

**STEM CELLS
IN
PREDICTIVE TOXICOLOGY**

*A report of a scoping study from the Stem Cells in Predictive
Toxicology Task Force,
for the Department of Health and the Department of Trade and
Industry*



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STEM CELLS IN PREDICTIVE TOXICOLOGY

1. SUMMARY

In December 2005, the UK Stem Cell Initiative (UKSCI) recommended the establishment of a public private partnership (PPP) to develop stem cell technology as a tool for predictive toxicology in drug development, to reduce the high attrition rate for drugs that fail during clinical trials on grounds of toxicity. Subsequently, in July 2006, Sir Keith O’Nions (Office of Science and Innovation, Department of Trade and Industry, DTI) and Professor Sally Davies (Department of Health, DH) held a consultation meeting on the proposed PPP initiative with representatives from the pharmaceutical companies. In order to inform recommendations to Government on funding for collaborative research, it was agreed that ABPI would prepare a strategy for developing tools for predictive toxicology, utilising in particular stem cell technology – by hosting and leading a project that would conduct an analysis of the gaps and opportunities in the area.

A Task Force was established to provide oversight to the project. The group comprised senior representatives from a number of ABPI member companies and key stakeholders in the industry, who have the appropriate expertise and substantial corporate activity in the area of predictive toxicology or stem cell technology; stem cell experts, UK Stem Cell Bank, representatives from BIA, MRC, DH and DTI. The study was project managed by ABPI, and consulted with a range of stakeholders (see Appendix 2).

The pharmaceutical industry spends over £3.4 billion per year on research and development (R&D) of new medicines. A key objective for the industry is a reduction in inefficiencies and high cost associated with taking compounds through to late stage development – many drugs fail due to an unacceptable safety profile. Availability of validated human cell-based *in vitro* toxicity screens may facilitate earlier attrition of compounds with unacceptable safety profiles, and therefore, also reduce the use of animals. Significant potential added value to the pharmaceutical industry from stem cell technology is many-fold: a reliable, consistent and unlimited source of cells for screening, avoiding sporadic and limited availability of human tissue, and enabling a closer phenotypic match than animal material. Improved drug safety will also provide a tangible benefit for patients and trial volunteers. The step change to current systems will be significant and can be exploited in emerging technologies.

Recommendations

i. This report from the Task Force recommends that a focused initiative should be established, with the vision of establishing open protocols and standardised systems in stem cell technology that will enable stem cells to be consistently differentiated into stable homogenous populations of particular cell types, especially liver cells, and of physiologically relevant phenotype suitable for toxicology testing in high throughput platforms. Universal protocols with general acceptance to the industry are unlikely to be developed in the absence of a joint collaborative effort and investment.

ii. The initiative should take the form of a public-private partnership: a funding consortium of government (to drive the science base and infrastructure, and ensure appropriate regulatory framework) and pharmaceutical partners (as end-users to drive the development of a shared, common resource for the development of safer

medicines). Activities of the initiative will be accessible to relevant stakeholders such as academic scientists and biotech companies. As there is related activity globally, the consortium should also engage with relevant centres and initiatives where appropriate.

iii. A 1-year pilot phase should first be initiated, during which a number of projects are carried out to inform on feasibility of the science and nature of the biology gap to be addressed in a full 4-year initiative. A consortium will also be legally established during this phase, and a detailed science programme and budget drawn up. The budget recommended for the pilot year is £1.2 million, including matching funding provided by government.

Benefits to the UK

The benefits to the UK of such an initiative will be substantial. It will strengthen the science base in stem cell biology and secondarily toxicology, and contribute to an international lead for the UK in stem cell biology. It will enhance academic-industry collaboration, and will open up exploitation opportunities for small and medium enterprises (SMEs), for example in assay and hardware development. A partnership with support of the government will highlight competitive advantage for pharmaceutical companies to locate their R&D and safety assessment activities in the UK, and with potential for leveraging future inward investment by enabling participation of companies whose toxicology base may be outside the UK.

As other countries and centres are building up their capacity and capability in stem cell research, the window of opportunity for the UK is narrow, and should be seized.

2. PREDICTIVE TOXICOLOGY

2.1 Significance in drug discovery process

Reduction of the rate of attrition of drugs in late stage development due to unacceptable safety profile is a key objective for the pharmaceutical industry. In order to achieve this, identification of the toxicity potential of candidate drugs at an early stage in the drug discovery pipeline is essential. The provision of validated (predictive) high-throughput cell-based *in vitro* toxicity screens may be highly beneficial in this respect. However, the effectiveness of these is currently severely limited by the lack of availability of relevant cell types (i.e. human, target organ phenotype) that are adequately validated.

2.2 Initiatives/activity underway in predictive toxicology/ safety assessment

- DTI Safety Biomarkers Technology Programme 2006;
- EU Framework 6 “Predictomics” project (Industry/Academic Consortium): Development of *in vitro* assays to predict chronic toxicity;
- EU Innovative Medicines Initiative: Predictivity of safety evaluation is one of four pillars in the strategic research agenda;
- ILSI Biomarkers (Industry Consortium): Identification and validation of clinical and pre-clinical biomarkers of toxicity;
- California Institute for Regenerative Medicine: the draft 10-year strategic plan includes the development of stem cell toxicology tools as a goal;
- FDA Critical Path Predictive Safety Testing Consortium: FDA-Industry consortium with multiple project arms charged with validating existing potential preclinical (and later, clinical) markers of toxicity via pooling of knowledge and resources. Includes teams on nephrotoxicity, hepatotoxicity, vasculopathy, carcinogenicity;
- Miscellaneous proprietary work undertaken or commissioned by individual companies.

2.3 Potential added value of stem cells

The aim of focusing on stem cells is to exploit this emerging technology and the current favourable environment created in the UK for human embryonic stem cell research, for the development and provision of a panel of fully differentiated toxicologically important human stem cell lines with specific and reproducible phenotype, in sufficient quantity so as to enable their use in high-throughput *in vitro* toxicity screens. This is seen to be a challenging aim in itself, and is very likely to have significant benefit for wider applications than the development of predictive toxicology assays and instrumentation alone.

Step changes that stem cell technology may bring, include:

- A long-term source of cells, indefinitely maintained, with capacity for differentiation into a range of specialised cells;
- Avoidance of donor and preparation variability, sporadic and limited availability of human tissue for screening compounds;
- Closer match to *in vivo* phenotype and physiology, particularly for organ systems where there is reliance on non-human material;
- Ability to investigate cell developmental effects;

- Mixed cultures of differentiated cells to assess effects of chronic exposure;
- Cells sourced from different donor genotype may provide pharmacogenetic information on susceptibility to specific toxicities;
- Reduce and replace usage of animals and animal tissue;
- Improve the predictivity of early toxicity screening of pipeline compounds and reduce later stage attrition of drugs;
- Reduce the industry costs and inefficiencies associated with late stage failure;
- Improve patient and volunteer safety (e.g. hepatotoxicity) via safer drugs.

2.4 Limitations of current systems, and potential for enhancement by stem cells

The predictivity of *in vitro* screens is dependent on the appropriateness of biological modelling, which may be limited by available cell types, simplicity of cell model, and technology platform. Furthermore, the toxic potential of a candidate drug may be substantially influenced by or solely dependent on interacting factors such as disease states, metabolic activation, other drugs, and patient phenotype. Mimicking such conditions therefore poses challenges to the *in vitro* toxicologist.

Additional constraints on the model design include:

- screen throughput: since high throughput screens usually require a simple cell monolayer;
- ready availability of cells;
- convenient end-point measurements and duration of exposure since chronic toxicity testing will require treatment replenishment:
- longer-term cell survival;
- demand for test compounds that may be limited during the early stages of drug discovery.

Developments that may facilitate model design include:

- advancements in technologies such as high content screening platforms which will enable multiple endpoint measurements and provide more mechanistic-based screening;
- the application of three-dimensional tissue models, housed in bioreactors to provide a holistic model(s) of target organs. Current systems are cumbersome, expensive, and do not lend themselves to any reasonable throughput.

However, the single most important component of a high throughput toxicity screen is the cell type used. For hepatotoxicity testing, the primary human hepatocyte is the natural choice since the more readily available hepatoma cell lines lack the differentiated phenotype that may be important for determining toxicity. Similarly, animal primary cells may not match the appropriate phenotypic characteristics. However, the availability of high quality primary human hepatocytes is limited and therefore not practicable for regular hepatotoxicity testing without donor-to-donor variability. Currently it is not possible to efficiently expand human primary hepatocytes and when available, these cells rapidly lose their key characteristics such as drug metabolising enzymes.

Differentiation and expansion of stem cells into cells approximating primary hepatocytes (or indeed any other required primary cell) therefore offers important potential to provide continuous and readily available supplies of cells with limited variability, which may also retain their differentiated phenotype for longer periods.

Current developments in progenitor cell biology (e.g. embryonic and adult stem cells) suggest that such a goal is achievable and should improve the quality of predictive toxicity testing.

A tabulation of the gaps and limitations of current models, and the potential added value of stem cells, is attached in Appendix 1. A survey of emerging technologies and the potential for their exploitation by stem cell tools is also attached (Appendix 1).

2.5 Cell types – Common priorities identified by the pharmaceutical industry as end-user of resource

A prioritised list of target cell types was agreed as follows, with hepatocytes and cardiomyocytes acknowledged to be of primary universal utility in safety assessment.

RECOMMENDATION

Priority:

1. Liver cells
 - a. hepatocytes
 - b. resident non-parenchymal liver cells (biliary, kupffer, SEC, stellate, etc) both directly and as hepatic co-culture contributors
2. Cardiomyocytes

Secondary priority:

3. Bone Marrow cells (erythroid, myeloid lineages)
4. Neuronal cells (particularly for use in developmental toxicity screening)

Should an initiative be successful in attaining its objectives with regard to the cell types above, consideration for follow-up activity may include cell types relevant to reproductive toxicity, and perturbation of developmental pathways.

Long-term objectives

Following *in vitro* screening, testing of novel compounds in animal models is typically the next step in toxicity screening, and is currently required by regulators in the preclinical phase of drug development. Whilst this initiative focuses on the initial development of characterised tools for early decision-making in discovery research the availability of reliable stem cell tools would nonetheless minimise and lead to more effective use of animals, as only compounds that have a positive *in vitro* safety profile will proceed to the *in vivo* stage. The validation of new characterised tools for use in preclinical safety packages would be a goal for the longer term and follow-up phase of the initiative, and will require engagement with UK, EU, and US regulators in terms of criteria for regulatory acceptance (MHRA, EMEA, FDA respectively).

3. STRENGTHS OF THE UK AND OPPORTUNITIES

Science and R&D

- Record of innovative pharmaceutical R&D, which has longstanding historic strength in toxicology. Excellent academic and biotech research capacity in stem cell science and tissue engineering.
- UK has relative strength in embryonic stem cell (ESC) research due to favourable research climate (40% of SC patent applications, compared to 15% in US).
- Positive, collaborative environment.
- Relatively well developed biotechnology and venture capital community for exploitation of science.

