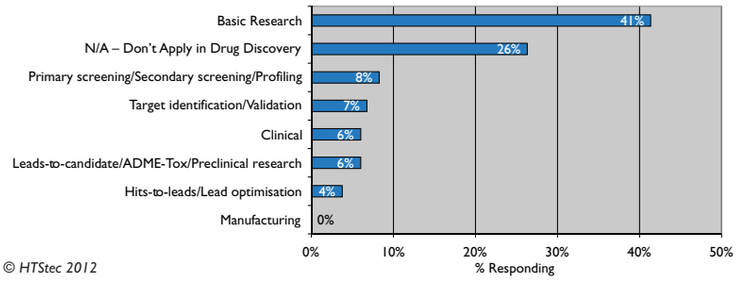
STEM CELLS
rapidly gaining traction in research and drug discovery

By Dr John Comley

A recent market survey on stem cells in research and drug discovery showed that despite a significant amount of hype and hope around stem cells, most drug discovery-related efforts today still fall into the category of basic research and the majority of that was directed towards the oncology/cancer disease area. Human-derived stem cells were of greatest interest and the full range of stem cell types were under investigation, with no single stem cell type predominating. The cell lineages respondents were most differentiating stem cells into were brain, neuronal and glial cells. Expense was the most limiting obstacle in stem cell research and cells that have been demonstrated to go down a particular lineage pathway only was the most relevant criteria when purchasing stem cells. Many new research tools are now obtainable which have the ability to jump start work on stem cells and the wider availability of off-the-shelf cells derived from induced pluripotent stem cells and embryonic stem cells is expected to catalyse a profusion of approaches ultimately leading to improved compound screening, toxicity assessment, disease modelling, and new target discovery.

Figure 1: Stages of drug discovery and development process using stem cells today



The much talked-about therapeutic applications of stem cells (eg regenerating damaged tissues or organs) although exciting, have for the most part, not yet reached fruition. However, many readers may not appreciate how broadly stem cells research has gained a foothold in drug discovery and development efforts and are expected to provide new approaches leading to improved compound screening, toxicity assessment, disease modelling, new target discovery and our understanding of disease mechanisms and pathways.

The pharmaceutical industry has been investigating the potential of stem cells for around a decade now. Initially, embryonic stem cells

(ESC) were seen as an unlimited source of multiple cell types for research. Those derived from mouse ESC are already being used for some small scale high-throughput screens. In addition, at least one commercial supplier now produces human cardiomyocytes on an industrial scale from ESC. More recently, tissue-specific cells derived from human induced pluripotent stem cells (iPSC) have become available. For example, iPSC-derived cardiomyocytes have enabled therapeutically-relevant modelling of cardiovascular diseases, neurodegenerative disorders and metabolic disorders. Scientists now have increasing access to human cells in sufficient quantities to develop new disease models and stem cell-based assay formats for use in *in vitro* screening and toxicity assessment. These assays when deployed earlier in the drug discovery process will significantly aid in the identification of more selective lead compounds.

Stem cells also possess the ability to morph into any of our body's cell types, including all the diseased tissues in the body. Diseased stem cells will be critical if researchers are to directly test, screen and derisk drugs on the relevant cell types.

Underpinning all these activities is the need for improvements in techniques used for culturing, expansion, characterisation and differentiation of human ESC and iPSC, as well as reprogramming techniques for the creation of iPSC.

This article is based on some of the findings of HTStec's Stem Cells in Research & Drug Discovery Trends survey and report, published in December 2012[1]. We attempt to explore the current use of stem cells by reporting on those key questions about the incorporation of stem cells into drug discovery and development efforts. Readers will see there are a multiplicity of objectives and applications for stem cells and although they are undertaken by researchers in Pharma and Biotech, many have more to do with basic research than actual lead discovery.

Key demographics of stem cell use in research and drug discovery

Survey respondents reported that 38% of their entire cell work being undertaken today (2012) involved stem cells. Only 17% of survey respondents were currently using stem cells in clinical applications.

The stage in the drug discovery and development process most using stem cells today by survey respondents was basic research (41% using). This was followed by 28% not currently applying in drug discovery. Use by survey respondents in other

Figure 2: Disease areas utilising stem cells for research or drug discovery applications

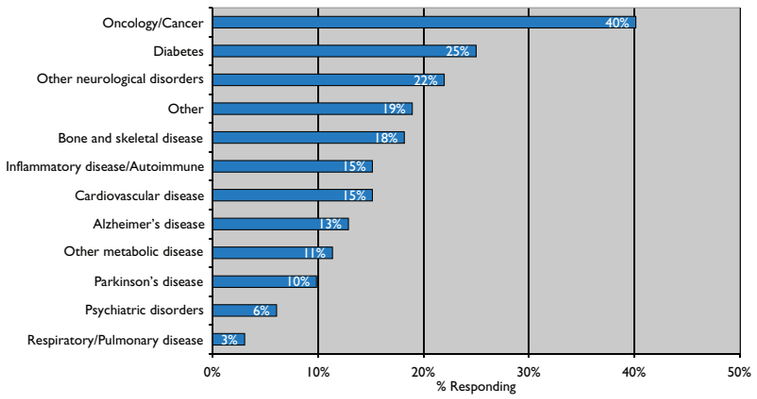


Figure 3: Percentage of stem cell research done with cells derived from the following species

