Cell Proliferation

*Cell Prolif.*, 2011, **44** (Suppl. 1), 70–84 doi: 10.1111/j.1365-2184.2010.00723.x

# From embryonic stem cells to iPS – an ethical perspective

J. Suaudeau

*Pontifical Academy for Life, Rome, Italy*

*Received* 26 *June* 2010; *revision accepted* 19 *July* 2010

## Introduction

Ethical debate on development of stem cell research focused from the outset on issues of methods of acquiring these cells. Human embryonic stem cells (hES) proved particularly promising for development of future regenerative medicine on which all hopes have been pinned. At the same time, obtaining the cells required sacrifice of already well-developed preimplantation human embryos, which would otherwise have given rise to healthy newborn infants if they had been naturally implanted *in utero*. Some researchers opted for hES cells as opposed to whole embryos, and some preferred to look for other, less problematic, types of stem cell. However, many pursued both avenues of investigation at one and the same time, that is, on the one hand viewing hES cells as the 'gold standard' to which everyone had to refer in any case, and on the other hand hoping to manage to obtain these cells, or their equivalent, in ways that showed more respect for human embryos and was accepted by all.

Thus, between human embryonic stem cell research and research into non-embryonic stem cells, from the adult organism or from the umbilical cord for example, a third avenue of stem cell research has been opened – alternative proposals, which seek to develop methods that are both biologically advisable and ethically acceptable for collection of pluripotent embryonic or embryonic-like stem cells.

Various proposals in particular include:

- alternative biologically oriented proposals involving destruction of human embryos;
- alternative ethically oriented proposals, based on use of defective preimplantation human embryos;
- alternative ethically oriented proposals not involving the destruction of human embryos.

Correspondence: J. Suaudeau, Pontificia Accademia per la Vita, 1 via della Conciliazione, Roma 00193, Italia. Tel.: (0039)(0)6 69 88 24 23; Fax: (0039)(0)6 69 88 20 14; E-mail: jsuaudeau@pav.va

## Alternative proposals involving embryonic destruction

Development of embryo cloning by somatic cell nuclear transfer into enucleated oocytes (SCNT) (1) provided researchers working in the stem cell field with an avenue of research leading to prevention of hES cells being rejected due to histoincompatibility, if they were going to be applied to patients. Two successive proposals were suggested to forestall this possible rejection, both using SCNT:

(a) – 'therapeutic cloning' or 'research cloning'.

(b) – creation of human cytoplasmic hybrid embryos (cybrids).

### *(a) Therapeutic cloning to produce immunocompatible human ES cells*

Therapeutic cloning, better termed 'research cloning', could provide a solution to the obstacle of immunological rejection, one of the factors limiting clinical application of ES cell technology (2). Nuclear transfer could procure human ES cells that would be immunologically compatible with the patient they were intended for, given that these cells would be obtained from embryos created by nuclear transfer of one of the patient's own somatic cells into an enucleated human oocyte (3). However, this proposal immediately encountered serious biological and ethical difficulties.

From biological standpoints, the nuclear transfer technique, even limited simply to production of blastocysts from which to obtain stem cells, has produced positive, consistent results in only some species (mice, cattle, rabbits). In primates, the Oregon National Primate Research Center team, after years of not very fruitful work in this field, managed to obtain two ES cell lines from 35 blastocysts of rhesus macaque created by SCNT from 213 prepared embryos (4), then, more recently, two other ES cell lines from six blastocysts created by SCNT from 71 prepared embryos (5). In humans, therapeutic cloning, despite repeated efforts and some false announcements (6), has

still not produced any consistent results. In May 2005, Stojkovic *et al.* (7) broke the news of creation of the first cloned human embryo in the United Kingdom, but they had not extracted stem cells from it. More recently (January 2008), a group of researchers at the Stemagen Corporation, La Jolla California (8) reported creation by cloning of 14 human embryos, derived from 29 oocytes. Five of these were said to have been able to develop to the blastocyst stage, but the authors had not tried to extract stem cells from them. More recently, Li *et al.* (9), Shandong, China, also reported creation of human blastocysts by SCNT (five out of 26 prepared embryos, produced from 135 oocytes from 12 donors), but again did not seek to derive hES cells from them. Therapeutic cloning in humans is therefore still a costly undertaking, severely limited by number of human oocytes it requires (10,11).

This procedure encounters two types of ethical objection (12). The first, which applies to all technologies that use embryonic stem cells, is related to the way in which they depend on destruction of incipient human life. The second type of objection is more specific to therapeutic cloning; embryonic stem cells, can be used from frozen embryos remaining after *in vitro* artificial fertilization procedures, while therapeutic cloning requires deliberate creation of early-stage human embryos, followed by disaggregation. Its ethical acceptability thus depends on degree of moral value the preimplantation embryo is recognized to possess (13). That some people refuse to call such embryos created by SCNT to be true embryos, use instead other expressions such as 'cells epigenetically reprogrammed by SCNT in oocytes with no spindle' (4), or, more simply 'clonotes' (14,15). This takes nothing away from their reality as human embryos.

Even if therapeutic cloning were to become more efficient and produce consistent results in humans, it will always be morally illicit as it makes a deliberate choice against life of a human being in favour of a possible health benefit to other individuals. This is a high point in 'commoditization' of human life, in use of that human life for specific interests. Such action would be contrary to ethical principles expressed by Emanuel Kant, according to which an individual human being should not be thought of solely as a means, but always as an end (16). In his reply to John Harris, who argued that human beings were often used as a 'means' in medical practice (for example when they provide blood for transfusions, or when they donate tissues or organs for transplants), Kahn replied that what was contrary to human dignity in therapeutic cloning was not simply that cloned embryos created in this way are used as a 'means', but that they are used 'exclusively as a means' (17).

Moreover, with this option, the good effect (health benefit for the patient) is only a remote possibility, with as yet no tangible guarantee, while the bad effect, destruction of a human life at its beginning, is very real. This shocking act does not come in association with the beneficial act, or as a side effect not sought as such, but is the basic condition for the good effect sought. This excludes application of the principle of double effect (indirectly voluntary) to therapeutic cloning.

### *(b) The human cytoplasmic hybrid embryo (cybrid) proposal*

Because of the difficulty in collecting sufficient numbers of human oocytes to be able to create human embryos by cloning, and to extract stem cells from them, some have suggested using the interspecies somatic cell nuclear transfer (iSNCT) technique that seeks to create cloned embryos by transfer of a human somatic cell nucleus into an enucleated animal oocyte (18). The dervivatives would be cytoplasmic hybrid embryos – cybrids (19) – with human nuclear DNA and animal cytoplasm containing animal mitochondrial DNA. This technique has been attempted to be used primarily to ensure survival of certain endangered species, but it has had very little success. The only positive result of all such attempts has been birth of the so-called gaur/cow hybrid given the name Noe (20).