Governance

- Good governance structure (regulatory agencies) and ethical framework, with a centralised regulated human embryonic stem cell bank and codes of practice.

Public support

- Government is supportive of science and stem cell biology, which additionally, aids establishment of a sound legal and regulatory framework and provides public assurance. Integrating basic and translational health research into a single health research fund, with proposed creation of a Translational Medicine Strategy Board (Cooksey Review 2006), which would be a natural home for support of an initiative.
- UKSCI recommendations for increased investment from government on stem cell research, capacity, facilities, and coordination.
- The majority of the UK public is in favour of the use human embryos in medical research (MORI Poll 2003).

Timely opportunity

- A window of opportunity is open. Other countries are building positions of strength, with substantial funding. E.g. Japan, Australia, California.

4. STEM CELL TECHNOLOGY

4.1 Stem cells

Developmentally, embryonic stem cells are the earliest precursors to distinct cells of mature phenotype that make up specific tissue and organs, while adult stem cells (ASC) (giving rise to restricted range of lineages) reside in somatic tissue such as bone marrow and mesenchymal tissue, and fetal stem cells in fetal umbilical cord blood and organs *in utero*. Embryonic stem cells are obtained from the inner cell mass of 5-6-day old blastocysts. They are considered to have characteristics of pluripotent stem cells (giving rise to all cell types) and to have a capacity for indefinite self-renewal, whereas the replicative properties of multipotent ASC and physiological characteristics of lineages derived from them tend to be more limited. ESC are therefore a powerful, virtually unlimited source of self-renewing cells, indefinitely maintaining an undifferentiated state in culture yet able to generate all the cell types of the human body.

The proposed use of human embryonic stem cells (hESC) for predictive toxicology will be *in vitro* and distinct from patient-oriented use in regenerative therapy, which is generally perceived to be a more achievable goal scientifically and in terms of public acceptability.

4.2 Strategy for application in predictive toxicology

RECOMMENDATION: A SHORT, MEDIUM, LONG TERM STRATEGY

In the short term (1-2 years), a universal set of acceptance criteria for each type of differentiated cell (e.g. hepatocyte, cardiomyocyte) should be developed and agreed with safety experts. This may include surface markers, gene expression, protein or metabolic profiles. If supporting data of existing lines are inadequate, promising lines should be characterised physiologically for indication of utility in toxicology. Currently accepted toxicology assays should then be used to comparatively assess available stem cell lines – available from commercial sources or not-for-profit repositories.

In the medium term (5 years), an initiative should seek to achieve the establishment of a repository of human embryonic stem cells that can be consistently differentiated into stable homogenous populations of particular cell types (hepatocytes and cardiomyocytes in this case, for toxicology), and of physiologically relevant phenotype suitable for toxicology testing in high throughput platforms. Basic stem cell biology, quantity scale-up, physiological and toxicological characterisation will be key to achieving this goal.

In the longer term (4-8 years), should medium term objectives be successful, follow up activity should ensue in developing other toxicologically important cell types (haemopoietic, germ cells), and validation towards regulatory acceptance.

4.3 Scientific and technological considerations.

Comments were sought from the academic and biotech scientific community, and a review was commissioned from Dr Tim Allsopp of Stem Cell Sciences, Edinburgh. These have fed into the report. The timing of this study over the summer vacation period was too short to hold a workshop meeting of scientists, and this could be held during the pilot stage of an initiative.

4.3.1 Cell type and source of stem cells

Choice of species. For practical reasons, the review explored the feasibility of researching mouse systems prior to human stem cell work. However, the scientific consensus was that the cell differentiation and biochemical signalling pathways are substantially distinct to limit ability to extrapolate from murine to human systems. As the human system is the most relevant for safety screening, it is proposed that the initiative focus on human stem cells.

Source of stem cells. The review explored all available sources: adult, fetal, and embryonic. Sourcing from somatic tissue will enable the collection of cells from donors who have suffered relevant adverse drug reactions for genetic analysis and the study of idiosyncratic toxicology. While socially more acceptable and avoiding IP barriers, ASC can be technically more challenging.

Nonetheless, for certain cell types, derivation from ASC may be achievable earlier, and could be a back-up in the event of failure of hESC work to achieve acceptance criteria. Therefore, to enable the generation of cells with suitable characteristics for toxicology screening, it is proposed that adult, fetal and embryonic sources are investigated for the cell types of interest – likely resulting in phased availability.

Ultimately, selection criteria should be made based primarily on utility of the cells for the intended purpose (this may need to be determined through experimentation).

Summary

A table of cell types against tissue source is attached, summarising factors impacting on their current status: feasibility of production, fidelity of fate specification, validation of functionality, scalability of manufacture and tractability of modification.

As shown in Table 1, an analysis of current state of art indicates that the establishment of liver and cardiac cell types are a more tractable option from embryonic than from fetal or adult stem cells. Across all systems, development of neuronal cell types may be achieved, arguably rather earlier than liver and cardiac cells, though difficulties may present for purified neuronal populations. On the other hand, obtaining haemopoietic cells is a far more challenging prospect in embryonic, fetal and adult systems.

Table 2 summarises priority areas for an initiative in improvements to stem cell technology, with the production of hESC lines in heart and liver systems of priority in the immediate term.

RECOMMENDATIONS

The initiative should focus on human rather than murine stem cells.

Adult, fetal and embryonic sources should be investigated as to the best source to generate cell types of interest, with immediate priority proposed for liver and cardiac cell types from human embryonic stem cells.

In addition to toxicological impact, greater added value will be gained from pursuing liver and cardiac lines as neuronal research is well under way.

TABLE 1. CURRENT ART STATUS FOR HUMAN STEM CELL TECHNOLOGY

Provenance	ES cell lines					Fetal [tissue]					Adult [tissue]				
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
Liver ^α	2 ¹	3	3	5	5	3	4	4	5	5	4	5	5	5	5
Cardiac ^β	1	3	1	1	2	4	4	5	5	5	4	3	3	4	4
Neural ^γ	1	1	3	3	2	1	2	1	3	3	1	1	3	4	3
Blood ^δ	2 ²	3 ²	4	4	4	3	4	4	4	4	4	4	4	4	4
Skin ^ε	3	5	5	3	5	3	5	5	5	5	1	2	3	1	1

Key

1 = established technology

2 = developed methodology, non peer reviewed

3 = methodology under development

4 = currently problematic

5 = no indication

A = feasibility of production

B = fidelity of fate specified

C = validation of functionality

D = scalability of manufacture

E = tractability of modification

α. Parenchymal cells only

β. No distinction made for atrial, ventricular cardiomyocytes or pacemaker cells

γ. Neural products from neuroepithelial fate; enrichment for subtype neuronal lineages possible to varying degrees

δ. Mixed common myeloid & lymphoid progenitors only

ε. Non follicular epidermis only; no evidence for sebaceous, follicular, sweat glands

¹ marked down on second opinion w.r.t. robustness of scientific literature² reviewed upwards

TABLE 2. PRIORITY AREAS FOR IMPROVEMENTS TO HUMAN STEM CELL TECHNOLOGY³


Provenance	ES cell lines					Fetal [tissue]					Adult [tissue]				
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
Liver ^α	1										↑	↑	↑	↑	↑
Cardiac ^β	1			1											
Neural ^γ	1	1	*	*	*	1	1	1	*	*	1	1	*	*	*
Blood ^δ						3->1					*	*	*	*	*
Skin ^ε						3->1					1	*		1	1

Key

1 = established technology
 3->1 = progression of discovery to technology

A = feasibility of production
 B = fidelity of fate specified
 C = validation of functionality
 D = scalability of manufacture
 E = tractability of modification

- α. Parenchymal cells (other liver cells are also prioritised)
- β. No distinction made for atrial, ventricular cardiomyocytes or pacemaker cells
- γ. Neural products from neuroepithelial fate; enrichment for subtype neuronal lineages possible to varying degrees
- δ. Mixed common myeloid & lymphoid progenitors only
- ε. Non follicular epidermis only; no evidence for sebaceous, follicular, sweat glands

 = high priority, 0 – 3 years (↑ marked up due to higher tox priority)

 = priority 2 – 5 years (* marked down due to lower tox priority)

 = priority 4 – 7 years

³ Tables 1 and 2 modified for prioritisation in toxicology, based on original prepared by Tim Allsopp

CHALLENGES IN STEM CELL BIOLOGY

Key challenges associated with the generation of stem cell lines are summarised below.

4.3.2 Generation of stable cell lines

ISCI1 (International Stem Cell Initiative I, report in preparation) has done some work on defining markers of the human embryonic stem cell state, which will help enrichment of stem cells from complex mixtures, and specification of protocols for the isolation of SC and for driving differentiation. Remaining issues centre on maintaining genetic stability of lines, rather than their derivation. Factors include standardisation of culture conditions, media, plastics, reliance on feeder cells - essential for meeting high standards in use by stem cell banks and for the reliable screening of compounds in industry. Development of universal methodology is therefore required. Some of these challenges, including genetic stability of hESC lines, will be addressed by the International Stem Cell Initiative and the FP6 ESTools consortium. Should specific conditions (e.g. culture) be required by this initiative, the UK Stem Cell Bank will have the ability to meet these requirements.

4.3.3 Efficiency of differentiation

Human ESC are advantageous in their efficiency of differentiation as well as in the subsequent separation and purification (differentiation protocols generate complex mixtures of cells). Challenges for efficient differentiation include: the standardisation of culture protocols, as many procedures are not reproducible by other users; insufficient understanding of lineage specification and markers; rigorous definition of terminally differentiated cells; and the influence of epigenetic modification in culture.

4.3.4 Consistency, reproducibility, homogeneity

The minimum quality control criteria would be the standards set by the UK SCB. ASC pose a particular challenge in terms of maintenance as few systems exist that describe ASC lines that symmetrically self-renew in culture. It is also generally recognised that SC from adult sources are far more limited in stability and homogeneity, in many cases arising from artificial immortalisation. Coherence in defining quality control aspects will be required for both ASC and ESC (see international efforts for ESC in 3.3.2).

4.4 Scale up and integration into high-throughput systems (HTP)

Most scientists agree that the validation of automated large scale of human cell lines is achievable (whilst maintaining consistent properties), particularly for adherent cultures. However this has not really been done yet and will require development work, including the use, or not, of feeder cells.

4.5 Cells and lines currently available

Globally, it is thought that over 200 hESC lines were derived in 2004 alone, though it is likely that only a fraction would have met rigorous ethical and technical standards; and hence only hESC lines from banks with proper governance mechanisms in place will be used in an initiative. Key repositories include the US National Stem Cell Bank (US NSCB, which will include those 22 lines eligible for US federal funding as well as lines listed from other countries); and the UK Stem Cell Bank (4 lines currently available, with 26 already approved for deposition). Lines derived from somatic tissue are available for purchase from commercial sources (eg Chemicon), mesenchymal or fetal lines from not-for-profit repositories such as ATCC, ECACC, Coriell Institute, NIH Center for Research Resources. Any cells used in an initiative must meet the ethical guidelines adopted by the consortium.