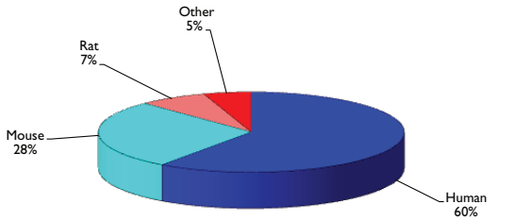


Figure 4: Percentage of work done with different stem cell types today

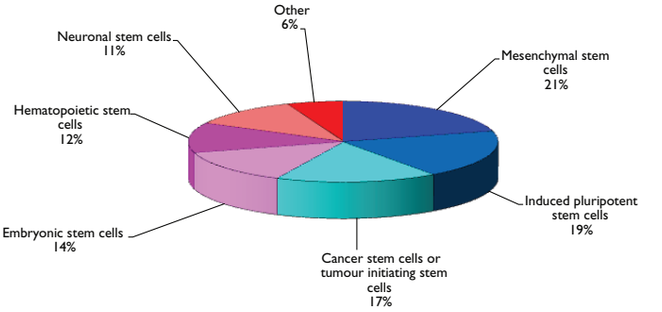


Figure 5: Key objectives of respondent's stem cell research

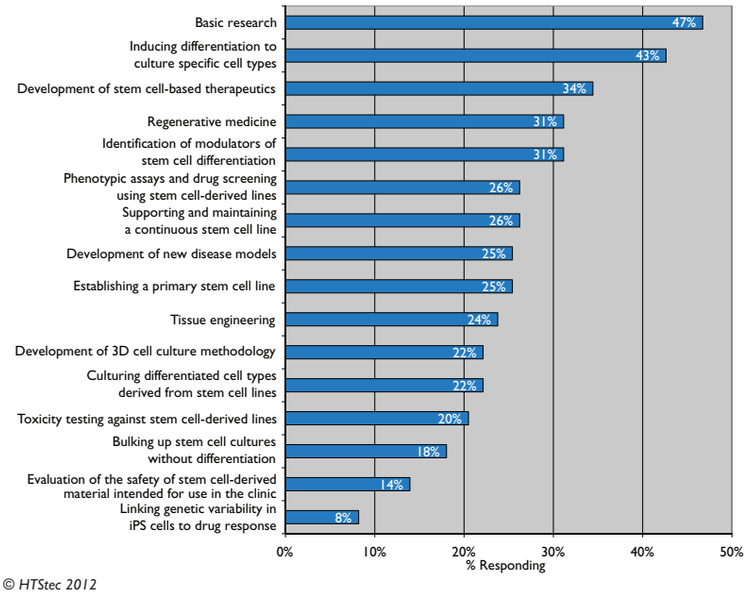
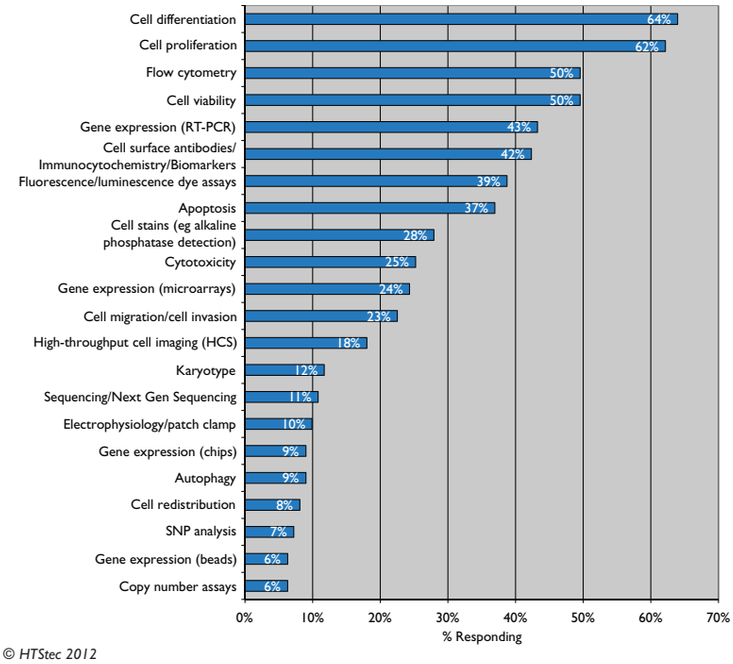


Figure 6: Phenotypic assays and characterisation methods performed on stem cells today



specific aspects of drug discovery was limited to 8% or less per area (**Figure 1**).

Most (40%) survey respondents were utilising stem cells in research or drug discovery applications in the oncology/cancer disease area. This was followed by diabetes (25% utilising) and other neurological disorders (22% utilising). Stem cells were least utilised in the respiratory/pulmonary disease area (**Figure 2**).

The majority (60%) of survey respondents' stem cells research was done with human-derived cells. This was followed by 28% mouse derived; 7% rat derived; and 5% other derived (**Figure 3**).

The stem cell type that survey respondents were making greatest use of today was mesenchymal stem cells (21% using). This was followed by iPSC (19% use); cancer stem cells or tumour initiating stem cells (17% use); ESC (14% use); hematopoietic stem cells (12% use); neuronal stem cells (11% use) and then other stem cells (6% use) (**Figure 4**).

The main key objective of survey respondents' stem cell research was basic research (47% investigating). This was closely followed by inducing differentiation to culture specific cell types (43% investigating); development of stem cell-based therapeutics (34% investigating); and then regenerative medicine and identification of modulators of stem cell differentiation (both 31% investigating). Least investigated objective was linking genetic variability in iPSC to drug response (only 8% investigated) (**Figure 5**).

Types of assays and characterisation methods most done on stem cells

The phenotypic assays or characterisation methods survey respondents are most performing or most planning to perform on stem cells was cell differentiation (64% performing). This was followed by cell proliferation (62% performing); flow cytometry and cell viability (both with 50% performing). The phenotypic assays or characterisation methods least performed on stem cells were gene expression (beads) and copy number assays (**Figure 6**).

Key obstacles in stem cell research

Survey respondents rated expense as the most limiting obstacle in stem cell research. This was followed by issues of assay sensitivity, robustness and reproducibility; difficulty of culture/propagation; and then difficulty of handling. Ranked least limiting was ethical issues (**Figure 7**).

The cell lineage into which survey respondents were most differentiating their stem cells was brain/neuronal/glial cells (35% differentiating).

This was followed by bone marrow cells (osteocytes) (21% differentiating), other (ie than those listed) (20% differentiating) and hepatocytes (19% differentiating). There was minimal interest in differentiating stem cells into thyroid and ovarian cells (**Figure 8**).

Impact on stem cell purchasing:

Survey respondents rated 'cells that have been demonstrated to go down a particular lineage pathway only' as the criteria of most relevance in the purchasing of stem cells. This was closely followed by 'cells that are totipotent (ie cells with the ability to divide and produce all the differentiated cells in an organism)' and 'cells have been be subject to cell line identity testing'. Rated least relevant to purchasing were 'cells that are partially differentiated or perhaps cryopreserved at key points in differentiation'. However, all criteria were rated very close to each other and overall of moderate relevance to purchasing (**Figure 9**).

Survey respondents ranked well characterised/validated as the most important reason that would influence their current or future expected purchasing of stem cells. This was followed by availability of specific stem cell type from desired species; price; and then internal data demonstrating value of stem cells. External pressure (eg legal changes/drive away from animal models/testing) was ranked least important (**Figure 10**).

Latest vendor offerings supporting research and drug discovery on stem cells

BD Biosciences (www.bdbiosciences.com) offers a diverse set of tools – above all, a range of flow cytometry systems – that enable researchers to characterise, analyse and sort heterogeneous stem cell populations, both hematopoietic and other stem cell types. With one of the widest selections of fluorochrome-conjugated, stem-cell relevant antibodies – to cell surface and intracellular biomarkers alike – they offer researchers a 'library' of tools for stem cell analysis, to verify that stem cells have maintained pluripotency or to monitor their changing expression patterns. BD's kits for the detection of key stem cell transcription factors by flow cytometry can be used to compare and optimise differentiation protocols. Containing optimised antibodies and buffer systems, these can be combined with surface staining and provide multimarker data on a cell-by-cell basis, thus capturing the diversity of phenotypes in heterogeneous cell samples. For the isolation of live cell populations, BD has worked out methods for fluorescence-activated cell sorting

Figure 7: Main obstacles in stem cell research

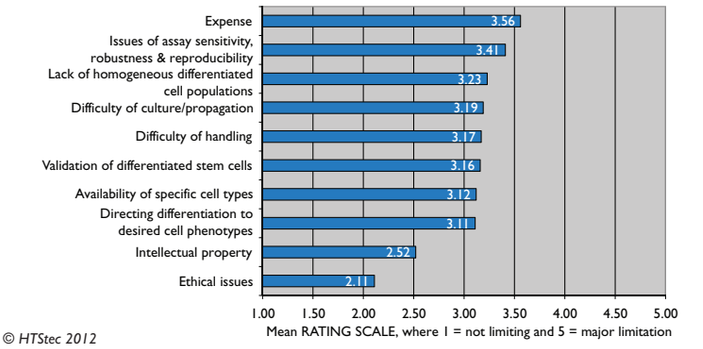


Figure 8: Cell lineages into which respondents are differentiating their stem cells

