Scientific and Clinical Advances Advisory Committee Paper

Paper Title:	**Update on alternative methods to derive ES and ES-like cells**
Paper Number:	SCAAC(06/13)01
Meeting Date:	12 June 2013
Agenda Item:	6
Author:	Anna Rajakumar
For information or decision?	Decision
Resource Implications:	None
Implementation	None
Communication	Information updates summarised in this paper and SCAAC's view will be used to update the paper 'Alternative methods to derive stem cells' used by the HFEA Licence Committee when considering research licence applications which involve the use of viable embryos for research purposes.
Organisational Risk	Low
Recommendation to the Committee:	Members are asked to: • consider the progress of research since October 2012, into alternative methods to derive embryonic or embryonic-like stem cells, • advise the Executive if they are aware of any other recent developments and • reflect on whether their views have changed in the light of recent research.
Evaluation	None
Annexes	None

1. Lay Summary

1.1. Human embryonic stem cells (hES cells) have the potential to form every other type of cell in the body. hES cells are important for research into cell biology, drug testing and disease modelling, and could potentially be used in therapies for patients.

1.2. hES cells are derived from the cells of human embryos. Currently the only way to derive hES cells involves using viable embryos but researchers are investigating alternative methods of deriving hES cells, or hES-like cells, without destroying viable embryos. The Committee considers the progress of research in this field annually. This paper highlights developments since October 2012.

2. Introduction

2.1. Section 3A(1)(c) of Schedule 2 of HFE Act 1990 (as amended) requires embryo research to be "necessary or desirable" for defined purposes. If alternative methods of deriving ES or ES-like cells are developed, it may not be necessary for research groups to destroy viable embryos. It is, therefore, important for the Authority to keep up to date with developments regarding these alternative methods so that the HFEA Licence Committee can bear them in mind when considering research licence applications.

2.2. In February 2012 SCAAC advised the HFEA that alternative methods to derive hES cells should remain a high priority for the Committee and the Authority during 2012/13. The Committee also asked to be periodically updated with relevant research developments, and last considered research in October 2012. This paper summarises key research since October 2012 and is therefore an update to SCAAC paper SCAAC(10/12)03.

3. Research

Induced Pluripotent Stem cells – reprogramming

3.1. Over recent years, pluripotent stem cells, derived from different types of somatic cells by nuclear reprogramming, have shown much promise. Since October 2012 further studies have explored the process of reprogramming. Some highlighted studies are detailed below:

- Sterneckert J et al. (2012) published a review highlighting that it has been possible to induce an alternative cell fate directly by the transdifferentiation of cells facilitated by the use of specific transcription factors. The study showed that Oct4 is a key factor in a reprogramming expressway that can be influenced by changing the experimental conditions. Further to this, Li et al. (2012) showed that it is possible to create induced pluripotent stem (IPS) cells from fibroblasts without adding SOx2 if mouse models were mutant in p27 (lacking the tumor suppressor) as this leads to a low level of

expression of the endogenous Sox2 gene. The researchers observed that cells lacking the tumour suppressor p27 can be reprogrammed into induced pluripotent stem cells (iPSCs) in the absence of ectopic Sox2. This study therefore highlights an important connection between p27 and Sox2 relevant for reprogramming.

- Reviews by Puri & Nagy (2012), Bilic & Izpisua Belmonte (2012) and Hussein et al. (2013) explored the current knowledge of the molecular and functional similarities and differences between iPS and ES cell types, emphasising the need for thorough characterisation of their properties as well as their differentiation capabilities in the pluripotent state. Puri & Nagi (2012) emphasis the need for further comparative studies in order to determine the preferable cell type for future therapies. Bilic & Izpisua Belmonte (2012) highlight the differences in epigenetic changes and DNA mutations between iPS and ES cells, noting the increase in these changes in iPS cells. Finally the review by Hussein et al. (2013) explored recent studies addressing genome integrity during the reprogramming process in IPS cells, examining the underlying mechanisms that have the potential to cause de novo genome damage. They also discussed the significance of an elevated mutation load, in a clinical context.

- Further to this, a study by Carey et al. (2011) suggested that difficult-to-control parameters influence epigenetic state and pluripotency during generation of iPS cells by transcription factors reprogramming. This work may highlight that generic comparisons of ES and iPS pluripotency "ground states" may be complex and need further consideration.