Activity in this area include companies such as Axordia which has produced 4 human hESC lines, two currently available from the UKSCB, CXR which is deriving hepatocytes from hESC, ReNeuron which has produced neuronal cells from adult brain and hepatocytes (data to be published). There are reports of hESC making hepatocytes but determining whether these cells are really hepatocytes rather than yolk sac has been based on comparisons with mouse data. Two to three academic groups in the UK are working on producing cardiac cells from hESC.

4.6 Capacity in the UK

Research in stem cell biology (phase 1) could conceivably be undertaken in academia and specialist stem cell companies. Subsequent phases (characterisation and evaluation) have a closer match to activities of specialist companies, with potential for in-kind participation by pharma partners.

Strong academic clusters can be found in Cambridge, Edinburgh, London, Manchester, Newcastle, Nottingham, and Sheffield. Additionally, five GMP facilities are in the process of being set up, the first almost ready in Sheffield with the remainder coming on-stream in the next two years. Academic capacity is thought to be highest in the UK for hESC, followed by fetal and adult SC. Following the allocation of funding for stem cell research in the 2002 Spending Review, the research councils and DTI have provided support for building the infrastructure (e.g. UK Stem Cell Bank) and skills base in stem cell biology, interdisciplinary networking – all essential in underpinning the proposed initiative. Further work will be required in establishing stronger links with system biologists, cell biologists, developmental biologists, geneticists and physiologists/ pharmacologists/ toxicologists. The provision of support for research personnel in the initiative's projects will help attract scientists from other fields with the right skills.

Examples of relevant small and medium enterprises (SMEs) in the UK biotechnology sector include Abcellute, Axordia, Cellcentric, CXR, Regentec, and Stem Cell Sciences; and abroad, ACT, Cellartis, ES International, Geron, Novocell, and Stem Cell Inc.

In all phases, an initiative may wish to access expertise outside UK if not available within, where it is necessary to achieve the goals of the initiative. Hence, these may be academic expertise or material, biotech companies, or pharmaceutical companies with a presence in the UK but whose R&D or toxicology activity is based abroad.

Gaps

Scientists consider that there are no obvious gaps in relation to stem cell biology in academia in the UK. However, overall capacity in the UK is spread rather thinly, and could be optimised by attracting scientists to the stem cell field from important relevant disciplines (such as developmental biology and genetics) and increasing numbers by providing appropriate training.

In terms of SME gaps, it is thought that the diversity of specialist stem cell activities directly relevant to drug discovery is low. Gaps include cell supply and formatting, metabolomics, tissue function replication and advanced imaging. Increased venture capital (VC) investment in the biotech sector would appear to be welcome.

On the whole, the UK is well placed to deliver this initiative, but access to expertise abroad will be necessary. If the UK is to maintain its lead in stem cell biology, or expand it in the longer term, the research base, both academic and commercial, will need strengthening.

RECOMMENDATION:

Parallel allocation of science budget to cross-research council stream, e.g. MRC or BBSRC, to build up academic capacity in stem cell biology and blue-skies research that may lead to advances in toxicology in the long term; and to facilitate scientific training and recruitment to the field from other disciplines particularly developmental biology and genetics.

An Initiative should be open to accessing expertise and materials outside the UK where necessary.

4.7 Recommendations: Focus for R&D

Based on the science and technological considerations above, the key areas of research required to achieve the aims of the initiative are:

- Greater acumen in the creation and isolation of human embryonic stem cells;
- Improved resolution in the requirements to generate the functionally differentiated state;
- More detailed understanding and characterising of phenotype, including gene expression and functionality, of differentiated cells;
- Automated scalable production of human embryonic stem cells;
- Validation of appropriate cell lines with tool compounds, ensuring high predictivity (equal or superior to existing assays) for clinical effects;
- Assay development to a suitable format for high-throughput *in vitro* screening (possibly beyond a joint initiative, as may be on an individual pharma basis).

5. PATENT LANDSCAPE AND FREEDOM TO OPERATE (FTO)

5.1 Patents on stem cell technology

Patents may cover stem cell lines and/or methods of deriving, maintaining or differentiating stem cell lines. Pragmatically, providers of existing stem cells of interest will advise on their patent status and any restrictions on use. The scope of these individual patents will indicate whether there is freedom to derive novel cell lines by similar or other means. Patents on methodology are likely to have a greater impact than those on composition of matter.

General points on patentability - patenting of stem cells from animal and adult human tissue and their application are not contentious, it is the human embryonic source that is contentious. The remit of the research encompassed in this report excludes discussion on patenting of the embryo itself.

A difference in approach exists between USPTO (US Patent and Trademark Office) and EPO (European Patent Office) – human ES cells are patentable in the US (unless “injurious to the well being of society”) but not EU. Note USPTO view independent of the US government and restrictions in federal funding. In the EU, processes involving human stem cells are patentable, isolated stem cells themselves are not if unmodified, but patentable if modified (e.g. by *in vitro* manipulation or genetic engineering) (2002). Operation of the morality clause is limited to human embryo derived SC, hence adult lines are not caught, neither are those from fetal/umbilical cord sources. This is illustrated by differential treatment of the WARF application (claims to a cell culture containing primate ESC) – accepted in the US, but refused by EPO (appeal under consideration in EU). Granting of a patent (Bruestle, for neural cells from hESC) earlier this year was therefore surprising, and uncertainty remains amongst patent attorneys as to whether this is the start of a trend for EPO (and indeed what, if any, impact it will have on consideration of the WARF appeal). The UKPO interprets the EU Biotechnology Directive more narrowly, and does not grant patents for process of obtaining SC from human embryos but will do so for pluripotent hESC which do not have the potential of developing into a human being (policy 2003). Not surprisingly, the US has by far the largest activity in priority filings and has granted 41 patents with a hESC claim. The ACT single cell embryo biopsy method will be a test case for the EPO as it has recently been successfully transferred from murine to human system (embryo is claimed not to be destroyed and cell extracted is pluripotent).⁴

A survey of the FTO in the two areas of stem cell technology and toxicology testing was conducted in May 2006 by Medical Research Council Technology (MRCT), focusing on the impact of research that may be conducted in the UK. Few pharmaceutical companies have performed as detailed analysis as MRCT, feedback from a couple of their patent attorneys is that they are content to adopt the MRCT analysis for this study, later pursuing any further analysis as necessary. While the WARF US patent restricts FTO in the US, the refusal of EPO to grant retains FTO in Europe. Nonetheless, in the event that an initiative's research were wholly carried out in the UK, new medicines developed using novel technology developed by the initiative is unlikely to be restricted to use by patients within the UK. The potential impact of patents granted by the USPO should therefore be assessed in this light.

⁴ Harmonisation in EU may be informed by an EC-funded project (Plomer, Nottingham) examining ethical and legal issues to arrive at a European roadmap without hindering competitiveness and investment.

Of significant patents to note, are the Thomson/WARF patents, which are broad in coverage of the composition of stem cells and lines, and processes for their creation. The patents will expire in 2015. Until then, material transfer agreements and commercial licences to use the WARF patents will entail reach-through rights. Of relevance to this study, is that hESC derivation of neural cell and cardiomyocytes are covered by exclusive licence to Geron; haemopoietic cells and hepatocytes are under non-exclusive licence. Interestingly, the patent specifies karyotypic normality, which might mean that karyotypically abnormal pluripotent hESC would not be covered.

A view expressed by patent attorneys and also quoted by MRCT is that it will be a matter of time before lawsuits are brought, for instance on grounds of prior art (not all prior art was acknowledged thereby removing novelty grounds). Of course, substantial funds could be required and granted patents tend not to be easily reversed.

Outcome of Bayer vs Housey 2003 indicates that any information derived from investigating stem cells can be imported into the US without risk of patent infringement, and many legal scholars agree that this strategy can be used to avoid patent claims that require steps of investigating, etc of stem cells (John Prince, Novartis).

Public benefit/interest is also one of the factors considered by courts in deciding whether to grant injunctions in infringement lawsuits. Recently, re-examination of WARF patents has been requested of the USPTO by two foundations, on the grounds that their existence is causing significant public harm and impeding hESC research in the US. The case is ongoing.

SUMMARY

In brief, WARF patents are unlikely to be showstoppers, licence agreements can be negotiated, and the patents are subject to challenge.

5.2 Patents on screening assays

A specific search may need to be conducted following identification of criteria to determine acceptance of new stem cell lines for use in toxicology, and therefore the physiological screens to be used. For example, particular cell markers may be patented. Pharmaceutical partners will have in-house knowledge in these areas.

5.3 Toxicology assays

Main findings from MRCT analysis of FTO in toxicology (May 2006):

- companies will be aware of Ames test and variations, and Long QT.
- familiarity with omics technologies as already in use by companies or adopted in parallel with their mainstream safety programmes.
- individual techniques or reagents are more likely relevant e.g. reporter constructs, cells from P450 knockouts or transgenics. Several reporter systems are claimed by CXR.

5.4 IP framework

It would be desirable to reliably capture IP of any invention arising from the initiative, with the aim of retaining freedom to use the IP. A framework should be designed to accommodate the stakeholders who may form an initiative (e.g. government, pharmaceutical companies). It should primarily enable equitable allocation of rights to new IPR while not restricting its exploitation and development. The framework will therefore cover IPR ownership, access rights, licensing and exploitation. An example of a high-level framework is the policy developed by the European pharmaceutical

industry for the Innovative Medicines Initiative (available on request). See also section 8.3.2 – *Principles of operation of a funding model*.

RECOMMENDATIONS

A watching brief should be maintained on the status of the WARF patents. Close attention to US patents if further detailed search is carried out for the initiative – majority of patents are filed here and it is by far the most complex system.

An IP framework will be developed alongside the consortium structure (section 8). In-licensing of background IP may require negotiation on a case-by-case basis.

6. REGULATORY AND ETHICAL-LEGAL-SOCIAL ISSUES

A consensus position of potential consortium partners is in development, and will reflect the framework and regulation of stem cell research in the UK, though may be more restricted, taking into account the positions of companies with an international presence (Appendix 3).

6.1 Sourcing and donors

Donor blastocyst cells are typically taken from surplus embryos generated and discarded in the process of IVF treatment. Rarer but possible sources are embryos created for use in research, oocytes from which hESC can be obtained by somatic nuclear cell transfer, and parthenogenic embryos. It should not be necessary to source from embryos created for the purposes of research (allowed in UK, not allowed in EU) as in any case such material will be limited. The consortium's policy will likely restrict sources to surplus embryos created for reproductive purposes, although this will be kept under review.

At present, the regulatory authorities relevant to this project are HFEA (Human Fertilisation and Embryology Authority) and the National Blood Service (for haemopoietic cells). The Human Tissue Act is also relevant in relation to adult and fetal stem cells. HFEA regulates the creation or keeping of embryos or gametes, donor insemination and research involving embryos. As of 2001, HFEA under the 1990 HFE Act, permits derivation for research that is necessary or desirable for understanding the development of embryos or understanding or treating serious disease. Advice has been provided, based on precedence (previous decisions), that new derivation for the purposes outlined in this initiative will be licensable for research. Serious disease is intended to mean impact on health and non-frivolous treatments. Subsequent commercial use is allowed for, as are patenting of cells or discoveries arising. Note this initiative will not involve new derivations of hESC at least in the pilot year, but this policy will be reviewed.

