specific hiPSC line. Hamaasaki et al. noted that hiPSC lines could only be made from fibroblasts taken from patients with fibrodysplasia ossificans progressiva when the mutated ALK2 gene is rescued [34] (Table 3). In a screen of 160 bioactive compounds that repress ALK2 gene expression or inhibit the constitutively active ALK2 gene product, three drugs were identified by their ability to allow the reprogramming of fibroblasts into hiPSC. Although this assay was not specifically designed to rescue this disease phenotype, it identified potential therapeutics for a devastating disease with limited treatment options.

iPSC-based platforms for drug discovery are not limited to disease models; wild-type cells can serve as a way to study canonical cellular pathways implicated in disease and normal growth (Table 4). Searching for novel neuropsychiatric drugs, 1,500 compounds were tested on wild-type hiPSC-derived neural progenitor cells for their effect on the Wnt/β-catenin signaling pathway, implicated in the mechanism behind drugs used to treat bipolar disorder, such as lithium [35]. Using a luciferase reporter assay system, 45 compounds were found to enhance Wnt/β-catenin activity, including some that potentiated the effects of lithium. Given the universality of the Wnt/β-catenin pathway, the potential use for these compounds extend beyond central nervous system pathologies.

A focused look at the “hits” from a large-scale compound screen can also implicate a novel aspect of the underlying disease pathway. Using an reverse transcription-polymerase chain reaction assay for expression of IKBKAP, implicated in familial dysautonomia, hiPSC-derived neural crest precursors were tested in a 7,000-compound screen [36]. In detecting eight compounds that rescued mutated gene expression, the assay further elucidated the role of the α2 adrenergic receptor activity in regulation of IKBKAP. Drug discovery using iPSC-derived cells can uncover disease mechanisms while identifying novel therapeutic compounds simultaneously.

### USE OF iPSC-BASED ASSAYS IN TESTING DRUGS FOR TOXICITY

A second use for hiPSCs is as a platform for measuring direct drug toxicity (Table 4). On a smaller scale, patient-specific hiPSCs can be used to identify side effects before the patient takes the drug itself—a sort of litmus test for safety. Personalized medicine in this form enhances patient care and optimizes the treatment of disease. Given that it is expensive to create and maintain pluripotent stem cell lines, they would be too costly to implement as a part of a routine patient health care paradigm currently. However, the creation of patient-specific hiPSC lines exists as a possibility for special circumstances and represents a future potential for personalized medicine.

A more feasible implementation of iPSCs in drug toxicity testing at this time is on a larger scale. iPSC-derived cells of various tissue types can be cultured in large grids and assayed for toxicity in a manner analogous to high-throughput screens for drug discovery. Using this approach, iPSC technology can be integrated into the current paradigm for drug development as part of safety testing in the early phases of clinical trials. This method offers an additional line of safety by testing in nontarget tissues, informing clinical trials before harm to human subjects or patients.

Testing drugs for cardiotoxicity is especially important since a substantial portion of new drugs are withdrawn from the market for this reason [37]. In an effort to address this need, hiPSC-derived cardiomyocytes were exposed to drugs with known cardiotoxic potential to calculate a “predicted proarrhythmic score” index, signifying a drug’s propensity to cause arrhythmia relative to its effective dose [38]. Another study characterized electrophysiological responses to a panel of drugs known to affect ion channels on a library of hESC- and hiPSC-derived cardiomyocytes from patients with various genetic cardiac diseases susceptible to cardiotoxicity (LQTS, hereditary dilated and hypertrophic cardiomyopathies) [39]. These data create a potential framework to evaluate new compounds for proarrhythmic side effects as part of a drug’s safety profile.

In addition to the safety of testing therapeutics on hiPSC-derived cells, there is also the benefit of cost savings. In recent years, the estimated cost of developing and testing a new drug has been $1.2–1.7 billion in the U.S. [40], spurring the search for less expensive alternatives to the conventional strategy. In fact, commercially available vials of hiPSC-derived cardiomyocytes are now being sold for $1,500 per unit for the purpose of drug testing, among other uses. hiPSC-based drug screening can potentially introduce savings in the costs of development, possibly generate more candidate compounds in a shorter amount of time, and test for life-threatening toxicity in a multitude of tissues. This body of knowledge may help prevent the costly recall of already-approved drugs and develop a new generation of safer drugs using an alternate, less expensive strategy.

