Human ES cell derived functional cells as tools in drug discovery

The drug discovery process is extremely-time consuming and expensive. Consequently, novel approaches for these processes and for reducing late-stage attrition are of great value for the pharmaceutical industry. In pre-clinical drug development, the lack of functional human cell systems leads to the extensive use of either cell systems with low clinical relevance, or complex and costly animal models. However, it is anticipated that the access to specialised cells derived from human stem cells will improve the quality of targets, hits, and leads, reduce attrition rate, and thus shorten the time and cost of drug development. The utilisation of stem cells in drug discovery spans from early target finding and evaluation studies, via the use of functional human cells in screening and pharmacokinetic studies, to the use of various stem cells technologies in toxicological testing. A prodigious future application of human stem cells is also to employ these cells as platforms and screening tools for the development of drugs that can activate and mobilise endogenous stem cell populations residing in various tissues throughout the human body.

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The area of drug discovery and development in the pharmaceutical and biotechnology industry consists of many dynamic processes. Each of these processes meets certain challenges that are costly and time-consuming. Thus, the R&D strategy within the individual companies needs to be optimised based on the complex interplay between several factors. In recent years R&D productivity has been declining, primarily due to long R&D cycles and approval times, drug attrition, and large clinical trial sizes. Out of these factors, the industry could address the long R&D cycles and high attrition rates by applying novel or improved technologies. For example, high-throughput technologies can be further developed and biomarker discovery and pharmacogenetics can be applied to a higher degree in order to increase R&D productivity. Furthermore, the compound attrition rate is negatively affected by the inability to predict toxicity and efficacy in humans. These shortcomings are in turn caused by the use of experimental pre-clinical model systems that have a limited human clinical relevance.

Cardiotoxicity and hepatotoxicity are the main causes of withdrawal of compounds during the development process and from the market. In order to reduce the very costly late stage attrition it is critical to assess potential toxicity or other

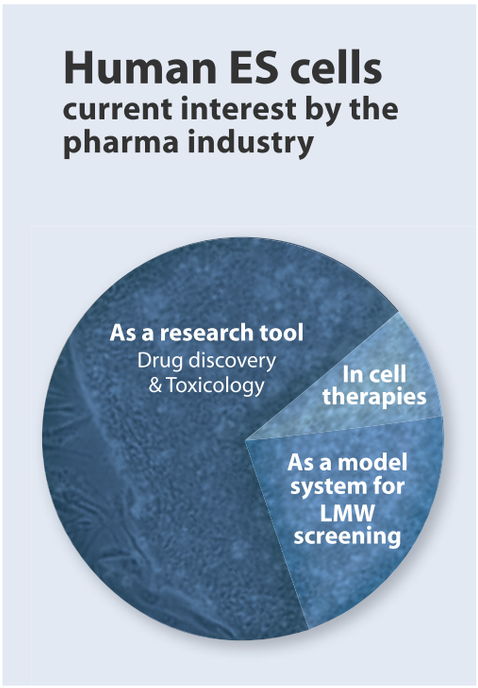


Figure 1
Schematic view of the pharmaceutical industry's interest on the use of hES cells and their derivatives. The figure illustrates an estimation by the authors

adverse effects of a compound as early as possible. Unfortunately, the toxicity of a new drug in humans is often not discovered until clinical trials are conducted. Animal models are today important tools to detect adverse effects of compounds but are costly and their clinical relevance is widely debated. In fact, animal models are about 50% effective in predicting human toxicity to the liver, heart and during development. Moreover, there are ethical and political concerns associated with the use of experimental animals. Although the *in vivo* studies are necessary to investigate the systemic effects of compounds, the pharmaceutical industry would clearly benefit from refining the use of animal models.

Many of the needs in drug development could take advantage of the use of human stem cell technologies. In the preclinical stage of drug development, a drug has to be tested extensively in the laboratory to make sure it will not be harmful when administered to humans. The common denominator in most *in vitro* drug discovery applications is the cell, whose functionalities and responses are being assayed. Stem cells are attractive to use for *in vitro* testing because they have the capacity of self-renewal and specialisation. Stem cells can be genetically modified to improve the throughput of the assays and can provide specific disease models useful for example in target identification and validation. In addition, they are important for understanding differentiation pathways and for identifying factors needed to manipulate cell lineages. In the adult, stem cells mediate tissue homeostasis and repair. There are a number of different types of human stem cells and these cell populations present varying degrees of developmental potency. Here we will illustrate some of the opportunities presented by human pluripotent stem cells (ie, embryonic stem cells), and discuss the different level of interest the pharmaceutical industry expresses for these possibilities (**Figure 1**). We will also illustrate some of the advantages these cells provide for the development of novel and improved tools for drug discovery.

