INNOVATION

Human stem cells and drug screening: opportunities and challenges

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Abstract | High-throughput screening technologies are widely used in the early stages of drug discovery to rapidly evaluate the properties of thousands of compounds. However, they generally rely on testing compound libraries on highly proliferative immortalized or cancerous cell lines, which do not necessarily provide an accurate indication of the effects of compounds in normal human cells or the specific cell type under study. Recent advances in stem cell technology have the potential to allow production of a virtually limitless supply of normal human cells that can be differentiated into any specific cell type. Moreover, using induced pluripotent stem cell technology, they can also be generated from patients with specific disease traits, enabling more relevant modelling and drug screens. This article discusses the opportunities and challenges for the use of stem cells in drug screening with a focus on induced pluripotent stem cells.

The drug discovery process is time-consuming and costly, in part owing to the high rate of attrition of compounds in clinical trials. Thus, modifications that could accelerate the advancement of promising drug candidates, or reduce the likelihood of failure, would be extremely valuable. High-throughput screening technologies already allow the rapid testing of thousands of compounds. For example, readily available genetically modified rodent or immortalized human cell lines that contain reporter systems are being used to assess whether compounds activate particular signalling pathways of interest. However, these cells do not necessarily provide an accurate representation of how the various drug candidates would initiate responses in humans. Furthermore, overexpressing reporter genes at high levels may not reflect the normal physiological condition. Other problems include biochemical differences between rodent and human cells, and changes in cellular response as a result of the immortalization of human cells to produce expanded lines. In most cases, it is desirable to screen drugs on the final tissue of interest, which is not often well represented by currently used cell lines (TABLE 1). Nevertheless, such immortalized cell lines are widely used for drug screening assays because they are cheap, easy to grow, reliable and reproducible.

Recent advances in stem cell research are now creating alternative possibilities. Embryonic stem cell (ESC) and induced pluripotent stem cell (iPSC) technologies provide a source of normal human cells that can theoretically be expanded to quantities necessary for drug screening and toxicology studies. These cells can be differentiated to generate specific cell types (for example, neurons, blood cells and cardiac muscle), and iPSCs can now be derived from patients with specific diseases. Does this provide an unprecedented opportunity for the pharmaceutical industry to improve drug screening? Or are the challenges to move to the widespread use of such cells too great? With these questions in mind, this article discusses the advantages and disadvantages for the use of human ESCs and human iPSCs (collectively referred to as PSCs) in drug screening for efficacy and toxicity, as well as the types of validation experiments that are required to move this nascent field forward.

Human ESCs for screening

Human ESCs are derived from the inner cell mass of early stage fertilized embryos[1]. These cells meet the two criteria that define a PSC. First, they continually self-renew to maintain the undifferentiated stem cell population. Second, they have the capacity to generate cell types residing in the three primitive germ layers: endoderm, mesoderm and ectoderm[2]. A wealth of knowledge about the transcription factors and chemical induction signals involved in specializing human ESCs to form these germ layers is now available. Fully differentiated neural tissue (including highly specialized dopamine and motor neurons), beating cardiomyocytes, blood cells and primitive endoderm with some signs of islet cell formation have all been generated from human ESCs in the cell culture dish[3]. As such, human ESCs have become a powerful experimental tool in the study of basic human development and hold promise for the treatment of disease through stem cell therapy[3].

What makes these cells interesting for drug screening? Simply put, from a single and potentially limitless starting source, most of the major cells within the human body that could be affected by a drug can be produced. We argue here that these cells would provide a better predictive model of both drug efficacy and toxicity than rodent cell lines or immortalized human cell lines. However, although the idea of using human ESCs for drug screening is attractive, controversy surrounds the use of human ESCs, as embryos are destroyed during the procurement of the cells. This has restricted federal funding for their use, and many large pharmaceutical companies have been cautious of investing in this technology. Furthermore, commercial use of human ESCs requires licensing agreements to be in place (see Further information and US Patent 20090011503 for examples). A further complication for the field revolves around the technical challenges associated with expanding and differentiating PSCs in large numbers (for a review see REF. 4).