Consent

The following principles will be applied: informed consent without undue pressure; no financial or medical inducement for embryo donation; privacy anonymity; researchers independent of the donation process; prospective (new cells) and retrospective (existing cells) patient consent. The initiative will take note of good practice developed elsewhere, e.g. UK Biobank. There is a national exercise underway to standardise patient information leaflets and consent forms for donation of eggs/embryos – advice from one of the co-investigators that the standardised documentation will cover the scope of this initiative.

Currently, the HFEA is seen as an effective regulator of hESC derivation. However it is critical that the incorporation of its role into the Human Tissue Authority maintains the confidence of the wider communities.

6.2 Stem cell research and resource curation

The UK Stem Cell Bank has policies and Code of Practice in place for deposition and access. Deposition in the Bank is a condition of granting of a licence from HFEA.

Lord Patel of Dunkeld, in his capacity as Chair of the Steering Committee for the UKSCB, has expressed support for this initiative and assurance that the UKSCB will ensure appropriate governance mechanisms are in place.

6.3 International research

There may be instances where stem cells derived outside the UK are imported for use within the UK. It is understood that this is acceptable in the UK provided the lines originate from countries with equivalent ethical frameworks as the UK (for example, Australia, Canada, Europe, Japan, Singapore, USA), and that the cells were obtained according to the ethical requirements of that country. China and India may be exceptions presently, but are planning to revise their regulatory frameworks for stem cell research, and the situation in both countries should be re-assessed in 1-2 years' time. The initiative for predictive toxicology should follow UK recommendations that imported lines are registered through the Steering Committee, and approved for deposit in the UKSCB, to ensure transparency and public assurance. The consortium will only undertake or fund research on lines deposited in the UKSCB or US NSCB. Provided the foregoing principles are also adopted for research abroad on stem cells derived outside the UK (using US NSCB or UKSCB lines), there is no reason why international research should not be included within an initiative.

6.4 Public engagement on public-private initiative

Thought should be given to potential public perceptions regarding the use of hESC-derived populations in an initiative, and it will be important to communicate the benefits of this consortium and ensuing applications.

MRC is considering commissioning a MORI survey through the UK Stem Cell Funders Forum on public attitudes to stem cell research. The findings could help inform communication activity.

RECOMMENDATIONS

An initiative could coordinate with the UK Stem Cell Funders Forum, in terms of positioning and communication and liaise with MRC on design and findings of its planned MORI poll. Alternatively, or in the event the survey does not proceed, a small focus group may be established to facilitate discussion, undertaken in the first year to inform on public attitudes.

An independent adviser on ethics will be appointed to assist the consortium's board. One of the work-streams in the pilot year will be to ensure appropriate governance mechanisms are in place, with a small working group if appropriate. The governance structure of the consortium will include an ethics advisory group to aid in monitoring policy, governance, and ethical aspects of external research funded by the partnership.

7. ANALYSIS OF FUNDING MODELS

To help inform on designing the most appropriate partnership funding model(s), and principles of best practice that should be adopted, an independent review of past and existing partnerships was commissioned⁵.

7.1 Principles of best practice

Successful partnerships share a number of features in common, including some of the following:

- Clarity of purpose
- Vision, developed and owned by all parties
- Commitment from and trust between all funding partners
- Clear, agreed focus
- A requirement for excellence of the science
- Recognition of benefits, balanced by risk, for all partners
- Independence of project selection
- Programme and project management and monitoring
- Transparency

7.2 Examples of multi-partner funding mechanisms

A few examples of funding mechanisms, reflecting a range of partnership arrangements and objectives, were reviewed (further details in Appendix 4).

- Individual government department funding for collaborative projects – The DTI Technology Programme
- Collaboration between research councils and government departments for funding collaborative research – the LINK Programme in Applied Genomics
- A shared overall goal, with separate funding streams – the NCRI (National Cancer Research Initiative)
- A common funding pool for collaboration between charity and industry on a specific focused project – The SNP Consortium
- Industrial support for a centre of excellence – The Division of Signal Transduction Therapy (DSTT) at the University of Dundee (more commonly known as the Kinase consortium)
- Creation of a new organisation – Medicines for Malaria Venture

RECOMMENDATIONS

Out of the six examples reviewed, the type of partnership with best fit was considered to be the SNP Consortium, which also shared focused objectives. This model was explored further and used to inform the design of the consortium described below.

⁵ A review was commissioned from Caulcott and Cooper.

8. RECOMMENDED MODEL – PRINCIPLES OF OPERATION

8.1 Research stage

The range of research and development encompassed within the initiative falls within the pre-competitive stage. On this basis, companies articulated their desired principles along which a partnership would operate. The consensus features are summarised below.

8.2 Features of funding mechanism

8.2.1 Duration

A full initiative totalling 5 years, preceded by a one-year pilot, i.e. 1+4 years. This does not preclude follow-on activity by the consortium beyond this period.

8.2.2 Entry strategy

Partnership mechanism should allow for subsequent entry of new partners.

8.2.3 Location

UK as the focus of the initiative, and collaboration with international groups where appropriate on scientific rationale (also see 3.6 Capacity). It was recognised that a major outcome of an initiative would be to build the stem cell base and leverage investment into the UK, strengthening the UK as preferred location for pharmaceutical R&D. International involvement will be considered within this context.

8.2.4 Payment

Partner contributions to the consortium will be committed upfront, as will allocation of funding for projects selected. Payment of project expenses will be retrospective according to agreed milestones. In some cases, partners may wish to contribute in-kind, e.g. access to expertise or equipment, carrying out validation assays.

8.2.5 Project management

An initiative with clear goals will require scientific and goals-driven programme management. Professional practical project management is seen to be essential, as is also highlighted in the review of funding models.

8.3 Outcomes of an initiative

8.3.1 Data sharing

Preferential early access to partners followed by wider release later, with realistic non-prohibitive pricing of materials and services. Protocols and standardised procedures will be published as soon as is practicable. The overall aim of an initiative would be to facilitate wide access to data, methodology and materials, to encourage wide uptake and standardisation across the industry. It may be that when ready, these could be supplied on a cost-recovery basis.

8.3.2 Ownership of intellectual property (IP)

The high-level IP framework recently developed by the pharmaceutical industry for the Innovative Medicines Initiative is seen to be a good exemplar of differential handling of foreground and background IP. A defensive IP policy would be built into the structure of a consortium. Briefly, IPR would be assigned to the PPP company, with the inventors given due recognition and named as such on any patents arising. The advantage of the company owning and handling all IP is that this can be dealt with on a coherent basis, avoiding multiple negotiation and licensing arrangements. Foreground IP may be available on a non-exclusive basis with third parties.

8.4 Structure of partnership/consortium

Based on the above review of funding models and desired principles of operation, the following model for a consortium on stem cells in predictive toxicology is proposed.

8.4.1 Structure and Governance

The proposal is for initiative to be structured as a not-for-profit company limited by guarantee and incorporated in the UK, with its objectives, terms of reference etc., formulated in the Memorandum of Association and Articles of Association. Funder partners will buy-in to the company at the start of the pilot year, and there will be mechanisms for new partners to join during the lifetime of the initiative.

The preferred option is for a small Company Board, to include an independent chair, CEO, and finance director. The Board will report to the Council which will comprise funder members, including government participation (eg DH, DTI), but which will probably not have directorship responsibilities. A Scientific Advisory Board (which includes external scientists with appropriate expertise) will make recommendations to Board and Council on setting scientific strategy, peer review and selection of research to be funded – its chair will be a member of the Company Board – though significant decisions will lie with the Council. An Ethical Review Board as discussed in more detail above, will also advise the Board. Again, the Chairman of the Ethical Review Board will be a member of the Company Board.

The Executive Board will:

- Comprise an independent chair, CEO, finance director, SAB chair, ERB chair, and possibly Government Board representative
- Oversee legal and company issues, including management of IP policy
- Operate the company on behalf of members
- Answer to the “Members’ Council” – regularly and annually at the AGM
- Function as a core “steering group”

The Members’ Council will:

- Feed in and provide advice to the Board
- Elect a chairman, who will be required to serve on the Board
- Have power to remove or amend Board membership and the CEO
- Make recommendations/nominations for Scientific Advisory Board
- Oversee IP policy implementation

The Scientific Advisory Board will:

- Include scientific experts, from academia and industry. Observers on the SAB or Council may include medical research charities, and possibly regulators on an *ad hoc* basis
- Define scientific programme and calls for proposals
- Make funding recommendations to the Council
- Assist with commissioning research where appropriate
- Help support peer review where appropriate
- Develop annual appraisal of progress and “state of the art”
- The Ethical Review Board (independent?)
 - To ensure compliance with UK ethical framework
 - Develop, propose and monitor ethical framework
 - Provide advice to the Board and Members’ Council

Further consideration will be given to the company structure beyond delivery of this report, including looking at a second option and weighing up pros and cons. A

second option may be a larger Board, with a small steering committee advising the CEO on a day-to-day basis (this was the structure for the SNP consortium foundation). The impact of the Company Law Reform Bill currently in Parliament will be monitored, however there is not expected to be any impact on the structure of the Board and constitution at this time.

9. PROPOSED PROGRAMME

A two-stage programme is proposed and described below. Should relevant enabling technology become available at any stage of the programme, the possibility of engaging with these will be explored.

9.1 Pilot

Government and pharmaceutical partners make an initial 1-year commitment to contribute to a seed fund to allow a year of pilot work, with the intention of subsequently progressing to a 4-year initiative. The purpose of the pilot phase will be to demonstrate proof of concept for an initiative, and the development of a detailed work programme, for consideration by pharmaceutical partners for decision on financial commitment to a full 5-year programme.

The pilot year will therefore aim to achieve (i.e. deliverables):

- i) Formation of a consortium as a legal entity/limited company
- ii) Conduct of pilot projects to understand the size of the biology gap to inform approaches in the 4-year programme. Specifically, this will entail a determination of the source population able to generate an optimal hepatic cell population, and the size of scale-up required;
- iii) Development of a detailed scientific work programme and budget;

Specifically, the pilot projects will be:

- To obtain presumptive hepatocytes from hESC lines (selected protocols) – with a possible minor element of cardiomyocyte progenitor cells if a suitable bid on cardiac progenitors is received;
- Establish functional readouts from lines – i.e. any necessary readout not addressed by the International Stem Cell Initiative;
- Assessment of current lines (adult, embryonic) for utility in predictive toxicology;
- Pilot staged scale-up of cells.

An additional criterion for success should also be joint working of the partnership/consortium.

9.2 Full 5-year initiative

Full detailed costings will be developed with the detailed work programme in the pilot phase. This work programme will encompass stem cell derivation, stable lineage differentiation into hepatocytes and cardiomyocytes, long-term maintenance in culture, scale up, cellular characterisation and evaluation. Scientists from industry and academia have all agreed that the initiative will require funding in the region of £10 - £15million. The final figure will be dependent on determination of the detailed work programme and may subsequently vary from these initial estimates.

