Could iPS cells be clinically useful?

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Introduction

In 2006, Takahashi and Yamanaka demonstrated, for the first time, that mouse fibroblasts can be reprogrammed into an embryonic stem cell-like state by introducing combinations of four transcription factors. These cells were termed "induced pluripotent stem cells" or "iPS cells".[1]

These experiments opened the door to the important field of cell reprogramming, so important that the journal Science considered it worthy to be chosen as its Breakthrough of the Year 2008.[2]

We recently published a review on the exciting course that led to the discovery of iPS cells.[3] In this article, we are going to refer exclusively to the possible clinical usefulness that these cells may have at present, since as Baker[4] recently commented, iPS cells are "are potentially far more useful than embryonic stem cells. They could eventually offer a method for taking cells from a patient's body, treating them, and turning them into therapeutic cells that can be returned to the same individual without the risk of rejection". Furthermore, unlike embryonic stem cells, their use has no ethical difficulty.

In this matter, we are going to refer specifically to: *1.* preclinicalexperiments conducted to date using iPS cells; *2.* the creation of cell lines from iPS cells obtained from the adult cells of patients with different diseases; and *3.* the obtaining of cloned animals from iPS cells.

Preclinical experiments

The therapeutic potential of iPS cells remains undefined; even in the field of animal experimentation it is not known whether mouse iPS cells obtained from adult fibroblasts can serve to restore physiological function of diseased tissues in vivo. However, there are some animal experiments that appear to indicate its likely clinical usefulness.

In 2007, Hanna et al.[5] were the first to conduct preclinical experiments with iPS cells. These authors, by using a humanized sickle cell anaemia mouse model showed that mice can be rescued after transplantation with haematopoietic progenitors obtained in vitro from autologous iPS cells. This was achieved after correction of the human sickle haemoglobin allele by gene-specific targeting. These results provide proof of principle for using transcription factor-induced reprogramming combined with gene and cell therapy for disease treatment in mice, while opening the possibility of using similar techniques in humans, once

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[1] TAKAHASHI K, YAMANAKA S. *Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors.* Cell 2006; 126: 663-676.

[2] VOGEL G. *Reprogramming Cells*. Science 2008; 322: 1766-1767.

[3] AZNAR J. *From stem cells to iPS cells. A passionate journey*. Studia Bioethica 2009; 2: 86-94.

[4] BAKER M. *Stem cells: Fast and furious*. Nature 2009; 458: 962-965.

[5] HANNA J, WERNIG M, MARKOULAKI S ET AL. *Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin*. Science 2007; 318: 1920-1923.

the problems associated with using retroviruses and oncogenes for reprogramming adult cells, especially adult fibroblasts, have been resolved; these are the primary difficulties in using iPS cells in human diseases.
In relation to these preclinical experiments, it seems interesting to note that they were published in Science on 21 December 2007, i.e. a few months after Wernig et al.[6] and Maherali et al.[7] confirmed Takahashi and Yamanaka's experiments on cell reprogramming in animals[8] and that these same authors had achieved this in humans,[9] which undoubtedly indicates the research power in this biomedical field.
Jaenisch et al.[10] also saw that iPS cells give rise to neuronal and glial cell types in culture. Upon transplantation into the foetal mouse brain, the cells migrated into various brain regions and differentiated into glia and neurons. Furthermore, iPS cells were induced to differentiate into dopamine neurons of midbrain character and were able to improve behaviour in a rat model of Parkinson's disease upon transplantation into the adult brain.
These results demonstrate the therapeutic potential of directly reprogrammed fibroblasts of neuronal cell replacement in the animal model and also open the possibility of being used in humans, although as previously mentioned, many important problems need to be resolved before this technique can be safely applied in man.
Another important step in the assessment of the clinical usefulness of iPS cells was when in January of the same year, 2009, Xu et al.[11] demonstrated that the haemorrhagic symptoms of haemophilic mice could be improved using these cells. Haemophilia A is caused by mutations within the factor VIII gene that lead to depleted protein production and inefficient blood clotting. Several attempts to treat haemophilic patients using gene therapy have failed for various reasons, including immune rejection.
In this paper the authors prepared murine iPS cells from tail-tip fibroblasts and differentiated them to both endothelial cells and endothelial progenitor cells using the embryoid body differentiation method. The endothelial progenitor cells derived from iPS cells secreted factor VIII. These iPS-derived cells were injected directly into the liver of irradiated haemophilia A mice. Non-transplanted haemophilia A mice died within a few hours, whereas transplanted mice survived for more than 3 months. In addition, plasma factor VIII levels increased

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[6] WERNIG M, MEISSNER A, FOREMANET R ET AL. *In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state*. Nature 2007; 448: 318-324.

