Technical and Bioethical Challenges

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# Technical and Bioethical Challenges Associated with using Stem Cells for Research and Therapy

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## Introduction: development of cell-based therapies for liver diseases

Millions of patients worldwide suffer from end-stage liver disease. Orthotopic liver transplantation has rapidly advanced and is currently the treatment of choice for patient with end-stage liver disease. However, the procedure requires major surgery, with many liver transplant recipients needing to spend time in intensive care units in the post-operative period, with considerable risks for infectious complications, acute renal failure and/or poorly functioning grafts (Razonable et al. 2011). Given the donor shortage and that only one or two patients at most may benefit from one donor liver, and the complexity associated with the transplantation procedure various

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alternatives have been evaluated, including cell therapies. The use of living cells as a therapeutic source to restore, maintain and/or enhance the liver function have numerous advantages when compared to organ transplantation as cells can be expanded *in vitro* to overcome the limits of organ shortage, cells can be genetically manipulated to correct functional and/or metabolic alterations, cells can be cryopreserved, transplanted without major surgical procedures and can be obtained from the same patients avoiding major risk of rejection and need for immunosuppressive treatments (Locke et al. 2009).Unfortunately adult hepatocytes cannot be expanded *in vitro* and cryopreserved cells are easily damaged during the freezing/thawing procedure

The transplantation of fresh isolated adult hepatocytes has been explored as an alternative to liver transplantation. Despite some encouraging results, demonstrating a clinical improvement for up to 12 mon following hepatocyte transplantation, this approach is hampered by the heterogeneity of patients treated, variety of transplantation reports with different setting and follow up studies (Sancho-Bru et al. 2009) and, importantly, the difficulties in harvesting and storing sufficient quantities of hepatocytes along with the significant cell loss following transplantation. All these factors have so far limited the potential applications of using adult hepatocytes for therapy (Han et al. 2009).

Alternative cell sources for hepatic cell therapy are being examined and of particular interest are both adult and foetal liver stem cells and pluripotent stem cells, with its great potential as an expandable and reliable cell source (Table 11.1). Stem cells are undifferentiated cells capable of proliferation, self-maintenance and are able to differentiate with plasticity into diverse mature progeny, including hepatocytes. Indeed, hepatocyte-like cultures have been generated *in vitro* from both Embryonic Stem Cells (ESCs), human peripheral blood monocytes and bone marrow-derived stem cells (Ruhnke et al. 2005, Agarwal et al. 2008, Chivu et al. 2009) and their administration in rodent models has been shown to support hepatic function (Sato et al. 2005, Moriya et al. 2008). Initial clinical pilot studies testing the direct administration of bone marrow derived stem cells have been encouraging, supporting and improving liver function in patients with chronic liver disease (Terai et al. 2006, Lyra et al. 2007). Indeed, Bone Marrow Stem Cells (BMSCs) have long been recognized as possessing potential to support hepatic population. A mobilization of the bone marrow derived hematopoietic stem cells fraction has been observed during hepatic injury and seems to play an important role in hepatic regeneration (Russo et al. 2006). However, the bone marrow derived mesenchymal stem cell fraction can potentially contribute to liver fibrosis (Forbes et al. 2004), highlighting the complexity of the injury/regeneration process in the liver. Further revisions on the role of bone marrow-derived hepatocytes in preclinical and

clinical studies have indeed highlighted remarkable differences in the way that cells may support this cellular regenerative process and this is likely to have an important impact in the development of therapeutic approaches (Stutchfield et al. 2010). Two main cellular regenerative processes to injured liver have emerged including a) a direct contribution to the resident hepatocyte population and progenitor cells and b) the supportive indirect role of bone marrow derived stem cells to promote endogenous processes. In the latter, investigations for therapies are largely focussed on investigating the paracrine mechanism by which bone marrow stem cells may promote tissue repair and how to mobilize the endogenous cell and/or paracrine factors resources in the patients.

In this chapter we will address the different technical and bioethical challenges associated with the development of stem cell treatments for hepatic disease. The main sources of stem cells that have been proposed for cell transplantation are described and the practical challenges for their use as models of human disease and their potential for clinical applications are discussed, with a particular emphasis on the use of induced reprogrammed pluripotent stem cells.

## Sources of stem cells for cell therapy

### *1. Embryonic stem cells*

ESCs are pluripotent cells derived from the inner cell mass of the blastocyst-stage embryos and possess potent differentiation potential as they can generate any differentiated phenotype of the three primary germ layers (endoderm, mesoderm and ectoderm), as well as germ cells (Thompson et al. 1998). Moreover, due to their capacity for self-renewal they can theoretically provide an unlimited supply of cells that could be differentiated into hepatocytes to support regeneration of the diseased liver. *In vitro* differentiation of ESCs towards the hepatic lineage is well documented, generating functional but immature hepatocytes (Yamamoto et al. 2003, Agarwal et al. 2008) and when they are transplanted in animal models of hepatic disease, these ESCs-derived hepatocytes were able to engraft in the damaged liver and support differentiation towards hepatocytes but with limited regenerative and function capacity (Heo et al. 2006). Ongoing studies are focussed on improving the differentiation protocols to generate more robust hepatocyte-like cells from ESCs with greater functional and regenerative properties (Hay et al. 2008, 2011, Payne et al. 2011).

However, bridging the therapeutic potential of human Embryonic Stem Cells (hESCs) towards its clinical applications has raised one of the most controversial debated areas in scientific research. This debate largely revolves around the ethical implications of using human embryos as the

main source for obtaining these promising stem cells, mostly from surplus embryos created for the purpose of assisted reproduction. Such controversy also derives from the potential application of somatic cell nuclear transfer to generate blastocysts in order to produce immunologically compatible hESC lines for therapeutic use in patients. The most controversial argument against the research with human embryonic stem cells is that this technique involves the use of human embryos and, as largely argued, this represents the destruction of human life for those who considers life begins at conception (Green 2007). Similarly, the creation of embryos specifically for the sole purpose of deriving stem cells may have more considerable moral implications than using surplus embryos that would eventually be destroyed. The main point of argument is that there are two approaches to assess the embryo, the biological one, by which that embryo is part of the development process as proven scientific consensus and the metaphysic-theological approach which debates the issue strictly in terms of absolute moral values. In this vein, any regulatory system should be based on scientific facts and integrating the real facts that affect society, not in specific moral beliefs (Casado and Egozcue 2000).

Opponents to the use of embryonic stem cells cite the advantages of adult stem cells and, more recently, induced pluripotent stem cells. The moral objection to ESCs has had the effect of driving forward the research in these alternative areas more strongly. On the one hand, this can be beneficial in developing new lines of enquiry, but it may also have the consequence of accepting a lower standard of human material.

Furthermore translational research with human ESCs has also raised other controversies related to the mixing of human and animal cells or DNA. Studies using chimaeras are common in biomedical research and introduction of human DNA into animal cell lines (or vice versa) is an everyday occurrence. Vaccines and xenotransplantation research represent some of the more visible examples in which animal-human mixing has been routinely applied. Similarly, human ES cells are implanted into immunodeficient mice to test for teratoma formation as the standard to assess stem cell quality and developmental potential (Lensch et al. 2007). Indeed, the International Society for Stem Cell Research (ISSCR) endorse the use of these forms of human/animal chimeras on which a limited number of human cells are introduced at any stage of pre-or post-natal development, and where incorporation into any lineage or tissue is likely to be minimal. However, those protocols in which human cells may contribute a significant degree of chimerism to the central nervous system and/or germ line raised serious ethical concerns as prospects that they may develop human features. This is especially concerns when human ES cells might be incorporated into the brain or gonads of a closely related primate (Hyun et al. 2007). The National Academy of Sciences (NAS) and the ISSCR have

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formulated guidelines for research involving human-animal chimeras and recommend that "all research involving the introduction of human ES cells into nonhuman animals at any stage of pre/post-natal development should be reviewed by the Embryonic Stem Cell Research Oversight committees (ESCRO; NRC-U.S., NAS) and stated their opposition to research in which human ES cells are introduced into non-human primate blastocysts (pre-implantation embryos), as well as the breeding of any animal into which human embryonic ES cells have been introduced.

include dates after (ESCRO; NRC-U.S 2005; NAS 2007) as references are quoted in the list

Another aspect associated to the clinical use of human ESC-derived therapies into patients is the potential risk of teratoma formation and the immunocompatibility issues following transplantation. Several preclinical studies are addressing the safety issues related to phenotype stability of the derived ESCs but the real proof remains to be validated through long-term trials (Wu et al. 2007). Similarly, there are concerns about the compatibility between ESCs-derived tissues between patients. Even though ESCs seem to display a certain degree of immune privilege due to their minor expression of histocompatibility antigens, it has been shown to be sufficient to induce an acute rejection in differentiated tissues derived from ESCs (Robertson et al. 2007). Several approaches have been proposed to overcome the immune barrier including the use of somatic cell nuclear transfer from given donors to generate blastocysts and their derived patient-specific hESCs lines that are immunologically compatible for therapeutic use. However, as mentioned there are also serious ethical concerns with the use nuclear transfer technique in human embryos and the targeted "creation" of human embryos for the sole purpose of deriving hESCs.