The ability of iPSCs to recapitulate the process of development from embryo to adult tissue can also be used to test for drug toxicity in developing tissues, for which there is currently no standardized human protocol. Researchers have used partially differentiated hESCs to examine the teratogenic effects of known substances (e.g., ethanol, lithium, retinoic acid, and valproic acid) [41, 42], a paradigm that can easily be extended to hiPSCs. Aside from having an unrestricted source of pluripotent cells, the use of hiPSCs has the additional benefit of being easily scaled up for high-throughput testing, opening a new arm of safety testing for teratogenicity.

Using hiPSCs in phase I clinical trials may provide a more sensitive assay for a candidate drug’s toxicity and safety compared with conventional clinical trial phases. However, the relatively new nature of iPSC testing should keep these results in the arena of “guiding” rather than “determining” evidence. If a new compound were to demonstrate safety in the conventional phase I trial but show toxicity in
the hiPSC-based arm of the study, this might not be deemed sufficient to abort continued investigation. At a minimum, a finding of toxicity in hiPSC-based assays would warrant surveillance of the predicted toxicity during the next phase in drug development and allow for exploration of the mechanism. Additionally, the hiPSC arm of clinical testing can be used to evaluate drug-drug interactions that cannot be tested prospectively, potentially identifying toxic interactions before they would be noted in a trial population. In principle, the addition of a hiPSC-based auxiliary arm would help to provide a more fine-tuned and multipronged approach to understanding the risks and benefits of a new therapy.

THE CHALLENGES AND FUTURE DIRECTIONS OF iPSC-DERIVED DRUG TESTING

In the field of computer science, Moore’s law describes the doubling every two years of the number of components able to fit on an integrated circuit. It seems that an analogous trend can be observed in the field of personalized medicine with the amount of genetic information being integrated into an individual patient’s care. The limited set of genetic tests for disease-carrying alleles and drug metabolism polymorphisms that serves as the basis for personalized medicine currently will eventually be supplanted by whole genome sequencing of each individual. Although this is a vast and valuable body of knowledge to incorporate into a patient’s care, it only draws on a patient’s genotype. The companion body of knowledge—a phenotypic model of the patient—might soon be made available through the array of patient-specific medical tests that iPSC technology can offer (Fig. 2). Connections made between genetic databases and the corresponding response to drugs on relevant hiPSC-derived cells will present a renewable source of pluripotent stem cells. However, it chamber in culture. These ECTs beat spontaneously, exhibit the Frank-Starling mechanism, and eject fluid [43]. More recently, investigators were able to implant rat mesenchymal stem cells into these ECTs to demonstrate the interaction between multipotent progenitor cells and cardiomyocytes in a three-dimensional culture [44]. Similar models using ECTs from neonatal rat cardiomyocytes have additionally been demonstrated to be a viable platform for drug screening [45]. hiPSC-derived cardiomyocytes have also shown the ability to integrate into three-dimensional tissue constructs and graft successfully in vivo mouse models [46]. Although much of the iPSC-derived tissue engineering has taken place in cardiomyocytes, other researchers have been able to generate cerebral organoids. These three-dimensional collections of cells demonstrate discrete brain regions and have even been used to model human microcephaly [47]. Techniques that emulate the native tissue structure are recreating a more physiological setting with which to study human disease and test new therapies. Considerable challenges remain in the integration of iPSCs into drug discovery—namely the variable and possibly incomplete reprogramming of iPSCs from somatic cells. The process of reprogramming involves TET enzymes that mediate the generation of 5-hydroxymethylcytosine (5hmC). A study comparing the activities of TET enzymes in hESCs and hiPSCs suggests that hiPSCs represent an incompletely reprogrammed pluripotent state. This difference in 5hmC detected between hESCs and hiPSCs is also present between clones of hiPSCs, which may account for the variation between hiPSC lines [48]. In scaling up the hiPSC disease model for in vitro drug testing, this variability will likely affect the reproducibility of the results from these studies. The implications of incomplete reprogramming and interline variability on translating from hiPSC in vitro arms to clinical trials represent a technological hurdle that may delay this development or hinder its utility. Thus, it will be of increasing importance to develop robust differentiation protocols and reliable assessments of the functionality of an hiPSC-derived disease model before fully integrating these cells into the process of drug development. Upon their debut, hiPSCs held the potential and promise of hESCs without the ethical restrictions facing the latter. In comparing the utility of hiPSCs to hESCs, hiPSC lines are patient-specific and present a renewable source of pluripotent stem cells. However, it