Phased programme

I. Pilot Year 1

1. Obtain presumptive hepatocytes from h lines using selected protocols. Associated purification of cells with two goals:
 - i) Evaluation of methods to differentiate highly enriched hepatocytes from hESC;
 - ii) how to purify the hepatocytes from other cells (methods exist but generally the approaches need developing, e.g. transfecting cells with reporters or drug resistance markers).
2. Establish preliminary data on functional readouts from lines – i.e. any necessary readout not addressed by the International Stem Cell Initiative;

Assessment of current lines (adult, embryonic) for utility in predictive toxicology. Four hESC lines are currently available from the UKSCB, with more being processed and therefore becoming available with time.

3. Pilot staged scale-up of cells.

The pilot projects are described in further detail in section 9.5.

II. Full initiative Years 2-3

1. Continue to generate first phase bank of ethnically diverse, quality controlled, stable stem cell lines, including hESC derived populations and tissue stem cells.

- (i) Develop knowledge on fate specification process for hESC lines (Year 1-2)⁶
- (ii) Optimise current state of the art for liver and cardiomyocytes from hESC lines, and resources permitting, liver then neural and haemopoietic cells from adult tissue stem cells (Year 1-3)
- (iii) Optimise current state of the art for liver and cardiac from human fetal tissue, and blood from hESC lines (resources permitting) (Year 2-3).

2. Improve definition of functional requirements from differentiated state (Year 1-2).

3. Establish procedures for the scalable automated production of human stem cells (Year 1-3).

4. Validation of differentiated cells phenotype, stability and purity (Year 2-3).

- (i) Critical path phenotyping measurements (e.g. for hepatocytes: CYP P450 expression and induction profile, albumin synthesis and secretion, urea production). Success criteria should be pre-defined and met in order to progress to the next phase. Estimated turn-round time for analysis is approximately 1 month. This would be employed in an iterative manner to monitor and facilitate improvement of phenotype.
- (ii) Non-critical path phenotypic analysis to include, for example, expression profiling.

5. Critically assess suitability for standardization, scale up of differentiated cell numbers, and consistency of cell line production (Year 3).

III. Full initiative Years 3-5

1. Validation of '*in vitro*' tissue' phenotype (Year 3-4).

2. Link specified, functional phenotyping (pharmacology) of cells with simple toxicology assays (Year 3-4).

3. Evaluation of toxicological relevance (Year 4-5). Benchmarking with current gold standard assays based on primary human tissue. Response to key reference compound set (including approximately 10 well-characterised hepatotoxins and non-toxic drugs, with simple cytotoxicity end-point). Compound set and cell response and end-point measurements should be selected to demonstrate human specificity/

⁶ Range of time indicated in brackets (e.g. Year1-2) reflects staggering of anticipated milestone dates for different cell type, as some may be achieved more quickly than others.

selectivity, mechanism and consistency with human lesion. Estimated time required 3-6 months.⁷

IV. Beyond the initiative, Year 6

Activity may continue beyond the 5-year initiative, possibly as a follow-on collaborative effort, with the aim of maximising exploitation of stem cell development work and ultimately gaining acceptance of their use by regulatory authorities. These are also potential stretch goals for the 5-year initiative.

1. Evaluation of sensitivity and specificity data using a broader compound set (100+) containing human selective and non-human selective toxins, mechanism/pathway specific toxins (with measurement of appropriate mechanism specific response e.g. cholestastics (biliary transport), steatotics (mitochondrial oxidation)).

2. Validation and performance of cells in user assay format. Database development, including multi-site trials and diversification of assay system. Incorporation of cells into individual partner's own *in vitro* systems in order to demonstrate robustness of cell, scope and limitations of their application and predictive value in a range of assay formats. Data will be shared within the consortium.

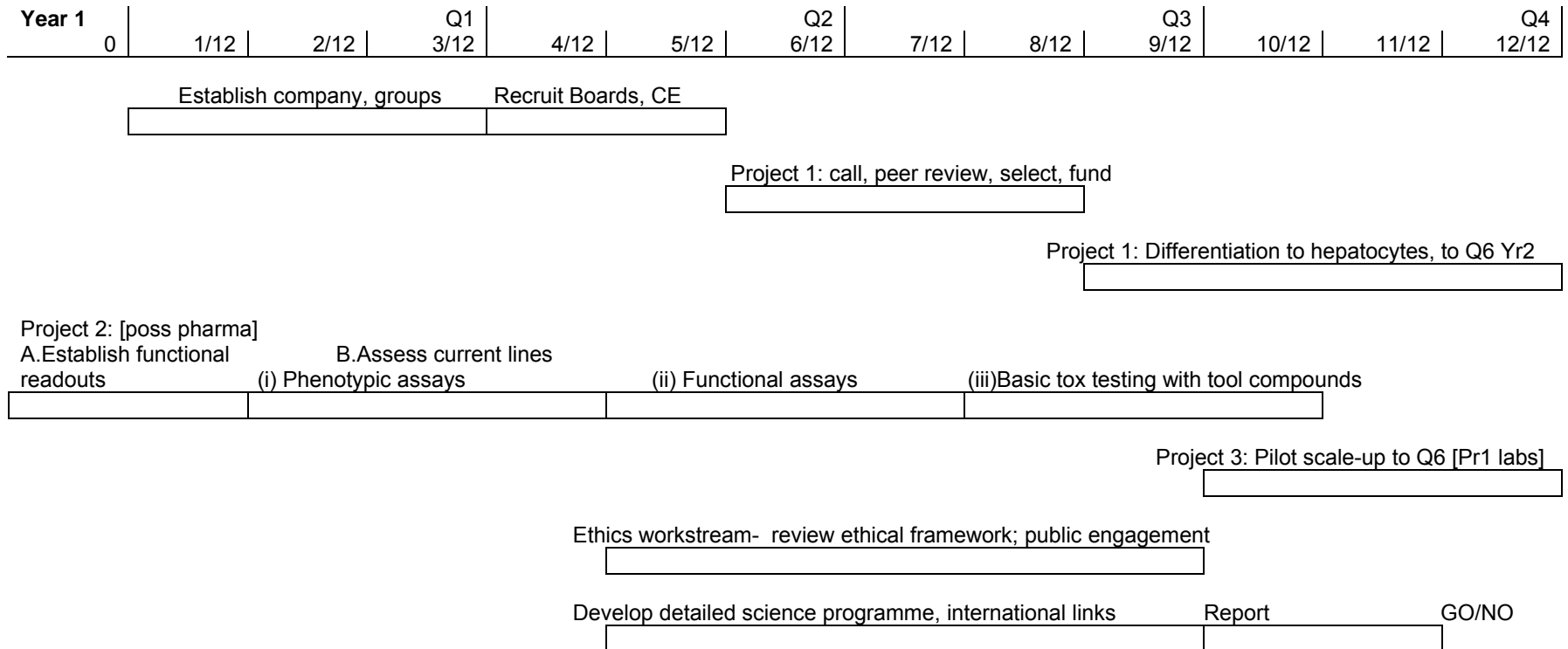
3. In the longer term, toxicogenomics, the differential sensitivity to toxicity based on underlying genetic differences between hESC lines, may be explored.

Provision of the stem cell resources developed for intended use in drug discovery may be undertaken by SME as a commercial service, contracted by the consortium (not-for-profit basis), with fees from third-parties covering the cost of provision.

⁷ Applies primarily to hepatocyte validation work. Other cell types (cardiomyocytes, neuronal etc) will likely have distinct and different acceptance criteria and timelines.

9.3 Timelines for the Pilot Year 1

Interim activity prior to pilot: Draft structure and TOR of groups; IP policy; communications



9.5 Pilot year: Rationale for resources required, and expected deliverables

Project 1: To obtain hepatocytes from hESC lines, and establish functional readouts

This pilot project focuses on evaluating differentiation and isolation protocols for hepatocytes, and is likely to be most appropriately undertaken by academic laboratories or stem cell companies. There will probably be two approaches: mostly comparing existing protocols, but also an element of developing new protocols. With the latter, the deliverable at the end of a year would at best be *prima facie* evidence that hepatocytes can be obtained from hESC.

The work can be divided into subprojects:

- a) Comparison of existing proposed protocols across different hESC lines.
- b) Development of reporter lines for assessing hepatocyte differentiation, including developing various marker tests to identify hepatocytes.
- c) Using developmental biology knowledge, to devise and test potential new protocols for inducing hepatocyte differentiation based on:
 - i. control of gene activity either by transfecting appropriate regulatory genes or RNAi techniques, and
 - ii. control of hepatic differentiation by specific cocktails of growth factors etc.

Deliverables - timescale and milestones

Q3-4 Commence

- Q3 Comparison of hESC in standard scale protocols across different lines, and proposals for deselection of poor performing cells or protocols:
- Secure appropriate stocks of acceptable quality controlled hESC lines and control cells.
 - Confirm characteristics of undifferentiated cells including antibody marker and RT-PCR methods and establish assays for hepatocyte functional characteristics.
- Q4
- Demonstrate isolation/differentiation of cells for further use by each proposed protocol in partner centres.
 - Initial screen of differentiated cultures for hepatocyte including cytochrome p450, albumin and alpha fetoprotein expression.
 - Improved methods of purifying hepatocytes from other differentiated cell types.
- Q5
- Demonstrate reproducibility of effective differentiation protocols and characterisation of cell populations produced.
 - Carry out early toxicology evaluations.
 - Optimisation of differentiation/isolation protocols and purification.
- Q6 Data on scalability of optimised method:
- Early scale up to at least 5-10X standard protocols and characterisation of cultures using hepatocyte functional assays.
 - Hepatocytes obtained from hESC with new protocols.

Note: Due to an anticipated lag in establishing a company, recruiting of staff, and project selection, this project may commence 6 months from the start of the pilot year.

Resources required

Stem cell differentiation is tissue-culture heavy, labour intensive and expensive. Skilled staff are required particularly for establishing or adapting stem cell protocols, hence the need for experienced postdoctoral scientist (PDRA, rather than PhD student) supported by a technician/research assistant (RA). A contribution to overheads is added at 46% of salary costs.

Consumables required are culture media, additives, plasticware, antibodies, PCR primers and reagents, growth factors. Many of these are specialist products and expensive.

Tissue culture equipment specifically required for the project includes a Class II tissue culture hood (£5,000), CO₂ incubators (£7,000), and inverted microscope (£3,000) per pair of PDRA + technician, on the assumption projects will be carried out in labs with appropriate expertise and other standard equipment.

It is estimated that 5 teams (PDRA + technician) will be required to undertake cell differentiation protocols and initial assays. The initiative may be viable with a minimum of 4 teams, but this reduces the chances of success. This configuration of teams is for budgeting purposes only, to estimate the number of personnel required; there will be flexibility in how teams are actually put together.

Project 2: Establish readouts – the characterization of hepatocytes

The longer term aim (into the full initiative) will be to demonstrate that hepatocytes derived from hESCs have a hepatic phenotype and functionality that is superior to existing lines such as HepG2 and Huh-7. In the pilot, baseline analysis will be carried out on differentiated hESC compared with undifferentiated hESC and differentiated control cells (e.g. primary cells, HepG2), thereby producing preliminary data on differentiated hepatic systems. As novel presumptive hepatocyte cells will only be available later in the pilot year, this project will focus initially on existing non-hESC lines, then hepatocytes derived by existing protocols.

