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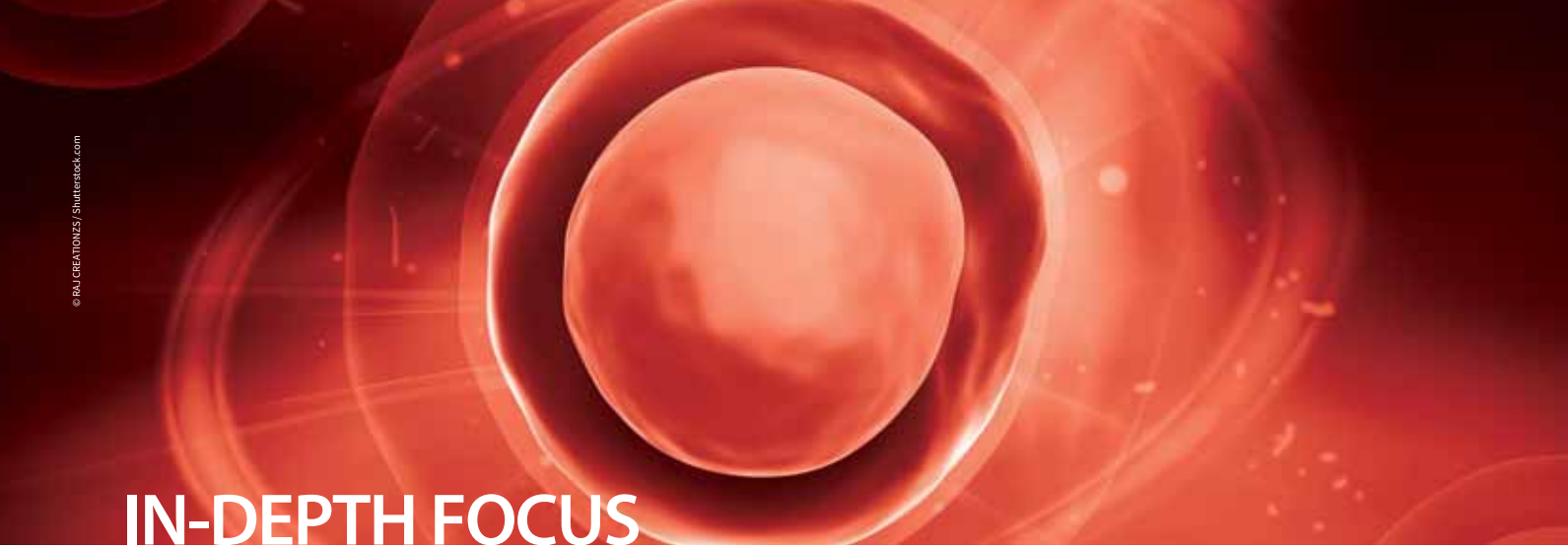

The promise held by induced pluripotent stem cells for research and regenerative medicine

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Human induced pluripotent stem cells (iPSCs) have had an unprecedented impact on biomedical research, disease modelling, toxicology studies and drug development. In addition to circumventing ethical and moral disputes associated with the use of blastocyst-derived embryonic stem cells (ESCs), iPSCs provide new perspectives for personalised medicine in the future. In this review, we highlight mechanisms underlying the induction of pluripotency in somatic cells and discuss potential applications in the field of regenerative medicine.

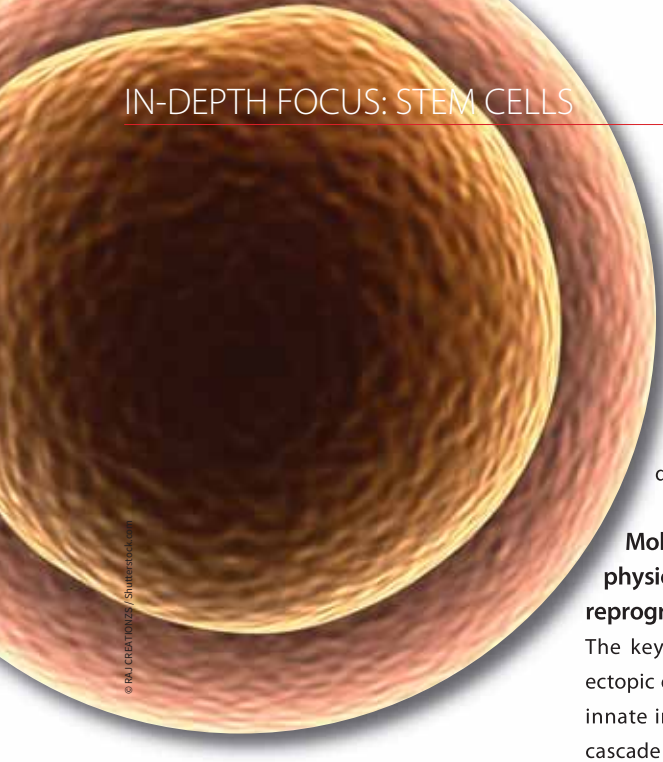
Induced pluripotent stem cells (iPSCs), like embryonic stem cells (ESCs), are inherently endowed with the ability to self-renew indefinitely and are pluripotent, which implies the ability to differentiate into cell derivatives of all three embryonic germ layers (mesoderm, endoderm and ectoderm) upon instructive cues. Pluripotency is induced in somatic cells by the over-expression of distinct combinations of embryonic transcription factors, namely, OCT4, SOX2, KLF4 and c-MYC (OSKM)[1], or OCT4, SOX2, NANOG and LIN28 (OSNL)[2]. Of these two combinations, the Yamanaka factors are the most frequently used by the majority of laboratories (**Figure 1**, page 4).

Since the initial publication of the protocol, my laboratory[3-6] and several worldwide have derived iPSCs from numerous cell types, such as melanocytes, hepatocytes and cells derived from cord blood, peripheral blood, adipose tissue, amniotic fluid, chorionic villi and, more recently, urine[7] – refer to Drews *et al* for an extensive review[8].

In this review, we briefly summarise recent advances and achievements made in the derivation of human iPSCs, highlight key molecular mechanisms underlying the induction of pluripotency in somatic cells and further pinpoint potential and promising applications of iPSCs, with emphasis on disease modelling, toxicology studies, drug screening and personalised medicine.