Figure 2. hiPSCs and personalized drug treatment. Patient-specific hiPSCs can be used as a platform to individually optimize drug treatments for safety and efficacy. The retained genetic background in each hiPSC-derived cell type can potentially serve as a way to anticipate individual, organ-specific reactions to medications. Abbreviation: hiPSC, human induced pluripotent stem cell.
has been shown that hiPSCs are not as similar to hESCs as initially thought. In some cases, it has also been demonstrated that the pluripotency of hiPSCs is limited compared with hESCs, as seen in some cell types, such as neuronal [49] and hematopoietic [50, 51] differentiation. Using genome-wide comparison, Chin et al. demonstrated that substantial epigenetic and genetic differences exist not only between hiPSCs and hESCs but also between hiPSCs of low (less than 20) and high passage number [52]. Similarly, it was found that the transcriptional profile of iPSCs is globally similar, but distinct from that of embryonic stem cells (ESCs) [53]. It is important to keep in mind that although both types of pluripotent cells have similar abilities, iPSCs carry a significantly different epigenetic and genetic profile than their embryonic counterparts.

Although eliminating integrating viruses in the creation of iPSCs reduces genomic aberration, it has been demonstrated that the act of reprogramming somatic cells to a pluripotent state introduces genomic instability not found in ESCs [54, 55]. High-resolution single-nucleotide polymorphism genotyping with subsequent copy number variant (CNV) analysis showed that both hiPSC and hESC lines carry more regions of recurrent duplication than nonpluripotent cell lines [56].

Table 5. Summary of advantages and disadvantages of iPSC-based drug discovery compared with other modalities

| Platform for drug studies | Advantages | Disadvantages |
|---------------------------|------------|---------------|
| Human iPSCs               | • Human cell lines | • Carry genetic and epigenetic changes |
|                           | • Can be patient-specific and/or disease-specific | • In vitro system, not a physiological body system |
|                           | • Can be scaled up | • Established differentiation protocol exists for only some cell lines |
|                           | • Can be used to test in multiple cell types | • Costly compared with animal studies |
|                           | • Can be used to simulate development | • Newer technology with shorter history than with ESCs |
| Human ESCs                | • Human cell lines | • Ethical concerns, leading to limited uses and quantities |
|                           | • Represent human pluripotent cells without risk of introducing genetic and epigenetic manipulations | • Not patient- or disease-specific |
|                           | • Can be scaled up | • In vitro system, not a physiological body system |
|                           | • Can be used to simulate development | • Established differentiation protocol exists for only some cell lines |
|                           | • Can be used to test in multiple cell types | • Costly compared with animal studies |
|                           | • More experience with ESCs than iPSCs | |
| Animal iPSCs              | • Can be scaled up | • Animal phenotype of disease may differ from human phenotype |
|                           | • Fewer restrictions on use than human ESCs | • Not patient-specific |
|                           | • Can be used to test in multiple cell types | • Carry genetic and epigenetic changes |
|                           | • Can be used to simulate development | • In vitro system, not a physiological body system |
| Animal studies            | • In vivo system | • Established differentiation protocol exists for only some cell lines |
|                           | • Can be scaled up | • Newer technology with shorter history |
|                           | • Established model for testing drugs | • Costly compared with conventional animal studies |
| Human clinical trials     | • Most relevant to potential patient population | • Animal phenotype of disease may differ from human phenotype |
|                           | • Established model for testing drugs | • Animal metabolism of drug may differ from human metabolism |
|                           |                         | • Not patient-specific |
|                           |                         | • Limited set of animal models for human diseases |
|                           |                         | • Higher risk of harm to human subjects |
|                           |                         | • Variables of study less well-controlled |

Abbreviations: ESC, embryonic stem cell; iPSC, induced pluripotent stem cell.

DISCUSSION

In the context of existing drug testing platforms, such as animal studies, human clinical trials, animal iPSCs, and ESCs, hiPSCs provide advantages that can augment the current approaches to drug discovery (Table 5). Although hiPSCs can be used for predictive low-throughput and unbiased high-throughput drug screening, they can also serve as a way to understand the biological mechanisms behind drug-drug interactions, an area that is currently not well explored. In this capacity, hiPSCs may eventually play a larger role in studying the effects of polypharmacy, both in the general population and in a patient-specific context. This is an increasingly important aspect of drug development, taking into account the commonality of multiple medication regimens and a patient population that will likely increase the number of medications as they age. Furthermore, the ability to simulate organ systems with hiPSCs may allow for studying the effects of a drug’s metabolites on target and nontarget cell types, an important part of a drug’s side effect profile that can currently be observed only in animal models or clinical trials.

