Stem Cells as an Enabling Technology in Drug Discovery

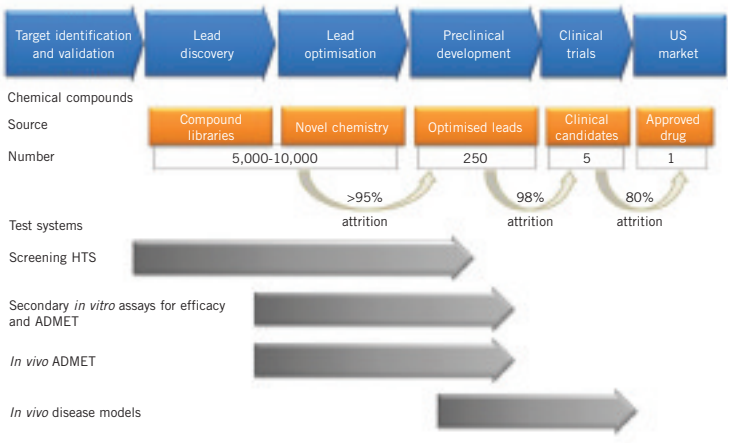
By Maria Webb at Ligand Pharmaceuticals

Stem cells (SCs) are emerging as a viable enabling technology for high throughput screening and lead discovery, and the replacement of mouse SCs with a reliable commercial source of large quantities of reproducible human SC populations will offer a major opportunity to improve the clinical predictability of preclinical drug discovery and development.

Current drug discovery and development endeavours require 10 to 15 years and cost $800 million to $1 billion for each successful drug. The Pharmaceutical Research and Manufacturers of America (PRMA) estimate that for each medicine approved in the US, 5,000 to 10,000 compounds are screened and, of these, five are entered into clinical trials (1). Over the period from 1991 to 2000, the 10 largest pharmaceutical companies reported an 11 per cent clinical success rate; nearly one third of these clinical failures were attributed to lack of efficacy and an additional 30 per cent to unacceptable toxicology or clinical safety (2). Since clinical trials consume most of the cost and time spent on drug discovery and development, new strategies that improve the predictive value of preclinical evaluations are needed (1).

Current drug discovery efforts commonly follow the generalised process outlined in Figure 1. The early stages of drug discovery – target identification and validation, screening for chemical compounds that 'hit' the target, secondary assays for activity and *in vitro* pharmaceutical and safety assessments to identify 'leads', and animal pharmacology and toxicology to optimise lead compounds to drug candidates – rely predominantly on engineered cell lines to 'humanise' the test systems or on animal sources for cells, tissues and disease models. However, the poor predictability of early tests contributes to more than 95 per cent compound attrition during selection of lead compounds for optimisation (1). Specifically, too many secondary assays correlate poorly with activity in disease models. Furthermore, animal disease models predict only 50 per cent of clinical drug toxicity (primarily cardiac and hepatic) (3). Therefore, more reliable preclinical models for screening, pharmacology, disease efficacy and safety assessment are needed to reduce the attrition of drug candidates (3,4). This article focuses on the potential for using stem cells (SCs) in high throughput screening (HTS) and secondary assays to improve the efficiency of early drug discovery.

Figure 1: Outline of the drug discovery process



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IMMORTALISED CELLS AND PRIMARY CELLS IN CURRENT HTS

HTS technologies are evolving to identify compounds that exhibit both the desired interaction with a target protein and favourable *in vitro* pharmaceutical ADMET profiles. As more than 50 per cent of HTS systems are cell-based, it is critical to work in physiologically relevant cellular systems (5).

Many HTS systems use immortalised cells derived from tumours or from cells transformed by onocogenes. Immortalised cells have the advantage of being easily engineered to express target proteins and reporters in a null background that maximises the signal-to-noise ratio. Although they may be grown in virtually unlimited quantities, such cells are genetically abnormal and may change in phenotype over time; most importantly, they lack physiological context by virtue of over-expression of the target protein and frequently lack a full complement of signalling pathways (6). As reviewed by Eglen *et al*, compounds that appear to be full agonists of an over-expressed receptor may behave as partial agonists and partial agonists may be antagonists in more physiologically relevant assays (7). Alternatively, an effective compound may be overlooked if it activates a

signalling pathway absent from the immortalised cell. Test results may also be confounded by constitutive activity of over-expressed receptors.

Primary cells isolated from appropriate human populations are presumed to be the ideal for HTS because they express the target protein and associated signalling pathways at physiologically relevant levels in a natural environment. However, the limited supply of donor tissues from human populations restricts the use of human primary cells. Moreover, supplies of primary cells are generally insufficient to support HTS because homogeneous lines are difficult to isolate, have short life-spans and unstable phenotypes in culture, and do not survive freezing. The most common current uses of primary cells are in tissue-specific toxicology assessments and secondary functional assays (8).