Despite these mediocre results, iSCNT has been seen as a possible solution to problems posed by difficulty in recruiting human oocytes for therapeutic cloning. The idea would be to use enucleated bovine oocytes as host and activator of human somatic cell nuclei. Human–animal cytoplasmic hybrid embryos would thus be created (99.9% human due to the nuclear DNA, 0.1% animal due to the mitochondrial DNA), from which human-like embryonic stem cells could be extracted, once these embryos have developed to the blastocyst stage. These ES cells would be genetically human, and could therefore be used therapeutically, without the risk of rejection.

The Advanced Cell Technologies, Massachusetts, team, led by Robert P. Lanza, Jose B. Cibelli and Michael D. West, was the first to study this possibility. In 1999, these authors reported that they had created embryos by nuclear transfer of human somatic cells into enucleated cow oocytes (21). Twenty-six per cent of these embryos ($n = 6$) were said to have been able to develop to the 4- to 16-cell stage and only one of them to have reached the 400-cell stage. Success of this operation was thus more than limited. In December 2003, K. H. Chang and coworkers of Seoul National University, South Korea (22), reported creation, using the same technique, of human/cow hybrid embryos, with modest results: out of 286

embryos prepared in this way, only four were able to develop to morula or blastocyst stage, and there was no mention of ES cell extraction from these embryos. The same authors published a follow-up to this study in October 2004 (23). Of 194 human/cow hybrid embryos created by SCNT, only two reached the blastocyst stage. Finally, in April 2006, Illmensee *et al.* (24) reported creation of 37 human/cow hybrid embryos, seven of which were said to have reached the blastocyst stage, but no ES cells could be extracted from the blastocysts. These authors, admitting the paucity of their results, explained the problem as being due to aberrant reprogramming of embryos created in that way.

Despite this repeated lack of success, the British Human Fertilisation and Embryology Authority (HFEA) declared on 5 September 2007 that it was in favour of creation of such cybrids, declaring them to be necessary and desirable in both scientific and ethical terms (25,26).

Pointless and with no future from a biological point of view, creation of cybrids is, however, a serious matter from an ethical point of view. Even if the individual resulting from this transfer into a bovine oocyte is genetically 99% human, the process itself does not respect humankind, of which the cybrid is a part, as a member of the human family (Universal Declaration of Human Rights, Preamble).

## Alternative proposals to obtain hES-like cells from embryos with no development potential

The second line of response to problems connected with obtaining hES cells is different from the first in that what is primarily sought here is not a solution to the biological problems posed by hES, but development of ethically acceptable methods to enable hES cells to be collected without viable embryos having to be destroyed.

Four proposals for acceptable alternative methods of obtaining hES cells have thus been put forward:

- H. A. Zucker and D. W. Landry proposal use of poor-quality embryos rejected by IVF centres;
- proposal to use parthenogenetically created embryos;
- proposal to use created embryos that are defective and unable to implant: altered nuclear transfer, OAR.

### *(a) Use of poor-quality embryos*

The first proposal, presented by Landry and Zucker (27), is to use hES cells from human embryos created in IVF centres and discarded due to their poor morphology – suggesting most potential subsequent embryonic death. Such embryos still contain live cells that are a potential source of hES cells, as shown by Byrne *et al.* (28). These authors proposed using the nuclear transfer cloning technique to create human embryos that were probably non-viable but could serve as hES cell donors (29). In 2003, Mitalipova *et al.* (30), University of Georgia, reported that they had been able to derive hES cells from surplus human embryos from IVF clinics that were offered for research because of their lack of development and morphological anomalies, and that they had been able to obtain four hES cell lines from such embryos. In October 2003, it was the turn of Pickering *et al.* (31) at King's College, London to report obtaining three hES cell lines from 58 human embryos created by IVF, subjected to preimplantation diagnosis, and judged to be defective and unfit for implantation. Chen *et al.* (32), University of Huazhong, Wuhan, China, also reported derivation of two hES cell lines from 130 embryos created by IVF but eliminated because of their poor quality. Zhang *et al.* (33) at the Centre for Stem Cell Biology and Developmental Genetics of the University of Newcastle (UK) found that arrested embryos, which had not succeeded in reaching morula or blastocyst stage, and which were considered dead from the IVF point of view, still had viable blastomeres capable of proliferation in culture, and from which hES cells could be derived. Finally, Lerou *et al.* (34) Harvard Medical School, Boston, USA showed that there was a possibility, albeit very low (0.6%), of deriving hES cells from 3-day embryos considered to be of poor quality, and with low probability of uterine implantation. hES cell derivation efficiency was clearly better (4.1%) when these poor-quality embryos had reached 5 days development, and was 8.5% when they had become blastocysts. This group therefore warned against the idea of being able to collect hES cells from 3-day embryos, and also emphasized that embryos considered to be of poor quality, but that had reached the blastocyst stage, could be considered a reliable source of good-quality hES cells.

From the biological standpoint, this proposal does not offer a real solution to the problem of limited numbers of human embryos available for obtaining hES cells, because to be effective, it requires blastocysts, and most arrested embryos or those presenting morphological changes did not reach this stage of development. Nor does it solve the problem of immunological rejection of hES cells, if these cells were to be used clinically.

The idea of using embryos facing certain death, as a source of hES cells, might be defended from an ethical standpoint based on the analogy of taking organs from brain-dead patients. However, to be able to justify taking the inner cell mass of these embryos, there would have to be certainty about their state of embryonic death. However, at present, there are no reliable, early criteria for declaring an embryo to be dead. Exposure to a period of 24 h observation to establish diagnosis of embryonic

death, solely based on absence of cell division during this period is judged by many to be inadequate (35). Under these circumstances, use of apparently non-viable human embryos to obtain hES cells does not look acceptable from an ethical standpoint, as there is no certainty about their imminent death.