Professional groups including the NAS in the US, the Human Fertilisation and Embryology Authority within the UK and the ISSCR clearly oppose to reproductively clone humans and prohibit *in vitro* culture of human embryos beyond 14 d. These guidelines allow the derivation of human ESCs lines from excess embryos from IVF, from embryos created explicitly for human ESC research, or from embryos created by therapeutic cloning, including the necessity for appropriately detailed and informed consent. Such approval would be only granted where the research is "necessary or desirable" and the use of human embryos is essential. It is of interest that these guidelines also include the possibility for interspecies mixing, in particular regarding the production of *cybrids*, in which human somatic cell nuclei are introduced into enucleated animal oocytes to induce reprogramming. Such procedures have been recently approved in the UK but only after a considerable debate (St John and Lovell-Badge 2007). While the use of this technology may overcome the difficulties of using human oocytes to carry out similar procedures, mostly compromised by the shortage of donated human oocytes (Holden 2005), there are important scientific concerns mostly associated with the presence of both animal

and human mitochondria (St John and Schatten 2004). The overall idea is that stem cells obtained from human-nonhuman cybrid embryos could be suitable for *in vitro* purposes of studying human diseases, despite the presence of animal mitochondria (St John and Lovell-Badge 2007). However, these cells may not be fully functional after transplantation *in vivo* into animal models as mitochondrial function would be essential in this setting. Similar to nuclear transfer, the complexity and poor understanding of the intrinsic mechanisms along with the limited results have critically compromised the further progression of this technique.

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## 2. Adult stem/progenitor liver cells and bone marrow stem cells

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The postnatal liver has an extensive regenerative potential grossly supported by the presence of a progenitor cell population and the plasticity of the hepatocyte. In response to an injury and/or regenerative stimulus, the normally quiescent hepatocytes will become activated and initially drive the regeneration response. However, such regenerative response of resident hepatocytes may be overwhelmed and/or compromised and then the Hepatic Progenitor Cell population (HPCs) will support the hepatic regeneration, as detected in several hepatopathies (Zhou et al. 2007). However, the identification and further characterization of this putative liver progenitor cell population remains controversial and warrants further research. To add to the complexity, the presence of such cell populations in the liver has been related to the existence of multiple stem cell niches within the liver that can become activated depending on the mechanisms and location of injury (Petersen and Shupe 2008). In rodents these liver stem cells have been termed oval cells and are capable of differentiation into both hepatocytes and biliary epithelia. In humans, the presence of a similar stem cell niche population in the adult liver and their role in hepatic regeneration remains uncertain. Despite all these hurdles, the potential application of these adult stem/progenitor cells remains interesting as it avoids many of the ethical issues related to ESCs and safety issues associated to teratoma formation, allowing the development of autologous transplantation with no need for immunosupression. Ongoing studies are focussing on the characterization of these liver stem cells and their functional regenerative role during liver disease.

Although liver regeneration is mainly an endogenous process, the supportive role of extra-hepatic Bone Marrow Stem Cells (BMSC), in particular the HSCs subpopulation, that migrate into the liver and contribute to its regeneration has long been recognized (Petersen et al. 1999, Forbes et al. 2004). Several hypotheses about the mechanism by which BMSC contribute to liver regeneration have included differentiation into hepatocytes, cell

fusion with hepatocytes to form cell hybrids and stimulation of paracrine effects (Stutchfield et al. 2010). Although transdifferentiation of progenitor cells into hepatocytes and the differentiation of hepatocyte like cells have been demonstrated, the mechanism that seems to be more favourable clinically is the role of BMSCs in modulating the endogenous repair mechanisms through their paracrine effects promoting angiogenesis which is crucial to liver regeneration (Beaudry et al. 2007).

HPCs and/or BMSCs have relevant benefits as cell sources for autologous transplantation in liver disease. However, there are some critical issues associated with, their identification and characterization, to obtain a well-standardized homogeneous cell population, and further concerns over the phenotypic stability of the engrafted BMSCs, their risk for contributing to liver fibrosis and whether the regenerative potential of cells derived from diseased patients may already be critically compromised. Despite all, autologous stem cells derived from bone marrow are the only stem cell type to have undergone clinical investigation to date. However, published studies have been small and mostly reporting safety, with limited information on efficacy of repair (Piscaglia et al. 2010). Further randomized controlled trials are needed to establish a genuine role of these cells in liver repair.

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## 3. Alternatives approaches to deriving pluripotent stem cell lines

There is a general agreement on the great potential for human health in cellular-based therapies. Through recent years we have seen extensive debates discussing alternative sources to obtain pluripotent cells, with the aim to reinforce the development of these cell-based therapies and to bring new resources that may help to somehow soften the stringent attitude overshadowed by all the debate on the use of human embryos as a source (Fig. 11.1).

One proposed method is a **modified approach for the nuclear transfer technique**, in which a single blastomere from an eight to 16-cell embryo is used to create a hESC line while not compromising the potential of the embryo to develop further. This has been successfully carried out in mice (Chung et al. 2006) and it is also clinically used for Preimplantation Genetic Diagnosis (PGD). The isolated cell can be cultured, expanded and used for genetic diagnosis and the derivation of embryonic stem cells line. With more than a decade of experience in this technology and hundreds of children born following PGD, the evidence suggests that the procedure does not impair the embryo's developmental capacity with no proven risk for higher malformations or related developmental problems in the born children (Verlinsky et al. 2004). Moreover, researchers have reported the successful development of pluripotent stem cells lines from single cells taken from

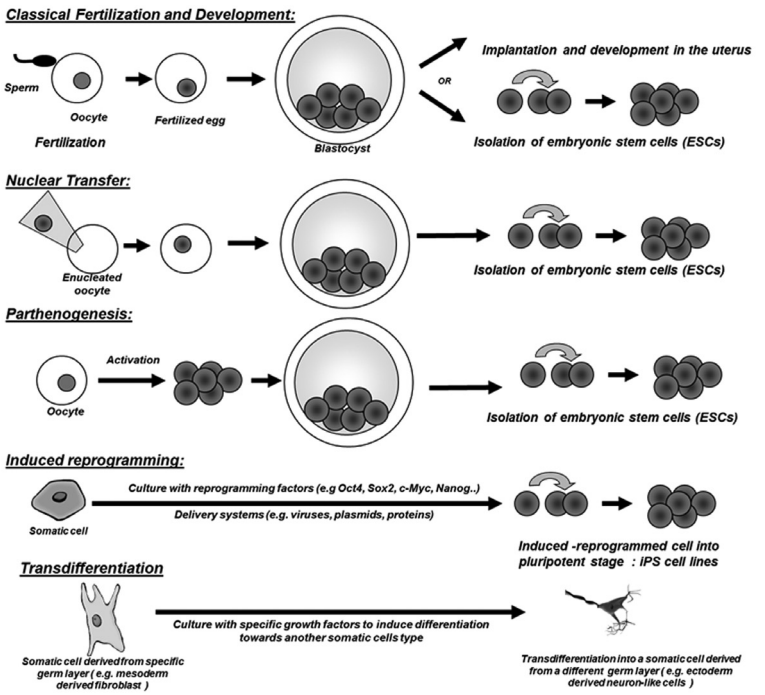


**Figure 11.1.** Approaches to generating pluripotent stem cells: Derivation of human embryonic stem cells from the inner cell mass of blastocysts that form after fertilization of the oocyte by spermatozoa, after somatic-cell nuclear transfer into an enucleated oocyte or by direct activation of the oocyte (parthenogenesis). Alternatively, pluripotent stem cells can be obtained by direct reprogramming of somatic cells by culture with factors such as Oct4, c-Myc, Sox2, Nanog (induced pluripotent stem cells: iPSCs). ESCs: embryonic stem cells.

thawed human embryos (Klimanskaya et al. 2006), providing the proof-of-concept for the feasibility of this approach. However, it is not clear that this could be implemented as a routine approach for couples undergoing ART procedures, as they would more likely opt for implanting an embryo that has not been exposed to such manipulations. Although evidence suggests the removal of a single blastomere imposes very little risk on a child that is born as a result of this procedure, there is much uncertainty about whether such an approach could become a standard procedure for isolating and developing personalized pluripotent stem cell lines from hESCs.

