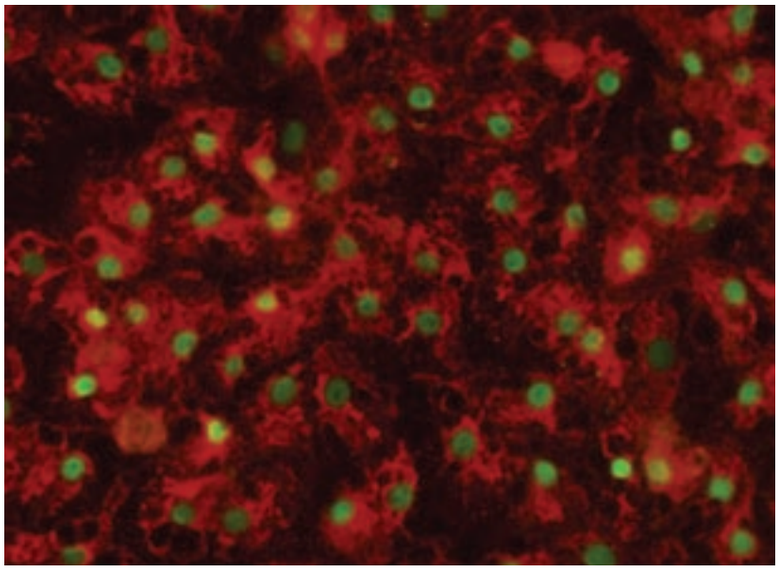
Induced pluripotent stem cells in high-throughput cellular screening

With the failure rates of drug candidates continuing to present phenomenal costs to the pharmaceutical industry, this article discusses how newly emerging induced pluripotent stem cell (iPSC) technologies have the potential to be an effective tool in weeding out low quality candidates early in the process, reducing attrition costs and, ultimately, improving the percentage of new drugs to market.

By Dr Carolyn E. Peluso

Less than 10% of clinical phase drugs make it to market[1]. This would be deemed an efficient use of resources if most drug candidates failed early in process, engendering a comparatively small expense; but nearly a third of drug candidates end in costly, disappointing failures at Phase III. The loss in capital and reputation, not to mention the risk to patients, when a drug candidate fails at this stage of development can be staggering. For example, the Bristol Myers Squibb (BMS) candidate BMS-094 (a hepatitis C drug) failed at Phase III, after the death of a patient, at a cost of $1.7 billion[2], and this is not an isolated case. In 2012 alone, Pfizer, Johnson and Johnson, Eli Lilly, Roche and AstraZeneca all suffered high-profile Phase III failures of this nature. Some of these drugs, such as BMS-094, fail due to unexpected toxicity, but others are simply ineffective in clinical trials. Thus, it is evident that pharmaceutical companies need to re-evaluate how drug candidates are developed and screened, so that the least worthy candidates are screened out early. Here, we will review how newly-emerging induced pluripotent stem cell (iPSC) technologies may be an effective tool pharmaceutical companies can use to make the pre-clinical phase of drug discovery more efficient and improve the number of drugs that make it on to the market.

The decision to promote a drug to the clinical phase is based in large part on data from pre-clinical phase testing, which relies on high-throughput screening methodology and cell-based models. And, it goes without saying, that the reliability of the data generated by the assay is only as good as the starting material. Traditionally, continuous cell lines have been the preferred substrate to support pre-clinical testing because they represent a homogenous population of cells that can be grown in sufficient quantities to support a high-throughput screening campaign. Continuous cell lines, however, are often derived from oncogenic or non-human tissue (eg, mouse, rat), and may not provide a physiologically relevant model of the disease being studied; and this is particularly true if the disease aetiology is genetic. Patient-derived primary cells can also be used, but sufficient quantities are generally not able to be grown to prosecute a large scale screening programme. Cell heterogeneity between patients can also confound results due to genetic variations and other factors. In contrast, iPSCs are generated by expressing key transcription factors (The Yamanaka Factors: OCT4, SOX2, KLF4, and c-MYC) in adult somatic cells, which results in their being 'reprogrammed' to a pluripotent state[3]. These cells can be generated from the skin cells of normal healthy individuals or