### *(b) Parthenogenesis*

Parthenogenesis is the process by which a new individual develops from a non-fertilized oocyte. It can be induced in mammals by artificial, chemical or electrical stimulation of an oocyte, which then becomes a zygote, and develops to form an embryo that only has the genetic programme of the mother and is called a parthenote. In primates, embryos created in this way cannot develop correctly and are generally lost before they can implant (36,37), probably because of a lack of expression of the paternal imprinted genes. Recourse to parthenogenesis has proposed (38,39) as a way of obtaining immunocompatible hES cells from a female donor. Moreover, this approach would be ethically acceptable, as it does not lead to destroying normal human embryos. These authors had derived embryonic-like stem cells from four monkey (*Macaca fascicularis*) embryos at the blastocyst stage, obtained through development of metaphase II oocytes that were not fertilized but activated by a calcium wave, *ionomycin* [according to the process described by Mitalipov *et al.* (40)]. These ES cells were capable of multiplying *in vitro* for more than 10 months (41). Lin *et al.* (42) derived pluripotent stem cells from human blastocysts obtained by chemical stimulation of non-fertilized metaphase II oocytes, and showed that these cells had epigenetic, cellular and differentiation characteristics comparable with those of hES cells derived from fertilized oocytes. These results have since been confirmed by various other teams (43–45). Revazova *et al.* (46,47) derived HLA homozygous hES cell lines from parthenogenetically created human embryos, and found that ability of these cells to differentiate was identical to that of hES (48). These cells proved to be able to differentiate into myogenic, osteogenic, adipogenic and endothelial cells lines, and to form muscle- and bone-like tissues *in vivo*. They integrated into damaged muscular tissue (49). Parthenogenetic dopamine neurones derived from parthenogenetic primate hES cell lines and transplanted into the right striatum of rats made them hemiparkinsonian, and treating them with 6-hydroxydopamine restored motor function of these animals (50).

From the biological standpoint, these studies indicate that stem cells harvested in parthenote embryos have characteristics similar to those of hES cells collected from viable embryos created by fertilization. Moreover, these cells are histocompatible with the oocyte donor, which to a certain extent solves the issue of immunological rejection. However, the parthenogenetic origin of these cells gives us reason to think of abnormal expression of imprinted genes (51), casting some doubt on safety of using such cells if they were to be transplanted into patients. However, it appears that these anomalies could be corrected to a certain extent in pluripotent cells compared to parthenogenetic embryos from which they came (52). Jiang *et al.* (53) found an expression of certain paternal imprinted genes in ES cells derived from parthenogenetic embryos, and this expression increased with culture time. Thus, with cells derived from partheogenetically created embryos, we would have pluripotent cells of quality equal to that of ES cells, which also would be histocompatible with the recipient when the parthenogenetically activated oocyte came from that same person.

However, it is specially from an ethical standpoint that attempts have been made to underline interest in parthenogenesis, compared to fertilization, as a method of obtaining hES cells. Authors of these studies on obtaining parthenogenetic stem cells consider that derivation of these embryonic-like pluripotent cells from non-fertilized human oocytes activated to parthenotes would be ethically acceptable, as the product of this activation, the parthenogenetically created embryo, should not be regarded and treated as a real embryo. In their view this parthenote, incapable of developing beyond the blastocyst stage, with no future potential, should be considered potentially dead, an apparent organism breaking down, and treated as such. This opinion seems questionable however, as these activated human oocytes behave exactly like normal embryos until their epigenetic imbalance curbs their development and stops them implanting *in utero* (54).

### *(c) Altered nuclear transfer*

The proposal presented by William Hurlbut (55–57), Stanford University, Program in Human Biology entitled Altered Nuclear Transfer (ANT) aims to create by cloning (SCNT), an altered human embryo (58), that is to say, an embryo incapable of implanting or developing after implantation, which could become a morally licit source of hES cells. This embryo would have been created with a genetic defect preventing it from implanting, but could provide good quality embryonic stem cells. Hurlbut's plan is to create embryos deficient in the *cdx2* gene needed for trophoblast individuation by RNA interference (59,60). *cdx2*-deficient blastocysts are unable to implant but develop an inner cell mass from which embryonic stem cells can be derived. Once these cells have been obtained, they would have their *cdx2* gene re-expressed, which would turn them into normal pluripotent hES cells. According to Hurlbut, the biological result of altered

nuclear transfer with *cdx2* deficiency would not be an embryo, as it would not have the ability to develop, rather a group of cloned cells, comparable with a teratoma, the tumour that forms from embryos arrested in development, a disorganized mass with no future. Hurlbut thinks that it would be morally licit to destroy such altered embryos to collect their inner cell mass (61).

Meissner and Jaenisch (62) showed that it was effectively possible in mouse, to neutralize *cdx2* gene in mouse fibroblasts and use these cells to create (through SCNT) a *cdx2*-deficient embryo, unable to implant, but from which it was possible to derive ES cells, to which a normal *cdx2* function could be restored afterwards by transferring the gene. However, this idea of Hurlbut, which Jaenisch showed to be a real biological possibility, is not without its weak points, scientifically and ethically (63).

- From a scientific point of view, it is not known what might happen to human embryos if they were made *cdx2*-deficient, given that this is a homeobox gene, of the Hox essential gene family (64), which plays a number of important roles in embryonic development.
- From an ethical standpoint, comparison between a defective embryo that does not implant and a teratoma is not correct. The teratoma is a cell formation that has no organization, no internal drive to develop, and is therefore not an organism or a biological individual. The *cdx2*-deficient embryo behaves like an organism with a development plan, through to the blastocyst stage. That it is unable to implant takes nothing away from its quality as a biological individual. A defective embryo, unable to develop, remains an embryo until dissolution of its organic unity.
- Again from an ethical standpoint, the proposal to manipulate the genome of human embryos created by cloning to make them incapable of implantation is perplexing. That many embryos produced through sexual relations have chromosomal or genetic anomalies that prevent them from developing properly or implanting does not justify deliberate creation of such anomalies. We do not have the right to do something bad, with a view to a potential good, and silencing *cdx2* cannot be considered good for the embryo concerned. On this point, Turnpenny (65) asks whether, from an ethical standpoint, intentionally downgrading the moral status of human embryos, in order to render them suitable for research that was otherwise deemed immoral, would be dissimulation.

### *(d) Oocyte-assisted reprogramming*

Grompe and George took up William Hurlbut's idea of ANT and modified it (66). Their idea is to make *Nanog* gene overexpress, a central requirement for acquisition of pluripotency by preimplantation embryo cells (67). Grompe thinks that by artificially inducing *Nanog* to overproduce in the somatic cell nucleus, that is to be transferred to an oocyte so as to produce a cloned embryo, embryos resulting from such transfer would not be real embryos rather groups of pluripotent cells, from which hES cells could be derived without troubling the conscience.

Grompe's proposal calls up some reservations:

- from a biological standpoint, the scientific basis on which Grompe builds his project is very uncertain. *Nanog* overexpression in the zygote may cause cells of the embryo at segmentation phase to become pluripotent too soon, compromising survival of the embryo. However, this has not been proved, specially as *Nanog* does not act alone and requires combined expression of at least *Oct4*, *SOX2* and *STAT3* (68).
- from an ethical standpoint, Grompe's proposed operation necessarily involves creation of an embryo by SCNT; extracting hES, if this is possible, presupposes prior destruction of the embryo. Here we find the same objections as those directed earlier at Hurlbut's ANT proposal.