- Recent work analysing differences in protein expression and protein phosphorylation between embryonic stem cells and induced pluripotent cells (Phanstiel et al. 2011) has reported deep proteomic coverage of human embryonic stem cell and induced pluripotent stem cell lines. Merging these results with RNA-seq analysis data, they found functionally related differences across each tier. This research group has also introduced the Stem Cell–Omics Repository (SCOR), as a resource to collect quantitative information, such as mRNA, protein and post-translational modifications.

- Further research by Zhou et al. (2012) has recently reported the development of a protocol for generating human iPS cells from exfoliated renal epithelial cells present in urine. Derivation iPSCs from urine cells may be advantageous as the isolation of urinary cells is straightforward, cost-effective, and relatively quick (approx. 2 weeks for the urinary cell culture and 3-4 weeks for the reprogramming). The yield of iPS cell colonies has also been

shown to be high at up to 4% using retroviral delivery of exogenous factors. Urinary iPS cells were also found to demonstrate effective differentiation potential (ability to create pluripotent cells).

Induced Pluripotent Stem Cells - Disease Modelling

3.2. Since October 2012 further studies have also explored the potential for disease modelling for induced pluripotent stem cells.

- Mahru et al. (2012) reported that human iPSCs derived from Huntington's Disease patient fibroblasts can be corrected by the replacement of the expanded CAG repeat with a normal repeat using homologous recombination. The study highlighted that the correction remains in iPSCs differentiation into DARPP-32-positive neurons. The correction of the patient's iPSCs was shown to result in normalised pathogenic signalling pathways and, hence, reversed disease phenotypes (eg, tendency towards cell death and changes in mitochondrial bioenergetics in neural stem cells).

- A recent study by Mekhoubad et al. (2012), exploring the modelling of Lesch-Nyhan syndrome, showed that low-passage female iPSCs retain the inactive X chromosome of the somatic cell they are derived from. However on further culture they undergo an erosion of X chromosome inactivation. This is characterised by a transcriptional derepression of genes on the inactive X and a loss of XIST expression that cannot be reversed by differentiation or reprogramming. This study therefore demonstrates that erosion of XCI has a significant effect on the use of female human iPS cells for modelling Lesch-Nyhan syndrome.

- Vitale et al. (2012) found that variability in transgene expression and pluripotency marker levels did not prevent iPS cells from demonstrating other characteristics for pluripotency, including teratoma formation (although teratoma formation in itself may not deifine pluripotency). The study found low interindividual and interclonal variability in iPSCs fulfilling the criteria for pluripotency, with high correlation in their gene expression profiles. This work suggests that it is possible to define a similar "ground state" for each cell line therefore creating a marker for comparing patient vs control cells.

- Recent research (Kondo et al. 2013) in Alzheimer's Disease (AD) patients was conducted, exploring differentiated iPSCs that were developed into neural cells, from patient cells. Aβ oligomers accumulated in iPS cell-derived neurons and astrocytes in cells from patients with a familial amyloid precursor protein. This resulted in endoplasmic reticulum (ER) and oxidative stress. The accumulated Aβ oligomers were not proteolytically resistant. Docosahexaenoic acid (DHA) treatment subdued the stress

responses in the patient neural cells. Demonstrating this differential manifestation of ER stress and DHA responsiveness may provide an explanation for variable clinical results obtained with the use of this treatment and suggests that DHA may in fact be effective for a specific group of patients.

Induced Pluripotent Stem Cells - Immunogenicity

3.3. Another potential problem with induced pluripotent stem cells (iPS cells) being used in a clinical context relates to immune response upon transplantation. A number of studies, highlighted below have explored this response:

- In 2013 Araki et al. examined the immunogenicity of differentiated skin and bone marrow tissues derived from mouse iPS cells. The group observed no differences in the rate of success of transplantation when skin and bone marrow cells derived from iPSCs were compared with ES cell-derived tissues. Further to this the research suggested limited immunogenicity of transplanted cells differentiated from iPS and ES cells. A limited response for tissues derived from either iPS or ES cells was observed, with no increase in the expression of the immunogenicity-causing (Zg16 and Hormad1) genes in regressing skin and teratoma tissues.