Human pluripotent stem cells

Human embryonic stem (hES) cell lines were originally isolated about a decade ago. Prior to this scientific breakthrough, researchers had been employing mouse ES cells for various purposes that are continuously important, such as genetically engineered mice, which are used routinely for pharmaceutical target validation. However, there are substantial differences between humans and rodents and this has left a gap in our understanding of human embryonic development and tissue specification.

To date, hES cell lines have been derived in a number of independent laboratories worldwide and >400 hES lines have been reported by various investigators although the level of characterisation of these lines varies considerably. Under appropriate culture conditions, hES cell lines can be maintained in culture indefinitely and exhibit a stable developmental potential to differentiate into all the cells of the human body. The application of hES cells has been surrounded by ethical and legal considerations which, in most parts of the world, have led to the establishment of clear guidelines and regulations concerning hES cell research (for details see: The international Society for Stem Cell Research, www.isscr.org; The National Academy of Sciences, www.nasonline.org; the National Institutes of Health, www.nih.gov; The UK Stem Cell Bank, www.ukstemcellbank.org.uk).

Scientific breakthroughs relating to the generation of human induced pluripotent stem (iPS) cells were recently published by Japanese and American researchers. These cells were derived by viral transduction of certain stem cell-associated genes into non-pluripotent human cells. By selecting a small set of critical genes, the investigators could successfully reprogramme adult cells to obtain an apparently pluripotent phenotype. The results suggest that iPS cells may be possible alternatives or

complements to hES cells in the future. However, much research remains since it is still not clear whether iPS cells are perfect substitutes for embryonic stem cells. In addition, the differentiation potential of the human iPS cells and also issues related to effects caused by the genetic manipulation of the cells need to be addressed.

Human ES cells have the capacity for extensive, possibly indefinite, self-renewal but the most important feature of hES cells is their ability to differentiate into virtually any cell type present in the adult human body. In order to specifically derive functionally differentiated cells, investigators are working intensively to improve protocols beyond the spontaneous differentiation of hES cells. Of particular note, however, is the diversity of different cell types that have been derived from hES cells (**Table 1**).

To date, one of the most important issues of hES cell research is the proper maintenance and expansion of the undifferentiated cells. Pluripotent hES cell lines are traditionally propagated in co-culture with a feeder layer which provides unknown factors, which support undifferentiated growth of hES cells. It has been shown that conditioned media or defined culture additives can replace supporting feeder cells to a certain degree in their ability to support undifferentiated hES cell expansion. However, no universal protocol has been developed so far. Co-ordinated international efforts, such as the International Stem Cell Initiative (ISCI) have been initiated to address this issue. The European Union's 7th Framework Program (http://cordis.europa.eu) is addressing the challenges regarding hES cell cultivation and scale-up of hES cell production. Cellartis is participating in focused developments towards the application of bioreactor and automation technologies (ITI life sciences, Stem cell programme; www.itilifesciences.co.uk) that are required to lift hES cell production up to a robust and industrially adequate level. These and other structured efforts make it likely to believe that the challenges of industrial hES cell manufacturing will be at least partially solved within the coming years.

Hepatocytes derived from hES cells

The human liver controls a number of key functions such as the conversion of cholesterol to bile acids, production of many serum proteins, and metabolism and disposition of xenobiotics. Hepatocytes are the dominating cell type of the liver and are therefore seen as a key cell-type in the drug discovery process. These cells may provide new targets for drugs affecting liver-related diseases, and will have broad uses in studies of liver metabolism and pharmacokinetic properties of novel compounds. Furthermore, liver toxicity and alterations of hepatic physiology are the most frequently occurring reason for preclinical failure among new chemical entities. A reliable source of functional and handy hepatocytes would substantially facilitate the development of new drug discovery strategies and provide possibilities to perform improved *in vitro* testing. Current liver cell systems are hampered by the fact that primary hepatocytes and available cell lines either rapidly lose important functional systems, such as metabolic competence, or already

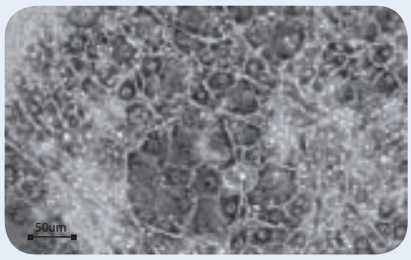
Summary of specialized cell types derived from hES cells