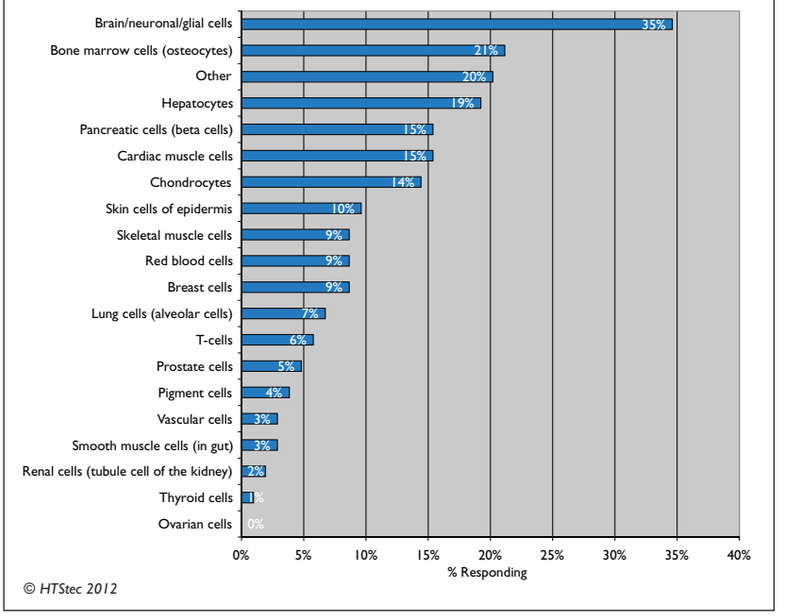


Figure 9: Influence of criteria on purchasing of stem cells

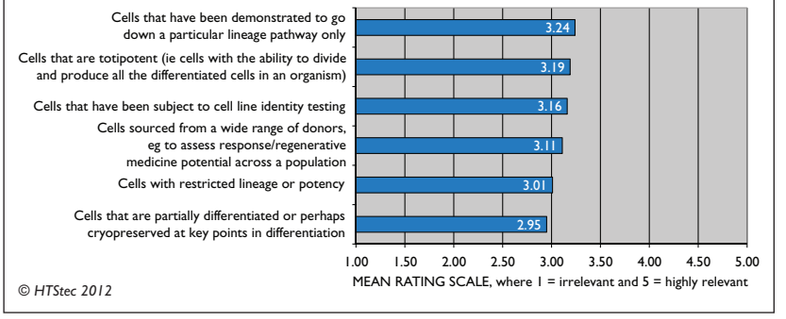
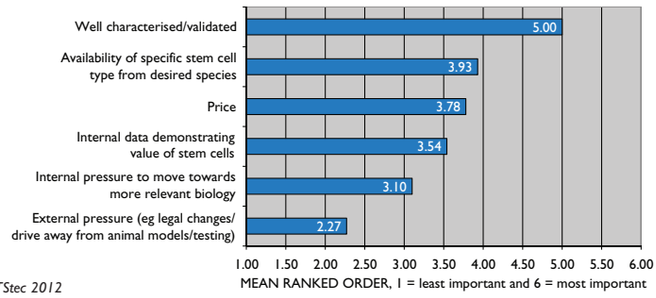


Figure 10: Most important factors on purchasing of stem cells



(FACS) to isolate stem cell populations in bulk, based on their characteristic marker signatures. The new BD Stemflow™ human iPSC sorting and analysis kit includes antibodies to TRA-1-60 and SSEA-4 (mark reprogrammed cells), and to CD13 (marker for the starting fibroblasts). BD's newer cell sorters, the BD FACSJazz™ and BD FACSAria™ Fusion, can also sort in single-cell depositions to support downstream applications such as next-gen sequencing. To help researchers define unique marker signatures for new stem cell populations and develop strategies for their analysis and isolation, BD Lyoplate™ cell surface marker screening panels facilitate profiling of cell preparations for hundreds of cell surface markers by flow cytometry or cellular imaging (**Figure 11**).

Figure 11
BD FACSJazz™ cell sorter

Cellular Dynamics International (CDI) (www.cellulardynamics.com) is a leading manufacturer of terminally differentiated human cells derived from induced pluripotent stem cells (iPSCs). CDI has industrialised the manufacturing of human cells in high purity, quantity and quality, which has led to iPSC-derived cells steadily replacing cell lines, animal models and primary cells. Manufactured human iPSC-derived cells not only increase the reproducibility of, but also the biological relevance for, applications in toxicity testing, drug discovery and disease modelling and will ultimately revolutionise regenerative medicine and stem cell banking. The company's current iCell® product offering includes cardiomyocytes, neurons, endothelial cells, hepatocytes, astrocytes and hematopoietic progenitor cells. CDI also offers MyCell Products, which are custom cells manufactured from any individual, including those with genetic mutations, adverse drug responses and/or diseases of interest to pharmaceutical and academic researchers. MyCell Products leverage iPSC technology to make stem cells prior to directing the differentiation towards terminally differentiated cell types and thus can be genetic engineered, for example, to introduce or correct a specific mutation to create human disease models and isogenic controls. Current MyCell Products provide access to a number of disease models, including cardiomyopathies and arrhythmias, vision disorders, neurological disorders and muscular dystrophies, and CDI is currently expanding its MyCell catalogue products to include neurodegenerative disorders and drug-induced liver injury (DILI). In summary, CDI is leveraging iPSC, genetic engineering and robust directed differentiation manufacturing to revolutionise the study of human health (**Figure 12**).

EMD Millipore (www.millipore.com), the Life Science division of Merck KGaA of Darmstadt, Germany, provides tools for stem cell research including cells, culture media, reprogramming kits, growth factors, small molecules and characterisation tools. The company has recently developed optimised media formulations using a combination of small molecules and proteins for the expansion and directed differentiation of human pluripotent stem cells. Its recently launched PluriSTEM™ Human ESC/iPSC Medium is comprised of low concentrations of Activin-A, TGFβ1, and b-FGF to

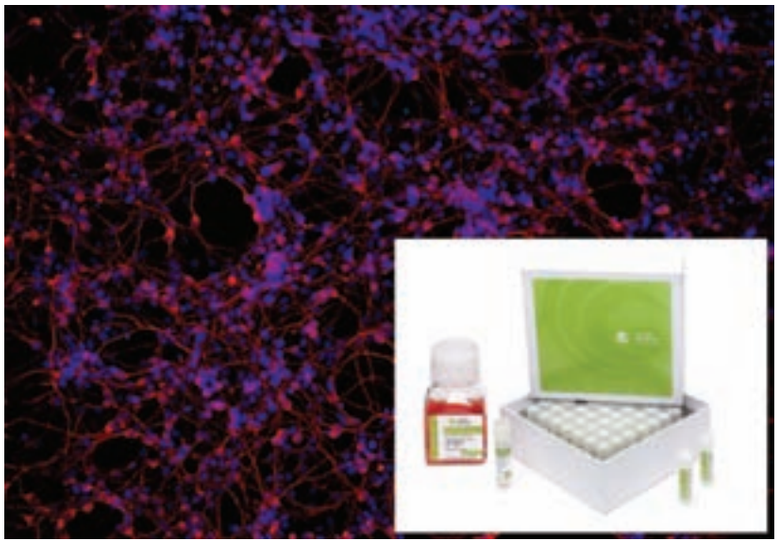


Figure 12: iCell Neurons differentiated from iPSCs derived from T cells indicate neuronal marker beta-III tubulin (red) with nucleus stained with Hoechst (blue). Inset of iCell Neurons kit