- Guha et al. (2012) differentiated mouse iPSCs into embryoid bodies (EBs) or representative cell types spanning the embryonic germ layers and examined their immunogenicity, firstly in vitro and after their transplantation. The research concluded that there was no evidence of increased T cell proliferation in vitro, rejection of syngeneic iPS cell-derived EBs/tissue-specific cells after transplantation, or an antigen-specific secondary immune response. The research suggested that iPSCs do not appear to be rejected after transplantation. The group also found no significant evidence of an immune response to undifferentiated, syngeneic iPSCs. This data supports the idea that differentiated cells generated from autologous iPS cells could be used for cell replacement therapy without triggering a negative immune response.

- Gaucher's disease is caused by mutations in the GBA1 gene, which encodes acid-β-glucosidase. While the non-neuronopathic aspects of the disease can be treated with enzyme replacement therapy (ERT), the early-onset neuronopathic form cannot be treated effectively by therapeutic options. Tiscornia G. et al. (2013) have created an iPS cell model using d fibroblasts of a patient with Gaucher’s Disease (GD). These cells were reprogrammed and iPSC lines derived. The GD iPSCs expressed pluripotent markers, differentiated into the three germ layers, formed teratomas, had a normal karyotype and showed the same mutations and low acid-β-

glucosidase activity as the original fibroblasts they were derived from. This work shows that this system could be used as a platform to test chemical compounds capable of increasing acid-β-glucosidase activity.

- Recent research by Homma et al. (2013) looked at replacement of dysfunctional photoreceptors, exploring the potential to alleviate retinal neurodegenerative diseases. This research examined the physiological properties of developing rod photoreceptors driven by the promoter of rod differentiation factor, Nrl. GFP-tagged developing rods show Ca (2 +) responses suggesting an immature developmental state. The article concluded that Nrl-promoter-driven donor photoreceptors exhibit physiological characteristics of rods and that iPS cell-derived rods (in vitro) could potentially be a viable cell therapy.

- Jin et al. (2012) generated iPSCs from Retinitis Pigmentosa (RP) patients and a Sendai-virus vector was used with specific reprogramming gene factors (POU5F1, SOX2, KLF4, and c-MYC) in skin cells from an RP patient. Selection of the iPSC lines was carried out by karyotype analysis and other in vitro and in vivo pluripotency tests. This study demonstrated ER stress showing its potential utility for disease modelling in vitro. Further to this, a paper by Car et al. (2013) discusses the various methods used to differentiate retinal pigment epithelium from human embryonic stem cells (HESC), and details the surgical approaches to transplanting these cells.

Primordial germ cells

3.4. The primordial germ cells in an embryo develop into embryonic germ (EG) cells that, in an adult generate the reproductive gametes (sperm or eggs). These EG cells have similar properties to embryonic stem cells. There is currently research looking into the reprogramming of the primordial germ cells into a pluripotent state, as detailed below:

- Nagamatsu G. et al. (2012) studied the expression of reprogramming factors in primordial germ cells (PGCs). PGCs expressed reprogramming factors Oct3/4, Sox2 and c-Myc, but not Klf4. However, other factors expressed in PGCs, could compensate for Klf4 during somatic cell reprogramming. This study showed that Primordial germ cells could be converted to a pluripotent state by infection with any of the identified reprogramming factors (Oct3/4, Sox2, Klf4 and c-Myc). The research specified candidate genes involved in the regulation of tumorigenicity and the optimisation of reprogramming.

Amniotic stem cells

3.5. Many different cell types have been found within amniotic fluid with much

potential for pluripotent cells isolated from this source. It has been suggested that amniotic epithelial (AE) cells in particular possess ES- and iPS cell-like pluripotent differentiation characteristics (De Sacco et al.2010). Amnion cells also have additional advantages of being retrieved in a non-invasive way and can be frozen and stored easily. Recent studies have suggested that stem cells derived from this source have increasing potential to maintain genetic stability and possess pluripotent characteristics.

- Moschidou et al. (2012) demonstrated that amniotic fluid stem cells (AFSCs) can be fully reprogrammed to pluripotency without ectopic factors, using a specific culture protocol. This shows that amniotic stem cells have the potential to produce patient-specific pluripotent cells for potential use in cell replacement therapies and disease modelling. The cells showed an ability to form embryoid bodies (EBs) in vitro and teratomas in vivo. Furthermore the study reported that these cells maintained genetic stability, protein level expression of key pluripotency factors, high cell-division kinetics, telomerase activity and repression of X-inactivation.