Another potential source of pluripotent embryonic stem cells is through **parthenogenesis**, which involves the development of an embryo directly from an oocyte without fertilization by sperm. Parthenogenetic oocyte activation can be induced in the absence of spermatozoa by exposure to certain chemicals (e.g., ionomycin, cycloheximide) and physical stimuli (e.g., electric stimulation) in various mammalian species including humans

(Revazova et al. 2007). Although parthenote embryos do not produce viable offspring (both maternal and paternal gene imprinting are required for development), the derived ESCs can differentiate into cellular derivatives *in vitro* and form teratomas *in vivo* (Lengerke et al. 2007). Despite such characteristics, the potential clinical application of parthenogenetic human ESCs has not been very attractive mostly due to observations that parthenote-derived cells are not genetically identical to the oocyte donor raising immunocompatibility concerns for clinical transplantation. Also there is the question whether the absence of paternal imprinting will affect the normal development of these cells, and the fact that the source of oocytes are very restricted and limited to the patients that are able to donate an oocyte. For all these issues parthenogenesis remains a controversial approach for deriving pluripotent human ESCs, mostly associated to its "artificiality" and their lacking of full developmental capacity.

Taking into account the ethical, social and legal implications involved, and with new discoveries in the area of **cellular reprogramming** research, seeking to reprogram adult somatic cells to become pluripotent represents a very promising approach and hence the large amount of public support and high number of research groups working in this field. With the pioneering work of Yamanaka's group (Takahashi and Yamanaka 2006) reporting the generation of ESCs from an adult fibroblasts by forcing the expression of specific genes and changing the epigenetic status of the adult differentiated cell to become a pluripotent cell, has brought a whole new perspective in the stem cell field. Undoubtedly, this opens whole new perspectives for continuing the development of stem-cell derived therapies, as such technology might obviate the need for destroying the embryo and have great potentially facilitating the derivation of an immune compatible cell-based therapeutic products from patients, overcoming the need for immunosuppressive treatments. However, all the gene manipulation work also raises some other safety and bioethical concerns regarding the epigenetic stability of the differentiated cell state.

Finally another approach of remarkable growing interest is the induction of **transdifferentiation** of somatic cells into other differentiated lineages. Indeed, this approach argues whether cells can be induced to "transdifferentiate" directly into another state of differentiation, converting from a somatic cell type to another type without first reprogramming into pluripotent cells (Graf and Enver 2009). This transdifferentiaton between somatic cell lines of cells it is well reported in several studies, with examples of conversion within the same germinal layer such as fibroblast into muscle cells (Weintraub et al. 1989) within the hematopoietic lineage converting T and B-cells into macrophages (Xie et al. 2004) and cardiac fibroblasts into cardiomyocytes-like cells (Ieda et al. 2010). Recent studies have reported the transdifferentiation potential between different germ layers, Vierbuchen et

al. (2010) converted fibroblast (mesoderm) to neurons-like cells (ectoderm) and Efe et al. (2011) converted mouse fibroblast to cardiomyocytes, shedding some new light on the biology underlying cell differentiation and cell-fate, suggesting new avenues for induced reprogramming protocols obviating the need to pass through a pluripotent state.

Overall, this approach for inducing differentiation is increasingly being investigated and ongoing research is providing good evidence of cell plasticity; however it also brings with it the risk of aberrant gene expression patterns and therefore serious consequences for its clinical use.

## Prospects of iPSCs for disease modelling and cell transplantation

Recently, the generation of Hepatocyte-Like Cells (HLCs) has also been demonstrated to be feasible with human-induced pluripotent stem cells (iPSCs) (Song et al. 2009, Sullivan et al. 2010, Touboul et al. 2010, Si-Tayeb et al. 2010). IPSCs appear to be a promising source for the generation of hepatocyte-like cells that could provide a defined and renewable source of human cells relevant for cell therapies and pharmacological *in vitro* testing. But there are several technical challenges regarding the reprogramming procedure and the expansion and derivation of pluripotent-induced cells and their differentiation towards functional hepatocytes. These issues will be discussed below.

### *1. Introduction to cell reprogramming and induction of pluripotency: technical issues*

Cells are characterized by their gene expression patterns and function. For a cell to achieve its differentiated status during development a series of changes which take place in a tightly regulated manner to allow the adequate modifications in the genetic profile and function. To investigate these epigenetic changes is crucial to understand how cell fates are regulated and thus how can this be controlled physically.

During development, cells proceed from a state of totipotency, pluripotency to a more differentiated and tissue restricted fate. As the embryo develops, forming the trophoblast lineage and the inner cell mass, cells are characterized as pluripotent because they can be differentiated into all somatic cells and germ line cells of the developing embryo. Such changes through the cell potency to differentiate into specific lineages depend on tightly regulated intrinsic molecular signalling pathways. However, cell fates during development are neither restrictive nor irreversible. Initial studies

in cell reprogramming, using nuclear transplantation of somatic nuclei into eggs (Gurdon 1962) showed that the epigenome of differentiated cells can be reset to a pluripotent state. Thus to elucidate the factors that maintain the pluripotent state of the early embryonic cells and their modulation during differentiation is the key to understand the potential application of reprogramming cell technology in basic and translational research.

Reprogramming of somatic cells to pluripotency is accompanied by extensive remodelling and epigenetic modifications. Despite the research much remains to be understood about the nature of gene regulation during iPSCs formation. Although several strategies are used to induce reprogramming, only a small fraction of the initial cells will become iPSCs, with the first reprogrammed cells appearing no earlier than 5–10 d after expression of the reprogramming factors (Jaenisch and Young 2008). The determination of efficiency of *in vitro* reprogramming is typically based upon the arbitrary appearance of iPSCs colonies and being calculated by the cells that expressed pluripotency markers (Hanna et al. 2010). This methodology, while informative, remains purely descriptive and provides very limited information on intrinsic cell changes and their expansion and differentiation potential. Moreover, it does not account for differences in cell population size, cell division times, fluctuations in the number of cells that are lost or undergoing apoptosis during passaging and cell culture raises variability among the cell population undergoing reprogramming.

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Many studies suggest that somatic cells can be reprogrammed effectively to a pluripotent state with molecular and biological characteristics indistinguishable to those for ESCs. However, it is important to realize that accurate and quantifiable assessment of these characteristics remains challenging mostly due to the methodological limitations and the complexity of the dynamics of the cellular reprogramming process. Some of the main limitations that may affect the gene expression of the iPSC and their biological characteristics are the presence and incomplete silencing of the reprogramming transgene used to induce the reprogramming the cells of origin (Kim et al. 2010, Sullivan et al. 2010). These residual transgenes can perturb the identity and functionality of the induced cells. Another factor is that the *in vivo* developmental competency of the iPSCS may also depend on the original genetic background of the cells of origin, and they may respond differently to the induced reprogramming. Other critical parameters relate to the effects of the expanding protocols as reprogrammed cells may adapt differently to culture conditions. Overall these constraints may affect the epigenetic state and biology of the iPSCs which can translate into serious genetic deregulation events and abnormal developmental potential.

Sulliva n et al. 2010a

One of the main issues regarding the potential use of iPSCs in the clinics as a good alternative to hESCs is how similar these pluripotent reprogrammed cells are to ESCs. Are iPSCs equivalent to ESCs? Have the

iPSCs the same differentiation potency as their embryonic counterparts? Can the genetic and/or epigenetic profile of the reprogrammed cells be affected or destabilized during differentiation to targeted lineages and/or during transplantation, generating cells that are different from those derived from ESCs? All these questions raise important biological issues regarding the safety and the efficacious use of this cell source for developing cell-based therapies, and also its ethical and legal implications.