iCell® Hepatocytes. Immunostaining of iCell Hepatocytes expressing albumin (red) and hepatocyte nuclear factor 4-alpha (green). *Image courtesy of Cellular Dynamics International*

patients with genetic conditions such as cystic fibrosis and Downs syndrome, and then differentiated into mature cell types. This technology is allowing investigators to generate physiologically relevant cellular substrates, complete with age-matched controls, tailored to the disease the drug candidate is meant to treat. In addition, because these cells can be differentiated into a multitude of mature cell types like cardiomyocytes or hepatocytes, they are ideal for estimating the toxicity of drug candidates. Thus, pre-clinical decisions about which drug candidates are most likely to be effective and safe when they reach Phase III clinical trials can be significantly improved by using iPSC-based assays.

The factors used to reprogramme adult somatic cells into iPSCs were discovered in 2006[3], and an amazing amount of progress has been made in a short period of time. As with any emerging technology, however, the benefits of using iPSCs for *in vitro* disease modelling, drug discovery and toxicity testing are becoming apparent as the research community finds solutions to the hurdles commonly associated with these cells. First, iPSCs were originally reprogrammed using viral vectors. Viral vector-based reprogramming raises the concern that viral integration into the host genome might occur and lead to alterations in gene expression, which would make them less representative of the donor cell. In response, several commercial entities, such as ATCC and Cellular Dynamics International (CDI) now offer 'footprint-free' iPSC-lines generated with episomal vectors that eliminate the need for viral vectors, and thus the potential for viral integration. Second, there are concerns that the *in vitro* microenvironment may results in variability in gene expression between laboratories[1]. Much of the variability between lines can be addressed, however, by careful experimentation and by developing standardised culture systems; and again, commercial entities, such as ATCC and Vitro Biopharma, are actively developing ways to culture iPSCs in a standardised way. Lastly, there are concerns that the production of iPSCs would be difficult to scale up to high-throughput quantities. However, CDI is already generating industrial quantities of high-quality iPSCs, using the non-viral, 'footprint-free' episomal reprogramming techniques.

In addition, both the academic and industrial segments of the drug discovery community have committed to establishing a role for iPSCs in drug discovery. In fact, in December 2012, Roche and Innovative Medicines Initiative announced the formation of an academic-industry union, StemBANCC. This initiative seeks to generate and characterise 1,500 high quality iPSC lines derived from 500 patients to support drug discovery and testing. It will be managed by Oxford University, include contributions from 10 pharmaceutical companies and 23 academic institutions and have funding of 55.6 million euros over five years[4]. Thus, the pieces are falling into place to establish iPSC-based *in vitro* disease models as a tool to identify appropriate disease targets, design effective screening assays and improve toxicity testing. These initiatives will work to reduce Phase III failures by ensuring only the highest quality candidates graduate from the pre-clinical to the clinical phase of development.