- Nishino K. (2012) suggested that the amnion-derived iPS cells show a more rapid decrease in the number of aberrant methylated sites during additional cultivation than endometrium-derived iPSCs and menstrual blood cell-derived iPSC. This study further supports the hypothesis that iPSCs derived from the amnion have the potential to be used clinically.

Somatic Cell Nuclear Transfer

3.6. Finally it is important to note that there has been some recent developments in research exploring the potential to derive human embryonic stem (hES) cells from somatic cell nuclear transfer. This technique involves skin cells, which are taken from an adult. The nuclear genome from this cell are then placed inside an egg, stripped of its own DNA. Until recently researchers have struggled to encourage the egg to divide (meiotic arrest takes place inducing a rapid exit from the metaphase stage) and reach blastocyst stage.

- A research team in the US (Tachibana et al. 2013) have recently published data suggesting they are able to use somatic cell nuclear transfer to successfully reprogramme human somatic cells into ES cells. By refining steps in the spindle removal, donor cell fusion, and cytoplast activation the group were able to develop to the blastocyst stage.

- This research (Tachibana et al. 2013) also suggests that embryo quality was improved when super-ovulation resulted in fewer eggs. This could highlight that a yield of >10 eggs may not be conducive to good quality ES cells derivation and be applicable to methods

used across IVF. Furthermore, the study outlined a method of egg activation using caffeine, electroporation and excluding inomycin that may also be relevant in to IVF/ICSI methods.

4. Conclusions

4.1. When SCAAC last considered the progress of research in October 2012, the Committee raised interest in recent research looking at a systematic comparison with iPS and ES cells. The Committee discussed the concerns that techniques to reprogrammme iPS cells have not been sufficiently refined, detailing the problems with direct reprogramming.

4.2. Furthermore, Committee members highlighted that the rate of acquisition of new mutations in somatic cells are unknown and the mutation load of iPS and ES cells remain unknown. There is little information about an acceptable level of mutations in order to use cells produced for therapy. A member suggested that immunogenicity might also be an issue when considering the therapeutic application of iPS cells, which has been highlighted in this paper.

4.3. SCAAC concluded that, despite promising developments in the iPS cell creation process and the potential to derive stem cells from other sources, there is still no viable equivalent to embryonic stem cells and therefore the creation of stem cells from embryos may still be considered “necessary or desirable” for defined purposes

4.4. In this paper the Committee is asked to consider developments in the reprogramming methods used to create iPS cells and to consider other the research highlighted, such as work using amniotic stem cells. The Committee should also carefully consider the progress recently made in developing the process of somatic cell nuclear transfer to produce ES cells. Recent work has shown that previous obstacles (suboptimal activation and premature exit from meiosis) have been circumvented, optimising the approach and demonstrating that a more efficient reprogramming process of somatic cells to a pluripotent state.

5. Recommendations

5.1. Members are now asked to:

- consider the progress of research (since October 2012) into alternative methods to derive embryonic or embryonic-like stem cells;
- advise the Executive if they are aware of any other recent developments; and

- reflect on whether their views have changed in the light of recent research.

5.2. Information summarised in this paper and SCAAC's view will be used to update the paper 'Alternative methods to derive stem cells' used by the HFEA Licence Committee when considering research licence applications which involve the use of viable embryos for research purposes.