Table 1 | **Advantages and disadvantages of human cells available for drug screening**

Cell type	Advantages	Disadvantages	Refs
Immortalized cell lines	• Growth and maintenance low in cost • Homogeneous cell population	• Lack important aspects of native function (e.g., metabolic function and electrical properties) • Not representative of all cell types	4
Primary cell lines	• Fully differentiated cell types • Close approximation of native function	• Not easily accessible or available for all cell types • Require fresh preparation • Questionable reproducibility	4
Embryonic stem cells and induced pluripotent stem cells	• High quantity • Readily available source of all cell types • Fully differentiated cell types • Close approximation of native function	• Growth and maintenance expensive • A lot of time needed to obtain fully differentiated cell types • Require more effort to achieve purified populations	6,7, 34,37

Human ESCs are extremely difficult to grow in comparison with typical cell lines because they require daily changes of growth medium and are susceptible to spontaneous differentiation. Although automated systems are being developed, most researchers manually remove the clusters of differentiated cells. This is a time-consuming and laborious task. Furthermore, human ESCs are prone to chromosomal abnormalities[5], which necessitates regular karyotype screening. Finally, reproducibility may be an issue as variations in growth and differentiation properties may arise among human ESC lines[6]. Techniques for deriving purified populations of specialized tissues (for example, heart or brain) have been reported[7,8], but standard, validated techniques are far from established. Most protocols are challenging, variable, inefficient, expensive and require long periods of time — sometimes up to 10 weeks — to produce the desired cell types.

Such concerns raise the question of whether the potential benefits of investing in human ESC technologies for drug screening are going to justify the cost. We will return to this question later, but first, we describe the new opportunities offered by the derivation of PSCs from adult tissues.

Human iPSCs

The discovery of iPSCs has revolutionized the stem cell field, and has also resulted in interesting and new avenues of exploration for drug discovery. iPSCs are generated by expressing a small set of powerful pluripotency genes in somatic cells (fibroblasts or other adult cell types) to force genetic reprogramming. These newly reprogrammed cells lose their previous somatic cell properties and adopt similar characteristics to human ESCs with regards to morphology, growth properties, gene-expression profiles and differentiation potential.

In 2006, a team of scientists in Japan first reported the conversion of mouse somatic cells into iPSCs using four genes: *Oct4* (also known as *Pou5f1*), *Sox2*, *Klf4* and *c-myc* (also known as *Myc*)[9]. A year later, three studies used similar techniques to reprogramme human fibroblasts[10–12] and, since then, various groups around the world have reprogrammed human and mouse somatic cells into iPSCs using slightly different techniques and pluripotency genes[13]. However, reprogramming efficiency is low (ranging from 0.001% to 1%) and the use of transgene integration methods has been shown to alter gene expression, even after transgene excision[14–16]. Therefore, new methods are being developed, which include using non-integrating viruses or plasmid transfection[17–20] (or simply the proteins themselves[21–23]), or small-molecule induction and/or complementation[24] that will reduce these gene-dosing issues and should lead to more stable cell lines.

The advantages of human iPSCs over human ESCs are that no embryos need to be destroyed to produce them and that many lines can be established from a single donor. But, perhaps most importantly, it is possible to derive them from patients after the disease has been diagnosed and so the severity and age of onset is known. However, some of the issues that limit the application of human ESCs also apply to iPSCs. The generation of iPSCs is not simple. As mentioned above, the efficiency of generating clones is low. Once clones have been established, a considerable amount of time and effort is involved in manually selecting clones and in fully characterizing their reprogrammed pluripotent state. Furthermore, the issues associated with their growth and differentiation, including daily changes of growth medium, maintenance, chromosomal abnormalities and complex differentiation protocols are similar to those described above for human ESCs[25]. Further confusing this emerging field is the current intellectual property status for iPSCs. Although Kyoto University has been granted a patent for iPSCs by the Japan Patent Office[26], and more applications are pending, it is unclear at present how far-reaching their effects will be on future commercialization. Clearly, iPSC intellectual property issues will be of great interest for those considering investing in this evolving field.