Derivation protocols

The initial protocols for deriving iPSCs relied on retro and lentiviral based ectopic expression of the reprogramming factors. Although these protocols are reproducible, viral-derived iPSCs harbour integrated viral DNA. Continuous efforts are being made to derive iPSCs employing non-integrative methods such as episomal plasmids, mini circle DNA, sendai viruses, bacterial expressed OSKM proteins, embryonic microRNAs (miR-302-367 cluster or miR-200c plus microRNAs of the miR-302 and miR-369 families) and *in vitro* derived OSKML mRNAs (reviewed by Drews[8]). Of these,



the episomal-based approach is the preferred method[9]. Numerous small molecules, capable of enhancing the efficiency of reprogramming, have been described; for example, vitamin C[10], valproic acid (VPA) – a histone deacetylase inhibitor[11], and also VPA in combination with 8-Bromoadenosine 3', 5'-cyclic monophosphate (8-Br-cAMP), an analogue of cyclic AMP[5]. However, supplementation with a combination of several other chemicals has been shown to be more effective. For instance, transient supplementation with defined cocktails consisting of substances such as the MEK inhibitor PD0325901, the ALK4/5/7 inhibitors SB431542 and A-83-01, the GSK-3 inhibitor CHIR99021, the inhibitor of lysine-specific demethylase 1 Parnate (also known as tranylcypromine), the allosteric activator of 3′-phosphoinositide-dependent kinase-1 (PDK1) PS48, the histone deacetylase inhibitor sodium butyrate (NaB), thiazovivin, and ROCK inhibitor HA-100 enhance distinct reprogramming protocols in various somatic cell types (see Wang *et al* for a detailed list of small molecules[12]).

Molecular and physiological hallmarks of the reprogramming process

The key molecular events induced by the ectopic expression of OSKM are activation of innate immune responses[13,14] followed by a cascade of events such as inflammation and activation of reactive oxygen species (ROS). Increased ROS leads to both DNA and protein damage and ultimately the activation of p53 (gatekeeper of genome integrity), which is responsible for arresting cell cycle and inducing apoptosis and senescence[14]. Activation of the p53 machinery is seen as a roadblock to the induction of pluripotency. Accordingly, stable or transient down-regulation of p53 enhances the efficiency of reprogramming somatic cells[5,15-20] but at the expense of maintaining genome integrity.

The reversal of epithelial-to-mesenchymal transition (EMT), hence, inducing mesenchymal-to-epithelial transition (MET) is another crucial step when inducing pluripotency in cells of mesenchyme origin[21-24]. This transition has been shown to be rapidly activated by the initial inhibition of TGFβ pathway by supplementing with the ALK4/5/7 inhibitors SB431542 and A-83-01 or by over-expressing mir200[9,25-28].

Chromatin remodelling, CpG methylation and histone modifications also play key roles in activating pluripotency supportive-gene regulatory networks. This has been demonstrated by the supplementation of somatic cells with agents known to activate the chromatin remodelling machinery, these include VPA, sodium butyrate and vitamin C[10,11,28].

Reprogramming of energy metabolism which is a characteristic feature of cancer cells[29], is also operative during reprogramming[3,28,30-34]. There is a reduction in the number of mature mitochondria within iPSCs unlike the parental somatic cell and, likewise, reduced oxidative phosphorylation. This translates into increased glycolysis and, hence, reduced production of ROS. Accordingly, derivation of iPSCs under hypoxic conditions or by the modulation of oxygen levels using the allosteric activator of 3′-phosphoinositide-dependent kinase-1 (PDK1) PS48 has been shown to significantly enhance the efficiency of cellular reprogramming[28,35].

The importance of maintaining genome integrity

A normal karyotype is obligatory if we are to derive clinical grade iPSCs for future applications in cell replacement therapies. There is accumulating evidence suggesting that the derivation of iPSCs can adversely affect the integrity of the genomes of the parental cells. The occurrence of chromosomal aberrations within human ESCs and iPSCs have been demonstrated[36,37]. Furthermore, the reprogramming process has been shown to be associated with high mutation rates[38-40]. By next generation sequencing-based analysis of mitochondria DNA (mtDNA) from four distinct human iPSC lines, Prigione *et al* identified the occurrence of both hetero and homoplasmic point mutations, some of which were induced by the reprogramming process[32]. A recent PCR-based screen for mtDNA deletions in as many as 16 human ESC lines at distinct cell passages (33-334) identified 20 distinct large deletions ranging in size from 3.7 to 11.8 kb[41]. Although these deletions did not affect the differentiation potential of these lines, the long term effects on functionality has yet to be assessed. Interestingly, Cherry *et al* have shown that iPSCs carrying a high burden of deleted mtDNA

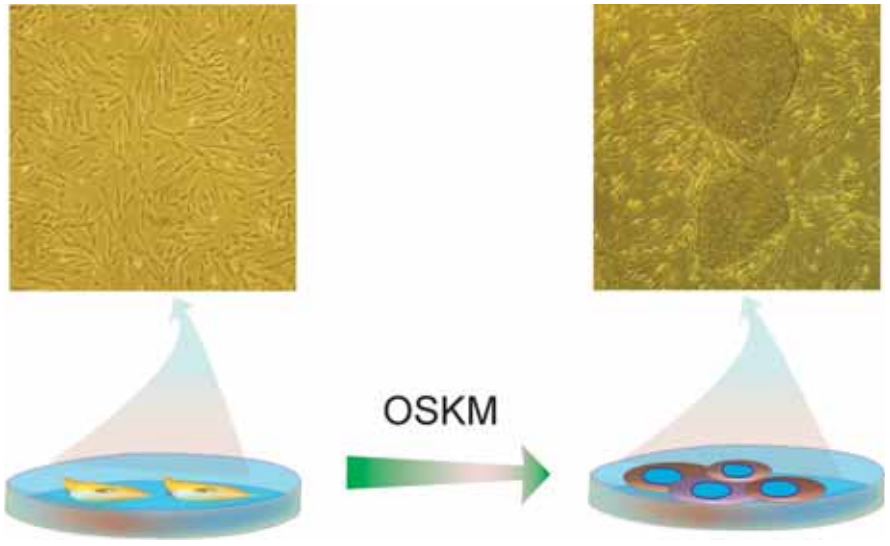


Figure 1: Cellular reprogramming – the generation of induced pluripotent stem cells
The limited developmental potential of somatic cells (left), such as skin fibroblasts or cells obtained from amniotic fluid, can be reset by forced over-expression of reprogramming factors like OCT4, SOX2, KLF4 and c-MYC (OSKM). As a result, these somatic cells form colonies of induced pluripotent stem cells (iPSCs, right) that closely resemble *in vitro* cultures of human embryonic stem cells (hESCs)

display differences in growth, mitochondrial function, and hematopoietic phenotype when differentiated *in vitro*, compared to isogenic iPSCs without deleted mtDNA[42]. This is the first demonstration that iPSCs derived from patients with mtDNA disorders can be useful tools for studying mitochondrial diseases. Indeed, whether the described point mutations and deletions within mtDNA will have adverse effects for future therapeutic applications of iPSCs is open for discussion and further investigations.

Proposed *in vitro* and *in vivo* applications of human iPSCs

Due to their differentiation potential and availability, we envisage a broad range of applications of iPSCs both *in vitro* and potentially *in vivo* in years to come. The scheme presented in **Figure 2** illustrates some of these applications which are further discussed in the following paragraphs.