The following tabulated list of assays will enable characterization of existing and novel hepatocyte-like lines, and comparison with established lines (e.g. HepG2, Huh-7). A sequential plan of characterization (stages: I basic phenotype, II cell function, III toxicity) will be employed.

Stage	Feature	Assay technology	Comment
I	Morphology	Light microscopy	
I	Protein expression	ICC/ELISA	AFP* (for foetal phenotype), Albumin, alpha-1 antitrypsin, LFABP, transferrin, urea
I	Transcription factors	Taqman	CEBPa & b, HNF4, FOXA2, TCF1, ONECUT, OATP2, Alb
I	Phenotypic stability**	Various	Assess at D1, 3, 7 of culture
II	Drug Metabolism enzymes		P450 isoform identification and levels
II	Functional metabolism (e.g. testosterone)	HPLC	Functional assay
III	Response to test	Cellomics or	Nuclear morphology, plasma

	compounds	similar	membrane permeability, Ca flux, mitochondrial membrane potential
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* Including AFP:CYP450 and AFP:Alb ratios

** Include measure of proliferative status

Deliverables - timescale and milestones

- Q1 Establishment of assays and training for stages I and II.
 Q2 – Q3 Characterization of existing cell lines via stage I and II assays (allow for 3-5 lines plus HepG2, Huh-7 comparator controls).
 Q4 Toxicity response-characterization of hepatocyte lines using c18 tool compounds (stage III assays)

Resources required

One PDRA and one RA are likely to be needed for this work. Other costs incurred will include the purchase of existing lines from commercial sources.

Notes:

1. Novel cell lines emerging from the project's biology laboratories can be evaluated in the characterization programme upon availability. Once established, the stage I and II characterization should be achievable within a 3-month period, with a further 2-3 months for basic toxicity testing. It may be possible to accommodate an additional 3 to 4 lines via this staggered process.

2. The Q1 target may vary significantly depending on the capability of the laboratory. Consolidation of these assays (for stages I and II at least) in a single laboratory will promote optimal consistency and best use of resources. Most large pharmas will have capabilities to perform these assays, whilst academic and contract labs may have some gaps, and in these cases allowance for training will have to be made. CXR labs in Dundee are likely to have most if not all stage I and II assays.

The stage III work is more likely to be performed within a pharma lab.

Project 3: Pilot staged scale-up of cells

This project will not seek to develop new approaches in the pilot year, but will involve basic experiments that would give an indication of scale up potential. Hence, using 5-10X bench scale in flasks would inform on the yield of utilisable cells, thereby indicating practical issues that would be dealt with in later work.

Deliverables - timescale and milestones

- Q4 Commence
 Q5-6 - At least 5-10X scale-up in standard or modified culture flasks from selected standard established protocols.
 - Assessment of efficiency of scale up using different protocols or systems.
 - Measurements of key hepatocyte functional expression following scale up.

Resources

One PDRA and RA will be required for this project, over 6-9 months. Although this is costed separately to the differentiation work, it is likely that staff will be part of a Project 1 laboratory or a Project 1 team, rather than a standalone team.

10. RECOMMENDATIONS

- i. Stem cells in predictive toxicology: Stem cell technology brings potential added value in advancing current systems for predictive toxicology. Better tools will facilitate elimination of drug candidates with a poor safety profile earlier in the drug discovery process; enable a more efficient process for developing new safer medicines; and reduce animal usage.
- ii. Vision: A focused initiative should be formed, with the vision of establishing a bank of stem cells that can be consistently differentiated into stable homogenous populations of particular cell types, particularly liver cells. Universal protocols with general acceptance to the industry are unlikely to be developed in the absence of a joint collaborative effort and investment.
- iii. The initiative should take the form of a public-private partnership: a funding consortium of government (to drive the science base and infrastructure, and ensure appropriate regulatory framework) and pharmaceutical partners (as end-users to drive the development of a shared, common resource for the development of safer medicines). Activities of the initiative such as R&D, will be accessible to relevant stakeholders such as academic scientists and biotech companies on the basis of merit and appropriateness. Potential pharmaceutical partners are AstraZeneca, GlaxoSmithKline, GE Healthcare, Novartis, Pfizer, and Roche. The initiative should have a strong UK focus but as the area is competitive, should also engage with appropriate expertise internationally.
- iv. A short, medium and long-term R&D strategy is outlined as follows. It should focus on human rather than murine stem cells. In the short term (1-2 years), a universal set of acceptance criteria for each type of differentiated cell (e.g. hepatocyte, cardiomyocyte) should be developed and agreed upon with safety experts. This may include cell surface markers, gene expression, protein or metabolic profiles. If supporting data of existing lines are inadequate, promising cell lines should be characterised physiologically for indication of utility in toxicology. Currently accepted toxicology assays should then be used to comparatively assess available stem cell lines.

In the medium term (5 years), an initiative should seek to achieve the establishment of a repository of human embryonic stem cells that can be consistently differentiated into stable homogenous populations of hepatocytes or cardiomyocytes, and of physiologically relevant phenotypes suitable for toxicology testing in high throughput platforms. Basic stem cell biology, quantity scale-up, physiological and toxicological characterisation will be key to achieving this goal. Specifically, an initiative will generate:

- Greater acumen in the creation and isolation of human embryonic stem cells;
- Improved resolution in the requirements to generate functionally differentiated cells;
- More detailed understanding and characterisation of cell phenotype, including gene expression and functionality, of differentiated cells;
- Automated scalable production of human embryonic stem cell-derived populations;
- Validation of appropriate cell lines with tool compounds, ensuring high predictivity (equal or superior to existing assays) for clinical effects;
- Assay development to a suitable format for high-throughput *in vitro* screening.

In the longer term (4-8 years), should medium term objectives be successful, follow up activity may ensue in developing other toxicologically important cell types (haemopoietic, germ cells), and validation towards regulatory acceptance.

v. Patent landscape and freedom to operate: The key stem cell patents (WARF) are seen to be unlikely showstoppers, and are under challenge – a watching brief should be maintained on the status of these. Close attention should be given to US patents if further detailed searches are carried out in future. An IP framework should be developed alongside the consortium structure, within this, in-licensing of background IP may require negotiation on an individual basis.

vi. Ethical-legal-social issues:

An ethicist or social scientist should be included as adviser on the initiative's executive board. A consensus position of potential consortium partners is in development in draft form, and will operate within the framework and regulation of stem cell research in the UK. One of the work-streams in the pilot year will be to ensure appropriate governance mechanisms are in place, forming a small working group if appropriate. The governance structure of a partnership initiative may include an ethics advisory group to aid in monitoring policy, governance, and ethical aspects of external research funded by the partnership.

vii. Public engagement: The initiative should coordinate with the UK Stem Cell Funders Forum, in terms of positioning and communication and liaise with MRC on design and findings of its planned MORI poll; alternatively, a small focus group discussion may be considered for the first year to inform on public attitudes.

viii. Of the range of several partnership funding models reviewed, the type of partnership with best fit was considered to be exemplified by the SNP Consortium, which also shared focused objectives. The SNP model was explored further and used to inform the design of the consortium outlined in the report. This is proposed as a not-for-profit company limited by guarantee incorporated in the UK, with the management board reporting to a Funders' Council and advised by a Scientific Advisory Board. The review also recommends a set of principles of best practice for successful partnership.

ix. Investment required for a 5-year initiative is estimated to be at least £10 million. The budget recommended for the first year is £1.2 million, with contributions of £100,000 each from six pharmaceutical partners, and matching contribution (£600,000) from a mix of government and research council funds.

x. A 1-year pilot phase should first be initiated, during which a number of projects are carried out to inform on feasibility of the science and nature of the biology gap to be addressed in a full 4-year initiative. A consortium will also be legally established during this phase, and a detailed science programme, milestones and budget drawn up for consideration by partners towards the end of the pilot year, on decision to proceed to a full initiative.

xi. Additionally, there should be parallel allocation of the UK science budget to research council streams, e.g. MRC or BBSRC, to build up academic capacity in stem cell biology and blue-skies research that may lead to advances in toxicology in the long term; and to facilitate training of new scientists, as well as recruitment to the field from related disciplines particularly developmental biology and genetics.

APPENDICES

APPENDIX 1: Gaps and limitations of current toxicology systems; Potential for enhancement by stem cells

APPENDIX 2: Stakeholders consulted

APPENDIX 3: Draft consensus position on stem cell research

APPENDIX 4: Analysis of funding models

APPENDIX 5: Stem Cells in Predictive Toxicology Task Force

APPENDIX 1. A. Gaps and limitations of current toxicology cell models

HEPATOXICITY

Cell Type	Major Limitation	Comments
Standard Cell Line	<ul style="list-style-type: none"> ▪ Poor phenotypic and functional match to <i>in vivo</i> hepatocytes ▪ Low basal and/or inducible drug metabolising capacity* 	*Cell line constructs with specific human CYPs available under licence
Primary non-human hepatocyte	<ul style="list-style-type: none"> ▪ Poor phenotypic and functional match to <i>in vivo</i> human hepatocytes ▪ Low basal drug metabolising capacity ▪ Limited duration of differentiated phenotype (allowing short term exposure only) ▪ Preparation to preparation inconsistency ▪ Animal usage ▪ Purity of cell populations* 	*Including non-parenchymal cells and hepatocytes with limited viability
Primary human hepatocyte	<ul style="list-style-type: none"> ▪ Low basal drug metabolising capacity ▪ Preparation to preparation inconsistency ▪ Limited and/or sporadic availability ▪ Limited duration of differentiated phenotype (allowing short term exposure only) ▪ Donor variability ▪ Purity of cell populations* 	*Including non-parenchymal cells and hepatocytes with limited viability

HEPATOXICITY

Differentiated Stem Cells	Potential added value	Comments
	<ul style="list-style-type: none"> ▪ Close match to <i>in vivo</i> phenotype and functionality, including basal and inducible drug metabolising capability ▪ Differentiated phenotype expected to be maintained for longer period ▪ Eliminates animal use for primary cell prep. ▪ Highly consistent between experiments ▪ All cells derived from known, highly characterised donors ▪ Readily available on demand 	<ul style="list-style-type: none"> ▪ Incorporate into acceptance criteria at early stage ▪ Allows modelling of chronic exposure ▪ Assuming robust protocols are developed this should increase assay precision ▪ Enables a reproducible panel of donors to be used thereby increasing confidence when use in screening cascade ▪ Increased efficiency