### *(e) Summary: moral status of defective embryos*

Hurlbut's ANT project, Grompe's OAR and the proposal to take advantage of parthenote embryos have in common the idea of creating an abnormal, defective human embryo, unable to implant, which could be considered, as a result, simply as a biological artefact, while still being capable of delivering good-quality pluripotent cells to researchers.

A criticism of Hurlbut's project, which moreover applies to all other protocols in which there is a question of creating and using embryos without development potential, was presented by Melton, Daley and Jennings, in the *New England Journal of Medicine*, 30 December 2004 (63). These authors point out that reversible silencing of the *cdx2* gene to create an embryo with no trophoblast and unable to implant, is not ethically so different from exporting inner cell mass blastocysts to harvest embryonic stem cells. Silencing this gene, they say, destroys. ahead of time, the embryo one has created (like someone who leaves a bomb ready to explode in the hold of an aircraft about to depart). Moreover, these scientists add, Hurlbut's argument about the supposed ethical superiority of his proposal compared to conventional extraction of hES from the inner cell mass of blastocysts, is misleading. Hurlbut judges acceptable, the fact of destroying an embryo that carries the *cdx2* mutation, while judg-

ing unacceptable to destroy a normal embryo, because the former, says Hurlbut, has no inherent principle of unity, would have no coherent drive in the direction of mature human form. However, such an opinion is based on an error of judgement, as at the start, the *cdx2*-deficient embryo still has the principle of unity and of coherent development conferred on it by its genome. Melton, Daley and Jennings conclude their critique quite rightly by saying that nor do they see why expression or not of *cdx2* should be seen as a reference point in order to work back to a judgement about the embryo and its moral status. On this point, Byrnes (69) stresses that an embryo whose *cdx2* gene has been silenced by RNA interference for example, was probably developing well at least up to the 16- to 32-cell stage, the time when the gene normally begins to be expressed. This embryo would be normal at that stage, in the same way as a person is normal up to the moment when a genetic defect in his genome makes Huntington's disease, for example, appear in his phenotype.

## Alternative proposals that do not involve the destruction of embryos

Alongside these alternative proposals on obtaining hES cells from viable blastocysts, which all involve use of cloned or defective human embryos, and which do not therefore really solve the ethical problem associated with obtaining hES cells, other proposals have been made to obtain hES cells without having to destroy human embryos. These are:

- using germ stem cells;
- using blastomeres harvested by embryo biopsy; and
- reprogramming somatic cells to embryonic-like cells.

### *(a) Embryonic germ cells*

Embryonic germ cells (EGCs), are derived from foetal primordial germ cells (70). They are the functional equivalent of embryonic stem cells, in terms of their capacity for *in vitro* proliferation and broad cell differentiation. However, they are more difficult to harvest and tend to differentiate spontaneously in culture (71). Use of these cells for regenerative therapy, in patients, is theoretically possible, but has never been tried. From an ethical standpoint, it would involve harvesting germ cells from aborted human foetuses (5–9 weeks after fertilization), which poses ethical problems of the link between projected therapy (or study) and abortion of the foetus from which the cells were to be taken. This would only be morally acceptable if there was a proportional therapeutic interest in these cells, if there were no other therapeutic tactic of equal value that could be used to combat the subject's pathology and if it was possible rigorously to make a separation of time, space and surgeons between the act of abortion and the recovery of foetal tissue in order to extract germ stem cells.

### *(b) ES cells derived from a single blastomere collected by embryo biopsy*

A further proposal to obtain hES-like cells without embryo destruction is to remove by biopsy one blastomere from an embryo in the segmentation phase. This would be cultured and would multiply to produce hES cells. For example, Takeuchi and Bahia, Center for Reproductive Medicine and Infertility, Weill Medical College, Cornell University, New York, reported at the 21st Annual Conference of the European Society of Human Reproduction (June 2005), that they had obtained nine ES cell lines from 46 blastomeres obtained by biopsy from mouse blastocysts (72). This report was followed in July 2005 by the publication by Sills *et al.* (73) on obtaining ES cells from mouse blastomeres collected by biopsy from four- to six-cell embryos. In 2007, Wakayama *et al.* (74) showed that ES cell lines could be derived from blastomeres of early-stage mouse embryos; very first stages of cleavage, two-cell embryo blastomeres having the highest success rate. This group had established 112 ES cell lines, with a 50–68% success rate for two-cell embryo blastomeres, 28–40% for four-cell embryo blastomeres and 14–16% for the eight-cell embryo blastomeres.

The idea was taken up by Lanza and co-workers at Advanced Cell Technology, Worcester, Massachusetts, USA, who proposed this method of obtaining hES cells without having to destroy human embryos. Initially (October 2005) (80), these authors showed that it was possible to obtain hES cell lines from a single blastomere taken from a seven-cell mouse embryo. In a second stage (August 2006) (80), they reported creation of two hES cell lines from blastomeres obtained by biopsying human embryos (one blastomere per embryo) supplied by an IVF centre, which had not been used and were destined for research (75). However, the procedure proved to be very inefficient, as only 2% of the blastomeres collected in this way had given rise to hES cell lines. In 2008, the same authors published a follow-up to this report, with improved results (76), as 29 out of 32 cell aggregates derived from blastomeres collected by biopsy had proliferated and generated hES-like cells in 20–40% of cases (groups 1 and 2). In the group in which the blastomeres had been cultured on a medium with laminin and fibronectin, three blastomeres out of 15 had generated stable hES cell lines. Geens *et al.* (77), (Brussels, Belgium) reported derivation of two hES cell lines from blastomeres from two hES cell lines from blastomeres collected from

embryos at the four cell stage77 (success rate: 12.5%). These two lines came from two different embryos. Other work has not only confirmed these results, but also shown the inefficiency of the process, as very few human blastomeres, after being removed by biopsy, have proved to be capable of cell division *in vitro* to produce hES. These studies also reported frequent chromosomal and genetic anomalies affecting the hES cells in these lines (78,79).