[7] MAHERALI, SRIDHARAN R, XIE W ET AL. *Directly reprogrammed fobroblasts show global epigenetic remodelling and widespread tissue contribution*. Cell Stem Cell 2007; 1: 55-70.

[8] TAKAHASHI, YAMANAKA. *Induction of pluripotent stem cells…*

[9] TAKAHASI K, TANABE K, OHNUKI M ET AL. *Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors*. Cell 2007; 131: 861-872.

[10] WERNIG M, ZHAO J-P, PRUSZAK J ET AL. *Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease*. PNAS 2008; 105: 5856-5861.

[11] XU D, ALIPIO Z, FINK L ET AL. *Phenopypic correction of murine hemophilia A using an iPS cell-based therapy*. PNAS 2009; 106: 808-813.

in transplanted haemophilia A mice during this period to 8% to 12% of wild type and corrected the haemophilia A phenotype.
These very interesting experiments, in which simply using endothelial cells, in turn derived from iPS cells derived from mouse tail-tip fibroblasts, showed that the severe symptoms of haemophilia A mice can be corrected, which in turn opens the door to treat human monogenetic disorders, assuredly a great prospect.
According to our information, until January 2009 only three disease models have been treated by iPS-derived strategies,[12] but a few months ago, Nelson et al.[13] expanded the therapeutic indications of iPS cells by providing the first evidence for repair of heart disorders.
The authors demonstrated that murine fibroblasts were transduced with human stemness-related factors through an efficient vector system to generate iPS clones with inherent cardiogenic potential. iPS progeny engrafted in the context of immunocompetent allogeneic transplantation and rescued post-ischaemic myocardial structure and function.
These extremely interesting experiments open the possibility of using iPS cells for the recovery of cardiac tissue damaged after a myocardial infarction. Moreover, the fact that in these experiments the fibroblasts were reprogrammed with human stemness factors, creating animal and human clone iPS cells, raises the exciting possibility of their future use in human disease.
In short, as Carpenter et al.[14] recently noted, "although iPS cells offer exciting opportunities for stem cell therapies, many questions must be addressed before these technologies will be suitable for clinical applications". In this same respect, Yamanaka, in a recent review on iPS cells,15[15] expressed his hope in the possible clinical use of these cells, stating that "the potential of the iPS cell technology in medicine, drug discovery, toxicology and technologies is enormous…
I sincerely hope the technology will contribute to the development of new cures for people suffering from various diseases and injuries".
David Cyranoski16[16] was even more optimistic, commenting that "if the researchers are right, clinical trials on the induced pluripotent stem (iPS) cells, which can turn into virtually any cell type and potentially used to treat disorders ranging from spinal cord injury to diabetes, could start within two years".

Different disease cell lines obtained from human iPS cells

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[12] HANNA, WERNIG, MARKOULAKI ET AL. *Treatment of sickle cell…*; WERNIG, ZHAO, PRUSZAK ET AL. *Neurons derived from reprogrammed fibroblasts…*; XU, ALIPIO, FINK ET AL. *Phenopypic correction…*

[13] NELSON T, MARTINEZ FERNANDEZ A, YAMADA S ET AL. *Repair of acute myocardial infarction by human stemness factors induced pluripotent stem cells*. Circulation 2009; 120:408-416.

[14] CARPENTER M, FREY-VASCONCELLS J, RAO MS. *Developing safe therapies from human pluripotent stem cells*. Nature Biotechnology 2009; 27: 606-613.

[15] YAMANAKA S. *Ekiden to iPS cells*. Nature Medicine 2009; 15: 1145-1148.

[16] CYRANOSKI D. *Stem cell therapies closer to the clinic*. Nature News doi: 10.1038/news.2009.525.