References

- Araki, R. et al. (2013) Negligible immunogenicity of terminally differentiated cells derived from induced pluripotent or embryonic stem cells. *Nature* 7;494*(7435):100-4.*
- Bilic, J. and Izpisua Belmonte, JC. Concise Review: Induced Pluripotent Stem Cells Versus Embryonic Stem Cells: Close Enough or Yet Too Far Apart? *Stem Cells* 33-41
- Carr AJ, et al. (2013). Development of human embryonic stem cell therapies for age-related macular degeneration. *Trends Neurosci.* 2013 Apr 16. pii: S0166-2236(13)00053-2. doi: 10.1016/j.tins.2013.03.006. [Not yet printed]
- Carey, BW. et al. (2012) Reprogramming Factor Stoichiometry Influences the Epigenetic State and Biological Properties of Induced Pluripotent Stem Cells. *Cell Stem Cell* 9, 588–598, December 2, 2011
- Conrad, S. et al. (2009) Generation of pluripotent stem cells from adult human testis. *Nature* 2009 456:20
- Golestanch, N. et al. (2010) Pluripotent stem cells derived from adult heman testes. *Stem Cells Dev.* 2009;18 1115-1126
- Guha, P. (2013) Lack of Immune Response to Differentiated Cells Derived from Syngeneic Induced Pluripotent Stem Cells. *Cell Stem Cell,* 12;4 407-412
- Hayashi, K. et al. (2012) Offspring from Oocytes Derived from in Vitro Primordial Germ Cell–like Cells in Mice. *Science* 338:6109 971-975
- Hussein, SM. et al. (2013). Genome damage in induced pluripotent stem cells: assessing the mechanisms and their consequences. *Bioessays. 35,152-62.*
- Homma, K. et al. (2013). Developing rods transplanted into the degenerating retina of crx-knockout mice exhibit neural activity similar to native photoreceptors. *Stem Cells.* 31, 1149-59.
- Jin. ZB, et al. (2012). Integration-free induced pluripotent stem cells derived from retinitis pigmentosa patient for disease modeling. *Stem Cells Transl Med.* 1, 503-9.
- Kondo, T. et al. (2013) Modeling Alzheimer's disease with iPSCs reveals stress phenotypes associated with intracellular Aβ and differential drug responsiveness. *Cell Stem Cell.* 12, 487-96.
- Leitch, HG. et al. (2011) Embryonic germ cells from mice and rats exhibit properties consistent with a generic pluripotent ground state. *Development and Stem Cells* 2010 Jul;137(14):2279-87.
- Li, H. et al. (2012). p27(Kip1) directly represses Sox2 during embryonic stem cell differentiation. *Cell Stem Cell.* 11, 845-52.
- Mahru, CA. Genetic Correction of Huntington's Disease Phenotypes in Induced Pluripotent Stem Cells. *Cell Stem Cell* 11:2: 253-263, 28
- Mekhoubad, S. et al. (2012) Erosion of dosage compensation impacts human iPSC disease modeling.Cell *Stem Cell.* 2012 4:10(5):595-60
- Moschidou, D. et al. (2012) Valproic Acid Confers Functional Pluripotency to Human Amniotic Fluid Stem Cells in a Transgene-free Approach*. Molecular Therapy* (2012); 20 10, 1953–1967.
- Nagamatsu, G. et al. (2012) Induction of Pluripotent Stem Cells from Primordial Germ Cells by Single Reprogramming Factors. *Stem Cells* 31(3):479-87.
- Nishino, K. (2012) Induced Pluripotent Stem Cells from Human Extra-Embryonic Amnion Cells: Role of DNA Methylation in Maintaining Stemness. *Stem Cells and Cancer Stem Cells* 4:59-65

- Phansteil, et al (2011) Proteomic and phosphoproteomic comparison of human ES and iPS cells. *Nat Methods.* 2011 Sep 11;8(10):821-7.
- Piccoli, M. et al (2012) Amniotic Fluid Stem Cells Restore the Muscle Cell Niche in a HSA-Cre, SmnF7/F7 Mouse Model *Stem Cells* 30:8:1675–1684
- Puri, MC. and Nagy, AC. et al. (2012) Concise Review: Embryonic Stem Cells Versus Induced Pluripotent Stem Cells: The Game Is On. *Stem Cells* 2012;30:10–14
- Sterneckert, J. et al (2012) Concise Review: Oct4 and More: The Reprogramming Expressway. *Stem Cells*: 30:1:15–21
- Tachibana, M. et al Human Embryonic Stem Cells Derived by Somatic Cell Nuclear Transfer. *Cell* 153, 1–11, June 6, 2013
- Tiscornia, G. et al (2013). Neuronopathic Gaucher's disease: induced pluripotent stem cells for disease modelling and testing chaperone activity of small compounds. *Hum Mol Genet.* 22, 633-45.
- Vitale, AM. et al (2012) Variability in the Generation of Induced Pluripotent Stem Cells: Importance for Disease Modeling. *Stem Cells Trans* 1:9 641-650
- Zhou, T. et al (2012). Generation of human induced pluripotent stem cells from urine samples. *Nat Protoc.* 7, 2080-9.