The iPSC technology has been used to model disease (for example, Alzheimer's, Parkinson's, spinal muscular atrophy and Rett's syndrome) phenotypes *in vitro*. Due to limitation of space, we refer you to Table 1 in Drews *et al* where we present a list of published iPSC-based

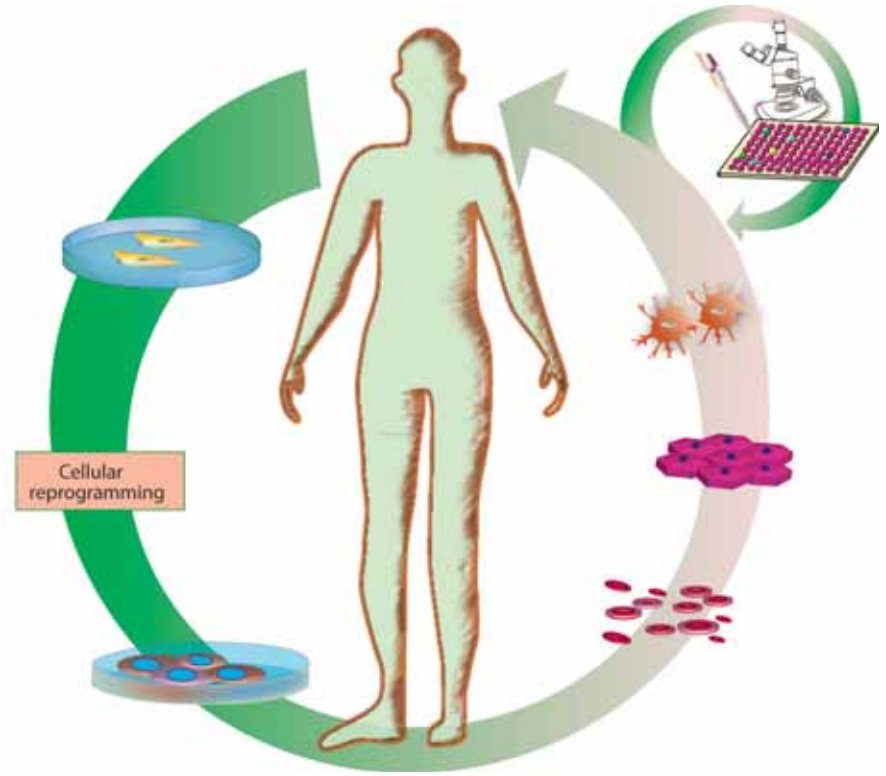


Figure 2: The promise of human induced pluripotent stem cells

Cellular reprogramming technology can be used to induce pluripotency in somatic cells obtained from healthy or diseased individuals. Once de-differentiated, the developmental potential of the resulting induced pluripotent stem cells (iPSCs) can be exploited to differentiate them into a specialised cell type of interest, such as hepatocytes, neurons, cardiomyocytes or blood cells with unprecedented potential for personalised medicine. Based on their genetic background these specified cells can be used, for example, to study the etiology of a disease or they can be applied in toxicity tests or drug development *in vitro*. Ideally, such terminally differentiated cells could be employed in therapeutic approaches involving stimulation and repair of damaged tissues *in vivo*

disease models which have been shown to mimic the disease phenotype[8].

Human iPSCs are also a useful *in vitro* tool for drug development and toxicology studies. Hepatocytes are central for characterising the metabolism of chemicals or candidate drugs. Various hepatocyte models have thus been developed for use in safety pharmacology and toxicology research to understand the mechanisms of drug induced liver injury (DILI) and to screen new chemical entities (NCEs) for their potential to cause adverse reactions. Freshly-isolated hepatocytes, cryopreserved hepatocytes, immortalised cancer cell lines (e.g. HepG2), liver tissue preparations (slices, microsomes and S9 fractions) and animal models broadly categorise the numerous hepatocyte models available for studies into the pathophysiology of DILI. However, the utility and relevance of these models are also limited. The gold standard *in vitro* model for the study of DILI in human is primary cultured freshly-isolated human hepatocytes. However, the use of human primary hepatocytes is impeded by their limited availability, inter-donor differences, variable viability following isolation and rapid de-differentiation of the hepatocyte phenotype in culture, particularly in the loss of cytochrome P450 (CYP) enzyme expression. The limited lifespan and phenotypic instability also limits the utility of the primary hepatocytes model to short-term studies only, and compromises their use in mechanistic studies of DILI which often occurs following prolonged exposure to drugs. Immortalised cancer cell lines such as HepG2 have been used to overcome these problems as they have an infinite life span and are readily available. However, they suffer from a deficit in metabolic activity[43-45].

"Human iPSCs are also a useful in vitro tool for drug development and toxicology studies"

An iPSC-based strategy would enable large scale studies impossible to perform on primary cell cultures and also studies on hepatocytes genetically susceptible to DILI as *in vitro* models with genotypic relevance for toxicology screening. Furthermore, iPSC-derived cardiomyocytes or neurons can be used for identifying pathways that can be modulated by drugs. There has been a surge in companies offering a variety of iPSC-derived terminally differentiated cell types, e.g. cardiomyocytes, hepatocytes and neuronal cells, which are useful for assessing safety and efficacy of potential new drugs and to evaluate toxicity *in vitro*, thus, also decreasing the need for animal models.

The success of transplanting human iPSCs

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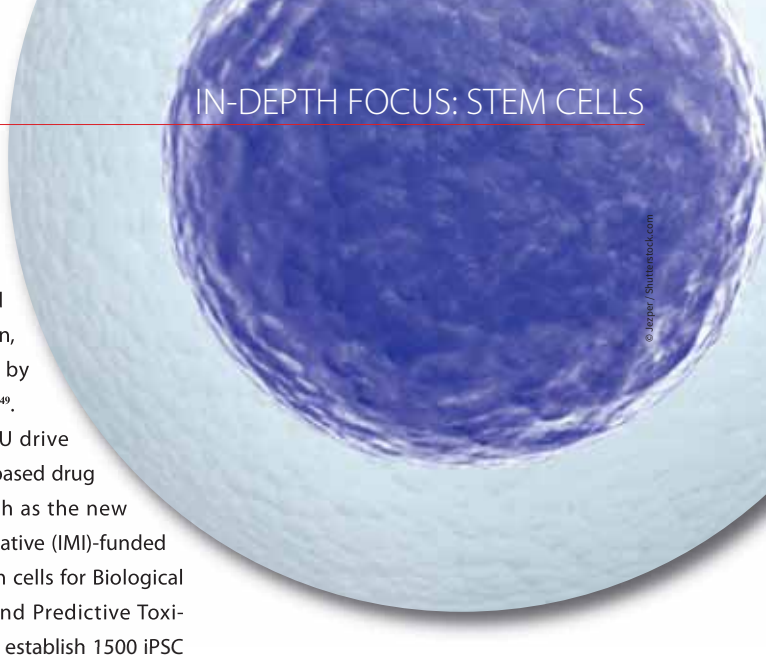

into rodent models[46,47] underscores the therapeutic potential of human iPSC-derived cells in regenerative medicine. Recently, the company Advanced Cell Technology and Moorefields Eye Hospital in London undertook a cell replacement trial to assess the safety of treating macular dystrophy by transplanting human ESC-derived retinal pigment cells[48]. Despite this hopeful and promising human ESCs-based trial in the eye, which is an immune-privileged organ, many experimental obstacles have to be bypassed before specific donor cell types derived from iPSCs can be applied to humans.