From an ethical standpoint, Lanza, Chung and Klimanskaya stressed the moral aspect of their enterprise when they presented blastomere biopsy for the derivation of hES cells (80). They expressed that what they had done, for the first time was to create human embryonic stem cells without destroying the embryo itself (81). This declaration caused great excitement at the time in the world's media, especially in Germany where the law prevented scientists from working on new hES cell lines derived from blastocysts. But the presentation was misleading as it gave the impression that the authors had simply biopsied human embryos, that they then left to develop, when their work was in fact based on the taking apart of these embryos to use all the blastomeres available. These statements left the false impression that embryo biopsy to obtain a blastomere that is a source of a stem cell line was relatively easy, could be done as part of a preimplantation diagnosis, and would not harm the embryo. The reality is very different; most often, blastomeres taken from preimplantation embryos do not develop *in vitro*, and to achieve some success, all blastomeres available in two- to four-cell embryos had to be taken, implying fact destruction of the embryo. There is therefore no question, at least for the moment, of such a technique being introduced as part of a preimplantation diagnosis, for example, where the biopsied embryo could have been allowed to live. Blastomere biopsy therefore does not solve the ethical problem of hES cell harvesting at all.

### *(c) Somatic cell reprogramming*

The third proposal for obtaining embryonic-like stem cells without having to destroy human embryos is reprogramming, inspired by results of nuclear transfer cloning. Surani *et al.*, Cambridge, UK, had expressed the principle in November 2001.

The epigenetic reprogramming by hybridization (82) approach proposes to use ability of human ES cells to reprogramme somatic stem cells to pluripotency. This would be performed by fusion of a somatic cell and an ES cell (83). The result of such reprogramming would be tetraploid cells with capabilities comparable to those of ES (84). The promoters of this approach, Eggan and co-workers, indicate that this somatic stem cell reprogramming should not raise ethical difficulties as the new stem cell lines obtained in this way would not require use of embryos or oocytes for their creation (85). However, from an ethical standpoint, this method presupposes the use of an ES cell line, and as a result implies illicit cooperation in the unacceptable act of collecting these ES cells from human embryos. It would also be morally illicit to wish to use the product of this hybridization, that is tetraploid cells, on patients, given the uncertainties connected with presence of such cells in the organism (may be a possible risk of cancer).

A further approach was to seek to individuate factors in the cytoplasm of reprogrammed cells (oocytes or ES cells) that induce reprogramming of somatic cell nuclei during nuclear transfer cloning. The possibility of dedifferentiating nuclei of the specialized cells to make them return to an undifferentiated state has been demonstrated for many types of cells (86). This kind of dedifferentiation happens spontaneously in simple organisms, such as the salamander, which can regenerate a lost limb (87) and has been successfully carried out on some human somatic cells, such as myotubes (88,89), myogenic cells (mediated by the purine reversine) (90–92), myoblasts (mediated by CNTF, *ciliary neurotrophic factor*) (93) and fibroblasts (mediated by incubation in an extract of T-cell cytoplasm) (94). From this research, it was clear that to obtain reprogramming of a somatic cell nucleus without involving an enucleated oocyte, factors in the cytoplasm of ES cells that can convert a differentiated cell to a pluripotent embryonic-like cell, thanks to transient expression of specific genes, had to be individuated (95,96).

The solution to the problem of reprogramming somatic cells without using nuclear transfer to an enucleated oocytes was presented in July 2006 by Yamanaka and colleagues, University of Kyoto, Japan (97–99) at the Congress of the International Society for Stem Cell Research (ISSCR) in Toronto. Using a technique of transferring some of the genes responsible for pluripotency of stem cells (*Oct4*, *Sox2*, *c-Myc* and *Klf4)*, Yamanaka *et al.* had been able to change phenotypically normal, somatic skin-differentiated cells (fibroblasts) of mouse into undifferentiated ES-like cells, called induced pluripotent stem cells (iPS), by their creator. Takahashi and Yamanaka published their results on 25 August 2006, in the journal '*Cell*' (100). One year later, Takahashi *et al.* (101), with the same team published identical cell reprogramming results, but this time from human fibroblasts. The scientific world realized the importance of this work, and its value, when these results were confirmed by three different teams of researchers, in Japan (102) and in the United States (103,104). With the help of cell selection effected by inserting an antibiotic resistance gene into the cells as they were reprogrammed, these authors were able to isolate the small number of reprogrammed cells and then

multiply them electively. At the end of 2007, Yu and colleagues (University of Wisconsin-Madison) published their own cell reprogramming results (105), which agreed with those of theYamanaka team. Demonstration by these various authors that somatic cells can be reprogrammed into iPS was greeted as the most important scientific breakthrough of 2008 (106).

## IPS cell new deal

*(a) Cells comparable with ES cells*

Human iPS cells (hiPS), as produced by Yamanaka *et al.*, Yu *et al.*, Thomson *et al.*, and Park *et al.* (107) and Lowry *et al.* (108), from human fibroblasts, present all the characteristics of embryonic stem cells with regard to morphology, self-renewal, and abundant, stable and unlimited *in vitro* proliferative capabilities. From the genetic point of view, these cells provide expression of pluripotency-related transcription factors, which are the characteristic and specific hallmarks of ES cells (presence of 27 specific epigenetic marker genes of ES cells, and signature of the ES state, specially *Nanog*, *Oct3/4*, *Sox2*, *Cripto* and *GDF3*). ES-specific surface antigens are found on iPS. By restoring pluripotency of these fibroblasts, reprogramming resets their biological age to its starting point, which can be seen in an activation of telomerase, causing lengthening of the telomeres (109). iPS are capable of producing such derived cells *in vitro*, which can represent all three embryonic germ layers, and can subsequently produce all cell types of the organism, including germ cells (102). Like ES cells, iPS cells form embryoid bodies *in vitro* and develop into teratomas when injected subcutaneously into laboratory mice. When iPS cells are introduced into embryos at the blastocyst stage, they take part, as do ES cells, in development of the three primitive embryonic germ layers, producing chimaerical embryos in which descendants of injected iPS cells, which originated in these three germ layers, are of all cell types, including gametes (110). The property of ES cells of being able to generate a whole animal when aggregated into tetraploid embryos (embryos incapable of developing by themselves), had not until recently been found to be the case with iPS cells. This has now been achieved as indicated in international publication of the work of a Chinese team (111) which, through tetraploid complementation, was able to obtain a mouse to developed from iPS cells (Xiao...).