In vitro disease modelling

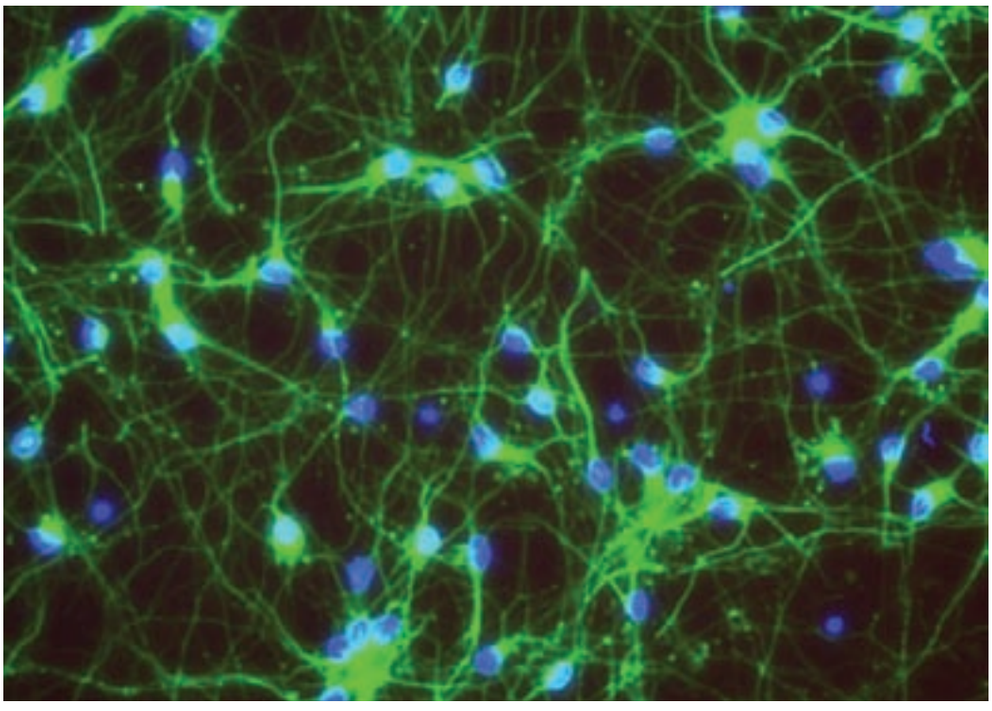
A good *in vitro* disease model supports the drug discovery process by allowing screening against relevant drug targets. Some of the first diseases to be modelled with iPSCs have been neurodegenerative diseases that affect children, for example Retts Syndrome and Spinal Muscular Atrophy (SMA). In Retts Syndrome, the underlying mechanism of this disease, a debilitating, neurodevelopmental disorder caused by a mutation in the MECP2 gene, is not currently known. Researchers, however, have made important strides forward by comparing neurons differentiated from the iPS cells of Rett Syndrome patients with those of healthy control subjects. These studies show that the Rett Syndrome neurons have reduced synaptic potential when compared to neurons generated from the iPS cells of healthy control patients. Additionally, in

this study the Rett Syndrome iPSCs were used to screen, on a small scale, compounds that might improve the synaptic character of the diseased neurons to provide a proof-of-concept for moving these studies on to a high-throughput platform[5].

Second, iPSC-based disease models are becoming especially useful in fields where there is a lack of patient samples, such as in SMA. SMA is a neurodegenerative disease caused by a mutation in the SMN gene that leads to the loss of motor neurons in the spinal cord. Onset of the disease is at about six months of age and death inevitably occurs within two years. Mutation of the SMN gene undermines the long-term survival of the motor neuron, so although the neurons mature, they quickly die[6]. In this case, having the ability to generate motor neurons from the fibroblasts of SMA patients is providing investigators with the materials they need to study the underlying causes of neuronal death, provide good drug targets and develop screening assays. As a first step, investigators have shown that neurons derived from iPSCs derived from the cells of SMA patients have a protein aggregate phenotype consistent with their *in vivo* counterparts that could easily be exploited for drug discovery screens[7].

Both the Retts Syndrome and the SMA iPSC-based models feature the potential for a clear-cut screening assay, and they both lend themselves to the generation of an appropriate age-matched or isogenic control. These features make them ideally suited for incorporation into a high-throughput synapse microarray system, such as the Galenea's Multiwell Automated NeuroTRansmission Assay (MANTRA). This apparatus uses sensitive fluorescent reporter to measure synaptic vesicle cycling as a way to assess pre-synaptic functionality[8]. Another high-throughput system to measure synaptic character is being developed at MIT. Their system allows neurons to form synaptic connections with a feeder layer of non-neural cells (engineered to express synaptic proteins) in a confined space, so that the effect of compounds on neurite length and synapse formation can be measured in a reliable, uniform and scalable (ie, high-throughput) manner[9]. Therefore, the screening assays for compounds to treat Retts Syndrome, SMA and diseases like them, stand to improve both from having better high-throughput platforms and more physiologically relevant iPSC-based substrates for testing.

iPSC-based models are also valuable when modelling diseases that affect multiple systems, such as Friedreich's Ataxia (FRDA). FRDA is a genetic disorder that causes progressive neurodegeneration of the sensory and cerebellar pathways, as well as cardiac dysfunction. The root cause of the disease has



iCell® Neurons. Immunostaining of iCell Neurons for the neuronal marker -III tubulin (green) showing extensive neurite outgrowth. *Image courtesy of Cellular Dynamics International*