Banking

To overcome time-consuming and high costs associated with the derivation, characterisation and safety validation of individual clinical-grade iPSC lines and also the immune rejection of non-autologous cell transplants, the establishment of HLA-haplotype banks of iPSCs has been suggested and even initiated in Japan, an effort spearheaded by Professor Shinya Yamanaka[49].

There has been an EU drive towards promoting iPSC-based drug screening platforms, such as the new Innovative Medicines Initiative (IMI)-funded project StemBANCC: Stem cells for Biological Assays of Novel Drugs and Predictive Toxicology which proposes to establish 1500 iPSC lines encompassing healthy and disease for specific use by pharmaceutical industries for drug screening (www.stembancc.org/Research.html) and also the IMI call for applications on establishing an EU-wide iPSC bank (www.imi.europa.eu/content/6th-call-2012). Though not yet reality, it is now accepted that iPSCs have far reaching potential for regenerative medicine[50].

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Biographies

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Professor Dr. James Affram Adjaye has a BSc (Honours) degree in Biochemistry from University College of Cardiff, Wales. He then obtained an MSc in Biochemistry from the University of Sussex, and then PhD (genetic manipulation and molecular biology) from King's College, London. He is now Director of the Institute for Stem Cell Research and Regenerative Medicine within the Faculty of Medicine at the Heinrich-Heine-Universität, Düsseldorf. He is also Group leader of the Molecular Embryology and Aging Group at the Max Planck Institute for Molecular Genetics in Berlin. He is involved in systems biology-based projects both at the national and international level where iPSCs are used to model human diseases.

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WORKSHOP PREVIEW

Practical workshop – cell based assays for screening

DATE: 11-13 June 2013
LOCATION: The European ScreeningPort GmbH

European ScreeningPort

Recent years have witnessed an expansion in the disciplines encompassing drug discovery outside the pharmaceutical industry. This is most notable with a significant number of universities worldwide that now host infrastructure such as compound libraries and automated screening centres[1-3]. An archetypal small molecule drug discovery project will aim to identify chemical starting points that modify the functions of genes, cells or biochemical pathways. In some but not all instances, these functions may be linked to disease processes, and an opportunity will exist to further develop the chemical starting points into novel therapeutic agents. In small molecule drug discovery, the ultimate aim is to identify new therapeutics, an activity that for reasons of high risk and cost has historically been conducted within the commercial sectors[4].

The expansion of small molecule drug discovery outside the pharmaceutical industry has coincided with increasing numbers of exploratory molecular targets and mechanisms, both therapeutic and non-therapeutic in origin[5]. Screening using miniaturised microtitre plate formats remains the most widely utilised methodology for identifying novel chemical starting points that are capable of modulating target function in a meaningful, biologically relevant manner[6]. The first practical steps in drug discovery include the selection of a target (followed by its cloning, expression and purification), development of an assay to monitor the activity of the target, and the synthesis and management of molecular libraries. The second practical steps include the use of the above in screening campaigns to identify primary hits, followed with their validation. In the context of drug discovery projects that make use of biochemical assays with purified targets, the activities of selected primary hits would typically be further evaluated in biophysical assays such as surface plasmon resonance and isothermal titration calorimetry. This effort would be expected to lead to the identification of validated hits with some of these selected for optimisation using multiple criteria including structure activity relationships, selectivity, physicochemical properties and liability[7,8]. The typical workflow described above was arrived at subsequent to the completion of the sequencing of the human genome where a wealth of new targets were identified and considered worthy of exploration for drug discovery purposes.

Despite the successes that have been reported in the literature[6] where the above approaches have led to the identification of potent and selective compounds, their activities often fail to translate *in vivo* and this may well be due in part to the target based assays being non-physiological in nature, e.g. the target protein being a truncate of the protein and the assay using a substrate that is non-physiological.

We are now witnessing a resurgence of cell based assays including phenotypic assays where a particular change is monitored, in some cases without knowledge of the underlying target(s) upon which compounds are acting upon. Some of the successes using these approaches for drug discovery have been reviewed[9]. It is interesting to note that in some cases where efficacious compounds were identified, the target(s) they act upon were identified subsequently. However, their efficacy may still be due to a poly-pharmacological effect that includes the effect of compounds upon additional targets that may still be unidentified. Advances in cell based assays are also being made, for example using human induced pluripotent stem (iPS) cell-derived cells that better recapitulate normal human biology compared to transformed cell lines and non-human primary cells.

It is in light of the above and the increase in adoption of cell based assays for drug discovery that a partnership was established between *European Pharmaceutical Review* and the

European ScreeningPort in order to set up a unique **practical workshop – cell based assays for screening**. This workshop is being held subsequent to two successful **practical workshops – biochemical assays for screening** that were held between 4 – 6 December 2012 and 7 – 9 April 2013. The **practical workshop – cell based assays for screening** will be held between 11-13 June 2013 at the European ScreeningPort facility in Hamburg and will be part lecture based with a significant practical component.

Participant profile, learning objectives and contents of the *practical workshop – cell based assays for screening*

The **practical workshop – cell based assays for screening** is designed for scientists at all levels (undergraduates, postgraduates and laboratory based scientists within academic and industrial research organisations) engaged in early stage drug discovery and have an interest in the development, validation and utilisation of cell based assays for screening against small molecule libraries. The **practical workshop – cell based assays for screening** is equally well suited to technically focused staff from core facilities or contract research organisations who may wish to extend their expertise. The evening dinner on the first day will offer the opportunity for the participants to network and establish relationships that would be mutually beneficial.

The main learning objectives of the **practical workshop – cell based assays for screening** will be to examine by way of practical sessions and lectures, the design and application of cell based assays for small molecule screening campaigns in drug discovery. All participants will take part in the practical sessions and these will involve the development of screening compatible cell based assays, Primary screening using a small molecule library, and Profiling of compounds in dose-response experiments. Participants in this workshop will discuss and demonstrate practically: (1) the appropriate steps in selecting suitable assays in light of the fact that a multitude of assay technologies are currently available for a given target; (2) how to select an appropriate technology; which criteria should be examined during the early stage drug discovery process; (3) whether a generic, flexible set of assay methodologies or customised solutions should be applied to the targets being investigated; (4) annotation of hits using cell health assays (e.g. cell viability, proliferation, apoptosis, mitochondrial toxicity) as well as cardiac hypertrophy and neurite outgrowth assays using human iPS cell-derived cardiomyocytes and neurons.

The specific aspects of the lectures will cover general concepts in drug discovery, the role of biochemical and cell based assays for drug discovery purposes, their advantages and disadvantages and how to incorporate them into a drug discovery workflow.

The practical workshop – cell based assays for screening will include the following laboratory sessions:

1. General concepts for cell based assays exemplified using luciferase reporter and High Content imaging assays
2. IC_{50} determination for inhibitor, signal stability, choice of liquid handling and Z' calculation
3. Screening of cell based assays against a small molecule library (proof-of-concept screen)
4. Application of cell health, cardiac hypertrophy, and neurite outgrowth assays using human iPS cell-derived cardiomyocytes and neurons.