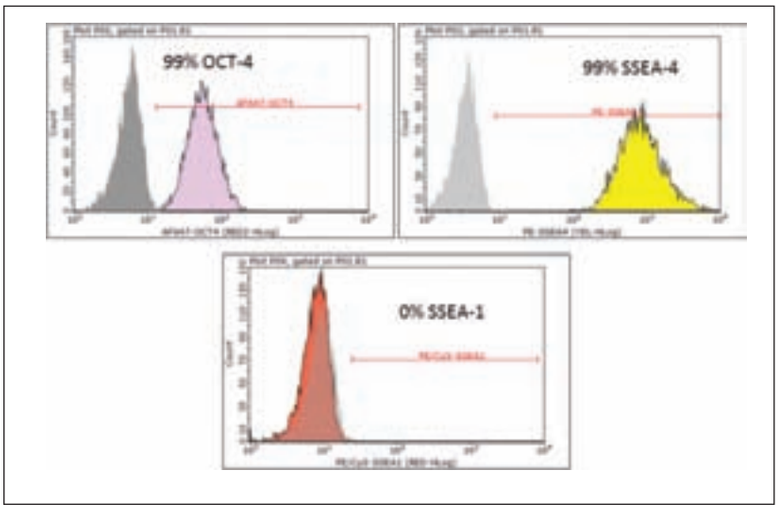
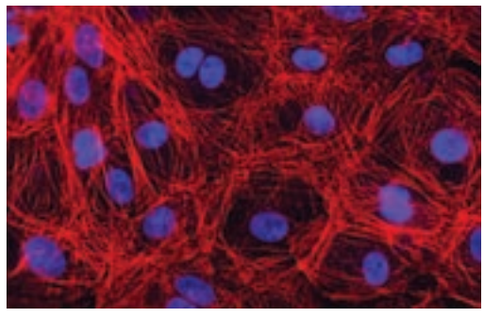


Figure 13: Human pluripotent stem cells maintain proper marker expression when cultured in PluriSTEM™ Human ES/iPS Cell Medium. H9 human ES Cells grown in PluriSTEM™ medium for 22 passages with feeding three days per week were analysed for Oct-4, SSEA-4 and SSEA-1 expression by FACS analysis. Note the high expression of human pluripotent stem cell markers Oct-4 and SSEA-4 and negative expression of SSEA-1, a human differentiation marker not expressed by human ES/iPS cells

Figure 14
Image of Cytiva cardiomyocytes from GE Healthcare Life Sciences which are differentiated human cells for drug safety testing and are stained for DNA (blue) and Troponin I (red)



promote stem cell self-renewal and potent small molecule combinations to inhibit unwanted spontaneous differentiation, as well as human serum albumin (HSA) to aid in overall colony morphology. PluriSTEM medium addresses current challenges with routine expansion of human embryonic and induced pluripotent stem cells, including the necessity for everyday feeding and requirement for significant technical expertise. This new defined medium provides a simpler and robust feeder-free culture method that allows for feeding every 2-3 days and provides high viability and proliferation rates in single cell passaging. To enable the development of neuronal cell systems, EMD Millipore has released differentiation media that allow researchers to generate expandable neural progenitor cells and terminally differentiated neurons from pluripotent human ESC and iPSC. Neural progenitors are generated 10 days from starting cultures of traditional feeder-based and/or feeder-free cultures of undifferentiated human ESC/iPSC and can be expanded for over 3-5 passages, resulting in at least 20-fold expansion. These new cell culture media, in combination with its STEMCCA reprogramming technology and cell permeable TAT-Cre recombinase for efficient generation of transgene-free iPS cells, provide a workflow solution to researchers establishing model cell systems (**Figure 13**).

Many drugs still fail at a late clinical stage, often due to cardiotoxicity, and making improvements in predictive toxicology is a major industry challenge. To address this, **GE Healthcare Life Sciences** (www.gelifesciences.com/cardiomyocytes) developed Cytiva cardiomyocytes which are derived from human embryonic stem cells (hESC) as a biologically relevant cell model for early-stage toxicity testing with the aim of failing potential therapeutics earlier and thereby reducing the cost of the drug discovery and development process. Cytiva cardiomyocytes are produced on an industrial scale, highly characterised, functionally verified and comprise mainly ventricular myocytes. GE Healthcare has used Cytiva cardiomyocytes, in both electrophysiological and high content assays, to investigate functional and structural effects of compounds and gain insight into mechanisms of toxicity. GE Healthcare recently launched the Cytiva Cell Integrity and Cell Health high-content assays for monitoring multiple indicators of toxicity, including apoptosis, nuclear morphology, DNA content, mitochondrial health and calcium levels, in a rapid and more convenient format than traditional cellular assays. The assays improve upon

single endpoint toxicity assays by offering greater sensitivity and information through monitoring multiple parameters simultaneously. By combining the predictive nature of human cell models with the analysis capabilities of high-content imaging systems, such as the GE Healthcare IN Cell Analyzer, the resulting data provide more detailed and integrated surveillance of toxic responses (**Figure 14**).

Life Technologies (www.lifetechnologies.com/stemcells) provides a wide range of tools and services that allow scientists to manipulate pluripotent stem cells (PSC) using novel approaches for reprogramming, long-term culture and propagation, and characterisation. Focused on innovations to streamline stem cell research experiments and obtain results in a consistent, reproducible manner, Life Technologies recently introduced the new TaqMan® hPSC Scorecard™ Panel, which evaluates pluripotency and detects germ layer bias by using real time qPCR assays and intuitive data analysis software. The Scorecard gene panel was developed in collaboration with Dr Alex Meissner and follows from his landmark publication[2] to allow researchers the ability to score cell lines relative to a standard. Novel culture media like Essential 8™ Medium and Essential 6™ Medium, can be used together to simplify the generation, expansion and differentiation of pluripotent stem cells (PSCs) by offering a feeder-free, xeno-free system for all three steps. For scientists looking to differentiate PSCs into terminal neural lineages, the new Gibco® PSC Neural Induction Medium eliminates the need for embryoid body formation and rosette isolation, reducing the timeline for neural induction from 14+ days down to seven days. Research labs needing to reach their desired outcomes faster can utilise Life Technologies' CellModel™ Services for reprogramming human fibroblasts or blood cells, characterisation, differentiation, screening and custom assay development (**Figure 15**).