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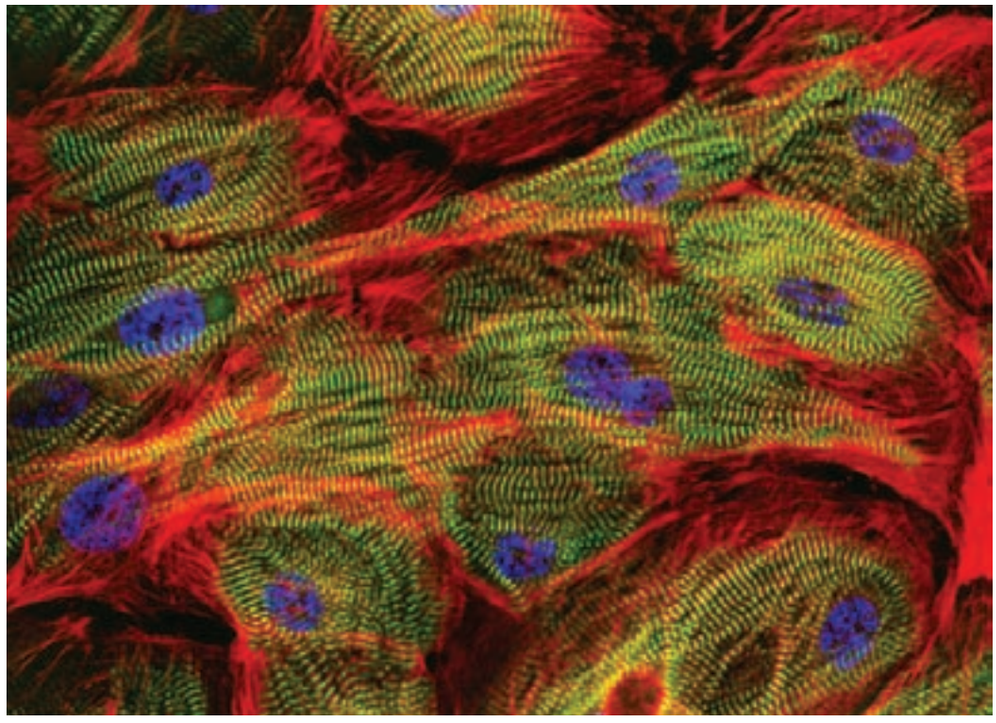

iCell® Cardiomyocytes. Immunostaining of iCell Cardiomyocytes showing actin (red), myomesin (green), and nuclei (blue). *Image courtesy of Cellular Dynamics International*

been linked to the reduced expression of the mitochondrial protein, frataxin, which results in mitochondrial dysfunction that disproportionately affects the brain and the heart. Although, cell models for this disease exist, these lines are not derived from the cells that are most affected by the disease (ie, cardiomyocytes or neurons), and so they do not faithfully represent the *in vivo* condition[10]. iPSC lines on the other hand can be generated from individual patients and then differentiated into the cell types affected by the disease. In this way, the effect of drug candidates on the mitochondrial function of different cell types can be examined in the same genetic background.

The preceding examples demonstrate the progress that has been made in modelling certain diseases. However, these diseases all have clear genetic causes and symptoms that manifest early in childhood or early adolescence. More difficult to model are diseases such as Alzheimer's or Parkinson's disease, which may or may not have an identified genetic component, and that are heavily influenced by age or environmental factors. In cases like these, iPSCs generated from patients with genetic forms of these diseases may still be used to develop new biomarkers. In addition, models developed with iPSCs from patients with genetic forms of the disease may still offer insights that will help in the development of new leads for compounds that may be effective in the sporadic forms of the disease. For example, one of the most commonly associated with Parkinson's disease is in the Leucine-rich repeat kinase-2 (LRKK2) gene. Dopaminergic neurons (the class of cells affected in Parkinson's disease) have been generated from patients with the LRKK2 mutation and microarray analysis shows that they have higher levels of oxidative stress proteins than their control counterparts[11]. These studies indicate that the neurons affected in Parkinson's disease may be more susceptible to oxidative stress and this may help investigators model the disease and predict appropriate drug targets that are applicable to both the genetic and sporadic forms of the disease.