The *practical workshop – cell based assays for screening* will include the following lectures:

1. Introduction to drug discovery and the design and development of biochemical and cell based assays for drug discovery purposes – what can be achieved and learning from past successes and failures
2. Screening jargon and terms
3. Selection of assays which will ensure translation of hits between formats
4. Data analysis and reduction – going beyond the Z'. Discuss methods to analysing *in vitro* biological assays data including false positive / negative rates, dose-response curve fitting and correlations
5. Analysis of images from High Content Screening assays
6. Integrating your research program, design of project critical paths which integrate *in-vitro, in-vivo* and *in-silico* elements.

The **practical workshop – cell based assays for screening** is pending approval from the Society of Biology for purposes of Continuing Professional Development (CPD) and may be counted as 72 CPD credits if registered on the Society of Biology CPD Scheme.

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Biography

Sheraz Gul is Head of Biology at European ScreeningPort, Hamburg, Germany where he manages the assay development and screening of academic targets and is the scientific coordinator of the first **practical workshop – cell based assays for screening**. Prior to this, he worked for GlaxoSmithKline for seven years where he developed biochemical and cellular assays for High Throughput Screening as well as hit characterisation. In addition, he has worked in academia for five years on proteases and kinases. He is the co-author of the Enzyme Assays: Essential Data Handbook. He is also involved in many European drug discovery Initiatives involving government, Pharmaceutical industry and academia (e.g. EU Framework 7 and IMI). His research interests are directed towards maximising the impact of HTS for drug discovery.

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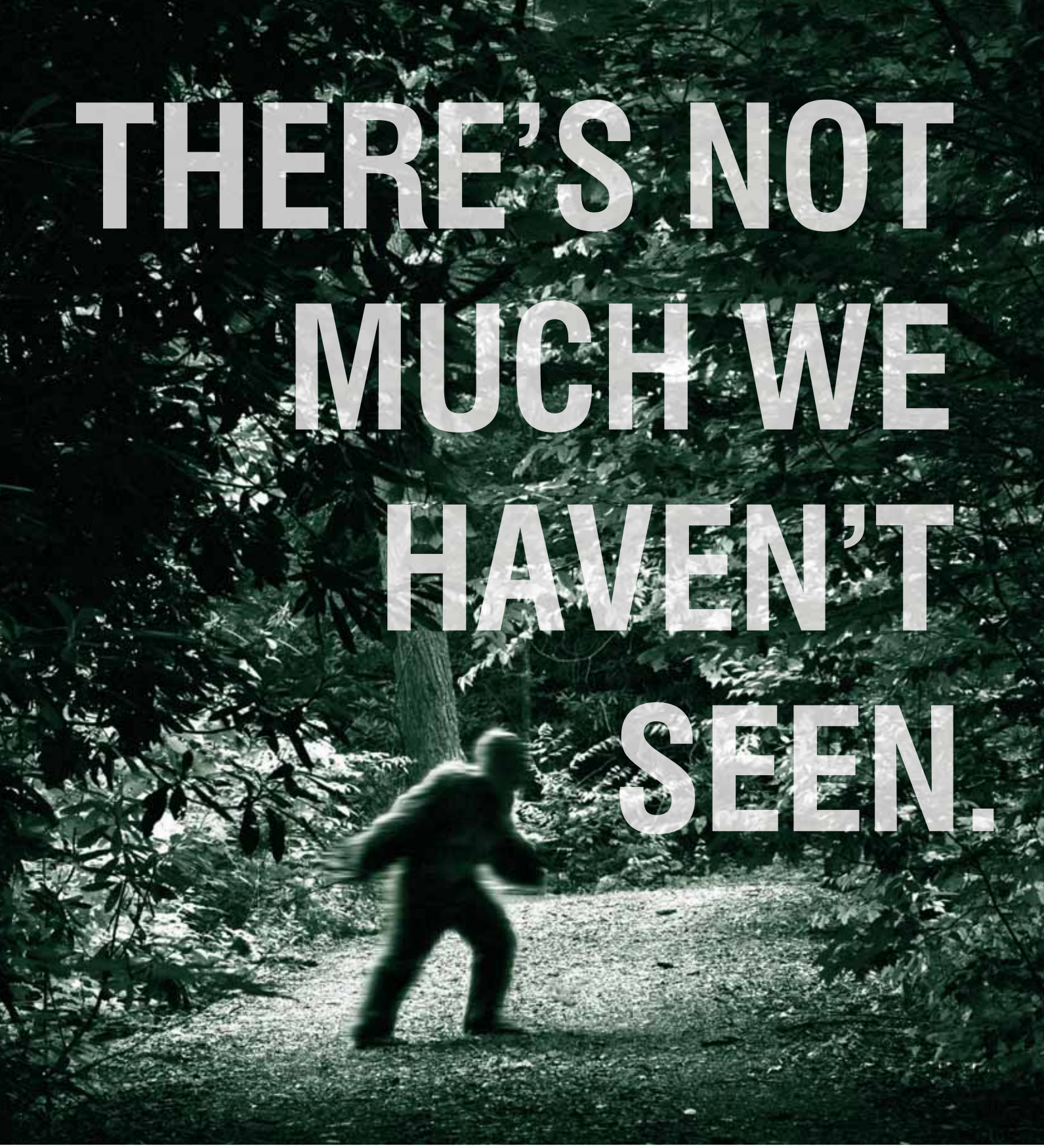

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Cardiac stem cells

Annette Meeson
Institute of Genetic Medicine, Newcastle University

For many patients with heart failure, treatment options include optimised medical management, surgical intervention, the use of devices such as pacemakers, ventricular assist devices (VADS) or total artificial hearts (TAH) or if available, a heart transplant. These approaches are not without their limitations and not all are suitable for all cardiac patients. Pharmacological management carries with it the risk of drug-related side-effects. The use of devices such as pacemakers, TAH and VADS may come with increased risks of infection, bleeding and device failure. Heart transplantation is a highly invasive procedure carrying the added risk of transplant organ rejection and the side-effects of long term immunosuppression, and remains a limited treatment option due to the shortage of suitable donor organs.

Other alternatives for the treatment of cardiomyopathy are urgently needed and since the acceptance that the heart is capable of some level of cell renewal in the last decade, the spotlight has turned on the use of cellular strategies to bring about cardiac repair. In this review, we discuss cardiac derived stem cells, the promise they hold, the current limitations, and questions we need to address, now that these cells have begun their journey into the clinical setting.

Types of cardiac stem cells – the cell of choice?