There is no doubt that cell lines affected by a certain disease are a very useful instrument in biomedicine, since their use may provide more in-depth knowledge of those diseases.
Preimplantation genetic diagnosis is presently used to select embryos for this purpose; these are generated by in-vitro fertilisation, children of a couple who suffer from a genetic disease, preferably monogenetic, to derive the disease cell line from them.
I do not believe it is necessary to emphasise here the serious ethical problems arising from the destruction of human embryos that these techniques entail and their explicitly eugenic nature. To that end, it would appear to be of great experimental and ethical interest to have other ethically correct techniques, by which these types of cell lines can be generated, especially if they are human cell lines.
In relation to this, Park et al.,[17]showed that murine models of human congenital and acquired diseases are invaluable but provide a limited representation of human pathophysiology. Murine models do not always faithfully mimic human diseases, especially for human contiguous gene syndromes such as trisomy 21. A true murine equivalent of human trisomy 21 does not exist.
Therefore, disease-specific iPS human cells capable of differentiation into the various tissues affected could undoubtedly provide new insights into disease pathophysiology by permitting analysis in a human system under controlled conditions.
Obtaining iPS cells from adult somatic cells of patients with various pathologies was achieved for the first time by Dimos et al.,[18] from the universities of Harvard and Columbia. They were able to generate iPS cells from an 82-year-old woman diagnosed with a familiar form of amyotrophic lateral sclerosis, a neurodegenerative disorder in which motor neuron loss in the spinal cord and motor cortex leads to progressive paralysis and death, and successfully directed these cells to differentiate into motor neurons, the cell type destroyed in amyotrophic lateral sclerosis.
The patient-specific iPS cells produced in these experiments will be important tools for further studies of mechanisms by which familial disease arises.
In September the same year, Park et al.[19] published a study in which they had achieved the derivation of human iPS cell lines from patients with a range of human genetic diseases with either Mendelian or complex inheritance. These diseases included adenosine deaminase deficiency-related severe combined immunodeficiency, Shwachman-Bodian-Diamond syndrome, Gaucher disease type III, Duchenne and Becker muscular dystrophy, Parkinson's disease, Huntington's disease, juvenile onset, type 1 diabetes mellitus, Down's syndrome (trisomy 21) and the carrier state of Lesch-Nyhan syndrome.
It is not necessary to highlight the importance that obtaining cell lines from such a wide group of diseases has for their study.

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[17] PARK I H, ARORA N, HUO H ET AL. *Disease-specific induced pluripotent stem cells*. Cell 2008; 134: 877-886.

[18] DIMOS J, RODOLFO K, NIAKAN K ET AL. *Induced pluripotent stem cells generated from patients with ALS can be differentiated into motor neurons*. Science 2008; 321: 1218-1221.

[19] PARK, ARORA, HUO ET AL. *Disease-specific induced*…

These types of experiments have continued in 2009, producing new and interesting results. In fact, in January this year, Ebert et al.[20] managed to generate iPS cells from skin fibroblasts taken from a child with spinal muscular atrophy, one of the most common inherited forms of neurological disease leading to infant mortality. These cells expanded robustly in culture, maintained the disease genotype and generated motor neurons that showed selective deficits compared to those derived from the child's unaffected mother. This was the first study to show that human iPS cells can be used to model the specific pathology seen in a genetically inherited disease.

Along the same lines, in March 2009, Soldner et al.,[21] from Rudolf Jaenisch's group, reported that fibroblasts from five patients with idiopathic Parkinson's disease could be efficiently reprogrammed and subsequently differentiated into dopaminergic neurons.

Some months later, in July 2009, Raya et al., from Juan Carlos Izapisua's group,[22] took a step forward on managing to obtain healthy iPS cells from the skin fibroblasts of six patients with Fanconi anaemia. Given that Fanconi anaemia occurs due to the mutation of a single gene, it is possible, using gene therapy, to correct the defect and to generate healthy iPS cells from the fibroblasts obtained.

These cell lines appear indistinguishable from human embryonic stem cells and iPS cells from healthy individuals. However, more importantly if at all possible, is that corrected Fanconi anaemia-specific cells can give rise to haematopoietic progenitors of the myeloid and erythroid lineages that are phenotypically normal, that is disease-free. These data offer proof-of-concept that iPS cell technology can be used for the generation of disease-corrected, patient-specific cells with potential value for cell therapy applications.

Ye et al.[23] also reported derivation of iPS cells from postnatal human blood cells and the potential of these pluripotent cells for disease modelling. Indeed, multiple human iPS cell lines were generated from previously frozen cord blood or adult CD34+ cells of healthy donors, and could be re-directed to hematopoietic differentiation. Multiple iPS cell lines were also generated from peripheral blood CD34+ cells of two patients with myeloproliferative disorders.

These iPS cells provide a renewable cell source and a prospective haematopoiesis model for investigating the pathogenesis of myeloproliferative disorders.