Cell type
Trophoblast
Endothelial cell
Cardiomyocyte
Smooth muscle cell
Hepatocyte
Insulin producing endocrine cell
Keratinocyte
Oligodendrocyte
Neuron and astrocyte
Glia
Germ cells
Adipocyte
Chondrocyte
Osteoblast
Natural killer cell
T cells
Dendritic cell
Megakaryocyte
Erythrocyte
Macrophage
Melanocyte
Retinal neurons
Motor neurons
Type II pneumocytes
Prostate tissue
Lung alveolar epithelial type II cells

Table 1: A large number of different cell types have reportedly been derived from hES cells and are summarised in the table

Figure 2
Micrograph illustrating hES cell derived hepatocytes differentiated for 21 days *in vitro* using a novel proprietary protocol. The cells display a typical hepatic morphology, are large, rhombic, bi-nucleated, highly granulated and have started to form sub-structures. Potential areas of applications for these cells in the drug discovery process are indicated as bullet points

hES cell derived hepatocytes
in drug discovery



- Target identification & validation
- Lead validation & optimization
- Cell screening
- Drug metabolism
- Safety assessment - hepatotoxicity

lack these properties, respectively. In fact, commercially accessible hepatic cell lines contain very low levels of metabolising enzymes and they have a distribution of other important proteins that differs markedly from the human hepatocyte. Moreover, the usefulness of human primary cells is relying on repeated sourcing, which is a bottleneck and major limitation. Thus, there is a clear need for alternative cell sources that emulate primary human hepatocytes but prove to have fewer obstacles. Therefore, stem cell differentiation into hepatocytes has a great potential to offer additional value to the pharmaceutical industry.

Several independent laboratories have reported on the derivation of hepatocyte-like cells from hES cells. The cells obtained display appropriate morphology (**Figure 2**) and express several hepatocyte-associated markers as well as some liver-like functions. For example, the glutathione transferase system has been shown to be active in these cells, at levels comparable to human hepatocytes. Importantly, there is also recent proof of drug-metabolising capacity in hES cell derived hepatocytes from our laboratories. Critical functions to be further established are broad metabolism and biotransformation capacity as well as transportation of exogenous compounds, because these are functions that are of outmost importance for industrial use of hepatocytes. One of the major challenges will be to improve, optimise and stabilise maturation of hES cell derived hepatocytes for functional use *in vitro*. We anticipate bioreactors to be very useful systems for the development of hepatocytes from hES cells in a way that mimics the *in vivo* maturation processes. Among the challenges when using hES cell derived hepatic cells for industrial testing is also to apply the cells in formats for drug discovery use. We have in our laboratories been able to reseed the hepatocyte-like cells into 96-well plates and maintain the morphology of the cells for several weeks. The 96-well format is expected to enable a useful throughput of novel assays. Clearly, hepatocytes derived from hES cells have the potential to combine a high degree of specific differentiation with an excellent availability for *in vitro* testing of potential new drug candidates.

Finally, the human liver is an organ consisting of many cell types besides the hepatocyte. For example Kuppffer cells, stellate cells and cholangiocytes are adding important pieces to the complex architecture of the liver. Therefore, to be able to better understand and predict positive and negative effects of new drugs *in vitro*, more complex models are required. This further underscores the potential for hES cells as a source for human hepatotoxicity models, since basically any cell type can be generated from the pluripotent stem cells. Although speculative, there is a great hope that hES cell research will pave the first way to mimic simple liver tissue, thereby dramatically improving the chances to accurately predict human toxicity *in vitro*.