Biological assays and gene expression studies are the main criteria generally used to assess the quality of the iPSCs. The chimera formation and germ line contributions assays by which iPSCs derived cells prove capable of forming all cell types of the body are crucial to prove that the iPSCs are pluripotent. In fact in mice it has been proven that the iPSCs have the same developmental potential as ESCs (Zhao et al. 2009). However, these types of assays are impossible in the human system (Lensch et al. 2007, Dolgin 2010). In this field most tests rely on *in vitro* pluripotency assays and teratoma formation. Indeed, the teratoma assay is currently the only established means of demonstrating how human iPSCs possess pluripotency when placed in an *in vivo* system. However, this approach remains a qualitative test and it is difficult to have a quantitative comparative approach with this type of assay. Other tests relying on identifying the genetic signature of these cells allow for a more comparative approach between iPSCs and ESCs. Several studies have indicated the similarities between these cells but the gene expression signatures remain controversial, in particular in early passage iPSCs lines in which larger variation in chromatin structure and gene expression are observed (largely associated with the residual epigenetic memory of the cell of origin (Polo et al. 2010)), and this may affect their differentiation potential (Sullivan et al. 2010). The fact that such patterns are not seen in ESCs derived from nuclear transfer suggests that reprogramming *in vitro* with transcription factors may be suboptimal.

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Sullivan et al., 2010b

Several studies have reported differences in gene expression, patterns of DNA methylation and differentiation potential (Chin et al. 2009, Doi et al. 2009, Hu et al. 2010). Many of these studies have focussed on few cell lines and therefore it is difficult to systematically study the role of epigenetic and transcriptional variation. In a recent study researchers tested a large panel of 16 iPSCs lines derived from multiple donors of varying age, sex and health status and examined their pluripotency and their ability to generate terminally differentiated cells, in particular, motor neurons (Boulting et al. 2011). Most of the iPSCs were capable of expressing similar pluripotent markers and generating functional neurons under a stringent standard protocol in a very efficient manner. These procedures were highly reproducible between laboratories, indicating the robustness of the standardized protocols as quality control for this stem cell resource. This extensive study found that human iPSCs could be differentiated on

average as well as hESCs, supporting their similarities. Although there were some variations in the differentiation efficiency from individual human iPSCs, these differences were highly reproducible suggesting that this may be related to intrinsic characteristics of the cell lines and not their reprogramming.

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Interestingly, in a recent study Sullivan et al (2010), showed that iPSCs lines derived from both female and male sexes and from two different ethnicities (North-American Indian and Caucasian) could be successfully differentiated to hepatocytes-like cells at efficiency similar to that for human ESCs (iPSCs efficiency of 70–90% vs. hESCs efficiency of 90–95%). It was suggested that iPSCs may prove a more uniform starting cell pool for derivation hence the better response to the differentiation protocols but iPSC-derived hepatocytes seem to be less functional that those derived from hESCs. Further research is required to clarify these possible differences in susceptibility to differentiation and function between pluripotent cell lines. Such studies are the key to the progression of these iPSCs derived cells towards modelling and clinical use.

Sullivan et al (2010a)

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## 2. Applications for modeling human diseases

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Human pluripotent stem cells have the potential to generate all tissues of the body (Thomson et al. 1998, Park et al. 2008) which present exciting opportunities for *in vitro* modelling of specific human diseases. They have great potential for investigating pathogenesis, aiding therapeutic discovery and exploring functional genomics (Colmann and Dreesen 2009, Freund and Mummery 2009), in particular for those disorders without suitable animal models and/or those previously lacking lineage specific cells for *in vitro* studies (Fig. 11.2).

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It is hoped that human iPSC-derived cells can provide complementary information even for diseases in which animal models are available. The limitations of animal models are being realized, especially by pharmaceutical companies who have experienced high attrition rates when compounds are transferred from animal experiments to human. The initial approach evolved from researchers isolating cells from preimplantation embryos used for genetic diagnosis purposes and deriving "disease-specific" human embryonic stem cells from the embryos affected by some genetic disorders such as cystic fibrosis and Huntington's disease (Mateizel et al. 2006). This allowed for the generation of mutant hESC lines that could be used as disease models. In this vein, the development of pluripotent stem cells derived from reprogrammed adult somatic cells harvested from patients with specific disorders provides new scope for creating disease-specific cell lines for modelling (Saha and Jaenisch 2009). These techniques have been applied successfully to blood and skin derived cells and their potential to

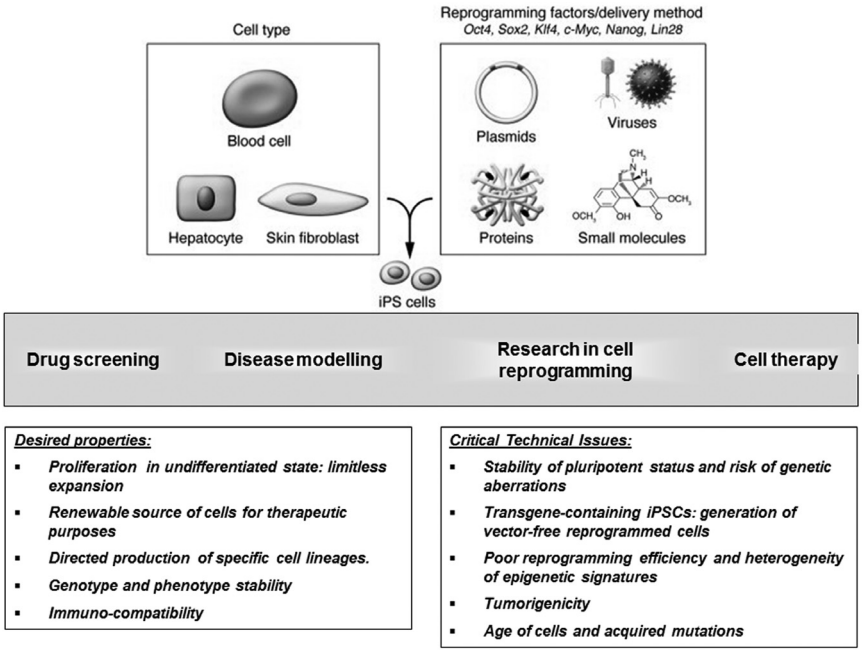


**Figure 11.2.** Schematic representation of the generation process of iPSCs and their research and clinical applications: different tools can be used to reprogram different adult cells inducing it to become an "undifferentiated" and to regain pluripotency; A number of desirable factors influencing the properties of induced pluripotent stem cells and constraints that may affect their biology and potential clinical use.

progress towards a disease phenotype has been demonstrated (Ebert et al. 2009, Raya et al. 2009).

We will now address the practicalities and critical issues associated with the development of cell sources for modelling from human iPSCs.

## *2.1 Harvesting cells from a patient and induction of pluripotency*

Somatic cells are harvested from patients; this is usually done by tissue biopsy or blood sampling (e.g., a cell fraction expressing the surface protein CD34 from the general blood sample or fibroblasts from skin samples). Then reprogramming protocols will be implemented to generate cell lines that will be critically screened for their pluripotent phenotype.

The direct reprogramming strategies currently available are using viral vectors generating human iPSCS with multiple integrated copies of these viral transgenes. The possibility of persistence of these integrated reprogramming

vectors in the disease specific human derived iPSCs poses the risk of being reactivated during further development and/or differentiation during disease modelling leading towards abnormal and/or cancerous phenotypes. This is of lesser relevance for modelling than for clinical studies, but still has the potential to disrupt experimental results. A well reported example is the use of the transcription factor c-Myc which studies have shown that led to high incidence of tumours in chimeras generated from mouse iPSCs (Nakagawa et al. 2008). Thus it is vital to develop alternative methods to induce cell reprogramming without using viral vectors and c-Myc. Studies using lentiviral vectors with the Cre-recombinase enzyme have shown to induce a higher reprogramming efficiency and vector deletion, but viral elements still remain. Other strategies such as the use of peptide or small molecules to induce reprogramming in the cells without the integration of any viral derived factors have extremely low efficiency and are still to be fully validated (O'Malley et al. 2009~~-see chapter on reprogramming).~~

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## 2.2 Induced differentiation to specific cell lineages

In order to fully investigate the disease cell phenotype we will require protocols to differentiate iPSCs to functional somatic cells. Several studies have successfully reported the differentiation of HiPSCs into different cell lineages (neuronal: Chambers et al. 2009, hepatocytes: Sullivan et al. 2010~~-see Fig 11.3~~; fibroblasts: Hokemeyer et al. 2008). However, such protocols are not well standardized, with different time courses and varying effectiveness between lines. Differentiated cells will undergo an extensive functional and biochemical analysis to identify their phenotype and how its matches the specific phenotype associated to the disease pathology. Due to the complexity of multigenetic disorders, this approach is mostly valuable for those monogenic disorders with well characterized phenotype disorder patterns (Raya et al. 2009, Ebert et al. 2009). The application of this approach for disorders with more complex genetic phenotypes associated to multifactorial disorders remains rather difficult. Indeed, disease associated to complex and significant epigenetic modifications pose a serious challenge for modelling. It is clear that obtaining a disease related phenotype from a given patient will critically depend on the characterization of the disease phenotype and its comparison with healthy control induced pluripotent cells. In addition, most derived cells will display immature phenotypes compared to authentic adult cells. Certain aspects of pathology are likely only to be observed in the adult phenotype. To complicate matters, development of disease can be associated with regression to a foetal phenotype, for example in heart, making it difficult to distinguish between immaturity and disease-related characteristics.