Thus, it stands to reason that by combining powerful experimental platforms, such as those that measure synaptic features or mitochondrial metabolism, with physiologically relevant iPSC-models of disease, pharmaceutical companies will be able to generate high quality drug targets and screening assays. Armed with such tools they should be able to make better decisions about which drug candidates to advance through the pipeline.

Toxicity testing

Much of the cost associated with pre-clinical testing is due to regulatory requirements that mandate a minimum of two *in vivo* animal models (at least one rodent and one non-rodent) be used for estimating the bioavailability of a new drug. There are several reasons for estimating toxicity in a whole animal model; among them are estimating safe clinical starting dosages or predicting drugs that may be metabolised and accumulated to toxic levels in the liver. However, given the number of drugs that fail due to toxicity in the clinical phase, animal models may not provide the best representation of human bioavailability. Once again, these assays are only as good as the starting material, so it is necessary to ensure that only the best candidates make it to the animal model phase. Importantly, some forms of toxicity can be estimated using a cell-based assay. For example, cardiotoxicity often manifests as ion channel block, arrhythmia, altered contractility, etc, and many of these issues can be examined using biochemical markers[12]. Therefore, the best way to make the process more effective is by making cell-based estimation of toxicity as accurate as possible prior to taking the candidate into animal trials; and iPSC are perfectly suited to the task.

As mentioned previously, CDI is generating industrial quantities of iPSCs, using episomal techniques that do not affect changes in the genome. In particular, CDI's iCell®Cardiomyocytes are derived from iPSCs, have the electrophysiological and biochemical properties of human heart cells, and can be made in sufficient quantity and quality to test against cardiotoxicity in a high-throughput platform[13]. Recently, scientists at Pfizer, Inc compared the ability of iCell®Cardiomyocytes to predict the cardiotoxicity of drug candidates against a traditional model of rat-derived cardiomyocyte cells, and the iCell®Cardiomyocytes were found to be the more sensitive and accurate model for predicting potential cardiotoxicity[14]. The value of these cells has been realised by Roche, which is currently using the iCell®Cardiomyocytes to monitor drug candidates for cardiotoxicity and this step has been integrated into its drug development decision-making process[13]. Additional evidence exists that iPSC-based models of cardiotoxicity are making their way into the decision-making process of other companies as well. Recently, Merrimack Pharmaceuticals promoted a novel liposomal doxorubicin formulation for the treatment of HER2+ breast cancer to Investigational New Drug status and Phase I clinical trials, based on pre-clinical cardiotoxicity data from an iPSC-based model[15].

Importantly, CDI is expanding the availability of its iCell®Cardiomyocytes technologies to the smaller pharmaceutical companies by partnering with the toxicity screening specialists at CeeTox to develop the Cardiac Arrhythmia Assessment Screen (CAAS). The CAAS combines iCell®Cardiomyocytes with CeeTox's *in vitro* toxicity screening technologies to provide a cardiotoxicity screening service that firms can utilise to profile a new compound early in the drug development process. Similarly, a partnership has formed between Vitro Biopharma and HemoGenix® to develop liver toxicity testing platforms that rely on Vitro Biopharma's adult stem cells and media and HemoGenix's® industry-accepted bioluminescent assay system. Thus, this system is well placed to streamline liver toxicity assays for drug development and design. Moreover they are in the process of expanding their partnership to add similar systems for the toxicity testing of kidney, heart and neural cells (Press Release, Vitro Biopharma, April, 2012). Therefore, iPSC technology is rapidly changing the way that drug companies of all sizes screen their candidates for potential toxic effects.

Concluding thoughts

The potential value of iPSCs to the drug discovery process is wide reaching. iPSC-based disease models are streamlining the bench-to-bedside transition by reducing the need to use less relevant animal cell models. Additionally, commercial entities have the ability to generate high-quality, physiologically-relevant cells in quantities that promise to make toxicity estimations more accurate and sensitive. These efforts will make drug discovery and toxicity estimation more effective and will help ensure that more low quality candidates are weeded out early in the process. Thus, iPSCs-based assays will improve the quality of the candidates selected for promotion from the pre-clinical to the clinical phase of testing, which will reduce costly Phase III failures and significantly improve the percentage of drug candidates that make it on to the market. DDW

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