*(b) Critique*

One of the main criticisms of Yamanaka and his initial work on iPS cells concerned inefficiency of the cell reprogramming process he had developed. Indeed, fewer than 0.1% of the skin fibroblasts that Yamanaka had treated in this way demonstrated effective reprogramming. This obstacle was overcome by transferring a neomycin-resistant gene into the cells to be reprogrammed, which enabled the effectively reprogrammed cells to be selected and subsequently multiplied in culture. However, this procedure could not be used if iPS were to be applied to patients. Meissner *et al.* (112) showed that it was not necessary to resort to such a procedure and that it was possible to isolate iPS based on simple morphological criteria. Various strategies have since been developed to increase the efficiency of cell reprogramming (113), such as addition of SV40LT and hTERT (114), inhibition of DNA methyltransferase (DNMT) (115) or silencing of p53 and introduction of UTF1, into human fibroblasts to be reprogrammed, multiplying by 100-fold the reprogramming efficiency (116). A further way to improve reprogramming efficiency would be to resort to embryonic stem cell-specific microRNAs. These, *mir*-291, 294, 295, increase reprogramming efficiency by *Oct4*, *Sox2* and *Klf4* and can replace *c-myc* in this respect (117).

Another debate opened by Yamanaka's initial work concerned use of *c-myc* transgene for cell reprogramming. This oncogene causes cancers in host animals (101) and Blelloch *et al.* (118) showed it was possible in the Yamanaka protocol, to substitute *n-myc* gene for *c-myc* oncogene, without adversely affecting reprogramming efficiency. Thomson and co-workers have successfully used other reprogramming factors than those chosen by Yamanaka and colleagues, specially *Nanog* and *Lin28,* without using *c-myc* oncogene and *Klf4* gene (105). Nakagawa *et al.*, of theYamanaka team, published a study in November 2007 (119) showing that it was possible to reprogramme somatic cells according to their own protocol, without resorting to *c-Myc* oncogene. However, the process was less efficient, as was also found by Wernig *et al.* (120).

A third point of interest from the work of Yamanaka and colleagues concerned use of multiple retroviral vectors – one for every gene transferred – to reprogramme skin fibroblasts by pluripotency gene transfer. Indeed, since 1999 and the death of Jesse Gelsinger (121), these vectors have had a bad press, and more recent findings of insertional mutagenesis leukaemias caused by retroviral vectors used in Necker Hospital, Paris, France, to treat children suffering from severe immunodeficiency (SCID-X) with gene therapy, did nothing to improve this verdict (122). This implied that Yamanaka *et al.*'s use of these multiple viral vectors would cause patients receiving iPS to run a disproportionate risk of incurring insertional mutagenesis. Again, more recent work has shown that this risk can be reduced, and moreover efficiency of gene transfer improved at the same time. Efficient reprogramming has

been obtained for example by using adenoviral vectors (123), a single lentiviral, polycistronic vector carrying four reprogramming genes (124,125), a non-viral polycistronic plasmid vector (126,127), a transposon (128) and a vector carrying transgenes excised once reprogramming had been completed – transposon (129) or non-integrating episomal plasmid vectors, whose presence depend on antibiotic addition (130). However, efficiency of iPS cell generation when an adenoviral vectors or plasmids are used, is very low. A way to avoid such viral vectors being integrated into the genome with encumbent risk of mutagenesis, is to generate iPS using small molecules that promote or facilitate cell reprogramming. Various groups have already identified such molecules, which can replace one or two reprogramming factors during iPS cell generation (131–134). A third way, which has already been explored successfully, involves resorting not to gene manipulation to achieve cell reprogramming, but to delivering the reprogramming proteins directly to cells by combining them with peptides (135), Harvard Stem Cell Institute, with recombinant proteins as by the Ding team at the Scripps Research Institute (136).

### *(c) Advantages of iPS*

Human iPS cells not only present the same characteristics and same biological and therapeutic potentialities as hES cells, but also offer advantages over the latter in that they are free of all ethical problems thus solving some biological difficulties that militate against clinical use of hES.

hiPS enable pathology modelling with much greater technical facility than hES; Yamanaka (137) cites generation of *in vitro* disease models as the first practical future application of this technology. He recalls work already carried out with generations of iPS cells from patients affected by neurodegenerative diseases such as amyotrophic lateral sclerosis, Parkinson's disease and spinal muscular atrophy. In the minds of the experimenters, use of hES cells to produce such cell disease models would have to involve cloning (SCNT), a costly, long and difficult undertaking, which moreover has never produced results in humans. Appearance of iPS solves this difficulty. Given that hiPS can be obtained by simply reprogramming cells of patients who are carriers of Mendelian or complex genetic diseases or suffering from problematic acquired diseases with genetic predisposition, they offer pharmaceutical research a material of choice for screening of molecules that could potentially be used therapeutically against these diseases. Moreover, they constitute a cell model for study of genesis and development of these pathologies.

Third, from the clinical use perspective, hiPS cells offer the inestimable benefit of being obtained from cells taken directly from the subject to be treated, eliminating the serious problem of immune rejection, which limits use of hES.

### *(d) Limitations of iPS*

Despite these advantages, hiPS also have their limitations. First, and most obvious, is that the cell reprogramming technique is not very efficient, and results in some heterogeneity in the degree of effective reprogramming reached by cells that are qualified as iPS, within the batch of reprogrammed somatic cells. Only some of these cells have properties comparable with those of ES, which explains the rather variable results found by different authors in their evaluation of iPS properties. iPs cells share their second limitation with ES cells; when they are injected as such, without having started the differentiation process, into a subject, they produce tumours at points at which they were administered into the organism.

iPS also share their third limitation with ES; a great deal of work remains to be carried out to control their differentiation into different cell types. However, gaining such control is the prerequisite for their clinical application.

The fourth limitation of iPS cells has only been recently demonstrated. It concerns their efficiency at forming the various cell types by differentiation (138). Hu *et al.* (139) of the University of Wisconsin found that hiPS, whose capacity for differentiation they were studying, used the same transcriptional systems as hES cells to generate neuroepithelia and various types of functional neurones. They took the same time period to do so, but did it with less efficiency and greater variability. Feng *et al.* (140) of Stem Cell and Regenerative Medicine International, Worcester, MA, USA, also found a relative lack of iPS efficiency in their differentiation into haemangioblasts, endothelial cells and haematopoietic cells. Comparing capacity of eight hiPS cell lines and 25 hES cell lines to differentiate into these various types of cells, they found in a test, that hES cells generated a thousand times more of the desired cells than iPS. They also found that various types of cells produced by iPS began to show signs of cell ageing leading to death after only a short time in culture. These results must of course, be interpreted in the light of more specific studies, as Daley *et al.* (141) suggest, as they may simply be the result of incomplete reprogramming of cells regarded as being iPS. Considering results of other iPS studies, we may indeed imagine that when iPS are completely and homogeneously reprogrammed, they are practically identical to ES. However, we must be prudent in estimating iPS performance, and wait for full exploration of all their possibilities to shed light on these issues.