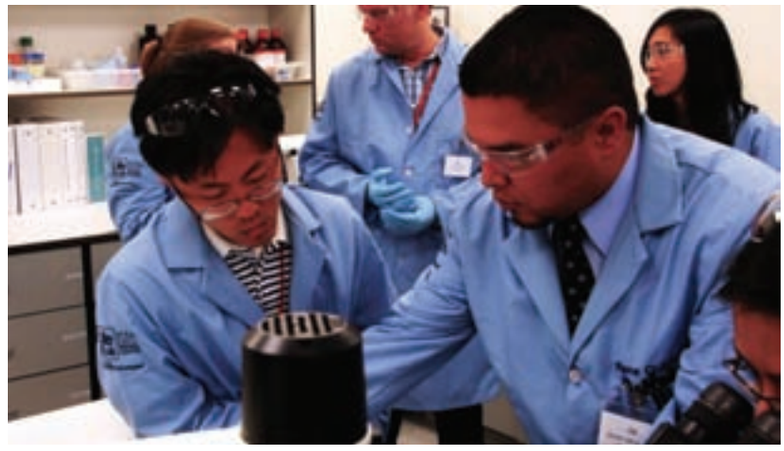


Figure 15: Life Technologies offers hands-on training to stem cell researchers through its LifeLab™ Pluripotent Stem Cell Workshops

Lonza (www.lonza.com) provides products and services that support the use of stem cells in basic research as well as in early stage drug and cell therapy development. Its pluripotent stem cell (PSC) service offering spans the full value chain from tissue acquisition to production of biologically relevant cell models used in screening applications. We have efficient protocols for generation of human induced pluripotent stem cells (hiPSCs) under defined conditions from both healthy and diseased donor tissue. The group is also well versed in process optimisation and scale-up of differentiation processes in order to provide high purity, functional cell types. Lonza's Poietics™ product line consists of both fresh and cryopreserved primary adult stem cells from a variety of donor tissues including bone marrow and adipose. New for 2013, it now has dental pulp stem cells and media which are scheduled to launch in July. It offers optimised media systems specific to each of its cell type for growth, expansion and differentiation. For certain cell types, Lonza also offers media systems for adipogenic, chondrogenic and osteogenic differentiation. Human progenitor cells from bone marrow and cord blood are available, as well as fresh unprocessed human bone marrow. Lonza

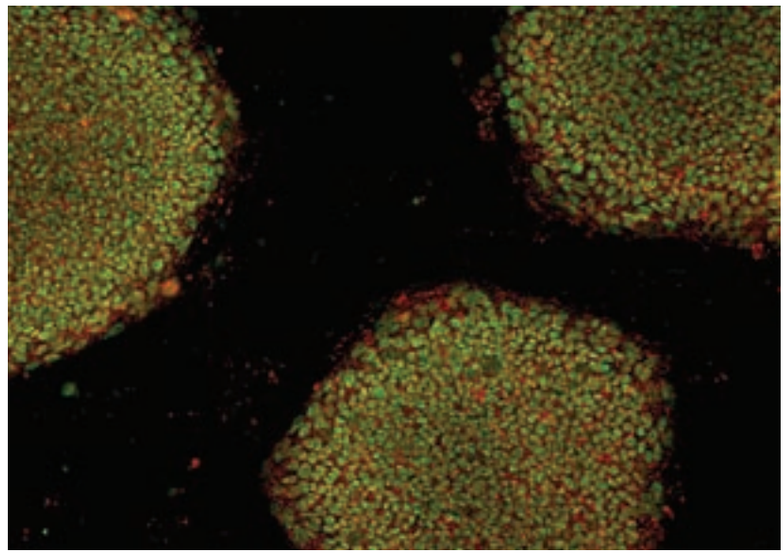


Figure 16: Lonza-generated hiPSCs exhibit characteristic expression of human embryonic stem cell associated markers POU5F1 (green) and SSEA4 (red)

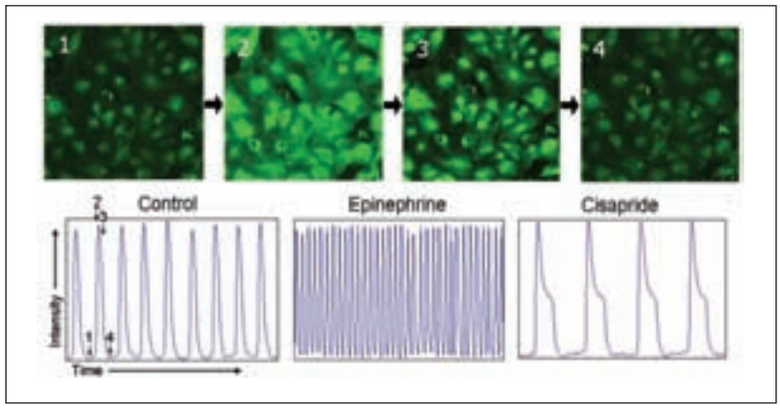


Figure 17: Top: Time lapse series of images taken with the Molecular Devices ImageXpress Micro XL System of contracting cardiomyocytes loaded with a Ca2+ sensitive dye. The relative timing of each image is indicated in the Control graph. Bottom: Integrated intensity versus time showing beating profiles for three different conditions. Epinephrine, a stimulant, speeds up the contraction rate, while Cisapride, a hERG blocker, slows down repolarisation and elongates the time of contraction

offers media systems that support both the proliferation of hematopoietic progenitors and their differentiation into erythroid cells, megakaryocytes and myeloid cells. It has human ESC derived motor neuron progenitors available pre-seeded in 96-well plates or cryopreserved, allowing researchers to move away from rodent models. Other PSC derived cell types are available on a custom basis (**Figure 16**).

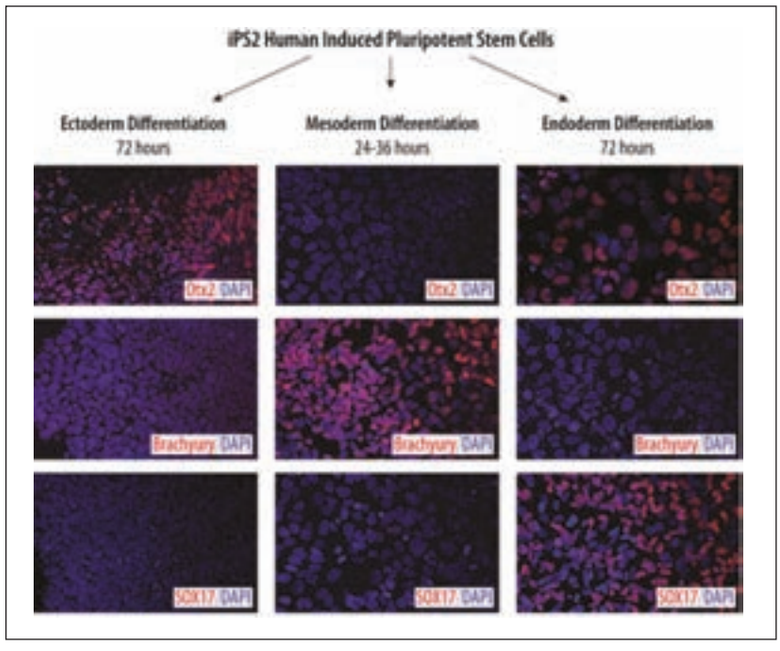


Figure 18: Verification of Human Stem Cell Pluripotency. iPS2 human-induced pluripotent stem cells were differentiated to ectoderm, mesoderm and endoderm using the media supplements included in R&D Systems' Human Pluripotent Stem Cell Functional Identification Kit (Catalog # SC027). The kit also contains antibodies for the confirmation of differentiation status (as indicated). This *in vitro* procedure ensures a healthy, pluripotent starting stem cell population which increases consistency between studies and reduces unwanted experimental variability