To our knowledge, the latest experiments published to date, which describe the possibility of obtaining iPS cell lines from fibroblasts of patients with a certain disease, are those by Maehr et al.,[24] who were able to produce iPS cells from

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[20] EBERT A, YU J, ROSE F ET AL. *Induced pluripotent stem cells from a spinal muscular atrophy patient*. Nature 2009; 457: 277-280.

[21] SOLDNER F, HOCKEMEYER D, BEARD C ET AL. *Parkinson's disease patient-derived induced pluripotent stem cells free of viral reprogramming factors*". Cell 2009; 136: 964-977.

[22] RAYA A, RODRÍGUEZ I, GUENECHEA G ET AL. *Disease-çorrected haematopoietic progenitors from fanconi anaemia induced pluripotent stem cells*. Nature 2009; 460: 53-59.

[23] YE Z, ZHAN H, MALI P ET AL. *Human induced pluripotent stem cells from blood cells of healthy donors and patients with acquired blood disorders*. Blood DOI 10.1182/blood-2009-04-217406.

[24] MAEHR R, CHEN S, SNITOW M ET AL. *Generation of pluripotent stem cells from patients with type 1 diabetes*. PNAS 2009; 106: 15768-15773.

patients with type 1diabetes by reprogramming adult fibroblasts with three transcription factors (OCT4, SOX2 and KLF4). The iPS cells, termed DiPS cells by the authors, have the hallmarks of pluripotency and can be differentiated into insulin-producing cells. In their opinion, these results are a step toward using DiPS cells in type 1 diabetes disease modelling, as well as for cell replacement therapy.

There is no doubt as to the importance that the creation of these cell lines may have for furthering the knowledge of these diseases and their possible treatment. However, there are still many technical aspects to be resolved before being able to use iPS cells in regenerative medicine.[25] As Yamanaka[26] says, the potential of iPS cell technology is enormous, but this technology is still in its infancy.

Up to this point we have referred to the possible clinical applications of iPS cells and the interest that this research has, since their use does not entail the ethical problems of the use of embryonic stem cells. However, iPS cells can also be used for non-ethical purposes. We will refer to this next.

Obtaining live animals from iPS cells

Although not directly related with the immediate clinical usefulness of iPS cells, the topic addressed here, an important aspect in the assessment of these cells, which in addition has a large ethical burden, is to know if these, as well as being able to obtain various human tissue cells, a step prior to their clinical use, can also obtain germ cells that could be used to generate a living being [27] or human beings directly.

With respect to the second question, in fact, this year, live animals have been obtained from iPS cells.

To our knowledge, Kang et al.[28] were the first to demonstrate that iPS cells can autonomously generate full-term mice via tetraploid blastocyst complementation, differentiating somatic cells into iPS cells by forced expression of the four transcription factors used by Takahashi and Yamanaka.[29] However, it has been unclear whether reprogrammed iPS cells are fully pluripotent, resembling normal embryonic stem cells, as no iPS cell lines have shown the ability to autonomously generate full-term mice after injection into tetraploid blastocyts,[30] although this has been achieved by Kim et al.[31]

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[25] YAMANAKA S. *A fresh look at iPS cells*. Cell 2009; 137: 13-17.

[26] ID. *Ekiden…*

[27] *(Editorial). New sources of sex cells*. Nature 2008; 452: 913.

[28] KANG L, WANG J, ZHANG Y ET AL. *iPS cells can support full-term development of tetraploid blastocyst-complemented embryos*. Cell Stem Cell 2009; 5: 135-138.

[29] TAKAHASHI, YAMANAKA. *Induction of pluripotent stem cells…*

[30] WERNIG M, MEISSNER A, FOREMANET R ET AL. *In vitro reprogramming of fibroblasts…*; MEISSNER A, WERNIG M, JAENISCH R. *Direct reprogramming of genetically unmodified fibroblasts into pluripotent stem cells*. Nature Biotechnology 2007; 25: 1177-1181.

[31] KIM J B, ZAEHRES H, WU G ET AL. *Pluripotent stem cells induced from adult neural stem cells by reprogramming with two factors*. Nature 2008; 454: 646-650.

In this paper, the authors[32] provide a demonstration that an iPS cell line induced by the aforementioned four transcription factors can be used to generate full term mice from complement tetraploid blastocysts.

After Kang's experiments,[33] two articles were published the following month, September 2009, in the same journal, Nature.[34] In the first, Zhao et al. [35] managed to produce 31 live mice from 37 iPS cell lines generated from skin fibroblasts. Using a technique similar to Kang's,[36] they generated viable, fertile mice from which they could obtain other live-born mice, using complemented tetraploid blastocysts.