In refs.--2010a and b

Sullivan et al. 2010a

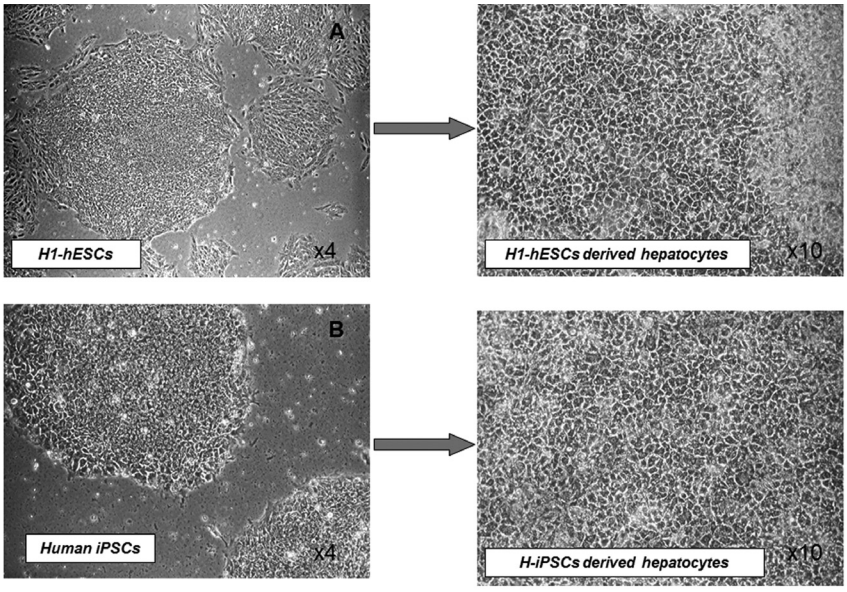


**Figure 11.3.** Phase Contrast microscopy images of hepatocytes derived from H1-hESCs (A) and hiPSCs human iPSCs cells. Images kindly provided by Dr Claire N. Medine (MRC CRM, University of Edinburgh).

## *2.3 Generation of disease-relevant phenotypes from the pluripotent iPSCs*

The main objective is to ensure that efficient differentiation methods are implemented to induce the differentiation of the pluripotent iPSCs derived from specific patients towards the disease-relevant phenotype (Fig. 11.2). The use of reporter genes is extensively applied to identify genetic modifications during cell differentiation to specific phenotypes, which may help to understand the dynamics of genetic changes associated to specific progression towards a disease phenotype. However, the integration of these reporter genes can also be quite inefficient for human ESCs, with limited transduction efficiency (Xia et al. 2007). Nevertheless, this is a growing area of research and new techniques are evolving to facilitate the incorporation of reporter genes for targetting endogenous genes in human iPSCS (Hockemeyer et al. 2009).

Remarkable progress has already been made with some cases of modelling monogenic diseases and some complex genetic disorders with early or short term developmental pathogenesis. Park et al. (2008) reported

the production of human iPSC lines for 10 diseases, ranging from simple Mendelian inheritance disorders (e.g., Adenosine deaminase deficiency) to complex conditions (e.g., Parkinson's disease and Type 1 Diabetes). Other groups have also reported the production of iPSC lines for other neurological, myeloproliferative and endocrine disorders (Dimos et al. 2008, Lee G et al. 2009, Soldner et al. cell 2009, Raya et al. 2009, Maehr et al. 2009). Importantly, the number of studies on human iPSCs in which the disease-specific *in vitro* phenotype has also been reported is also increasing (Carvajal-Vergara et al. 2010). There are also existing human iPSCs cell lines for myeloproliferative disorders (Ye et al. 2009) and endocrine disorders such as the juvenile diabetes mellitus (Maehr et al. 2009), and this list is growing fast. Hepatocyte-like cells (HLCs) have also been generated from human IPSCs (Sullivan et al. 2010, Inamura et al. 2011) opening whole new perspectives for developing new sources of hepatocytes from iPSCs derived from patients suffering form polymorphic metabolic disorders and other liver associated disease genotypes (e.g., alpha-1-antitrypsin), to model liver disorders *in vitro* allowing for the development of novel biomarkers and *in vitro* drug toxicity assays.

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In refs.--2010a and b

a

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reference was missing date- corrected now so this quote is ok

Another challenging factor is that the relevant –disease specific phenotype may also require specific environmental stimuli, from the neighbouring cells and surrounding tissues, cell matrix and mechano-physical properties. Therefore we need to provide an *in vitro* environment that will mimic this extracellular milieu during co culture of the differentiated disease-specific human iPSCs. Extensive work is ongoing in the field of biomaterial engineering to develop powerful supportive co culture *in vitro* systems to provide effective rich context for studying disease related cell-cell interactions and extracellular matrix effects (Guilak et al. 2009). The use of iPSCs derivatives with several bioactive materials and other evolving 2D and 3D scaffolds (Hay et al. 2011) may open new perspectives for cell culture, studying differentiation and disease modelling. A particular example for the liver is the development of micro patterned cell clusters from human hepatic cells to study liver function and hepatotoxicity assays (Khetani and Bhatia 2008). Recently Spence et al. (2011) efficiently directed the differentiation of human IPSCs into a three dimensional functional intestine which has great potential for generating intestinal tissue *ex vivo*.

Nevertheless, there are still some limitations when modelling human disorders, the latent period associated with some disorders such as Alzheimer's or late onset Parkinson's disease are especially difficult to mimic. Therefore for some diseases, an *in vivo* approach may be more suitable. Chimera assays provide a long-term access to complex and changing environment context for studying iPSCs; however there are some ethical concerns associated with this experimental approach. Human-animal interspecies chimeras can be generated by grafting pluripotent

CORRECT SPELLING :
feotuses to fetuses



cells into embryos, feotuses or adult animals (see Behringer 2007). The transplantation of human cells into immunocompromised animal models is extensively used in regenerative research. Similarly, humanized mouse models are being developed with successful hematopoietic, neural and hepatic reconstitution with human stem cell derivatives (Friese et al. 2006). In particular, a hepatocyte-humanized mouse has been generated for exhibition of human-type responses for drug-related cytotoxicity assays and studying the pathogenesis of some viral infections (Kneteman and Mercer 2005).

## 3. Perspectives for clinical applications of IPSCs: Bioethical, legal and social issues

Despite the rapid progress in understanding cellular reprogramming to induce pluripotency, many technical and biological hurdles remain before their translation into clinical applications. Summing up the main challenges already discussed, including the inefficient direct reprogramming methodology, the difficulties for expanding the induced pluripotent cells in a robust and stable manner and the standardization of the molecular profiling on the cells, it is obvious that researchers need to establish reliable and reproducible standardized protocols to test pluripotent cell lines in the laboratory. Despite extensive research efforts focussed in setting up reliable test set for these cells (Boulting et al. 2011), a recent study reported aberrant epigenomic reprogramming in five human iPSCs cells, showing significant reprogramming variability and aberrant DNA methylation profiles (Lister et al. 2011). Hence the importance of ensuring that extensive and thorough studies are carried out to characterize the genomic signature of the reprogrammed cells for their safe and effective use in the clinics. One would envisage the clinical applications of the iPSC as follows: a) harvesting of somatic cells taken from a living patient or a frozen tissue bank; b) generation of pluripotent cells carrying a disease-linked mutation by using standard well-characterized reprogramming protocols, and as already discussed, preferentially using methods that do not leave any genetic footprint in the derived cells (e.g., using non integrating vectors, excisable genetic elements , or using chemical or protein induced methods (Saha and Jaenisch 2009, Kim D et al. 2009, Hanna et al. Cell 2010); c) differentiation of the induced pluripotent cells into the target cell type of choice using robust differentiation protocols for the purpose of cell therapeutic source and/or as supportive cell source for the development of bioactive artificial devices. These differentiated cells should be thoroughly characterized for their genetic signature and phenotype as a quality test for their differentiation; d) investigation of their potential therapeutic interest through *in vitro* and

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Year needed

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delete Cell, and keep Hanna et al.2010

(e.g., using non integrating vectors, excisable genetic elements, or using chemical or protein induced methods; Saha and Jaenisch 2009, Kim et al. 2009, Hanna et al. 2010)

preclinical evaluation studies; e) long term safety studies to ensure that the cell source is safe and efficacious for human clinical use. The timescale of this process may pose a problem for the treatment of rapidly developing conditions.