Numerous putative cardiac stem and progenitor cell populations have been identified in the human heart, these include c-Kit positive lineage negative cells, and cardiac derived cells (CDCs) derived from cardiospheres (CS), cardiac side population cells (CSP) and cardiac mesenchymal stem cells (CMSCs)[1-5]. In animal models, several of these cell populations have been reported to display some level of ability to engraft, proliferate in response to cardiac injury and express markers of cardiac lineage commitment[6-9]. Some have also been reported to improve cardiac function following injury[8,9]. Comparison between the results of studies of these cell populations is however fraught with difficulties as they are isolated based on use of distinct but often different cell surface marker expression or differences in phenotypes. Even when studying the same cardiac stem cell population, there are differences reported (for example, CSP cells have been reported to be present in the hearts of mice by several groups but with variable numbers)[7,10,11]. There are also differences in stem cell isolation and culture protocols, transplantation methodology (e.g. number of injection sites used) delivery methods (e.g. direction intra myocardial injection or infusion via the circulation), differences in cell numbers being transplanted, timing of cell transplant following injury and even nature of injury model used and species used for these models, thus making direct comparisons between these cardiac stem cell populations difficult to evaluate which has most promise. This has also been the case where these cells have transitioned into clinical trials with both CS derived CDCs and c-Kit lineage negative cells having been trialled again, using not only different possible distinct cardiac stem cell populations, but using different protocols. In the Intracoronary cardiosphere-derived cells for heart regeneration after myocardial infarction (CADUCEUS) trial that used CDCs, cell numbers transplanted were 12.5, 17.3 or 25 million versus one million c-Kit positive lineage negative cells, used in the Cardiac stem cells in patients with ischaemic cardiomyopathy (SCIPIO) trial[1,2]. There also remains the issue of how distinct these cardiac stem cell populations actually are as it is known that they share expression of some markers, for example, CDCs have been reported to contain small populations of c-Kit expressing cells, as have CSP[6,10].

The promise

Due to limitation in options for cardiac patients, especially those with end stage heart failure, a cellular therapy has to be viewed positively, even if it only brings about small improvements in cardiac function. The recently completed

CADUCEUS Phase 1 trial and the SCIPIO Phase 1 trial, the former using autologous derived CS derived CDCs and the latter c-Kit positive lineage negative cells, are encouraging as they suggest generation and expansion of such human cells *in vitro* for transplant is possible and that they can be transplanted safely. Moreover, the initial results suggest improvements in heart function although the mechanisms behind these improvements have yet to be fully determined[1,2]. There is debate about the role of such transplanted cardiac stem cells; do they differentiate and contribute to cardiac regeneration? Or do they have a paracrine effect and secrete factors that support native cardiac cells spared by the cardiac insult enabling the resident cells to recover and give rise to new cardiomyocytes? This remains an area of debate but regardless of the outcome, the number of cells that can be generated and mechanisms to improve engraftment rates needs further exploration, as does furthering our understanding of the secretome of these cardiac stem cell populations if we are to harness their paracrine potential. However, this may prove challenging. In a study by Stastna and colleagues on cardiac stem cells and neonatal cardiomyocytes, they identified 15 proteins that might have paracine effects on cardiac cells[12], while in another study it has been reported that IGF-1 secreted by c-Kit positive GATA-4 high expressing cardiac stem cells appeared to promote cardiomyocyte survival above that of c-Kit positive GATA-4 low expressing cells from the same bulk culture[13]. This implies that we may also need to consider the inter-clonal variations within stem cell populations when considering how to harness the paracrine potential of such cells.

The challenges

Once past the problems of selection of native stem cell of choice for use as a cellular therapy, what issues lie ahead? Will cardiac cellular therapy be a 'one size fits all' approach to treating cardiac injury and disease? This seems unlikely. There is now evidence that stem cells can themselves be adversely affected in certain types of cardiomyopathy. In a study of aged diseased human hearts, higher numbers of c-Kit positive cardiac progenitor cells expressed the senescence marker p16 above that seen in non-diseased aged hearts[14]. While in a mouse model of diabetes, progression of disease appears to impact negatively on cardiac stem cell aging[15]. In order to obtain cells, biopsy tissue needs to be taken from the patients themselves, this could raise ethical issues unless the issue is generated as a product of necessary surgical procedures. The isolated cells then need to be expanded *in vitro* in a timely fashion and safely. There is controversy surrounding the effects of long term culture of stem cells with reports having been that in non-cardiac derived mesenchymal stem cells, cells undergo transformation changes that may or may not result in malignancy[16]. Therefore, the bulk expansion of cardiac stem cells must be shown to be safe before they are used in the clinical setting. Cardiac stem cells have previously been reported to be susceptible to changes often chromosomal in long term culture. The impact of these changes needs to be thoroughly examined[17]. Timing and method of delivery also remains a challenge. In animal models, cells are often transplanted immediately post injury, this is not an option in cardiac patients if cardiac stem cells are to be transplanted, unless one begins to consider the use to allogeneic cells and all the inherent issues with the use of this type of approach. Methods of delivery have impacts on cell viability. It has been reported that CSP cells delivered by direct injection into a mouse model of cardiac injury two weeks post injury as aggregates survived better when

compared to CSP delivered as cell suspensions under the same conditions[18]. Improvements in culture methods need to be explored more fully when human cardiac derived stem cells where cultured under low oxygen conditions they grew faster and the yield of cells was higher, more importantly the frequency of chromosomal abnormalities was reduced compared with cells from the same tissue samples cultured in the normal 20 per cent oxygen levels[17].

The future

The use of cardiac stem cells as tools for regeneration of the heart look to be possible and current evidence suggest they appear to be relatively safe (although some adverse effects were recorded in the CADUCEUS trial) and beneficial to some but not all patients and improvements in cardiac function were not sufficient to completely restore cardiac function to normal levels. Therefore, we may need to consider the use of cardiac stem cells in combination with other, in use, therapeutic approaches to treating heart disease. The use of VADS is on the increase as they appear to prolong the life expectancy of certain patients above that of optimised medical management and attempts are being made to determine if a combined approach using cellular therapy and left ventricular assist device (LVAD) implantation could support recovery in patients to a level that they could be taken off VAD support. Initially, trials took place using bone marrow cells along with LVAD implantation to try to determine this. While the number of patients treated was relatively small, in one trial of 10 patients given bone marrow cells at the time of LVAD implantation, one patient was successfully weaned from VAD support and in a single patient study, a patient post MI who already had a LVAD implant was given cells 99 days after LVAD implantation and was successfully removed from VAD support[19,20]. Further larger trials have followed and are taking place using bone marrow derived cells, but could this approach be improved by using autologous derived cardiac stem cells in place of bone marrow? Another approach might be to think of combining stem cells with biomaterials to improve engraftment rates of cells post transplant. CDCs used in combination with hydrogel injected directly into the heart of a mouse model of myocardial infarction showed enhanced retention above that of CDCs injected as a cell suspension[21], while bone marrow MSCs have also been shown to have an increased rate of differentiation to cardiac cells when cultured in hydrogel[22]. In light of the clinical potential of MSCs in treating a number of disorders, the recent identification and isolation of human CMSCs by Anzalone and colleagues[5] also warrants further investigation as a cardiac regenerative tool. The next few years bring the promise of a cellular strategy to treat cardiomyopathy, whether it is using the cells alone or in combination with other therapies or biomaterials or due to the identification of key proteins secreted by cardiac stem cells that promote cell survival and recovery of resident cells. Whatever the direction this takes, it appears more likely to succeed by taking a multidisciplinary approach, bringing together experts in the fields of cardiac and stem cell biology, cardiologist and cardiovascular surgeons, engineers, manufacturers of biomaterials and experts in proteomics.