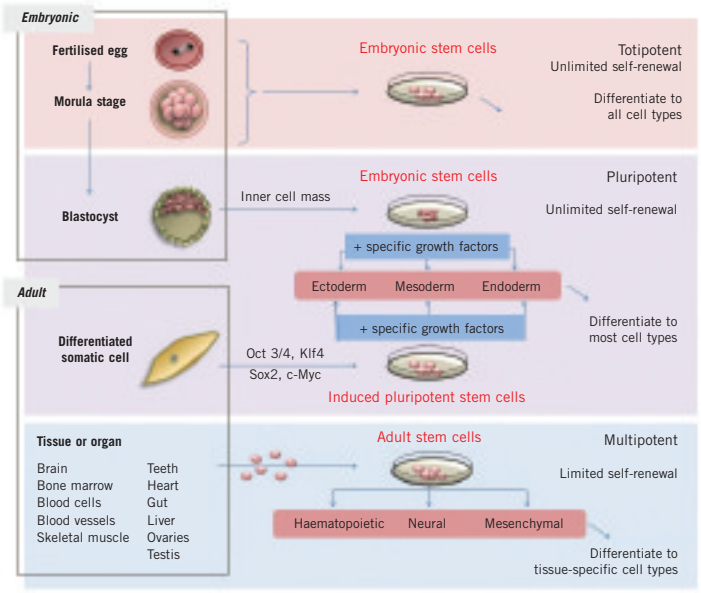

Figure 2: The development of 'totipotent', 'pluripotent' and 'multipotent' stem cells

STEM CELLS IN HTS AND DRUG DISCOVERY

The emergence of SC-based HTS in drug discovery has the potential to address the liabilities of immortalised cells, as well as current limitations of primary cells. The possible advantages and limitations of using SC in HTS to identify target hits, investigate functional activity and assess ADMET characteristics are outlined below. Recent advances in SC biology for the development of cell-based therapies are beyond the scope of this overview.

Stem cells, by definition, are unspecialised cells capable of self-renewal and differentiation into specific cell types; they are named according to their source as embryonic, adult (also known as nonembryonic or somatic) or cancer SCs. The recent discovery of induced pluripotent stem cells (iPSCs) – adult somatic cells reprogrammed to an embryonic SC phenotype – offers the prospect of a renewable source of large quantities of disease-specific SCs that may enable innovative HTS (9).

Embryonic Stem Cells

Embryonic stem cells (ESCs) are present in the fertilised egg through the morula stage of development and are 'totipotent', with the capability of differentiating into all embryonic and non-embryonic cell types (see Figure 2). Most ESCs currently used in research are harvested from the inner cell mass of blastocysts formed during development; these ESCs are 'pluripotent' with the capacity to form the three embryonic germ layers – the ectoderm, the mesoderm and the endoderm – that later differentiate into all adult cell types (9).

Mouse ESCs (mESCs) grow undifferentiated in culture for months, survive freezing and may be directed to differentiate into specific cell types (10); mESCs or the specialised cell types derived from their differentiation may be produced in quantities sufficient to support HTS.

Compared with mESCs, human ESCs (hESCs) grow more slowly, need unique culture conditions to remain undifferentiated and are more difficult to engineer genetically. In addition, reliable directed differentiation of hESCs to large populations of reproducible, homogeneous specialised cells requires optimisation of new induction and culture protocols (6). Furthermore, legal and ethical considerations restrict the use of hESCs in medical research (10). For these reasons, HTS based on hESC is unlikely to become an option in the near future (11).

Accordingly, current pharmaceutical HTS relies on cells differentiated from mESCs. The first reported ESC-based HTS for lead discovery employed mESCs as a consistent, easily scalable source of differentiated neurons that demonstrated the expected pharmacological responses to glutamine agonists (11). In another pioneering study, a phenotypic HTS in which differentiation of mESCs was the endpoint identified small molecules that induced neurogenesis (12). These innovative uses of ESCs – or cells derived from ESCs – in HTS offer a glimpse of the future for drug discovery and development.

In vitro toxicology tests are the most common use of ESCs in drug discovery (3). One such test for toxicity in developing cardiomyocytes derived from mESCs is championed by the European Centre for the Validation of Alternative Methods as a future standard for assessing teratology potential. This assay distinguishes strong teratogens from compounds with moderate, weak or no toxicity and shows 78 per cent correlation with *in vivo*

Table 1: Stem cells isolated from embryonic or adult sources

Stem cell type	Source	Differentiation potential
Totipotent ESC	Fertilised egg through morula stage	All cell types
Pluripotent ESC	Inner cell mass of embryonic blastocyst	Endodermal, mesodermal or ectodermal cell lines
Multipotent adult SC	Adult tissues and organs (brain, bone marrow, blood, blood vessels, skeletal muscle, teeth, heart, gut, liver, ovaries and testis)	Haematopoeitic (all types of blood cells) Mesenchymal (connective tissues, namely bone, cartilage, tendon, ligament, fat and marrow stromal cells) Neural (neuronal and glial cells) Epithelial (gut absorptive, secretory and enteroendocrine cells) Skin (keratinocytes)

models. With further optimisation – including the use of human rather than mouse SCs – and validation for HTS, this test has the potential to improve overall screening efficiency and reduce the need for some animal-based toxicity tests (3).