CARDIOTOXICITY

Cell Type	Major Limitation	Comments
Cell Line	<ul style="list-style-type: none"> ▪ Measurement of drug activity at specific target has only limited predictivity for <i>in vivo</i> response* ▪ Poor phenotypic match to <i>in vivo</i> human cardiomyocytes ▪ Limited functionality characteristic of heart 	* Single channel-expressing cell lines (e.g. hERG in CHO cells)
Primary non-human cardiomyocytes	<ul style="list-style-type: none"> ▪ Poor phenotypic match to <i>in vivo</i> human cardiomyocytes ▪ Limited functionality characteristic of heart ▪ Animal usage ▪ Purity of cell population ▪ No possibility for longer / chronic exposure 	
Human cardiomyocytes	<ul style="list-style-type: none"> ▪ Rarely/Never used 	
Differentiated Stem Cells	Potential added value	Comments
	<ul style="list-style-type: none"> ▪ Fully differentiated cell would enable overall electrophysiology to be determined ▪ Potential for close match to <i>in vivo</i> phenotype and functionality ▪ Reduces animal use ▪ Highly consistent between experiments 	<ul style="list-style-type: none"> ▪ Incorporate into acceptance criteria at early stage ▪ Incorporate into acceptance criteria at early stage ▪ Assuming robust protocols are developed this should increase assay precision

	<ul style="list-style-type: none"> ▪ All cells derived from known, highly characterised donors ▪ Fully differentiated cardiomyocyte layers allows for incubation / chronic exposure 	<ul style="list-style-type: none"> ▪ Enables a reproducible panel of donors to be used thereby increasing confidence when use in screening cascade ▪ Technology already exists.
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BONE MARROW TOXICITY

Cell Type	Major Limitation/Potential added value	Comments
Human haematopoietic stem cells	<ul style="list-style-type: none"> ▪ Limited availability ▪ Between donor variation 	CD34 ⁺ cells for expansion assays
Differentiated Stem Cells	Potential added value	Comments
	<ul style="list-style-type: none"> ▪ Obviates between-donor variation ▪ Reduce costs of regenerating cell supplies ▪ Enables investigation of compound effects on self renewal/maturation process 	

NEUROTOXICITY

Cell Type	Major Limitation	Comments
Cell Lines	<ul style="list-style-type: none"> ▪ Often derived from tumours ▪ Lacking important receptor/channels (i.e., glutamate receptors) ▪ Not fully differentiated or prone to differentiate <i>in vitro</i> ▪ Do not express complex synaptic activity ▪ Measurement of drug activity at specific target has only limited predictivity for <i>in vivo</i> response* ▪ Poor phenotypic match to <i>in vivo</i> human neuronal cells ▪ Limited functionality characteristic of neurons 	
Primary non-human neurons and astroglia cells	<ul style="list-style-type: none"> ▪ Limited numbers for large experimental setups and biochemical studies ▪ Time required for preparation and differentiation ▪ Animal usage ▪ No possibility to exactly mimic all cell-cell type of interactions 	
Human neurons	<ul style="list-style-type: none"> ▪ N.A. 	
Differentiated Embryonic Stem Cells	Potential added value	Comments
	<ul style="list-style-type: none"> ▪ Ready available pools of different cell types for individual cell studies or combination of different cell types. 	

	<ul style="list-style-type: none"> ▪ Potential for use as delivery systems after grafting in vivo ▪ <i>In vitro</i> studies can be carried out without animal use ▪ Highly consistent between experiments ▪ All cells derived from known, highly characterised donors ▪ Fully differentiated mixed cultures allows for incubation / chronic exposure 	<ul style="list-style-type: none"> ▪ Proof of principle ▪ Reduces animal usage ▪ Assuming robust protocols for culture and differentiation are developed this should increase assay precision ▪ Enables a reproducible panel of donors to be used thereby increasing confidence when use in screening cascade ▪ Technology already exists.
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One other potential advantage of using differentiated hESC lines is that each cell line will have distinct genetic characteristics which will be very useful for detecting genetic differences in responses to new compounds.

B. Emerging technologies and potential for stem cell exploitation

HEPATOCYTES

Technology	Key Advantage over established approach	Utility of stem cell(✓, ✗ or ?)	Est'd cells required (per test) ie single data point	Comments
Microtitre plate + fluorescence (ATP)	N/A	✓	25,000	For comparison
Cell line Reporter constructs	Simplified assay with increased throughput	✗	25,000	
Microtitre plate + Cell Imaging (single parameter)	Cell population-based measurements, potentially unlimited range of response signals can be measured	✓	25,000	Includes functional responses (e.g. biliary transport)
Microtitre plate + Cell Imaging (multiplex analysis)	Reduced cell numbers required/test	✓	5,000*	May be key factor if scale up is limiting for other platforms Assumes 5 tests combined
Microtitre plate + gene expression analysis	Increased selectivity of mechanism specific end-points	✓	25,000 ?	Eg CYP P450 isoforms
Microtitre plate + Cell Imaging (high content biology)	Increased information on cell response	✓	25,000	
Multicolour flow cytometry	Population-based multiplexed cell assays	✓	10,000	
Microfluidics +miniaturised cell	Miniaturisation reduces cell number/increases number of tests and	✓	Min 200?	May be key factor if scale up is limiting for other platforms

compartmentation	reduces compound requirements			
Bioreactor	Improved modelling of liver (e.g. oxygen gradients), capability for simulating exposure dynamics	✓	Variable	Not suitable for high throughput screening, but potential for miniaturisation
2 and 3D Co-cultures	Improved modelling of liver physiology important for some mechanisms	?	25,000 -	Requires complementary development of non-parenchymal stem cell types
Automated cell culture	Improved quality, quantity and continuity of supply	✓	N/A	Not suitable for isolated primary cells

CARDIOMYOCYTES

Technology	Key Advantage over established approach	Utility of SC in this system (✓, ✗ or ?)	Est'd cells required (per test)	Comments
Fluorescent measurement of action potentials	Higher throughput and greater predictive value	✓	1000+	
Laser microdissection + pressure catapulting	Purification/enrichment of differentiated cardiomyocytes	✓		Ref. Choudhary et al 2006
Isolated Single Cells: Hand crafted / traditional patch clamp	1. Preparation includes pore forming and accessory subunits for all cardiac ion channels. 2. Ion channels resident within their native environment.	✓	Min 10?	
Isolated Single Cells: Whole-cell current activities (eg.:	Ionic currents activated in their native environment.	✓	Min 10?	

I _{Ca} , I _{Na} , I _{Ks})				
Cell layer: Field potentials & Micro Electrode Array (MEA)	Higher throughput, can record conduction velocity and repolarisation delay. Can investigate effects on various cell types (ventricular, atria, pacemaker etc)	✓	5000	Technology already developed for human embryonic stem cells differentiated into ventricular cells

NEURONS

Technology	Key Advantage over established approach	Utility of SC in this system (✓, ✗ or ?)	Est'd cells required (per test)	Comments
Fluorescent measurement of action potentials, calcium fluxes organelle function	Higher throughput and greater predictive value	✓	1000+	
siRNA screen to identify the role of gene families in biological processes.	Higher throughput and greater predictive value	✓	1000-3000	
Isolated Single Cells: Hand crafted / traditional patch clamp	1. Preparation includes pore forming and accessory subunits for ion channels. 2. Ion channels resident within their native environment.	✓	10-50?	
Isolated Single Cells for subcellular studies and studies of synaptic function/activity	Ionic currents activated in their native environment. Mitochondrial function and synaptic function in neuronal networks	✓	100	

Cell purity requirement will be dependent upon the assay system employed (e.g. cell imaging based measurements analyse individual cell responses enabling discrimination between different cell populations). However, as a general rule, purity of SC derived cell lines should be equal to or better than routinely achieved in cell models currently in use for similar applications.

APPENDIX 2. Stakeholders consulted

Stem cell and biotech companies

- Dr Tim Allsopp, Stem Cell Sciences
- Dr Julian Braybrook, LGC
- Dr Julian Burke, Genetix
- Dr Ian Humphery-Smith, Bii
- Mr Ward Hills, consultant
- Mr Malcolm Rhodes, Bioprocess UK
- Dr Gareth Roberts, Novathera
- Dr Kevin Shakesheff, Nottingham University and RegenTec
- Dr John Sinden, ReNeuron
- Prof Roland Wolf, Dundee University and CXR Biosciences

ABPI R&D advisory groups

NIBSC Stem Cell Bank – Glyn Stacey (Task Force)

Scientists

- Prof Peter Andrew, Sheffield
- Prof Frank Barry, Galway
- Dr Emer Clarke, Stem Cell Technologies Vancouver
- Dr Chris Denning, Nottingham
- Dr Steve Dunnett, Cardiff
- Prof Tariq Enver, Oxford
- Prof Linda Griffiths, MIT
- Prof Sian Harding, IC
- Prof Tim Hardingham, Manchester Cell Matrix Centre
- Prof Douglas Higgs, Oxford
- Prof Chris Mason, UCL
- Prof Stephen Minger, KCL
- Dr Pierluigi Nicotera, MRC Tox Unit Leicester
- Dr Richard Oreffo, Southampton
- Prof Roger Patient, Oxford
- Prof Roger Pedersen, Cambridge
- Dr Chris S Potten, Epistem
- Dr Stefan Przyborski, Durham
- Prof Austin Smith, Cambridge/Edinburgh
- Prof Azim Surani, Cambridge
- Dr Catharine Verfaillie, Leuven
- Prof Fiona Watt, Cambridge
- Prof Ian Wilmut, Edinburgh

International consortia

- EuroStemCell consortium - Prof Austin Smith
- ESTools and ICSI – Prof Peter Andrews

NC3Rs – Dr Vicky Robinson and Dr Kathryn Chapman

Research funders

- MRC (+ Stem Cell Foundation)
- BBSRC
- CCLRC
- ESRC

- EPSRC
- BHF
- CRUK
- Wellcome Trust

Regulatory and ELSI

- Prof Anne McClaren, Cambridge
- Dr Angela McNab and Lord Richard Harris, HFEA
- Dr Adrian McNeil, Human Tissue Authority
- Lord Naren Patel, UKSCB Oversight Committee
- Mr John Prince, Novartis
- Dr Suzanne Watt, National Blood Service
- Prof Andrew Webster, York
- Dr Gareth Williams, Marks & Clerk
- Mr Richard Woodfield (tissue) passed to David Jones (toxicology), MHRA

APPENDIX 3. A draft consensus position on stem cell research

This statement is in draft form, and the position, when agreed, should be reviewed during the pilot year of an initiative, for example, by an ethics advisory group, and continue to be reviewed throughout the lifetime of the initiative. This draft is based on public statements from a number of pharmaceutical companies, applying principles agreed at a meeting of the Stem Cells in Predictive Toxicology Task Force.

The potential benefits of human stem cells

Our interest is in the potential of human stem cells (embryonic, fetal, adult) to differentiate into normal human cells, such as hepatocytes (liver cells) and cardiomyocytes (heart muscle cells). If this were possible, these could be used to evaluate what effect a potential new medicine has on the normal cell, and to provide a more accurate prediction of drug metabolism and toxicity outcomes in man. We believe this would represent a significant step forward in increasing the human relevance of studies at an earlier stage of development of a potential new medicine and would help us to overcome the current limitations that a restricted supply of normal cells presents.