PSCs in drug discovery

Cell and animal models contribute a wealth of information about the complexity of various disease processes. However, compounds that show a significant benefit in these models may fail to show effectiveness in clinical trials[27,28]. For example, use of a transgenic mouse that overexpresses mutant superoxide dismutase (*SOD*), a gene found to be associated with amyotrophic lateral sclerosis[29], enabled the identification of several compounds that alter disease characteristics, including vitamin E and creatine[30,31]. However, when these compounds were tested in humans, no clinical improvements were observed[32–34]. Furthermore, toxic effects of compounds are often missed in cell and animal models due to specific interactions with human biological processes that cannot be recapitulated in these systems. These data underscore the need for additional drug screening model systems that complement these systems or may better represent the human condition.

In this regard, human ESCs and human iPSCs (collectively referred to PSCs) may have a key role. However, it is necessary to stress the limitations of the PSC approach. Perhaps the most obvious and concerning is that the cells generated from PSCs are by definition developmentally immature. They may represent a good model of fetal biology, but it is difficult to relate these cells to fully mature cells found in adult humans that have been through years of development. Only further studies that directly compare the maturity of cells derived from PSCs with adult cells will address this issue, and many research groups are currently chasing this goal. With these caveats in mind, some of the major potential uses of the cells are discussed next.

Toxicity screens. Perhaps the most obvious use of PSCs would be in screens to evaluate the effects of novel compounds on specific human cell types that are relevant to potential toxicity. This approach involves

differentiating the PSCs to cells of the tissue that the investigative drug affects, and then performing dose–response toxicity studies (FIG. 1). One example of this type of study would be to assess the effects of drug candidates on functional cardiomyocytes, because drug development is often stalled by adverse cardiac effects[35]. Interestingly, human ESCs can be differentiated into cardiomyocytes that exhibit many of the electrical and physiological properties expected of cardiac cells[36–38]. Thus, measurable disruption of electrophysiological properties by known and novel compounds can be assessed in a clinically relevant, consistent and renewable cell source[39]. If cost-efficient and reliable, a simple screening tool such as this could allow potentially toxic compounds to be eliminated at an early stage of the drug discovery process, allowing efforts to be directed to more promising candidates. Clearly, carefully controlled experiments on cardiomyocytes with compounds known to cause arrhythmias, for example, are now needed. Although this capability may be developed in-house, these types of highly specific assays could also be contracted out to specialist companies. Both Geron Corporation and Cellular Dynamics International are generating human ESC-derived cardiomyocytes and assessing the cardiotoxicity of known and novel compounds. For example, the PSC-derived cardiomyocytes generated at Cellular Dynamics International are based on the founders' previous work showing spontaneous electrical activity, functional contractions and atrial, nodal and ventricular cell populations that all respond with appropriate electrophyiological alterations to arrhythmogenic-blocking drugs[36].