The iPS cells obtained maintain a pluripotent potential that is very close to embryonic stem cells generated from in vivo or nuclear transfer embryos. To test pluripotency, the authors randomly selected one or two cell lines from each of the experimental runs, and injected them into normal CD-1 blastocysts that were transferred to CD-1 pseudo pregnant recipient females. The mice produced have 5% to 80% chimerism.

Undoubtedly, a very interesting aspect of the experiments by Zhao et al.[37] is that from the mice produced, it was possible to obtain a second generation of live mice on mating the former with a female mouse. The mice from this second generation line continued to maintain the genetic characteristics of the first mouse which had been used to produce the iPS cells.

As previously mentioned, in the same edition of Nature, another group from the Scripps Research Institute in La Jolla, California, described how they obtained adult mice from iPS cells[38] also generated from skin fibroblasts.

In general, the technique used in the three previous studies was very similar, and consisted of obtaining blastocysts from genetically modified embryos, which only have the outer layer; blastocysts are generated which naturally lack the inner granulomatous mass, i.e. they only possess the capacity to generate the placenta. The iPS cells were injected into these modified blastocysts. They were then implanted in suitably prepared female mice. The embryos generated had the genetic characteristics of the mouse that had been used to generate the iPS cells.

Regardless of their undeniable biomedical interest, these experiments unquestionably merit a brief ethical reflection.

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[32] KANG, WANG, ZHANG ET AL. *iPS cells can support full-term development…*

[33] *Ibid.*

[34] ZHAO X, LI W, ZHUO L ET AL. *iPS cells produce viable mice through tetraploid complementation*. Nature 2009; 461: 86-90; FACKLER M. *Risk taking is in his genes*. The New York Times 11-XII-2007.

[35] ZHAO, LI, ZHUO ET AL. *iPS cells produce viable…*

[36] KANG, WANG, ZHANG ET AL. *iPS cells can support full-term development..*

[37] ZHAO, LI, ZHUO L ET AL. *iPS cells produce viable mice…*

[38] BOLAND M, HAZEN J, NAZOR K ET AL. *Adult mice generated from induced pluripotent stem cells*. Nature 2009; 461: 91-94.

There is no doubt that many scientific advances in themselves carry a negative ethical burden, for example human cloning or the use of embryonic stem cells since, as is known, a human embryo must be destroyed to obtain them.
Other techniques, however, are ethically neutral in themselves and others have been developed for a fundamentally ethical reason.[39]
This is what has happened with iPS cells, since using them to prevent the use of embryonic stem cells cannot have anything other than a positive ethical evaluation. However, using them to produce cloned human beings, if this becomes technically feasible, would not be ethically admissible. Therefore, in most cases, the moral judgement that a scientific advance merits will be a consequence of the purpose for which it is used. In other words, the ethical opinion of any experimental advance will depend on the use that the investigators and society make of it. The ethics of scientific advances are without question in the hands of their users.
This leads us to consider that the most important thing to safeguard the ethics of the use of scientific advances is the proper ethical training of the investigators and in the end, of the individuals who may use those advances. This is in keeping with that stated by Benedict XVI in his encyclical Spe Salvi,[40] in reference to human progress, which says: "the ambiguity of progress becomes evident.
Without doubt, it offers new possibilities for good, but it also opens up appalling possibilities for evil, possibilities that formerly did not exist. We have all witnessed the way in which progress, in the wrong hands, can become, and has indeed become, a terrifying progress in evil. If technical progress is not matched by corresponding progress in man's ethical formation, in man's inner growth, then it is not progress at all but a threat for man and for the world", a reflection which without making the slightest modification can be applied to scientific research and of course, to research with iPS cells.

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[39] Indeed, as Yamanaka himself said in an interview published in the New York Times (FACKLER. *Risk taking*…), after a colleague invited him to look at a human embryo down a microscope, "When I saw the embryo I suddenly realised there was such a small difference between it and my daughters. I thought, we can't keep destroying embryos for our research. There must be another way". The Japanese researcher reaffirmed this ethical criterion recently when he declared that "to overcome two major hurdles of human embryonic stem cells, ethical issues regarding the use of human embryos and immune rejection alter transplantation, I decided that nuclear reprogramming would be the goal" (YAMANAKA S. *Ekiden to iPS cells*. Nature Medicine 2009; 15: 1145-1148).

[40] BENEDETTO XVI. *Lettera Enciclica "Spe Salvi"* (30.11.2007).