There is a great interest to automated stem cell assays for use in drug development and evaluation of compound safety and toxicity. **Molecular Devices** (www.moleculardevices.com) has optimised its high content imaging (HCI), automated electrophysiology and fast kinetic fluorescence plate reader products to meet this need. It has developed HCI methods to perform assays on induced pluripotent stem cell (iPSC)-derived neurons, hepatocytes and cardiomyocytes. The large field of view camera on the ImageXpress® Micro XL system allows users to capture more cells and obtain better assay statistics. The new Custom Module Editor in version 5 of the MetaXpress® Software provides the ability to combine image processing modules for efficient, multi-parametric characterisation of stem cell differentiation and cell toxicity. Further studies have been done with iPSC derived cells using the IonWorks® Barracuda automated electrophysiology systems. The system can analyse 384 wells simultaneously and assess effects of compounds on cardiomyocytes with endogenously expressed ion channels. Molecular Devices has also developed a novel assay platform for studying cardiotoxicity using fast kinetic fluorescence measurements of spontaneously contracting cardiomyocytes loaded with its proprietary Ca2+ sensitive dye (**Figure 17**). This phenotypic assay, which has been optimised on both the FLIPR® Tetra System and the new SpectraMax® i3 Multi-mode Platform, can provide a sensitive measurement of toxic effects to cardiomyocytes. Its ScreenWorks® Peak Pro Software Module provides automated analysis of the temporal profiles to give characteristic outputs such as contraction rate, peak width, rise times and decay times. These features are also available in SoftMax® Pro Software.

In stem cell experiments, the ability to verify that the starting cell population is healthy and undifferentiated is an important methodological step that can reduce experimental variability, improve data consistency and provide valuable insight that may prevent weeks of wasted effort and reagents. To address this need, **R&D Systems** (www.rndsystems.com) offers a range of specialised kits that are specifically designed for stem cell research. For example, its Functional Identification Kits provide all of the reagents required to confirm the ability of

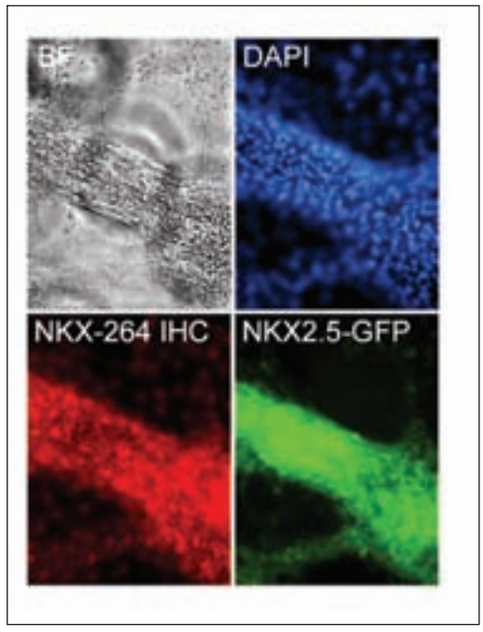


Figure 19
Sigma Aldrich iPSC clone 28 targeted (tagged) at the NKX2.5 endogenous locus with GFP at the c-terminus using ZFNs, showing cardiac differentiation at day 53

your starting population to differentiate into appropriate lineages *in vitro*. R&D Systems' Multi-Color Flow Cytometry Kits facilitate thorough characterisation of differentiation status via the simultaneous detection of up to four markers. The most referenced arrays in the industry, its Proteome Profiler™ membrane-based Pluripotent Stem Cell Array simultaneously detects 15 pluripotent stem cell markers. Although many researchers primarily consider R&D Systems a supplier of premium quality recombinant proteins, it also manufactures more than 12,000 high performance antibodies. To enable the detection of pluripotent marker expression in live, unfixed cells it recently developed GloLIVE™ azide-free antibodies that are optimised for live cell imaging. Since the acquisition of Tocris Bioscience in 2011, R&D Systems now provides bioactive small molecules to enhance reprogramming and investigate the underlying signal transduction mechanisms. Furthermore, to facilitate translational research, it supplies Good Manufacturing Practices (GMP)-grade proteins, which are manufactured in its ISO-certified facility. Collectively, R&D Systems stem cell products offer simple solutions to reduce unwanted variability and maximise the probability of experimental success (**Figure 18**).

Since the launch of the CompoZr™ ZFN platform at **Sigma Aldrich** (www.sigmaaldrich.com) in 2008, the field of targeted genome editing has exploded with many new developments and innovations in a vast array of application areas. Initially most scientists focused these tools on the genomes of traditional research models such as transformed cell lines, rodents and fish models. However, it did not take long before the technology showed significant utility in organisms and application areas that were quite different and innovative compared to mainstream research areas. Life science research geared towards drug discovery and development applications has pushed stem cells to the forefront in the last 5-10 years. Initially, human embryonic stem cells served as the major workhorse, but most recently the advent of induced pluripotent stem cells (iPSC) has catalysed a plethora of approaches for drug discovery and development. Using ZFNs researchers are able to 'tag' endogenous *loci* of iPSC to report a gene expression pattern allowing one to monitor pluripotency or some differentiated state. By using iPSC derived from disease patient tissues, these assays become much more relevant and help to mitigate risk associated with responsiveness or toxicology, eg in drug screening efforts. Sigma's cell engineering division, Cell Design Studio, offers gene targeting services in any cell type. These services range from simple gene knockout applications for target validation to more elegant gene knockin applications for purposes of high content screening or creation of isogenic cell lines derived from patients. By utilising these services, the bulk of effort may then be focused on the biology to accelerate the development pipeline (**Figure 19**).

The goal of Regenerative Medicine is to provide therapeutic solutions to a range of unmet clinical needs. In this respect, stem cells hold great potential as both direct therapeutic options and providing improved *in vitro* and *in vivo* preclinical assay systems for drug discovery. **StemCells, Inc** (www.stemcellsinc.com) provides a range of products and services to accelerate stem cell research and stem cell-based drug discovery and drug development. Specifically, under the **SC Proven®** brand (www.scproven.com), it markets products for the derivation, propagation, characterisation and validation of pluripotent stem cells and tissue stem cells. Leveraging experience and expertise, it also offers contract cell process development, scale-up and robotic production services. For neurodegenerative disorders, human stem cell-based assays represent a direct target to screen for modulators of endogenous neurogenesis, neuroprotection and for predictive neurotoxicology. For such applications, SC Proven offers a series of human neural stem cell (NSC) kits. Here, the NSCs are derived