As discussed above, HTS and secondary assays that reliably predict clinical cardiac and hepatic safety during drug discovery would substantially shorten time-lines, increase clinical success rates and reduce costs. To accomplish this goal, reliable sources of large, homogeneous populations of cardiomyocytes and hepatocytes that reproducibly predict toxicity in humans are essential. The studies described below represent some of the ongoing efforts to improve safety assessments by using ESC-based HTS and secondary assays.

Chaudhary *et al* studied a new technique – laser microdissection and pressure catapulting – for isolating functional cardiomyocytes derived from mESCs (13). The isolated cells continued to beat, retained operative calcium channels despite an immature phenotype, and were easily applied to an HTS format for assessing cardiac toxicity. In addition, Wu *et al* used beating cardiomyocytes derived from mESCs in a phenotypic screen to discover small molecules that induce differentiation (14). Furthermore, Norström *et al* demonstrated that cardiomyocytes derived from hESCs continued to beat and retained functional calcium channels and adrenergic and muscarinic receptors after freezing (15). These and other advances may enable ESC-based HTS for early cardiac safety evaluations.

Another goal is to utilise homogeneous human hepatocytes for assessing hepatotoxicity, drug metabolism and drug-drug interactions because of the considerable differences between murine and human liver physiology. Reports that hESCs can be induced to differentiate to cells with hepatocyte characteristics are promising (3). However, the routine use of ESC-derived cardiomyocytes and hepatocytes for ADMET assessments requires alternative sources of undifferentiated human SCs and protocols that reliably deliver functional cells representative of adult tissues (4).

Adult Stem Cells

Self-renewing SCs found in a variety of adult tissues and organs are considered multipotent because they can differentiate into a limited number of tissue-specific cell types (see Table 1). Thus, adult SCs are sources of haematopoietic, mesenchymal, neural, epithelial or skin cells, based on the type of cell generated during differentiation. However, the supplies of most multipotent SCs are limited because of the small numbers present in adult tissues and the limited capacity of adult SCs to grow in culture (9).

The advances most relevant to HTS have been achieved using mesenchymal stem cells (MSCs). Improved culture protocols now yield MSCs that survive many passages without losing their ability to differentiate (10). The basis for better induction protocols was established by using an optimised HTS to assess the effects of all combinations of four growth factors on MSC chondrogenesis. Using the same HTS to survey a small molecule library, five potential inducers and 24 potential inhibitors of MSC chondrogenesis were identified (16). In addition to increasing our understanding of adult SC biology, this demonstrates that adult SCs can eventually enable HTS for novel drug discovery. At present, adult SC offer the most practical source of human cells for enabling SC-based drug discovery.

Induced Pluripotent Stem Cells (iPSCs)

The discovery that pluripotent SCs may be created from adult somatic cells (see Figure 2) offers the hope of an unlimited supply of human SCs that no longer depend upon the availability of embryonic or foetal tissue. Two techniques are currently used to induce pluripotency in adult somatic cells: transfection of four oncogenic transcription factors (Oct3/4, Klf4, Sox2 and c-Myc) into a mouse or human somatic cell, and transfer of a mouse somatic cell nucleus to an enucleated oocyte with subsequent development into a blastocyst. Before iPSCs can be used widely in drug discovery, the efficiency (currently 0.01 per cent) and methods of conversion must be improved. Ongoing studies seek to minimise unwanted mutations or unintended reactivation of transfected transcription factors during differentiation by reducing the number of transcription factors or by replacing them with small molecules. Finally the recent discovery that iPSCs can be derived from individuals with inherited disease opens a new avenue for creating SC-based assays that reflect human disease (10).

FUTURE OPPORTUNITIES AND CHALLENGES

Stem cells, or specialised cells derived from SCs, are emerging as a viable enabling technology for HTS and lead discovery. Replacing the current use of mouse SCs with a reliable commercial source of large quantities of reproducible human SC populations offers the greatest opportunity to improve the clinical predictability of preclinical drug discovery and development. Before that

aspiration can be realised, new sources of human SCs must be found, and reproducible protocols for directed differentiation must be enhanced.

With reliable sources of more relevant cellular models of human physiology and disease, the use of SCs may be more widely applicable throughout the drug discovery and development process. In an early report, Allen *et al* (17) used engineered mESCs to elucidate the role of p38α kinase – the target for cytokine-suppressing anti-inflammatory drugs – in cytokine signalling pathways. Because p38α kinase deficiency is embryonically lethal, engineered ESCs provided the only opportunity for this evaluation. The creation of disease models from engineered ESCs remains another intriguing goal for HTS in pharmaceutical research. SC-based HTS may also open new avenues, such as identification of small molecules that induce cell differentiation and growth (12,14). In addition, SC-based HTS that detects biomarkers of human toxicity and intracellular signal transduction may allow early identification of compounds exhibiting both desirable and undesirable effects, increasing the efficiency of drug development (3).

In summary, humanised SC-based HTS and assays that reliably and accurately predict pre-clinical and clinical efficacy and safety have the potential to revolutionise drug discovery and development.

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