CONCLUSION

At present, iPSC technology is poised to become an important tool in the search for novel therapeutic strategies. The hiPSC-based human disease model has become a natural conduit into studies that examine potential therapies and cures for these diseases. The scalability of iPSCs allows for high-volume screening of drugs and for testing the toxicity of these same compounds in various tissue types. At the same time, the specificity of hiPSCs allows for the
study of rare diseases and personalized biological assays of drug response and toxicity. Together, these elements make iPSC technology a powerful and versatile instrument for the advancement of safe drug discovery and development.

ACKNOWLEDGMENTS

We thank R. Josowitz and S. Mullero-Navarro for their help in editing the manuscript. This work was made possible by support from the Doris Duke Foundation through the Clinical Research Fellowship (to H.C.K.) and the National Institutes of Health (Grant HL113499 to B.D.G.).

REFERENCES

1 Takahashi K, Tanabe K, Ohnuki M et al. Induction of pluripotent stem cells from adult human fibroblast cells by defined factors. Cell 2007; 131:861–872.
2 Yu J, Vodyanik MA, Smuga-Otto K et al. Induced pluripotent stem cell lines derived from human somatic cells. Science 2007;318: 1917–1920.
3 Gupta MK, Illich DJ, Gaarz A et al. Global transcriptional profiles of beating clusters derived from human induced pluripotent stem cells and embryonic stem cells are highly similar. BMC Dev Biol 2010;10:98.
4 Swistowski A, Peng J, Liu Q et al. Efficient generation of functional dopaminergic neurons from human induced pluripotent stem cells under defined conditions. STEM CELLS 2010;28: 1893–1904.
5 Okita K, Yamakawa T, Matsumura Y et al. An efficient nonviral method to generate integration-free human induced pluripotent stem cells from cord blood and peripheral blood cells. STEM CELLS 2012;31:458–465.
6 Yu J, Hu K, Smuga-Otto K et al. Human induced pluripotent stem cells free of vector and transgene sequences. Science 2009;324: 797–801.
7 Fusi D, Ban H, Nishiyama A et al. Efficient induction of transgene-free human somatic cells using a vector based on Sendai virus, an RNA virus that does not integrate into the host genome. Proc Jpn Acad Ser B Phys Biol Sci 2009;85:348–362.
8 Kaji K, Norrby K, Paka A et al. Virus-free induction of pluripotency and subsequent excision of reprogramming factors. Nature 2009; 458:771–775.
9 Weltner J, Anisimov A, Alitalo K et al. Induced pluripotent stem cell clones reprogrammed via recombinant adenovirus-mediated transduction contain integrated vector sequences. J Virol 2012;86:4463–4467.
10 Miyoshi N, Ishii H, Nagano H et al. Reprogramming of mouse and human cells to pluripotency using mature microRNAs. Cell Stem Cell 2011;8:633–638.
11 Anokye-Danso F, Trivedi CM, Juh R et al. Highly efficient miRNA-mediated reprogramming of mouse and human somatic cells to pluripotency. Cell Stem Cell 2011;8:376–388.
12 Nerbome JM. Studying cardiac arrhythmias in the mouse—a reasonable model for probing mechanisms? Trends Cardiovasc Med 2004;14:83–93.
13 Klunk WE, Lopresti BJ, Ikonomov MD et al. Binding of the positron emission tomography tracer Pittsburgh compound-B reflects the amount of amyloid-beta in Alzheimer’s disease brain but not in transgenic mouse brain. J Neurosci 2005;25:10598–10606.
14 Lahti AL, Kujala VJ, Chapman H et al. Model for long QT syndrome type 2 using human iPS cells demonstrates arrhythmogenic characteristics in cell culture. Dis Model Mech 2012;5:220–230.
15 Tanaka T, Takahashi K, Yamane M et al. Induced pluripotent stem cells from CINCA syndrome patients as a model for dissecting somatic mosaicism and drug discovery. Blood 2012;120:1299–1308.
16 Cherry ABC, Gagne KE, McLoughlin EM et al. Induced pluripotent stem cells with a mitochondrial DNA deletion. STEM CELLS 2013;31: 1287–1297.
17 Sepac A, Si-Tayeb K, Sedic F et al. Comparision of cardiomyogenic potential among human ESC and iPSC lines. Cell Transplant 2012;21: 2532–2530.