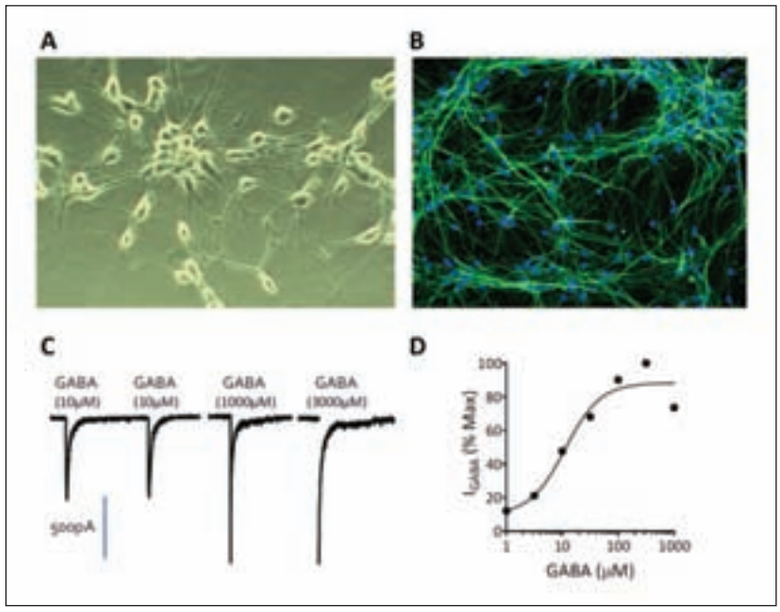


Figure 20: Morphology, immunocytochemical and pharmacological (R.F. Halliwell, unpublished) characterisation of SC Proven human NSCs. (A) Phase contrast image of mature neurons and (B), DAPI counter stain and β-tubulin/TUJ1 immunocytochemical marker with GABA ligand induced currents (C) and concentration-response curve (D)

from different brain regions and from the spinal cord. NSC are reproducibly expandable by automation, maintain long-term region-specific gene expression profiles, and retain an ability to differentiate into high percentages of physiologically functional neurons and glial cells *in vitro*[3] (**Figure 20**). These NSC cultures authentically mimic the morphology and pharmacobiology of their *in vivo* radial glia-type cell counterparts which are responsible for the majority of developmental and adult human neurogenesis. At the *in vivo* level, SC Proven high-resolution antibodies enable tracking of engraftment, migration and differentiation of human NSCs transplanted into animal models of human disease[4].

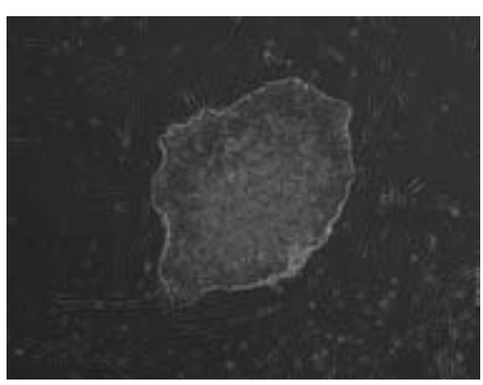


Figure 21
Human iPS colony derived from fibroblasts using STEMCELL Technologies TeSR™-E7™ reprogramming medium (coming soon) and an episomal vector system

In keeping with its goal of becoming a leading facilitator of regenerative medicine research, **STEMCELL Technologies** (www.stemcell.com) provides standardised, defined reagents that are designed to meet the unique needs of scientists in this dynamic field. Within the pluripotent stem cell field, STEMCELL Technologies offers defined reagents for feeder-free reprogramming, maintenance and differentiation. Recently, it has released a complete kit for derivation of definitive endoderm from human pluripotent stem cells. This is part of the STEMdiff™ differentiation portfolio that also includes reagents for differentiation to neural (STEMdiff™ Neural Induction Medium) and mesodermal lineages (STEMdiff™ APEL Medium). The derived endoderm can be used to generate terminally differentiated cell types, including hepatocytes and beta-cells, with downstream applications including toxicology assessment in drug discovery and studying developmental pathways. STEMCELL Technologies' product line for feeder-free maintenance of pluripotent stem cells now includes defined, low-protein TeSR™-E8™ medium, based on the simplified formulation published by the laboratory of Dr James Thomson (University of Wisconsin-Madison). TeSR™-E7™ (available August 2013) is a further simplified version of this medium that has been optimised for efficient reprogramming of somatic cells to induced pluripotent stem cells. The putative iPSCs generated in TeSR™-E7™ can be transferred to a maintenance medium where they readily establish thriving clonal cultures for further propagation as cell lines. STEMCELL Technologies also provide, xeno-free, defined media systems for other cell types used within the regenerative medicine field including mesenchymal and hematopoietic stem cells (**Figure 21**).

There are several techniques to use when reprogramming adult stem cells back to a pluripotent state. **Stemgent**'s (www.stemgent.com; www.asterand.com) proprietary mRNA reprogramming technology, Pluriton™ Medium and Custom Reprogramming Services, offers scientists the tools to address the challenges around deriving non-viral, non-integrating, clinically-relevant iPSC for use in regenerative medicine, drug discovery and basic research. Traditional reprogramming methods can lead to the integration of unwanted genetic material into the host genome and therefore can be disruptive to the reprogrammed cell's function. The advantage of the mRNA reprogramming method is that there is no risk for insertional mutagenesis that can later cause cancer. Other advantages include

speed and high efficiency. For example, mRNA reprogramming can result in iPSC colonies within two weeks as compared to 10 weeks with other methods which require screening. Stemgent's Custom Reprogramming Services offers iPSC generation using various patient lines and methods. In addition, Stemgent and Cellectis bioresearch have recently partnered to provide researchers with genome engineered iPSC lines. Stemgent's mRNA reprogramming portfolio and Cellectis bioresearch's TALEN™-based genome engineering technology enables the directed introduction of disease-specific genetic mutations to mimic disease and of reporter genes with fluorescent/luminescent tags to evaluate drug candidate efficacy, specificity and toxicity. Together these two powerful technologies pave the way for clinically-relevant applications in regenerative medicine. Through its Asterand brand, Stemgent also offers well characterised human tissue and human-based drug discovery services to pharmaceutical, biotech, diagnostic and research institutes (**Figure 22**).

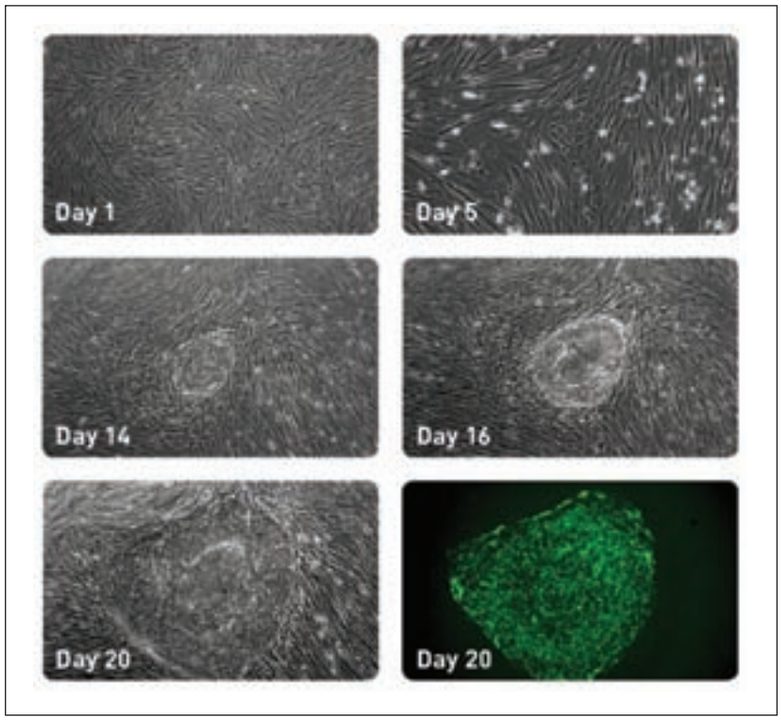


Figure 22: In collaboration with the laboratory of Dr Rudolf Jaenisch, human iPS cell lines were generated using Stemgent's mRNA Reprogramming System from dermal fibroblasts derived from an adult patient with Parkinson's disease. Time-course phase-contrast images show morphology changes up to Day 20 and expression of TRA-1-81 pluripotency marker at Day 20. All images taken at 10x magnification