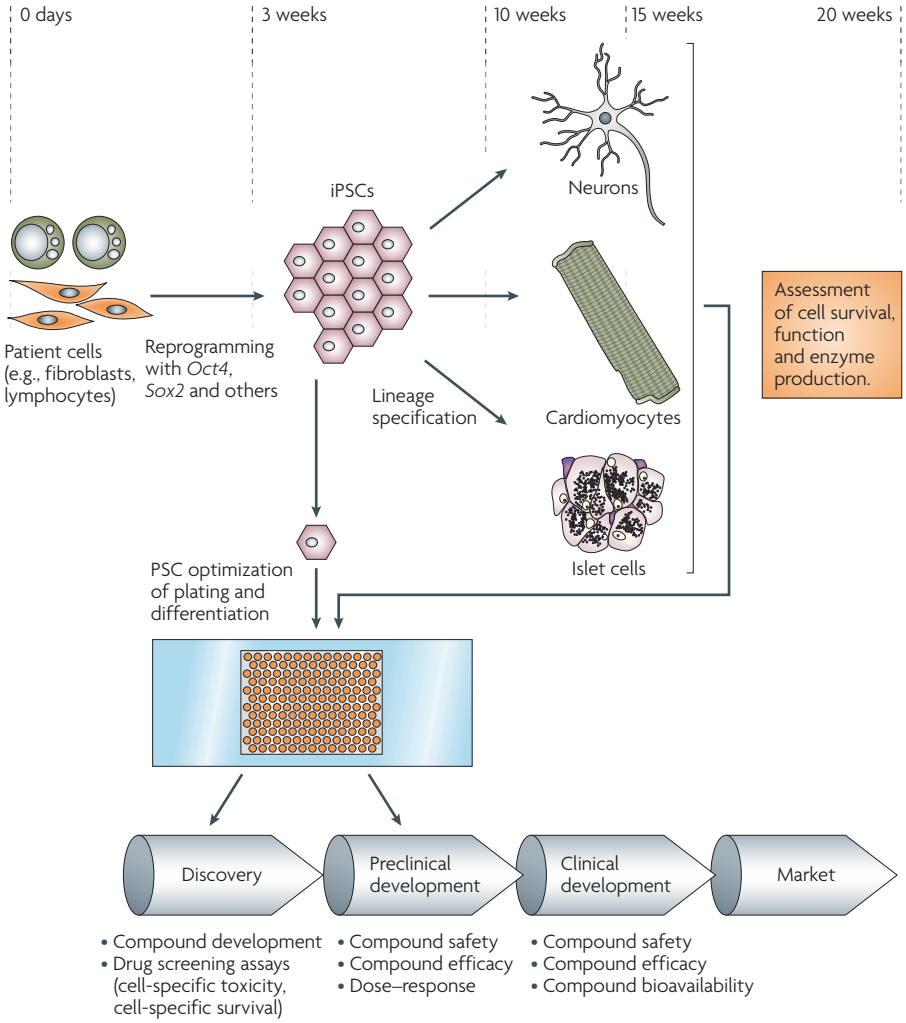

Figure 1 | **Generation and lineage restriction of induced pluripotent stem cells derived from somatic cells can be adapted for high-throughput screening.** Somatic cells (for example, fibroblasts or lymphocytes) derived from patients will form induced pluripotent stem cell (iPSC) colonies following exposure to reprogramming factors. Over the subsequent weeks, and given the correct nutrient-rich environment, iPSCs can be instructed to form the various cell types found in the human body, which could then be useful for assessing multiple cellular characteristics. Additionally, iPSCs could be lineage restricted to a multitude of human cell types (for example, neurons, cardiomyocytes or islet cells) that could then be used in the early stages of the drug discovery process to help identify novel compounds and to evaluate their safety and efficacy in these specialized cell types. These preclinical results could then be used to advance compounds through the next stages of drug development.

Even if the heart remains a primary target for this type of screening, the nervous system is another area of intense activity. For example, some groups have attempted to identify metabolic biomarkers in neural tissues derived from human ESCs after toxin treatment, although these studies were not performed in a high-throughput format[40]. In the future, it might be possible to develop a broad differentiation protocol from human PSCs that generates representative cells from all tissue lineages and then test novel compounds at different doses for toxicity on all the cell types in a single assay. For all of these studies, it remains to be determined whether these artificial *in vitro* models actually predict a drug's toxicity in a complex *in vivo* environment.

Differentiation screens. In addition to assessing the toxic effects of drugs, screens could also be performed to identify compounds that increase self-renewal or differentiation, promote maturation, or simply enhance cell survival from human ESC or iPSC lines. For example, *in vitro* cell culture conditions can sometimes be inadequate to maintain long-term survival of differentiated human cardiomyocytes or neurons. In these cases, the identification of drugs that promote differentiation or survival would be of interest. Traditional methods used to express receptors or to trigger relevant signalling pathways in cell lines could be adapted to work in PSC lines and would be expected to behave in a more physiologically relevant manner.