18 Gherghiceanu M, Barad L, Novak A et al. Cardiomyocytes derived from human embryonic and induced pluripotent stem cells: Comparative ultrastructure. J Cell Mol Med 2011;15:2539–2551.
19 Ananiev G, Williams EC, Li Het al. Isogenic pairs of wild type and mutant induced pluripotent stem cell (iPSC) lines from Rett syndrome patients as in vitro disease model. PLoS ONE 2011;6:e25255.
20 Farro N, Zhang W-B, Pasceri P et al. Rett syndrome induced pluripotent stem cell derived neurons reveal novel neurophysiological alterations. Mol Psychiatry 2012;17: 1261–1271.
21 Cheung AYL, Horvath LM, Grafodatskaya D et al. Isolation of MECPT2-null Rett Syndrome patient hiPSC cells and isogenic controls through X-chromosome inactivation. Hum Mol Genet 2011;20:2103–2115.
22 Yagi T, Ito D, Okada Y et al. Modeling familial Alzheimer’s disease with induced pluripotent stem cells. Hum Mol Genet 2011;20: 4530–4539.
23 Shi Y, Kirwan P, Smith J et al. A human stem cell model of early Alzheimer’s disease pathology in Down syndrome. Sci Transl Med 2012;4:124ra29.
24 Brennand KJ, Simone A, Jou J et al. Modelling schizophrenia using human induced pluripotent stem cells. Nature 2011;473:221–225.
25 Yang J, Cai J, Zhang Y et al. Induced pluripotent stem cells can be used to model the genomic imprinting disorder Prader-Willi syndrome. J Biol Chem 2010;285:40303–40311.
26 Chamberlain SJ, Chen P-F, Ng KY et al. Induced pluripotent stem cell models of the genotoxic imprinting disorders Angelman and Prader-Willi syndromes. Proc Natl Acad Sci USA 2010; 107:17668–17673.
27 Itzhaki I, Maizels L, Huber I et al. Modeling the long QT syndrome with induced pluripotent stem cells. Nature 2011;471:225–229.
28 Rashid ST, Corbinneau S, Hannan N et al. Modeling inherited metabolic disorders of the liver using human induced pluripotent stem cells. J Clin Invest 2010;120:3127–3136.
29 Jung CB, Moretti A, Mederos y Schnitzler M et al. Dantrolene rescues arrhythmogenic RYR2 defect in a patient-specific stem cell model of catecholaminergic polymorphic ventricular tachycardia. EMBO Mol Med 2012;4: 180–191.
30 Kobayashi S, Yano M, Uchinoumi H et al. Dantrolene, a therapeutic agent for malignant hyperthermia, inhibits catecholaminergic polymorphic ventricular tachycardia in a RYR2 (R2474S+/+) knockin mouse model. Circ J 2010;74:2579–2584.
31 Yazawa M, Hseuh B, Jia X et al. Induced pluripotent stem cells to investigate cardiophenotypes in Timothy syndrome. Nature 2011;471:230–234.
32 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.
33 Schenone M, Danicık V, Wagner BK et al. Target identification and mechanism of action in chemotherapeutics and drug discovery. Nat Chem Biol 2013;9:232–240.
34 Hamaasaki M, Hashizume Y, Yamada Y et al. Pathogenic mutation of ALK2 inhibits induced pluripotent stem cell reprogramming and maintenance: Mechanisms of reprogramming and strategy for drug identification. STEM CELLS 2012;30:2437–2449.
35 Zhao WN, Cheng C, Theriault KM et al. A high-throughput screen for Wnt/beta-catenin signaling pathway modulators in human iPSC-derived neural progenitors. J Biomol Screen 2012;17:1252–1263.
36 Lee G, Ramirez CN, Kim H et al. Large-scale screening using familial dysautonomia induced pluripotent stem cells identifies compounds that rescue IKKAP expression. Nat Biotechnol 2012;30:1244–1248.
37 Ferri N, Siegl P, Corsini A et al. Drug attrition during pre-clinical and clinical development: Understanding and managing drug-induced cardiotoxicity. Pharmacol Ther 2013;138:470–484.
38 Guo L, Abrams RMC, Babiare J et al. Estimating the risk of drug-induced proarrhythmia using human induced pluripotent stem cell-derived cardiomyocytes. Toxicol Sci 2011;123:281–289.

AUTHOR CONTRIBUTIONS

H.C.K.: conception and design, manuscript writing, collection and/or assembly of data, data analysis and interpretation; B.D.G.: conception and design, financial support, administrative support, final approval of manuscript.

DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

The authors indicate no potential conflicts of interest.

©AlphaMed Press 2014

508 Drug Discovery Using iPSCs