Tecan (www.tecan.com) is an established provider of laboratory automation for cell culture and cell-based assays, with many years' experience in providing advanced solutions for the culturing and maintenance of different stem cell types, from human embryonic stem cells (hESC) to induced pluripotent stem cells (iPSC). Precise regulation of culturing conditions is vital for reliable maintenance and differentiation of stem cells, and is ideally suited to automation on the Freedom EVO® workstation, using Freedom EVOware® to accurately define protocols and eliminate inter-user variability. Tecan offers a number of options for both feeder cell-based and feeder-free culture types, with maximum format flexibility to suit your workflow; for example, a process could be started in Millicell®-24 filter plates (Millipore), then transferred to a cell culture microplate or automation-friendly RoboFlask® (Corning). The open architecture of the Freedom EVO platform allows integration of all the modules necessary for fully automated culture growth – including CO_2 incubators, plate and flask handling modules and heating or cooling options – as well as incorporation of downstream processing and analytical instruments, such as Tecan's Hydrospeed™ plate washers and Infinite® readers, and high content cell analysis instrumentation. Tecan is also working closely with suppliers of 3D technologies – such as TAP Biosystems (RAFT™ matrix), Reinnervate (Alvetex®Scaffold) and InSphero (3D InSight™ technology) – to offer solutions for real three dimensional culture growth and analysis, allowing stem cells to be grown in an *in vivo*-like environment for improved assay performance and greater biological relevance (**Figure 23**).

Figure 23: Tecan Freedom EVO® workstation used in stem cell culture

References

1 Stem Cells in Research & Drug Discovery Trends 2012 Report, published by HTStec Limited, Cambridge, UK, December 2012.

2 Bock, C et al (2011). Reference Maps of Human ES and iPS Cell Variation Enable High-Throughput Characterization of Pluripotent Cell Lines. Cell 144:439-452.

3 Hook, L et al (2011). Non-immortalized human neural stem (NS) cells as a scalable platform for cellular assays. Neurochemistry International 59:432-44.

4 Uchida, N et al (2012). Human Neural Stem Cells Induce Functional Myelination in Mice with Severe Dysmyelination. Sci.Transl.Med. 4(155):155ra136.

Discussion

Table 1 summarises the stem cell-related vendor offerings discussed in this article currently available to aid research and for the pursuit of more relevant drug discovery approaches. This table includes a variety of essential tools to help researchers characterise, identify, isolate and analyse stem cells. Also highlighted in the table are a variety of human and mouse-derived stem cells that are now available in commercial quantities (both iPSC and ESC) for use in toxicity and screening assays. Fresh and cryopreserved primary adult stem cells can also now be purchased off-the-shelf from a variety of donor tissues. Many researchers have needs for a specific cell type or a custom cell and these are increasingly catered for by custom services that leverage vendors' expertise in iPSC or ESC technology to manufacture/engineer large industrial quantities of the desired cell enabling the end user to focus their efforts on the biology to accelerate their development pipeline. Not all labs want to buy in cells and to provide for their needs vendors now offer a range of tools to aid the manipulation of iPSC using novel approaches for reprogramming, optimised long term culture and propagation. Some of the latest culture media incorporate novel growth factors and small molecules for the expansion and directed differentiation of iPSC. With the greater availability of human iPSC- and ESC-derived cells, the previously used recombinant cell lines are gradually being replaced facilitating the development of new disease models for use in toxicity testing and screening, this is particularly noteworthy in the case of cardiomyocytes. Lab automation and analysis tools have a key role to play in the culture and screening of stem cells and vendors have started to address this need.

Finally, we should not ignore the fact that lack of technical expertise was perceived as a major barrier to the investigation of stem cells by 60% of survey respondents, and 72% expect to gain their expertise in stem cell work progressively on the job. To address this expertise gap, external training courses on stem cell techniques are likely to feature more prominently in the years to come.

To conclude, many new research tools are now available to jump-start work on stem cells and the wider offering of off-the-shelf cells derived iPSc and ESC is expected to catalyse a profusion of approaches ultimately leading to improved compound screening, toxicity assessment, disease modelling and new target discovery. DDW

	Application In Stem Cell Research & Drug Discovery						
Stem Cell Related Product Vendor	Characterization Tools e.g. Antibody Panels, Conjugates	Cell Identification Kits	Cell Isolation Tools e.g. Flow Cytometry, FACS	Cell Analysis Tools e.g. HCS Imagers	Primary Adult Stem Cells	hiPSC-Derived Cells	hESC-Derived Cells
BD Biosciences	✓		✓	✓			
Cellular Dynamics International						✓	
EMD-Millipore							
GE Healthcare				✓			✓
Life Technologies/Invitrogen	✓						
Lonza					✓	✓	✓
Molecular Devices				✓			
R&D Systems	✓	✓					
Sigma		✓					
Stem Cells Inc	✓	✓					
Stemcell Technologies							
Stemgent/Asterand						✓	
Tecan							

	Application In Stem Cell Research & Drug Discovery						
Stem Cell Related Product Vendor	Custom Services e.g. Assay Devpt., Manufacturing, Engineering	Cell Culture/ Propagation Media	Reprogramming Kits	Growth Factors & Small Molecules	Toxicity/ Screening Assays	Cell Culture Automation	Training Courses
BD Biosciences							
Cellular Dynamics International	✓						
EMD-Millipore		✓	✓	✓			
GE Healthcare					✓		
Life Technologies/Invitrogen	✓	✓	✓		✓		✓
Lonza	✓	✓					
Molecular Devices					✓		
R&D Systems			✓	✓			
Sigma	✓						
Stem Cells Inc		✓			✓		
Stemcell Technologies		✓	✓	✓			
Stemgent/Asterand	✓		✓				
Tecan						✓	

Table 1: Summary of stem cell-related offerings discussed in this article

Dr John Comley *is Managing Director of HTStec Limited, an independent market research consultancy whose focus is on assisting clients delivering novel enabling platform technologies (liquid handling, laboratory automation, detection instrumentation; assay methodologies and reagent offerings) to drug discovery and the life sciences. Since its formation nearly 10 years ago, HTStec has published more than 90 market reports on enabling technologies and Dr Comley has authored more than 45 review articles in* **Drug Discovery World**. *Please contact info@htstec.com for more information about HTStec reports.*