The most important question concerns risk associated with iPS of causing malignancy in the host, either because of inclusion of oncogenic transgenes, or because of persistence of undifferentiated cells in the differentiated batch to be administered to a patient, or due to still unknown factors connected with reprogramming. Detailed animal experimental studies will therefore be required to verify the harmlessness of hiPS, before going on to clinical application.

*(e) Ethical perspective on iPS*

From the ethical standpoint, development of iPS offers a valuable alternative method to collection of hES through harvest of the inner cell mass of human blastocysts. In effect, it enables stem cells to be obtained that are of comparable quality with hES in terms of stability, *in vitro* proliferation in the undifferentiated state and differentiation into all types of tissues, without any ethical barrier of embryonic destruction that at present paralyses research on ES cells and their clinical use. iPS cells thus solve the ethical dilemma that began in 1998 with demonstration by Thomson *et al.* that it was possible to culture hES cells. The way that iPS cells are obtained does not pose any ethical problem, they do not involve destruction of any embryo and their production fully respects dignity of the person who supplied the initial somatic cells. However, although iPS technology eliminates certain ethical questions proper to hES cell research, it raises new questions, and possibly new challenges (142).

A first issue must be mentioned, which already occurred to Yamanaka (143), when news media began to avail existence of iPS existence known to the public; this concerns possible generation of germ cells from iPS. As soon as the possibility of deriving hES cell lines from human blastocysts was demonstrated by Thomson *et al.* in 1998, researchers wanted to differentiate hES cells into gametes. Thus in 2003, Schöler and collegues of the University of Pennsylvania, USA (144) observed formation of oocyte-like cells from cultured murine ES cells. Again in 2003, Noce *et al.* From Tokyo, Japan (145), using bone morphogenetic protein-4 (BMP4) to stimulate differentiation of murine ES cells into primordial germ cells (PGS), observed that these cells could play a part in spermatogenesis when they were transplanted into seminiferous tubules. In 2006, Nayernia, Göttingen, Sweden, caused a wave of interest in the question of transformation of hES cells into gametes by showing that it was possible to obtain functional haploid male gametes from spermatogonial cells derived from ES cells (146). In 2009, Kee *et al.*, Stanford University, USA, identified the role of *DAZL*, *DAZ* and *BOULE* genes in successful differentiation of hES cells into primordial germ cells and haploid gametes (147). Although this work is still preliminary, it certainly opens the door to the possibility of obtaining gametes by managed differentiation of pluripotent cells, with all the consequences that may have for procreation (148).

The arrival of iPS drastically changes normal and ethical perspectives on stem cells (149); dominated until recently by the question of whether or not preimplantation embryos are respected, stem cells ethics must now confront an increasingly active field of research, at a time of great interest in perfecting iPS technology to produce cell disease models, but also at a time of renewed interest in hES cell research, to the extent that these cells serve as a sort of benchmark, mirror and counterpart to iPS cells.

Over and above the current buzz of excitement that for the moment only affects research laboratories and centres, possibilities are opening up for translation of this research into clinical applications on patients. The pressure exerted today by private clinics, operating in an uncontrolled manner in favour of immediate and indiscriminate clinical application of stem cells will make it tempting to apply iPS technology to patients, specially in terms of regenerative medicine, even though this technology is still in its infancy. iPS are certainly rightly regarded today as prime candidates for a major role in regenerative medicine; but the time has not yet come for them to be employed in the clinic. Too many obstacles remain to be overcome or clarified for it to be possible to envisage such application to patients at present (150).

## Conclusion

Development of induced pluripotent cells, or iPS, obtained by somatic cell dedifferentiation, constitutes considerable progress, not only in stem cell studies, but also for cell biology in general. It shows in effect that it is possible to reprogramme differentiated cells epigenetically, making these cells revert to their developmental starting point by erasing their epigenetic adult cell memory and re-activating expression of pluripotency genes in their genome. iPS cells offer a clear, simple and effective alternative solution to embryonic stem cells. Today they are as promising as embryonic stem cells in terms of *in vitro* self-reproduction-expansion capability and their clinical and ethical advantages cannot be ignored. However, these cells are not yet applicable in the clinical field, in the present state of knowledge.

Technical, biological and ethical progress represented by development of this cell reprogramming method does not eliminate (far from it) use of somatic (adult) stem cells, and umbilical cord blood cells. Individuation of stem cells capable of prolonged self-renewal and pluripotency, that are derived from already individuated stem

cells in umbilical cord blood and in various tissues of the adult organism is now well established, and has produced positive results in the clinical field. This does not require any embryonic destruction, any oocyte, and does not present any ethical problem. Moreover, it is safer and more reliable than reprogramming, which involves gene manipulation. It is to be hoped that in the future, scientists working in the adult stem cell field will succeed in developing stable lines of these pluripotent somatic stem cells, that will take their place alongside iPS for regenerative medicine of the future.

Certain clinics, taking advantage of local conditions, and banking on the distress of patients and on their hopes, use adult stem cells in an ill-considered way for purely commercial reasons. They risk damaging stem cell research in public opinion, either because of negative effects of the so-called treatments, or when falseness of their promises has become obvious. For that reason, establishing national and international regulations codifying use of stem cells is becoming a necessity (151–153).

To succeed in developing iPS, the other avenues discussed here have first to be explored, and iPS themselves are probably only a new frontier to be crossed. Stem cell research has already contributed greatly to science. It is to be hoped that tomorrow it will also do a great deal for patients.

## Conflict of interest

The author declares no conflict of interest.

## References


1 Wilmut I, Schnieke AE, McWhir J, Kind AJ, Campbell KH (1997) Viable offspring derived from fetal and adult mammalian cells. *Nature* **385**, 810–813.

2 Lanza RP, Cibelli JB, West MD (1999) Human therapeutic cloning. *Nat. Med.* **5**, 975–977.

3 Aldhous P (2001) Can they rebuild us? *Nature* **410**, 622–625.

4 Byrne JA, Pedersen DA, Clepper LL, Nelson M, Sanger WG, Gokhale S *et al.* (2007) Producing primate embryonic stem cells by somatic nuclear transfer. *Nature* **450**, 497–502.

5 Sparman M, Dighe V, Sritanaudomchai H, Ma H, Ramsey C, Pedersen D *et al.* (2009) Epigenetic reprogramming by somatic cell nuclear transfer in primates. *Stem Cells* **27**, 1255–1264.

6 Hwang WS, Roh SI, Lee BC, Kang SK, Kwon DK, Kim S *et al.* (2005) Patient-specific embryonic stem-cells derived from human SCNT blastocysts. *Science* **308**, 1777–1783.