Despite the general belief that the use of iPSCs raise fewer ethical concerns than those related to embryonic stem cells it must be acknowledged that, as reprogramming technology evolves, there are important dilemmas that have to be addressed (Fig. 11.4). These divide into donor-related issues such as the consent to donate material for iPSC derivation, and to matters of the safety of these untested therapies as they are translated towards clinical trials. It is crucial to realize the potential scaling prospect of the iPSCs derivatives and to understand whether the original donor will be

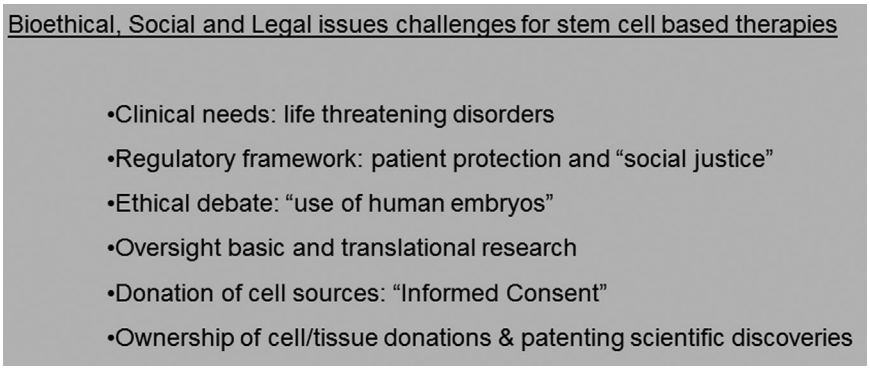


**Figure 11.4.** Factors influencing stem cell based therapies towards the clinics.

able to foresee a clear picture of their potential downstream use. These cells may be used extensively by researchers who will carry out a large variety of studies worldwide including genetic modification of the cells, preclinical testing in animal models, large scale genome sequencing, and sharing cell lines with other researchers. This raises issues of confidentiality protection and also as therapeutic applications evolve further issues with patenting, intellectual property and commercialization of the cell derivatives with/without proprietary right and share of royalties (Lo and Parham 2009). Several ethical, legal and social aspects must be considered for a responsible transition of stem cell research into appropriate clinical applications, as discussed in the Guidelines approved by the ISSCR (Hyun et al. 2008).

It may also be envisaged that selecting which disorders should be targeted for such experimental therapies is likely to raise a substantial bioethical debate. Clearly this is going to be a political decision that could be supported by legislation promoting specific areas of research. However, such legal response should also be supported by a social debate, where the scientific background, with its expectations and limitations,

are clearly discussed. It is important to not generate false expectations or misconceptions in patients affected by the disease being studied.

## *3.1 Oversight of iPSC research and translation*

First of all, it is imperative that individuals with stem-cell-specific expertise assist in the assessment of translational research, supporting an independent and thorough oversight of the studies that will lead to the clinical trials. This will involve a good scientific evaluation of the *in vitro* and *in vivo* preclinical studies that will form the basis for proceeding towards clinical application. This is particularly relevant when assessing potential treatment with iPSCs as there are technicalities that must be addressed as already discussed. Approved principles of quality control must be in place to promote maximal quality and safety of the cells to be used, and all is supervised by expert and independent oversight committee. It is important to acknowledge the complexities associated with some cell types and to ensure a systematic assessment of the integrity and potency of the cell products to minimize any risk to patients. Preclinical testing plays a key role in assessing the safety and establishing a proof of principle for therapeutic effect. Good preclinical studies must support the clinical strategy, providing convincing evidence of the safety of using these cells before advancing to human studies. Some of these studies can be particularly challenging as some human disease conditions may not be easily translatable to animal models and also there may be some existing physiological differences between species. Another challenge is that stem cells can act through multiple mechanisms and thus predicting their behaviour may prove difficult. Indeed, this is of particular interest for the iPSCs, in which it is crucial to prove that the reprogramming method worked well. The major stem cell banks such as the NIH's Human Pluripotent Stem Cell Registry requires the researchers submitting new cell lines of teratoma formation as it is currently considered the gold standard for assessing pluripotency. However, there is increasing debate on the efficacy and reliability of this test (Dolgin 2010). Some researchers argue that there may be no need to categorize for pluripotency as long as the cells can differentiate towards the required cell phenotype, e.g., hepatocytes for liver diseases. But others maintain that is crucial to ensure that that the cell line's developmental capacity is fully tested to ensure a safe and effective derivation of differentiated cells. There is the risk that if we attempt to shortcut the characterization of targeted derived cells from iPSCs we may miss important developmental pathways that are key for the "normal" differentiation of the cells towards a mature phenotype. Moreover, the *in vivo* teratoma assay has the added value that cells are injected into a living animal, thereby taking advantage of all the in situ growth factors that help to develop three-dimensional structurized tissues. Nevertheless,

In refs.--Müller et al. 2010? YES Agreed

extensive ongoing work is focused on the development of genomic and bioinformatics' methods as alternative methods for appraising a cell line's pluripotency (Müller 2010). However, until further consensus is achieved among the stem cells community, quantitative *in vitro* assays may so far remain as a good alternative in development.

These arguments demonstrated the complexity for developing reliable translational assays hence the importance of ensuring that individuals with stem cell expertise are involved in reviewing such translational research and to ensure that only when compelling preclinical data are available is there a justification for moving into clinical trials.

## 3.2 Consent for the derivation of human iPSCs

As with all clinical research, when planning for clinical trials of stem cell-based interventions the internationally accepted principles governing ethical conduct of clinical research and the protection of human subjects must be guaranteed. This requirement is identified as a fundamental international standard by the Helsinki Declaration (WMA 2004), the Council for International Organization of Medical Sciences (CIOMS and WHO 2002) , the National Commission for the protection of Human Subjects of Biomedical and Behavioral Research (The Belmont Report 1979) and the ISSCR among others (ISSCR 2006, US-FDA 2008). From the international legal perspective the Convention on Human Rights and Biomedicine, 1997 "The Oviedo Convention" and its Additional Protocol on Biomedical Research, 2004 (Council of Europe) and the Universal Declaration on Bioethics and Human Rights, UNESCO, 2005 should be taken into account.

Not listed in refs. CIOMS reference is page 183 reference 13 and deleted WHO

In refs.--2007 yes correct to 2007

Undoubtedly many of the research uses with iPSCs may evoke concerns about privacy and confidentiality. Donors may not be fully aware of the whole research potential and they might consider certain aspects a violation of their privacy: for example, in large scale genomic studies one could foresee complexity with DNA databases and confidentiality issues (Lowrance and Collins 2007). That is why it is crucial to give consent specifically about the future consequences; it is important to realize that to revoke the consent may not imply that the information generated until then will all be deleted. (Seoane et al. 2008). However, the Council of Europe permits research on identifiable bodily material without consent (but subjected to ethical review) in specific occasions where the research addresses important scientific interest, there is no evidence for an expressed opposed consent and it is not possible within reasonable efforts to contact the person for the consent.

In the initial stages of translating these iPCS-derived cell based therapies, we would envisage that the donation would be for allogeneic use towards the development of patient-specific iPSCs derived therapies. In this case it

may be useful that along with the voluntary informed consent, the donor is provided with all the information related to all the preparation and health screening of the cells, their storage and its duration and that the donor may be approached in the future to seek additional consent for new users. A relevant issue is the protection of donor privacy and the confidentiality of the acquired information, and the terms for their disclosure. This can raise some concerns on the future use of any resulting cell-derived products and the ownership of commercial and intellectual property rights. In some countries the regulatory bodies specifically mentioned the gratuity of the tissue/organ donation as a principle.

A key requirement for recipients of stem cells and cell products is also voluntary informed consent. This is particularly endorsed by the ISSCR guidelines and also by many of the jurisdictions regulating stem cell research in most of the westernized countries (Caufiled et al. 2007, ISSCR Guidelines 2008, McGuire et al. 2008). Special emphasis needs to be placed on the unique risk of stem cell-based clinical research during the informed consent process, including cell proliferation, phenotype stability and/or tumour development, exposure to animal source materials, risk associated with viral vectors and possibly other unknown risks. Indeed, the informed consent is particularly challenging for these stem-cell therapies, and thus patients must be told about the realistic potential for therapeutic benefits as, the consent must emphasize the novel and experimental aspects of these cell based interventions. Information related to risk and benefits are crucial to avoid misconception on the therapeutic outcomes. It should be noted that for an adult who lacks capacity to make a decision regarding his/her body material, participation in research is only lawful if the research has the capacity to benefit the person, or where the risk involved is negligible. Hence the importance of providing detailed recommendations for patient selection when seeking appropriate consent for future research.