Cardiomyocytes derived from hES cells

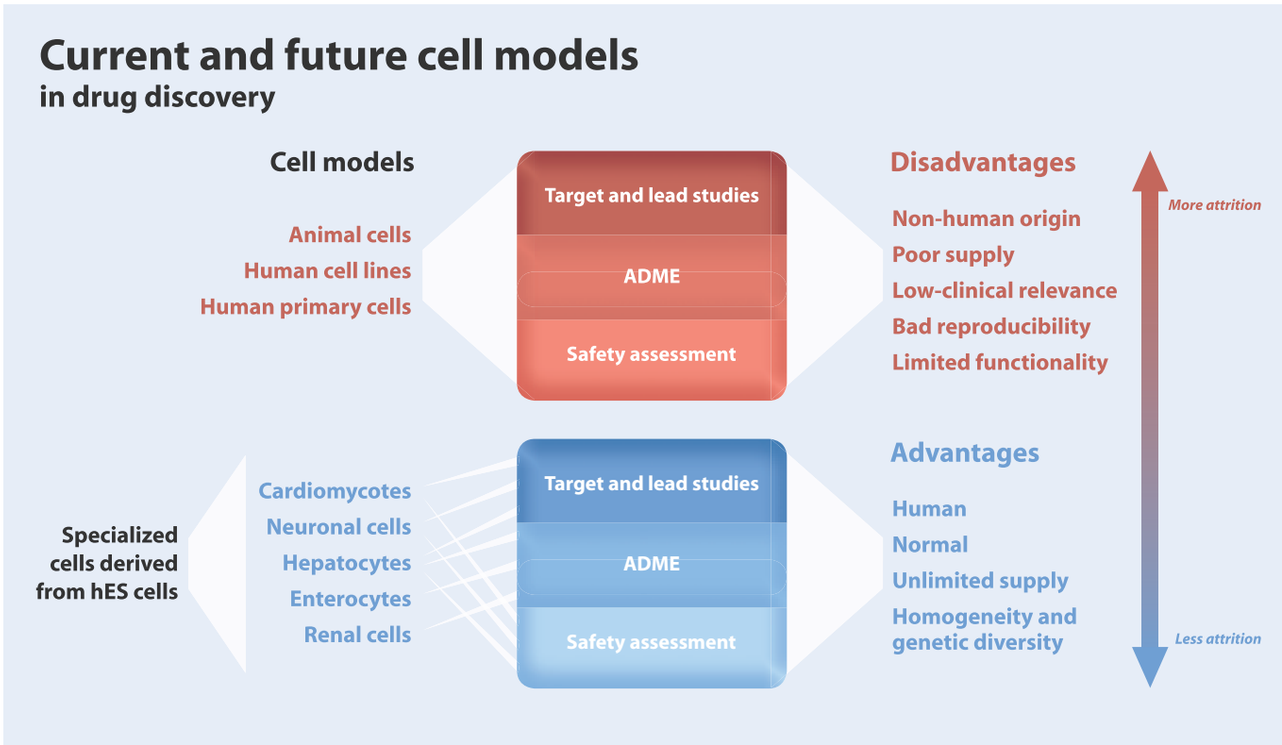
In the area of cardiac diseases, the pharmaceutical industry currently lacks human material for pre-clinical drug discovery and cardiac safety assessment. Based on the shortage of donor material and the problematic procedure of cell isolation, human primary cardiomyocytes are not currently available for pre-clinical drug discovery. However, there is great anticipation that the use of pluripotent human stem cells to derive functional cardiomyocytes for *in vitro* applications will lead to a

dramatic improvement of this situation. The initial observations that mouse ES cells readily differentiate into cells with cardiomyocyte-like properties was reported about two decades ago. Although the mouse system has proven very useful in certain aspects, it is clear that there are substantial discrepancies in cardiogenesis and cardiomyocyte function between mouse and human which are probably attributed to the species differences. Following the first isolations of hES cells, several studies have reported on the establishment and characterisation of spontaneously contracting cells derived from these pluripotent stem cells (**Figure 3**). The currently used techniques for cardiomyocyte differentiation from hES cells are, however, laborious and the yields are relatively low. Fortunately, recent reports have demonstrated new possibilities to improve the protocols for directed differentiation of hES cells to cardiomyocytes.

Several papers, including work from our laboratories, have described the basic characteristics of hES cell-derived cardiomyocytes. Cell analyses reveal that the morphology and ultrastructure of hES cell-derived cardiomyocytes share similarities with adult cardiomyocytes although the myofibrillar and sarcomeric organisation indicate an embryonic/fetal phenotype of the stem cell derived population. Interestingly, maturation of hES cell derived cardiomyocytes appears to occur at least to some extent during extended *in vitro* culture of the cells. On a molecular level, many markers expressed by mature cardiomyocytes are also expressed by hES cell-derived cardiomyocytes, including transcription factors, structural proteins, hormones, ion-channels, and tight junction proteins. Today, there is data enough to support the notion that current methodologies can give access to hES cell derived cardiomyocytes that can be useful for the pharmaceutical industry. It is likely that the access to functional human cardiomyocytes therefore can dramatically increase the strength of target identification and drug discovery in the cardiac disease areas. For target identification, validation and evaluation studies, the access to representative human cells will improve the precision of the assays. The possibility to evaluate a new target in a human close-to-physiologic environment is clearly advantageous to the use of animal models or transfected abnormal cell lines. The possibilities to introduce or delete genes in the stem cell derived cardiomyocytes, either already at the undifferentiated stage of the hES cells or conditionally in the cardiomyocyte-like cells, open up novel avenues for the development of *in vitro* cell based assays for initial target studies.