Ensuring high standards

Our commitment to ensuring high ethical standards in this area of research is reflected in the Human Embryonic Stem Cell Research Policy below, which demands compliance both with external legislation, regulations and guidelines, and with the consortium members' codes of research practice. The policy applies both to work funded or undertaken by the consortium, and includes essential criteria which must be met before any such research is undertaken, and draws from policies which govern inclusion in public stem cell registries, specifically the US National Institutes of Health (NIH) Registry⁸, and the UK Stem Cell Bank (UKSCB) (though in reflecting current corporate positions, may not be as broad as the UKSCB).

To take into consideration the fast-moving scientific, ethical and legal environment in this area, this policy will be reviewed in detail by an ethics advisory group in the pilot year of the initiative, and will continue to be monitored during the lifetime of the initiative. The group will also ensure that good governance mechanisms are in place for the initiative.

Implementation of the policy will ensure that all research effort by the consortium remains consistent with its strategy of developing more innovative, safer medicines for serious disease.

Human Embryonic Stem Cell Research Policy

We will only use human embryonic stem cell lines from and in countries where legislation and relevant external ethical approvals permit and will conform with the following principles.

- Before we engage in any research utilising cell lines derived from human embryonic stem cells, there must be a clearly defined purpose to increase knowledge about serious disease and/or to apply such knowledge in developing treatments for serious disease. Any proposal to engage in research using human embryonic stem cells or cells derived from them, must fall within the consortium's policy and standards for human embryonic stem cell research and banking, and use of human tissues and will be subject to peer review.

⁸ Formally known as the US National Stem Cell Bank

- The planned research must be conducted in accordance with any applicable local, national and international legislation, regulations and guidelines for stem cell research.
- Research undertaken or funded by the consortium will utilise only stem cell lines that are fully compliant with the following ethical criteria:
 - The stem cells must have been derived from a fertilised egg/embryo that was created for reproductive purposes by *in vitro* fertilisation procedures⁹;
 - The fertilised egg/embryo must no longer have been needed or intended for these purposes;
 - Fully informed consent must have been obtained to the donation of the fertilised egg/embryo for scientific research;
 - No financial inducements must have been provided for donation of the fertilised egg/embryo;
 - The initiative will neither undertake nor fund the use of cells from human embryos created for research purposes.
- Research will not involve the derivation of new embryonic stem cells (again, this will be reviewed in the pilot year). That is, only ethically scrutinised lines already deposited or approved for deposit in the NIH Registry or UK Stem Cell Bank will be used.
- Any relevant authorisation must have been obtained for the creation of the cell line and the consortium will comply with national guidelines for the importation and use.
- Regenerative medicine: This initiative is not involved in the cloning or use of human embryonic stem cells to repair damaged or diseased tissue. Human reproductive cloning: The initiative is also not involved in any research on human reproductive cloning on which there is an international ban as set out by UNESCO and most national legislations.

The consortium acknowledges the considerable scientific, ethical and public debate and takes very seriously the ethical and societal issues associated with research using stem cells derived from human embryonic tissue. We will conduct and support research that is performed in an ethically and scientifically responsible manner.

⁹ For consistency with current corporate positions, cells from embryos created for research or by somatic nuclear cell transfer or parthogenesis, as regulated in the UK by HFEA and accepted by UKSCB, are therefore not covered – although not currently a significant source of cells, numbers may increase in future. This aspect will be further reviewed in the pilot year.

Fetal and adult stem cells

■ The use of fetal or adult stem cells should comply with the Human Tissues Act where appropriate, and obtained under ethical consent without material inducements. See also the general principles below.

■ Any fetal stem cells used will have been derived from fetuses that have come to term, or from umbilical cord blood. Research using cells derived from aborted fetuses will not be supported.

General principles governing research using human tissue material

■ When genetic material and other tissue samples are collected from humans, neither the material itself nor associated data should reveal the identity of the subject to the investigators or consortium.

■ Subjects participating in such research will be given full information about the nature of the research and about the purpose of the specific investigations that will be performed and will be requested to give specific consent to this research.

■ If a study is expected to have the potential to generate findings of importance for the individuals in the study (e.g. a future risk for disease), the study protocol must outline in detail the process for handling such findings in relation to the individuals in the study. The process must be made known to the study individuals beforehand, and accepted by them in the consent form.

APPENDIX 4. Collaborative funding of research to support a focused research agenda

1. Introduction

Collaborative funding of research¹⁰, where different organisations come together in some manner to support research in a given area, offers a number of benefits over each body operating separately. Increased levels of funding can enable larger projects, or greater numbers, to be supported. Agreement as to common focus and direction can lead to a specific area of need being addressed in an intensive and timely manner. The involvement of a range of funding partners, such as industry as well as the public sector, brings a wider perspective on the issues. And there can be the opportunity to involve the best researchers, irrespective of their location in terms of sector or geography.

There are a variety of mechanisms that can be used when funding research in a collaborative manner. These range from true partnerships, where all the money is pooled and a new funding entity is created, through to agreements to collaborate and work together in an area in order to increase effectiveness. The choice of funding partnership is dependent on the focus and purpose of the proposed research programme. However, for any prospective funding model, there are various aspects which will be common, and can be described as best practice. These should be considered and incorporated into any collaborative funding scheme that is developed.

2. Best practice in collaborative funding

Experience of a variety of funding schemes, collaborative and otherwise, suggests that a number of factors are needed, and that if these are present, the likelihood of success is increased significantly. The list given here is not exhaustive, nor would all be essential in all cases.

A successful collaborative funding scheme is likely to have:

- Clarity of purpose
- Vision, developed and owned by all parties
- Commitment from and trust between all funding partners
- Clear, agreed focus
- A requirement for excellence of the science
- Recognition of benefits, balanced by risk, for all partners
- Independence of project selection
- Two stage application process
- Programme and project management and monitoring
- Transparency

3. Examples

Some examples of different collaborative funding schemes involving industry and public sector funders are given below. There are many variations on these options and the choice of approach would be determined by the nature and purpose of the science programme to be followed.

¹⁰ Collaborative funding is not the same as collaborative research. It may well be the intention of the former to support the latter, but it is not essential.

3.1 Individual government department funding for collaborative projects: The DTI Technology Programme

- DTI funding for collaborative research across all industrial sectors, divided into focussed six monthly calls. Overall funds committed to the Technology Programme, but no guaranteed commitment to any one area of subject.
- Research Council involvement on some occasions.
- Industrial funding essential for award, generally “in kind”, and on a project by project basis.
- Two stage application process, project selection by technical experts (but not expert scientific review) and ad hoc panel review, with DTI input.
- Projects managed by research team with high level DTI monitoring.
- Outcomes (including IP) entirely subject to formal agreement between partners.

Too early to assess, but issues relevant to this programme might be the lack of ongoing expert review panel and one-off nature of funding competitions in particular areas.

3.2 Collaboration between government departments for funding collaborative research: the LINK Programme in Applied Genomics

- DTI, MRC and BBSRC money committed upfront.
- Industrial funding essential for award, generally “in kind”, and on a project by project basis.
- Two stage application process, with review by established independent expert committee, no funding body input.
- Projects managed by research team with outside programme monitoring.
- Outcomes (including IP) subject to formal agreement between partners but the independent expert committee required measurable public benefit.

Successful programme, leading to excellent commercial and scientific outcomes.

3.3 A shared overall goal, with separate funding streams: the NCRI

- Partnership between government, charities and industry in cancer research.
- Research funding remains responsibility of individual bodies. NCRI provides opportunity for strategic overview.
- Outcomes managed through individual funder’s normal arrangements.

3.4 A common funding pool for collaboration between charity and industry: The SNP Consortium

- Single charity funder, several pharmaceutical and technology companies
- Companies could join later on.
- Narrow, specific focus set at the start.
- Single funding pool with award decisions made by a board of partner representatives.
- No open competition for awards, internationally excellent centres were already established.
- Project outcomes were regular release of data onto the web and publications. Industry did not have an early view of the data.
- No IP directly from the project.

Highly successful programme.

3.5 Industrial support for a centre of excellence: The Dundee Division of Signal Transduction Therapy

- Significant industrial funding over a five year period for basic research and some services.
- Public sector funding to support infrastructure and separate research programmes
- Overall focus of research and benefits established from the start
- Option for additional services
- Clearly defined ownership and use of IP by the parties
- Project Management Committee

A highly successful programme, renewed for a second five year period.

3.6 Creation of a new organisation: Medicines for Malaria Venture

- Multiple funders from all sectors, government, charitable, private and individual. Money is donated, and new partners are actively sought
- Board and office structure as with a company
- Overall focus was set at the start.
- Two stage application process with project selection by expert assessment including panel review. No direct funding body input.
- Highest quality projects sought, by a number of criteria.
- Project outcomes determined by agreements between MMV and partners but will be compliant with the aims of the MMV, which include to maximise the health impact of its supported research.

Too early to assess success, but there is evidence of increased development of medicines for a neglected disease area (malaria).

4. **Conclusions**

The choice of funding model for scientific research programmes is dependent on the nature of the proposed programme. Where industrial funding is a desired feature, it is important that industrial partners perceive real benefit in being involved, and it is likely that the research will be pre-competitive. The greatest successes will be achieved where there is a tightly-drawn focus and genuine commitment, including financial, from all the funding partners, coupled with a process for project selection that focuses on identifying excellent science.

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- http://www.efpia.org/4_pos/SRA.pdf (Innovative Medicines Initiative)
- <http://www.patentbaristas.com/archives/000497.php>

APPENDIX 6. Task Force for Stem Cells in Predictive Toxicology

- Dr Ruth McKernan (Chair)
VP Biology, Pfizer
- Dr Christopher Clarke (Deputy Chair)
Director and Head of Investigative Preclinical Toxicology, GlaxoSmithKline
- Professor Peter Andrews
Dept Biomedical Science, University of Sheffield, and Axordia
- Professor Ray Hill
Executive Director, Licensing & external research, Merck Sharpe Dohme
- Ms Jackie Evans
Marketing Director, Discovery Biosciences, GE Healthcare
- Dr Sandy Kennedy
Chair of ABPI Preclinical Drug Safety Advisory Group and Head of Safety Assessment GlaxoSmithKline
- Mr Clive Kind
Principal Scientist, Molecular Toxicology, AstraZeneca
- Dr Rose Maciewicz
Chief Scientist, AstraZeneca
- Professor Stephen Minger
Director, Stem Cell Biology Laboratory, King's College London
- Ms Natasha Neef
Senior Pathologist, Novartis
- Dr Glynn Stacey
Director, UK Stem Cell Bank, NIBSC
- Dr Thomas Weiser
Head of Toxicology Department, Non-Clinical Drug Safety, Roche

Observers

- Mr Mark Bale
Scientific Development and Bioethics Division, Department of Health
- Dr Barbara Blaney
Director, BioIndustry Association Scotland
- Dr Robin Buckle
Research Programme Manager, Molecular & Cellular Medicine, MRC
- Ms Sue Ellison
Office of Science and Innovation, DTI
- Mr Colin Pavelin
Scientific Development and Bioethics Division, Department of Health
- Dr Philip Wright
Director of Science and Technology, ABPI
- Dr Louise Leong (Project Manager)
R&D Policy Manager, ABPI

Other companies observing

- Eli Lilly
Johnson and Johnson