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### *3.3 Property rights associated to iPSCs and derived products*

Indeed, the use of personal genetic information by third parties has important ethical implications, mostly associated to the commercial use of these cells or the disclosure of personal information that could lead to discrimination towards the donor (e.g., disclosure of genetic predisposition could compromise employment and fairness assessment by insurance companies). To avoid such discrimination due to genetic causes and/or personal health, and to ensure the protection of sensitive personal medical information, the regulatory bodies encourage a strong level of protection and respect to such personal data as clearly stated in the article 10 of the Private life and right to information of the Oviedo's Convention (Council of Europe, Convention on Human Rights and Biomedicine 1997) and its

Not listed in refs: included in references line 15 see page 183

Additional Protocol on Biomedical Research (2004). Such guidelines and regulations tend to protect the privacy and confidentiality of the donor, avoiding any genetic or any kind of discrimination based on data obtained. While one could argue that the rational use of these derived cells should be only used for the benefit of the donor, it is important to ensure the benefits of such research are fairly and justly shared. In this vein, another key principle to consider is the benefit to other patients with similar disorders, not only to those directly involved in the donation and clinical trials; in agreement with the social justice values stated in the Universal Declaration on Bioethics and Human Rights by the UNESCO (2005).

Bodily material collected in the course of health interventions and/or collected and used for research purposes, with the consent of the patient concerned, may be stored in cell banks for long term. As the collected material (e.g., cells, tissue biopsies) have a unique identity, as they may be derived from the body of a person, this raises important bioethical concerns regarding the ownership and commercialization of this bodily material. Generally there can be no property rights in a human body, living or dead. The rights of individuals to their own bodies are not legally of "property ownership", as individuals cannot be owned as property by others. However, it is well established that where body parts "have acquired different attributes by virtue of the application of skill" then they may become property. For example any form of tissue that has been processed into a product, including modified tissues or cells, may be considered property and may legitimately be commercialized by those who have undertaken the procedure and not the person who originally donated the source of material (Rv Kelly and Lindsay 1999). Indeed, while The Human Tissue Act (UK 2004) prohibits any commercialism of human material for transplantation, this does not cover any parts that may have acquired the character of property by virtue of modification/application of human skill.

Not listed in refs. correct to 1998

Not clear see reference added below

Regarding the issue of commercialization and financial gain, The Oviedo Convention and Additional Protocols stated that "the human body and its parts shall not, as such, give rise to financial gain"; as also stated in the Convention on Human Rights and Biomedicine (Chapter VII-prohibition of financial gain and disposal of a part of the human body). However, as these body parts are "processed" they become property and potentially a lucrative product for sale and resale (European Group on Ethics in Science and new technologies, European Commission 2002) creating a whole new perspective regarding the ethical, legal and financial aspects of commercialization of these "processed" products.

Not listed in refs. added new reference

As stem cells technologies move forward, property and financial issues are becoming increasingly relevant, and it is up to the regulatory bodies and the oversight ethical committees to ensure that these are addressed with the

add the following references:
R v Kelly and Lindsay [1998] EWCA Crim 1578 (14th May, 1998).
The Human Act (UK 2004) http://www.legislation.gov.uk/ukpga/2004/30/pdfs/ukpga_20040030_en.pdf / Accessed on 23 January 2012
The European Group on Ethics in Science and New Technologies to the European Commission. Opinion on the ethical aspects of patenting inventions involving human stem cells http://ec.europa.eu/bepa/european-group-ethics/docs/avis16_complet_en.pdf / Accessed on 23 January 2012

appropriate proportionality, protecting the donor's rights and providing all the adequate information during the informed consent process but also protecting the research community and the industry interest for continuing advancing and investing in such technologies.

## *3.4 Sensitive research with iPSCs*

Although there are several areas of research with iPS cells that raised some socio-ethical concerns, the fields of transplantation into humans and reproductive manipulation remain particularly controversial (Aalto-Setälä et al. 2009).

Despite the technical issues associated with reprogramming, we can foresee that "virus-free reprogrammed" –iPSCs will develop in the near future making human transplantation possible. In addition to the development of allogeneic transplantation applications, it is very likely that with the development of stem cell banks access to a large number of iPSCs lines may be feasible. As previously discussed, this may raise concern on the control that a donor may be able to exert on the iPSCs derived and expanded from their own cells; some people may not want their cells to be transplanted into another person, or may want to restrict the use of these cells for particular areas of research and we should ensure that the donor's autonomy is thoroughly respected.

Therefore it is important that all the aspects regarding the "ownership" of bodily parts that have been manipulated are clearly reflected in the consent procedure and that during the informed consent procedure the donor is provided with sufficient information about the possibility for transplantation. Even in the case of autologous transplantation, it should be explained that the procedure may be long and thus regular screening of the derived cells through this period will be critical.

Furthermore, it is should be recognized that during this procedure the donor conditions may worsen towards a terminal state of disease or a life-threatening injury. In such conditions, it is very likely that end-of-life decisions may affect the potential use of the donors' stem cells and/or their derivatives. The merits of ensuring that that some kind of advance care directives have been previously discussed and agreed with the donor include the respect of the donor's autonomy in decision making, the respect to his/her personal values and the complexity of involving third persons such as family or health care providers. Advance care directives allow patients to provide instructions about their preferences regarding the care they would like to receive if they develop a terminal illness or a life-threatening condition. However, these directives cannot predict what situation may arise in the future or what new therapies may be available, which is particularly critical for stem cell derived treatment.

Critically ill and/or end-of-life patients may not want to go ahead with the stem cell treatment at that stage. A clear example is patients undergoing hematopoietic stem cell transplantation which is a high risk procedure that is performed with a curative intent in patients with certain hematologic malignancies, but it is associated with significant short-term morbidity and mortality. To confront the potential loss of decision-making capacity by advanced patients, it is important to encourage them to engage in advance care discussions (Joffe et al. 2007). Interestingly, some clinical studies have shown the relevance of confronting such discussions with the patients, indicating that the lack of engagement in advance care decision is associated with increased mortality suggesting that patients may well benefit from considering these issues (Ganti et al. 2007). The process of incorporating stem cell derived therapies with advance care planning may be difficult and complex as many of these techniques are still in development, especially for the iPS cell technologies.

Another sensitive area of research is the differentiation of stem cells into primordial germ cells that then can differentiate into mature gametes. (Ko and Scholer 2006). Although gametes derived from iPSCs would be useful for understanding the process of gametogenesis and towards the development of therapeutic approaches for infertility, their use also raises serious ethical concerns as they could potentially lead to the creation of another human being. Moreover, gametes derived from iPS cells would virtually have the same DNA as the somatic cell isolated from a specific donor. Indeed, there is a critical moral responsibility when attempting reproduction for the creation of embryos, since it raises all the ethical aspects related to the moral status of the embryo and its human entity (Green 2007). Indeed, such type of reproductive research while ethically arguable by many as a relevant experimental approach for infertility treatments would also create important social objections to the iPSC technology. At the early stages of the iPSCs technology the field should try to restrict this type of research through explicit regulations and appropriate consent from donors.

## *3.5 Social justice considerations*

Another potential issue is related to subject selection, ensuring fair access to well-designed clinical trials and effective stem-cell therapies without regards to patients' financial status, insurance coverage, social background or beliefs. It is important that stem cell research endorse the fairness of the social benefit of its progress. The ISSCR guidelines recommend that researchers and regulatory bodies attend to these issues of social justice and fairness when addressing the preparation of clinical trials and to ensure that benefits of research and discoveries are justly shared (Hyun et al. 2008). In this vein, the sponsor and the investigators have an ethical responsibility

to make good efforts to secure sufficient funding to ensure that no eligible candidates are prevented from enrolling to any trial because of their inability to cover the costs of experimental treatment. Groups or individuals must not be excluded from the opportunity to participate in clinical stem cell research without rational justification.