There are only a few examples of using high-throughput screening methods to assess differentiation. Desbordes *et al.* developed a method to seed human ESCs in a 384-well plate for high-throughput screening of compounds that are related to self-renewal and differentiation[41]. In an interesting differentiation assay, Borowiak *et al.* screened 4,000 compounds to discover two small molecules that drive human ESCs towards the endoderm lineage[42]. Finally, Schultz and colleagues used a high-content screen to identify a small molecule that works in synergy

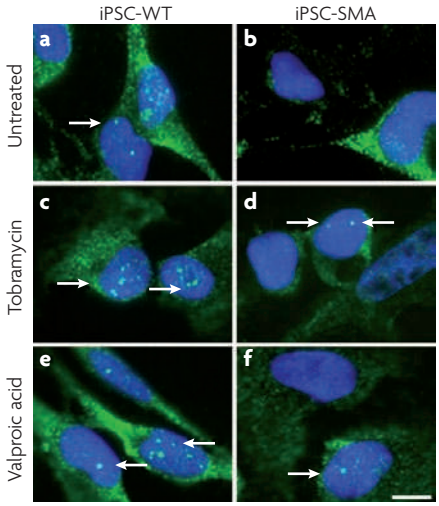

Figure 2 | **Drug treatment increases nuclear gems in induced pluripotent stem cells taken from a patient with spinal muscular atrophy.** Induced pluripotent stem cells (iPSCs) derived from a healthy individual (iPSC-WT) show nuclear aggregates of survival of motor neuron (SMN) protein, termed gems, in the absence or presence of drug treatment (**a**,**c**,**e**). Untreated iPSCs derived from a patient with spinal muscular atrophy (iPSC-SMA) generally lack the SMN nuclear gems (**b**). Both tobramycin and valproic acid will significantly increase the SMN protein and subsequent gem number (**d**,**f**), suggesting a possible therapeutic application. Gems are indicated by arrows. Scale bar represents 50 μm. Images are courtesy of V. Mattis, University of Missouri, Columbia, Missouri, USA.

with extracellular signalling cues to direct differentiation[43]. We anticipate that there will be many similar studies in the future that will allow the derivation of more uniform human tissues from these cell cultures.

Disease modelling and drug screening

Another way to use human PSCs for drug screening would be to isolate them from embryos or from patients with the disease, or to study cells that overexpress disease genes. The fundamental idea would be to expand the cells, differentiate them into the desired cell type, and then screen for drugs that may correct an observed disease phenotype. Although whole animal and cell-based systems have been vital to the understanding of disease genetics and mechanisms, human PSCs offer additional information. For example, human PSCs allow the study of human genes and downstream targets that are either absent or regulated differently in rodents. Furthermore, human PSCs provide a unique insight into human development that traditional human cell lines cannot. Finally, the fully specialized cell types that can be generated from human PSCs allow the investigation of cell types that may not be readily available.

There are some good examples of this approach for nervous system disorders. Stem cells isolated from either embryos or fetal brain tissue with developmental disorders (for example, Down's syndrome and fragile X syndrome) show specific deficits in neuronal production that mimic the disease process[44–46]. Although these cells have not yet been used in drug screening programmes, they could potentially be used for identifying compounds that reduce the neuronal loss seen in these models.

An alternative method of disease modelling would be to induce disease progression in cells using genetic manipulation or chemical toxicity. For example, Parkinson's disease primarily affects the dopaminergic neurons in the substantia nigra. Given the correct cell culture environment, human ESCs have been shown to effectively generate dopamine neurons with the typical chemical and electrical signatures of dopaminergic neurons observed *in vivo*[47]. Approximately 5% of Parkinson's disease cases have been shown to have mutations in various genes, including α-synuclein (*SNCA*), *PARKIN* (also known as PARK2) and *DJ1* (also known as *PARK7*), causing early onset of disease symptoms[48]. Overexpression of mutated synuclein in human ESC-derived dopamine neurons, for example, leads to the selective death of dopamine neurons in this cell culture system[49]. Thus, it is now possible to screen for drugs that may interact with this process, or simply reduce the accumulation of synuclein within cells. Information learned from these overexpression studies may not be relevant to the majority of patients, as the remaining 95% of Parkinson's disease cases are sporadic in nature with no known genetic component; however, synuclein accumulation in dying neurons is a common feature of every patient. There is good evidence that environmental toxins (for example, rotenone, paraquot or 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) also lead to the specific loss of dopamine neurons in humans and primates, so an alternative method of disease modelling would be to add these toxins to the PSC-derived dopamine neurons to examine the molecular processes involved in dopaminergic cell loss, and ways to prevent it.