In addition, there is a great interest in improved cardiotoxicity assays for safety studies. All novel drug candidates, including drugs targeting non-cardiovascular tissues, need to be evaluated for potential cardiac safety risks including the QT interval prolongation and direct arrhythmic effects. Human pluripotent stem cell derived cardiomyocytes may meet this need. At Cellartis, recent studies together with Multichannel systems GmbH have demonstrated important features of hESC-derived cardiomyocytes. The Micro Electrode Array platform (Multichannel Systems GmbH, Germany) enables straight-forward investigations of the electrophysiological properties of hES cell-derived cardiomyocytes and especially rhythm, activation and repolarisation can be readily monitored. In order to evaluate the usefulness of this system for safety pharmacology applications using stem cell derived cardiomyocytes, various compounds with known

Figure 3
Micrograph illustrating hES cell derived cardiomyocytes. The cells are grouped together in a spontaneously synchronically beating cluster which can be maintained in culture for many weeks without losing functionality. Potential areas of applications for these cells in the drug discovery process are indicated as bullet points



effects on cardiac cells have been tested. As an example, E4031, a hERG-channel blocker, was observed to inhibit the repolarisation of hES cell-derived cardiomyocyte at an IC50 of about 2nM. In similar cells, but by other investigators, D-sotalol was reported to induce delayed repolarisation, corresponding to QT-prolongations, providing further support for the possibility to use hES cell-derived cardiomyocytes in safety pharmacology. The hES cell derived cardiomyocytes, in combination with automated platforms for high-throughput electrophysiological recordings and novel *in silico* modeling approaches will provide cost-effective methods for investigating potential proarrhythmic risk of novel compounds.

Concluding remarks

Research on hES cells holds great promise for the understanding and treatment of human disease. In this regard, there are huge expectations on the future application of these cells for therapeutic interventions by permitting the creation of transplantable cells to be used in regenerative medicine. However, the pharmaceutical companies are reluctant to direct therapy with cells, since the cost of goods is high and the production of cells presents many practical hurdles. Another very interesting alternative to cellular therapy would be to search for pharmacological substances with a potential to influence the fate of endogenous stem cells in the human body. The drug industry is indeed also much more attracted to this approach, since the conventional strategy of low molecular weight drug development could be maintained. However, the currently most attractive application of hES cells and their derivatives is their use as improved tools to facilitate traditional drug discovery (**Figure 1**). In the context of this review we have therefore discussed some of the exciting novel opportunities for hES cells and specialised cells derived thereof for applications in the drug discovery process. The major reason for the great interest in hES cell-based systems it that they offer a way to obtain large numbers of different specialised cell types which otherwise are difficult or impossible to acquire from other sources. This will overcome many of the hurdles associated with current models, providing eg hepatocytes and cardiomyocytes that are human and normal, with an unlimited supply of homogeneous cells over time (**Figure 4**). Although progress has been made regarding directed differentiation of hES cells, there is still a need to improve the homogeneity and yield of the target cells by using enrichment and selection techniques. Furthermore, the generation of defined populations of specialised cells from hES cells usually

Figure 4
Schematic representation of cell models and their areas of applications in the drug discovery process. In red, currently used cell types and possible disadvantages by using them are illustrated. In blue, future possibilities and identified advantages by using hES cell derived specialised cells are illustrated. For each area of application, three important hES cell derived cell types have been chosen. Possible effects by using current and future cell models are also illustrated at the right hand side of the figure

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takes several weeks, making it advantageous to isolate and maintain intermediate precursor cells that can be cryopreserved and still have the capacity to proliferate and differentiate upon thawing.

It is anticipated that there will be a widespread use of hES cell-derived hepatocytes and cardiomyocytes in predictive toxicology since two of the leading causes of pre-clinical failure of new compounds are hepatoxicity and cardiotoxicity. Thus, novel improved models to assess adverse effects of new drugs early in the development phase are needed. It is encouraging to see that many new initiatives are addressing these new possibilities, such as the UK government and Pharma industry sponsored programme 'Stem Cells 4 Safer Medicines', launched in October 2007. However, it is also important to appreciate that hES cell research represents an emerging area of investigation, and there are still many fundamental issues related to hES cell culture and differentiation that need to be addressed. Nevertheless, the research community and biotech industry is now at the very early stages of putting into practice some of the new opportunities that hES cells provide. With enough resources, together with sound guidelines and regulations of stem cell research, the field of hES cells has a huge potential to revolutionise many aspects of human biomedicine and the understanding of normal and abnormal human development. Especially, the authors anticipate that development of many cell culture tools in the pharmaceutical industry will most likely emerge over the coming years due to access to many new hES cell-derived specialised cells. It is likely that pharmaceutical companies that successfully integrate hES cell technologies will gain a competitive advantage, and will produce better and safer medicines. DDW

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