Despite all these guidelines and the efforts of regulatory bodies, there are growing concerns that the development of customized cells and cellular products will remain unavailable to the vast majority of the population, mostly for financial reasons. While it is difficult to predict the future approach of the biotechnology industry and national health services regarding investment on these customized stem cell based therapies, at these early stages of translational development it is crucial that all the research community pool their efforts to facilitate international collaborations and universal access to stem cell-based treatment. These efforts may enable the implementation of more efficacious and cost-effective derivation and manufacturing procedures facilitating the establishment of large-scale banks for the benefit of the population.

## *3.6 Regulatory issues relevant to cell therapy*

A broad variety of stem- cell derived products are likely to be developed for therapeutic purposes, including simple biomaterials and autologous cells through pluripotent, viral modified cells with full spectrum of risk for the patients. Moreover, stem cell therapies are likely to be developed through very different routes than pharmaceuticals and therefore guidelines for safety and efficacy testing of pharmaceuticals and medical devices may not be suitable for regulating such stem cell-based products. Therefore to ensure that these are regulated in a proportionate, fair and adequate manner, several regulatory agencies are working together to establish an effective legislation. In particular, it is essential that countries work together to promoting good standards and importantly, a flexible approach facilitating the development of new treatments for the benefit of patients. It is therefore necessary to develop a regulatory framework capable to strike the balance between fostering research for the general benefit and the protection of the rights, well-being and security of those involved. Again, this would also be an active role of the oversight ethics and research committees as previously discussed.

Stem cell therapy is considered one of the Advanced Therapy Products (ATP), together with gene therapy and tissue engineered products. A regulatory framework is required for these ATP's to ensure patient accessibility to products and governmental assistance for their regulation and control. The guideline has to be multidisciplinary and address the

development, manufacture and quality control, as well as the preclinical and clinical development.

Legislation on cell therapy in ***Europe*** is based in three Directives: Directive 2003/63/EC (amending Directive 2001/83/EC), which defines cell therapy product as clinical products and specifies their requirements; The Clinical Trials Directive 2001/20/EC, which lays down the rules for conducting clinical trials to establish the safety, efficacy and quality of medicine products approved in the EU; and The Human Tissues and Cells Directive 2004/23/EC which establishes the standard quality, safety for the donation, harvesting, processing, preservation, storage and distribution of human tissues and cells. In addition, different levels of risk can be associated with specific types of stem cells, for example, the risk profile associated with induced pluripotent stem cells is expected to be different from those of adult stem cells for which substantial amount of clinical experience has already been gained. Therefore, a specific risk based approach according to Annex I, part IV of Dir 2001~~/b3~~/EC can be applied to such pluripotent stem cells containing medicinal products. These technical requirements stated in this Annex are included in the Regulation ~~9~~EC) (No) 1394/2007 on advanced therapy medicinal products which addresses general aspects of manipulated and genetically modified cells. The marketing authorization in Europe has to be prepared through the European Medicines Agency (EMA) which provides several guidance documents on cell-based medicinal products (Guidelines on human cell-based medicinal products EMEA/CHMP/410869/2006) which cover the general aspects of all cell-based products including stem cell advanced therapy medicinal products. In particular for stem cells that are genetically modified, of particular interest for iPSCs, see the draft for the guidelines on the quality, preclinical and clinical aspects of medicinal products containing genetically modified cells (EMEA/CHMP/GTWP/671639/2010). Recently the European Medicines Agency released a new document on drug products that are manufactured using stem cells (EMA/CAT/571134/2009). Titled, Reflection Paper on Stem Cell-Based Medicinal Products, the document advises manufacturers on quality-control issues regarding these products and provides an overview of the use of stem cells in drug development. The document applies directly to those companies pursuing marketing authorization for stem cell-based products.

In the USA, the Food and Drug Administration (FDA) has jurisdiction over the production and marketing of any stem cell-based therapy involving transplantation of human cells into patients. The FDA provides the regulatory structure regarding human cells, tissues and cellular and tissue based products, covering the wide range of stem cell-based products that may be developed for therapeutic purposes. This is subjected to the Public Health Act, Section 361, which sets the regulatory framework that

prevents the use of contaminated tissues or cells, limits the improper handling of tissues and ensures the clinical safety and efficacy of cells or tissues that are highly processed, are used for other than normal function, are combined with non-tissue components, or are used for metabolic purposes (FDA 1997). Stem cell-based products are also subjected to the Public Health Act, Section 351, which regulates the licensing of biological products to be used in studies involving humans (Halme and Kessler, 2006). This regulation envisions most, if not all, stem cell-based therapies to be considered biological products, and the manufacturer must ensure that product is "safe, pure and potent" (42 USCA § 262); the application for a new proposed stem cell-based product must provide data from preclinical studies on the likely safety and efficacy of the investigated product; and that approval and license of the product must be granted by the FDA once sufficient data demonstrates that the investigational product is safe and effective in humans. The key points of the current FDA regulation for cell therapy products include a) demonstration of clinical safety and efficacy; b) no risk for donors of transmission of infectious or genetic diseases; c) no risk for recipients of contamination or other adverse effects of cells or sample processing; d) specific and detailed determination of the type of cells forming the product and what are their exact purity and potency; e) *in vivo* safety and efficacy of the product. Moreover, specific recommendations are included regarding the use of cells or tissue that have been manipulated and may pose greater risk of disease transmission. Standardized procedures for processing and testing are required for the derivation, expansion, manipulation, banking and characterization of stem-cell products (21 C.F.R. § 610.12.). There have been concerns also about the potential use of stem cell products derived in xenogeneic feeder cells, and the FDA has established specific testing for adventitious agents according to the guidelines for xenotransplantation (U.S. Public Health Service Guideline on Infectious Disease Issues in Xenotransplantation). Another area which is becoming increasingly relevant relates to the safety concerns of cells that have undergone genetic alterations. The possibility that induced pluripotent cells could acquire genomic alterations that make them prone to transformation must be considered. New technologies are evolving to efficiently investigate genomic alterations and abnormalities. Recent studies have shown that karyotypically normal hiPSC lines possess small deletions and duplications that can be detectable by high profile genomic screening (Chin 2009). Although this screening process is fast improving and new reprogramming techniques using non-integrative techniques are being developed, it is very likely that an IPSC line that has to be expanded through culture for a long time will have some genomic changes. This is challenging for the FDA when addressing the monitoring of iPSC lines; it is very likely that their approach for approving the clinical use of these cells

Chin et al. 2009?

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may require a diligent monitoring of genomic alterations combined with all the other issues associated with transplantation (Lowry and Quan 2010).

There is still much work to be done and lots to be learned about the procedures to establish the safety and efficacy of iPSC based therapy products. The regulatory framework is very likely to evolve, as main regulatory bodies are actively trying to work in close collaboration with industry and academia to provide appropriate structure for ensuring the safety and efficacy of this new generation of stem cell-base products. Although at this stage the regulations may permit the use of xenobiotic reagents (based on their level of safety) and even viral integrative method for reprogramming cells (all under GMP grade approval), some effects of genomic instability or mutagenesis might not arise until several years of treatment. Indeed there are important hurdles to be addressed and such collaborations with the regulatory bodies are crucial towards the generation of safe iPSCs-based therapies.

## Conclusion

Despite all the technical, ethical, social and legal hurdles associated with the induced reprogramming technology and the derivation of iPSCs, research in the field is rapidly advancing toward the clinic. IPSCs hold enormous potential for fundamental stem cell research, disease modelling and clinical therapies. The generation of isogenic cells by direct genetic reprogramming with well defined factors through a standard and well defined protocol seems quite challenging, but exciting times to come are just around the corner. Recently, the field has taken an important step forward by giving the green light for two stem cell trials, one using cells derived from hESCs for the treatment of spinal cord injury and the other using adult stem cells that have been genetically engineered to be conditionally immortal for the treatment of ischemic stroke ~~(News, Nature Biotechnology 2011).~~ While it is very hard to predict what will happen with any of these therapies, it is very good to see that the fields are taking such steps forward. From this perspective, the prospects for implementing the iPS cell technology in disease modelling and patient specific cell therapies in the near future are very bright. And surely it will demand a multidisciplinary cooperation involving fundamental research, preclinical testing, clinical translation, bioethical considerations and economical policies.

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add (Mack. 2011)

It is important to address all the technical issues and challenges that are discussed to promote a responsible translation of stem cell research into safe and clinically effective stem cell- based therapies for various human disorders.

Mack GS, 2011. ReNeuron and StemCells get green light for neural stem cell trials. News , Nature Biotechnology 29, 95–97

Where more than three authors were listed in a reference entry these have been restricted to three followed by et al. as per the Editor's instructions
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