Similar models of degeneration for Alzheimer's disease using overexpression of pathogenic amyloid protein and cortical neurons, or amyotrophic lateral sclerosis using mutant SOD1 protein and motor neurons, could also be considered in the future. These types of screening assays could not have been imagined before human ESC culture techniques, as it was impossible to produce large numbers of human neurons in a cell culture dish. Thus, a new area of biological screening for neurological disorders is now available to explore.

Drug screening with patient-derived iPSCs

Perhaps the most important and most exciting potential application of human iPSC technology for drug discovery could result from the recent demonstration that these cells can be generated from adult patients with specific diseases[50–52]. Samples could be collected for which the onset, duration and severity of the disease was known. Human iPSCs could then be generated through reprogramming, and expanded and differentiated into cells that are affected by the disease. This differentiation process could be repeated using a potentially limitless source of starting tissue.

Although iPSCs can be used to model both sporadic and genetic diseases, they might be particularly advantageous for investigating diseases that have a solid genetic background and/or occur early in development. This is because sporadic diseases may be caused by epigenetic changes to cells that are subsequently removed through reprogramming. This is an area of intense investigation; for example, Jaenisch and colleagues reported that dopamine neurons could be derived from iPSCs generated from patients with sporadic Parkinson's disease[15]. Interestingly, there was no gross loss of dopamine neurons in this model, suggesting that it may not be a good system for screening anti-parkinsonian drugs. However, it is possible that a more detailed analysis of dopamine neuron anatomy and function may reveal other specific deficits in dopamine neuron function that are associated with the disease process. It is also possible that neurons derived from patients with adult onset diseases such as Parkinson's might not show any phenotype for many years in the cell culture dish. In this case, additional stressors may be required to trigger disease-specific traits.

In contrast to this example for Parkinson's disease, reports using iPSCs to model two different genetic diseases, spinal muscular atrophy and familial dysautonomia, have shown disease-specific phenotypes in cell culture that may be important for drug screening[51,53]. Spinal muscular atrophy is caused by a specific mutation in the survival of motor neuron 1 (*SMN1*) gene, which

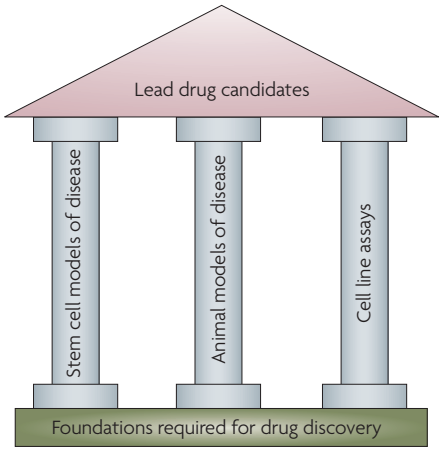

Figure 3 | **Pluripotent stem cells could become an important factor in the drug discovery process.** In combination with animal models and traditional cell-based assays, pluripotent stem cells (PSCs) will be key for developing clinically useful compounds. Drug screening assays using PSCs allow compound testing on differentiated human cells that may otherwise not be readily available from traditional cell lines. Moreover, PSCs can be generated from individuals that naturally harbour disease-specific traits, which could complement results from animal model testing and aid the development of more effective compounds.