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Stem Cells Roundtable

Moderator: George E. Plopper
Professor, Department of Biology, Rensselaer Polytechnic Institute

Chris Parker
Chief Commercial Officer
Cellular Dynamics International

Mark J. Powers
Senior Director, Research & Development – Primary and Stem Cell Systems
Life Technologies

What are the most significant advances in stem cell-based applications you expect to see in the next two years?

Powers: The field is moving at an incredibly fast pace, so this is an exciting time for novel technologies and applications. One area that will undoubtedly continue to advance is the development of tools and technologies for more efficient and effective cellular reprogramming, including improved methods for directly reprogramming somatic cells to desired cell types. Another important area is the optimisation of scalable culture systems to better integrate stem cells in screening, drug discovery and cell therapy applications. Finally, we expect advances in cell engineering and genomic editing to better enable the use of cell systems in modelling human disease.

Parker: I expect the commercial availability of iPSC-derived human cells via industrialised manufacturing will greatly expand the use of human stem and differentiated cells in life science research. Purchasing rather than making human cells enables laboratories to focus resources on experiments rather than on making reagents. Laboratories without stem cell capabilities can now access iPSC-derived human cells as well. I also expect research on specific human diseases will grow as scientists gain access to these cells. The formation of iPSC banks will provide starting materials for terminal cell differentiation of a number of disease models. Of recent note, the California Institute of Regenerative Medicine recently granted USD 32 million to collect samples from 3,000 patients with 11 different diseases to create and bank three iPSC lines for each patient.

A number of basic questions concerning stem cell function, such as the structure and function of stem cell niches, the mechanisms of maintaining stemness and the functional role of stem cell markers, remain largely unanswered. What are the top three questions in basic stem cell research that, if answered, would help you understand how best to use stem cells in pharmacology-based applications?

Parker:

1. What is the true measure of 'stemness' (i.e. stem cell lines can have variable behaviour, but identical markers for pluripotency)?
2. How do we cross the 'tertiary' maturity barrier for stem cell-derived terminal cells so that they fully recapitulate adult (mature) phenotype?
3. What is the appropriate clinical standard to ensure safety of stem cell-derived cellular therapies? What tests are needed, what animal models are acceptable proxies, and is immune-suppression acceptable?

Powers:

1. What are the facilitators and roadblocks in reprogramming and what is their engagement with the genome (small molecules, transcription factors, proteins)?
2. What is the role of epigenetic memory in the journey to pluripotency?
3. What is the predictive potential for iPSC disease models compared to current screening models that utilise engineered or primary cells?

In many instances, researchers in one subfield of stem cell research (e.g., basic, applied, preclinical or clinical research) fail to capitalise on the findings of those in other subfields. What are your suggestions for improving more active collaboration between these subfields of stem cell research?

Powers: The key to successfully fostering collaboration lies in ensuring that researchers, commercial entities and clinicians are part of the same discussion through conferences, workshops, etc. Beyond this, sponsoring research that requires the collaboration of scientists in different areas of focus will drive cross-disciplinary and cross-functional engagement. A common need for all stem cell researchers involves the ability to set and rely on standards for cell characterisation and safety. It is essential that these standards emanate from discussions relevant to the needs of researchers in each particular subfield, from basic research to drug discovery and clinical applications.

Parker: Within the stem cell field, I think the most important evolution is the newfound access to a consistent supply of human stem cells and differentiated cells due to their recent commercial availability. When researchers can concentrate on the data from their experiments rather than on creating the cells, or determining the confounding differences between cell batches, then discovery will take off. The skills needed to reprogram and differentiate cells will become less important as scientists can simply order the cells they need. How many scientists create their own PCR reagents today? Not many. Yet many scientists regularly run experiments with PCR tools. The stem cell field will likely follow the same path.

What emerging skill sets do you feel will be most in demand for those seeking jobs in the stem cell industry in the next five to 10 years?

Parker: CDI was one of the first commercial entities to set up an industrialised facility for manufacturing iPSCs and differentiated cells in high quantity, quality and purity with reproducible consistency, so we've learned valuable skills along the way. We see a need for researchers with a background in process sciences engineering, experience in making cell-based products in an industrial setting, hands-on work with real-time perfusion bioreactors and scale-up expertise in stem cell culture systems growing billions or trillions of stem cells.

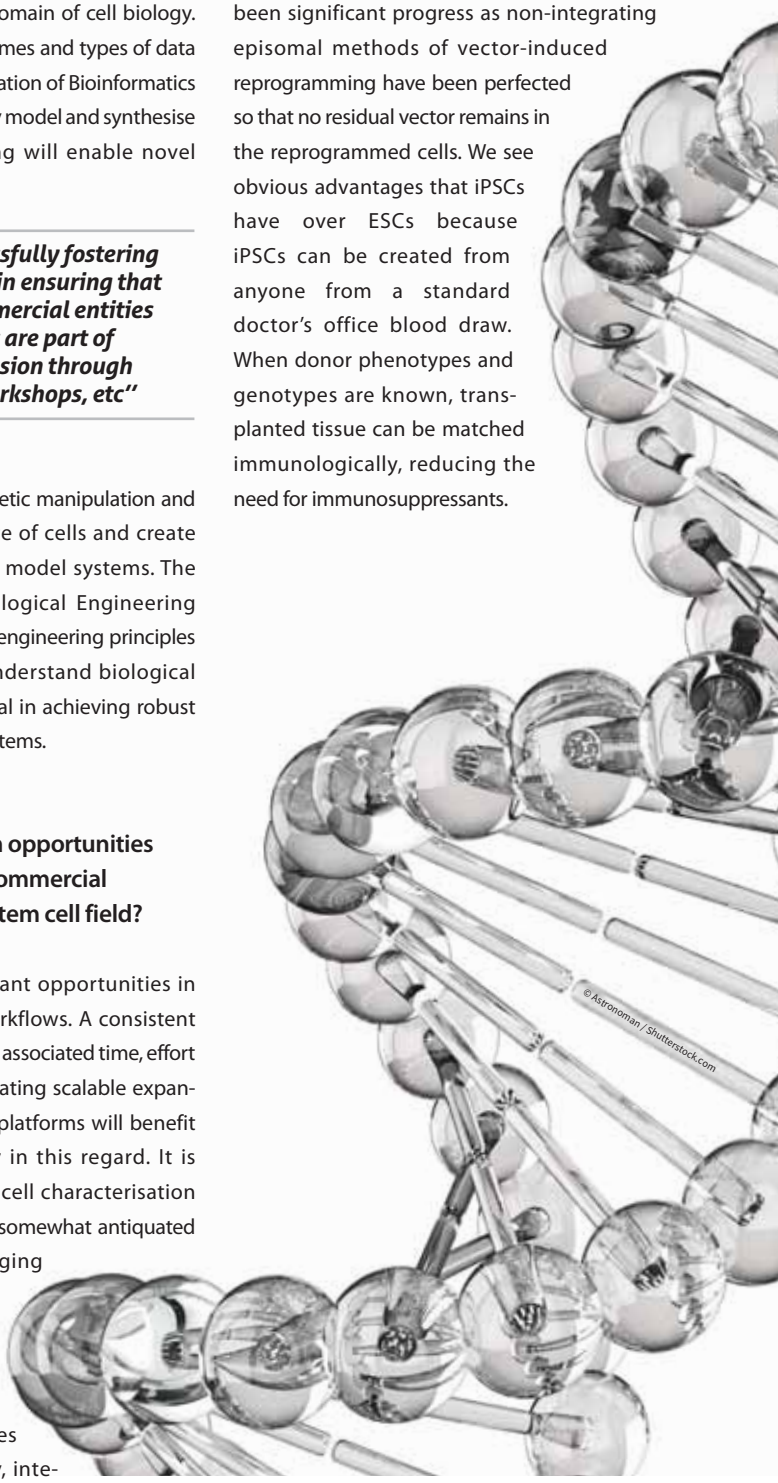
Powers: Stem cell research is a dynamic space, and critical needs are continually evolving beyond the traditional domain of cell biology. With ever-increasing volumes and types of data at our disposal, the integration of Bioinformatics will be critical to efficiently model and synthesise results. Cell Engineering will enable novel methods to leverage genetic manipulation and editing to direct the fate of cells and create more authentic cellular model systems. The implementation of Biological Engineering approaches – leveraging engineering principles to better model and understand biological systems – will be essential in achieving robust and predictive cellular systems.

"The key to successfully fostering collaboration lies in ensuring that researchers, commercial entities and clinicians are part of the same discussion through conferences, workshops, etc"

Where are the growth opportunities for developing new commercial opportunities in the stem cell field?

Powers: We see important opportunities in optimising stem cell workflows. A consistent need here is to reduce the associated time, effort and cost involved. Facilitating scalable expansion and differentiation platforms will benefit the field tremendously in this regard. It is also evident that many cell characterisation methods used today are somewhat antiquated and subjective. Leveraging genomic and genetic technologies (e.g., qPCR, RNA-seq, CHIP-seq, etc.) will enable faster and more objective analyses and predictions. Finally, integration of HTS and automation combined with iPSC-derived cell models will be truly enabling in enhancing the utility of stem cells in drug discovery and cell therapy.

Parker: The obvious growth opportunity is applying iPSC technology to human therapeutics. This will take several years to become a reality due to issues regarding the safety of the reprogramming process and differentiated cells. However, there has been significant progress as non-integrating episomal methods of vector-induced reprogramming have been perfected so that no residual vector remains in the reprogrammed cells. We see obvious advantages that iPSCs have over ESCs because iPSCs can be created from anyone from a standard doctor's office blood draw. When donor phenotypes and genotypes are known, transplanted tissue can be matched immunologically, reducing the need for immunosuppressants.



Show PREVIEW

Date: 12-15 June 2013 · Location: Boston, MA, USA

ISSCR INTERNATIONAL SOCIETY FOR STEM CELL RESEARCH

The ISSCR 11th Annual Meeting will be held on 12 - 15 June 2013 at the Boston Convention and Exhibition Center (BCEC), Boston, MA, USA, hosting stem cell professionals from around the globe.

The ISSCR's flagship meeting serves as the largest forum for stem cell and regenerative medicine professionals from around the world. Through lectures, symposia, workshops and events, attendees experience innovative stem cell and regenerative medicine research, advances and learn about what's on the horizon. The meeting features more than 2,000 abstracts, 150 speakers and provides numerous networking and professional development opportunities and social events.

Participants come from nearly 50 countries from academic, industry, government, non-profit, health provider and research settings. They represent a wide variety of disciplines and interests including: cell therapy, developmental biology, disease, genetics, ethics, regulatory and policy issues, transplantation, animal models and regenerative medicine.

Programs and events

The ISSCR 11th Annual Meeting is an unparalleled scientific event with top stem cell scientists from around the world sharing their research and perspectives. The sessions and events at the annual meeting are designed to provide attendees with a range of opportunities, including scientific enrichment, career development and professional networking. The meeting features lectures, symposia, workshops and events for scientists and researchers at all stages of their career.

Keynote, Featured and Presidential Symposium Speakers

- Keynote Speaker: Eric Lander, Broad Institute, USA
- Anne McLaren Memorial Lecturer: Elaine Fuchs, Rockefeller University, USA
- Ernest McCulloch Memorial Lecturer: George Q. Daley, Children's Hospital Boston, USA

Presidential Symposium Speakers

- **James Thomson**, Morgridge Institute for Research, USA
- **Edith Heard**, Institut Curie, France
- **Douglas A. Melton**, Harvard University, USA
- **Richard A. Young**, Whitehead Institute for Biomedical Research, USA

Plenary Topics

- Presidential Symposium
- Cell and Gene Therapy
- Disease Modelling
- Genomics and Epigenomics of Stem Cells
- Making Tissues and Organs
- Regeneration, Engraftment and Migration
- Stem Cells and Fate Control

Awards

Each year, the ISSCR honours innovative work to harness the potential of stem cells, celebrates the exceptional achievements of a young researcher, supports professional development and recognises extraordinary public service. The 2012 winners of the McEwen Award for Innovation and the ISSCR-University of Pittsburgh Outstanding Young Investigator Award will be presented at the annual meeting in June, as will the winners of the Poster Awards and the ISSCR Public Service Award.

Industry Wednesday symposia

The ISSCR Industry Wednesday Symposia is an opportunity for companies to present important scientific information to a highly engaged audience of leading stem cell researchers from around the world. These symposia will be held in the morning of the first day of the conference.

Focus Sessions

Focus Sessions are parallel, in-depth educational opportunities in science, society and education at the ISSCR Annual Meeting organised by members. The ISSCR is providing space at the Boston Convention and Exhibition Center (BCEC) on the morning of Wednesday 12 June, to non-profit organisations with a connection to stem cell research that are interested in discussing a topic of interest to ISSCR attendees. The goal of these Focus Sessions is to increase the representation of topics at the Annual Meeting that are of interest to constituencies within the ISSCR but are difficult to adequately represent within the main scientific program.

The global community of researchers, physicians, clinicians, ethicists and industry and government leaders will gather at the ISSCR 11th Annual Meeting in Boston in June to share information on, and explore new opportunities in, all facets of stem cell research. For further information regarding the meeting, please visit www.isscr.org/home/annual-meeting.

International Society for Stem Cell Research

The International Society for Stem Cell Research (ISSCR) is an independent, non-profit organisation established to promote and foster the exchange and dissemination of information and ideas relating to stem cells, to encourage the general field of research involving stem cells and to promote professional and public education in all areas of stem cell research and application.

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