leads to the death of motor neurons in the spinal cord and paralysis[54,55]. In mice, SMN is essential for motor neuron survival and knockout animals are embryonically lethal. Interestingly, humans, but not mice, have a telomeric duplication of the gene (*SMN2*), which, due to a single nucleotide transition and exon splicing alteration, only makes 10% of the normal SMN protein[56]. Children carrying only two copies of *SMN2* have the most severe disease course, with onset as early as 6 months and death by 2 years of age, whereas those with a higher *SMN2* copy number have a later onset and decreased disease severity[57]. Using patient fibroblast samples, many attempts are currently underway to find compounds that can increase the production of the SMN2 protein. However, motor neurons are the cells most severely affected by the disease and have yet to be studied. iPSCs generated from a child with spinal muscular atrophy were recently shown to continue carrying the genetic deficit while being expanded to large numbers. These cells can also be differentiated into motor neurons that then undergo disease-specific cell death in the cell culture dish[51]. This represents a powerful example of a model in which to explore both disease mechanisms and novel compounds that may block this disease-specific cell death pattern. Interestingly, drugs known to activate SMN protein in fibroblasts also worked in neural derivatives of iPSCs (FIG. 2).

Similar results were recently reported when studying iPSCs derived from a 10-year-old child with familial dysautonomia[53]. Familial dysautonomia is a developmental peripheral neuropathy caused by a point mutation in the inhibitor of κ-light polypeptide gene enhancer in B-cells, kinase complex-associated protein (*IKBKAP*) gene leading to loss of autonomic and sensory neurons. Interestingly, familial dysautonomia iPSCs showed a specific deficit of neural-crest-derived precursor cells and neurogenesis, characteristics that could be monitored following drug treatment[53]. The iPSC technology may be particularly well suited for early onset diseases such as spinal muscular atrophy and familial dysautonomia.

Many other disease models have been developed[58,59], but have either not yet been adapted for drug screening or are currently being explored. The key features for success will be that the phenotype being screened is reliable and consistent between clones. In some cases, while each iPSC clone or line might generate variable total numbers of specific cell types (as is true for different human ESC lines), there may be reliable longitudinal changes in the survival or functioning of specific cell types over time that define the disease process. It is this disease progression that may provide the most reliable assay with regard to compounds that could slow or prevent disease-specific cell death.

PSCs in drug screening: a look ahead

Although PSCs hold great promise for drug screening, and, as noted above, a strong case can be made for their use, enormous challenges still exist. The first is to convince sceptics that a drug effect on any artificial cellular system can accurately predict what will happen in humans. Our view is that these new technologies are not going to immediately replace current high-throughput assays or whole animal testing. Instead, they may provide the drug discovery field with an interesting new tool that may, in some cases, provide a strong predictive advantage when moving compounds from the bench to the clinic (FIG. 1). An important caveat to using PSCs is the need to generate a consistent source of cells that show reproducible and robust characteristics. PSCs are not immune to selective pressures exerted on them within tissue culture systems, so methods designed to extend the useful life of the cells may inadvertently cause adaptation, leading to changes in cell properties.

What might be the tipping point to convince research laboratories and industry to use PSCs for routine screening? There first needs to be a high-risk investment phase, which will require a number of retrospective studies. For example, drugs that have reached late-stage animal toxicity studies or even clinical trials, and then been found to have a specific toxic effect, need to be tested in PSC models to determine whether the toxicity could have been predicted with such models. This could be done rapidly, and a positive demonstration would certainly be encouraging. Indeed, the first example of a compound discovered through screening in a PSC model of that disease that went on to treat the same disorder would also increase interest significantly. At present, it is key to prove the validity of the PSC approach for drug development. The evolving balance between the cost of developing and using these new disease models and drug screening assays, and the possible benefit and savings that could result, will determine the extent to which the exciting potential of PSCs in drug discovery is realized. Finally, while PSC technology will be important, modern drug screening should use all available techniques to increase the chances of good, safe hits. In our opinion, the three major pillars in the drug discovery process will be iPSC testing, animal testing and cell line testing (FIG. 3). Alone, none of these may be a reliable predictor of drug efficacy or toxicity, but if a drug has appropriate effects in all three of these assays, its chances of reaching the clinic might significantly increase. Only time and validation experiments will determine whether this idea is correct.

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Competing interests statement
The authors declare no competing financial interests.

DATABASES

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