7 Stojkovic M, Stojkovic P, Leary C, Hall VJ, Armstrong L, Herbert M *et al.* (2005) Derivation of a human blastocyst after heterologous nuclear transfer to donated oocytes. *Reprod. Biomed. Online* **11**, 226–231.

8 French AJ, Adams CA, Anderson LS, Kirchen JR, Hughes MR, Wood SH (2008) Development of human cloned blastocysts following somatic cell nuclear transfer (SCNT) with adult fibroblasts. *Stem Cells* **26**, 485–493.

9 Li J, Liu X, Wang H, Zhang S, Liu F, Wang X *et al.* (2009b) Human embryos derived by somatic cell nuclear transfer using an alternative enucleation approach. *Cloning Stem Cells* **11**, 39–50.

10 Beeson D, Lippman A (2006) Egg harvesting for stem cell research: medical risks and ethical problems. *Reprod. Biomed. Online* **13**, 573–579.

11 Maher B (2008) Egg shortage hits race to clone human stem cells. *Nature* **453**, 828–829.

12 Lanza RP, Caplan AL, Silver LM, Cibelli JB, West MD, Green RM (2000) The ethical validity of using nuclear transfer in human transplantation. *JAMA* **284**, 3175–3179.

13 Kass LR, *Human Cloning and Human Dignity*, The Report of The President's Council on Bioethics, 2002, Public Affairs, New York, p. 53.

14 Jaenisch R (2004) Human cloning – the science and ethics of nuclear transplantation. *N. Engl. J. Med.* **351**, 2787–2791.

15 McHugh PR (2004) Zygote and "clonote" – the ethical use of embryonic stem cells. *N. Engl. J. Med.* **351**, 209–211.

16 Kahn A (1997a) Clone mammals...clone man? *Nature* **336**, 119.

17 Kahn A (1997b) Cloning, dignity and ethical revisionism. *Nature* **388**, 320.

18 Tecirlioglu RT, Guo J, Trounson AO (2006) Interspecies somatic cell nuclear transfer and preliminary data for horse-cow/mouse iSCNT. *Stem Cell Rev.* **2**, 277–287.

19 St John J, Lovell-Badge R (2007) Human-animal cytoplasmic hybrid embryos, mitochondria, and an energetic debate. *Nat. Cell Biol.* **9**, 988–992.

20 Lanza R, Dresser B, Damiani P (2001). Cloning Noah's Ark. *Scientific American* **283**, 84–89.

21 Lanza RP, Cibelli JB, West MD (1999b) Prospect for the use of nuclear transfer in human transplantation. *Nat. Biotechnol.* **17**, 1171–1174.

22 Chang KH, Lim JM, Kang SK, Lee BC, Moon SY, Hwang WS (2003) Blastocyst formation, karyotype, and mitochondrial DNA of interspecies embryos derived from nuclear transfer of human cord fibroblasts into enucleated bovine embryos. *Fertil. Steril.* **80**, 380–1387.

23 Chang KH, Lim JM, Kang SK, Lee BC, Moon SY, Hwang WS (2004) An optimized protocol of a human-to-cattle interspecies somatic cell nuclear transfer. *Fertil. Steril.* **82**, 960–962.

24 Illmensee K, Levanduski M, Zavos PM (2006) Evaluation of the embryonic preimplantation potential of human adult somatic cells via an embryo interspecies bioassay using bovine oocytes. *Fertil. Steril.* **85**(Suppl. 1), 1248–1261.

25 Hopkin M (2007) Britain gets hybrid embryo go-ahead. Human-animal embryos given green light after public backing. *Nature News* doi: 10.1038/news070903-12.

26 HFEA (2007) HFEA statement on its decision regarding hybrid embryos, Available at http://www.hfea.gov.uk/en/1581.html (accessed 5 September 2007).

27 Landry DW, Zucker HA (2004) Embryonic death and the creation of human embryonic stem cells. *J. Clin. Invest.* **114**, 1184–1186.

28 Byrne JA, Simonsson S, Gurdon JB (2002) From intestine to muscle: nuclear reprogramming through defective cloned embryos. *Proc. Natl. Acad. Sci. USA* **99**, 6059–6063.

29 Gurdon JB, Byrne JA, Simonsson S (2003) Nuclear reprogramming and stem cell creation. *Proc. Natl. Acad. Sci. USA* **100**(Suppl. 1), 11819–11822.

30 Mitalipova M, Cahoun J, Shin S, Wininger D, Schultz T, Noggle S *et al.* (2003) Human embryonic stem cell lines from discarded embryos. *Stem Cells* **21**, 521–526.

31 Pickering SJ, Braude PR, Patel M, Burns CJ, Trussler J, Bolton V *et al.* (2003) Preimplantation genetic diagnosis as a novel source of embryos for stem cell research. *Reprod. Biomed. Online* **7**, 353–364.

32 Chen H, Qian K, Hu J, Liu D, Lu W, Yang Y *et al.* (2005a) The derivation of two additional human embryonic stem cell lines from day 3 embryos with low morphological scores. *Hum. Reprod.* **20**, 2201–2206.

33 Zhang X, Stojkovic P, Przyborski S, Cooke M, Armstrong L, Lako M *et al.* (2006) Derivation of human embryonic stem cells from developing and arrested embryos. *Stem Cells* **24**, 2669–2676.

34 Lerou PH, Yabuuchi A, Huo H, Takeuchi A, Shea J, Cimini C (2008) Human embryonic stem cell derivation from poor-quality embryos. *Nat. Biotechnol.* **26**, 212–214.

35 Holden C (2006) Scientists create human stem cell line from "dead" embryos. *Science* **313**, 1869.

36 Loebel DAF, Tam PPL (2004) Mice without a father. *Nature* **428**, 809–811.

37 Surani MA, Barton SC (1983) Development of gynogenetic eggs in the mouse: implications for parthenogenetic embryos. *Science* **222**, 1034–1036.

38 Cibelli JB, Grant KA, Chapman KB, Cunniff K, Worst T, Green HL *et al.* (2002) Parthenogenetic stem cells in nonhuman primates. *Science* **295**, 819.

39 Trounson A (2002) The genesis of embryonic stem cells. Does parthenogenesis offer a more promising means of developing immune-matched ES cells. *Nat. Biotechnol.* **20**, 237–238.

40 Mitalipov SM, Nusser KD, Wolf DP (2001) Parthenogenetic activation of rhesus monkey oocytes and reconstructed embryos. *Biol. Reprod.* **65**, 253–259.

41 Vrana KE, Hipp JD, Goss AM, McCool BA, Riddle DR, Walker SJ *et al.* (2003) Nonhuman primate parthenogenetic stem cells. *Proc. Natl. Acad. Sci. USA* **100**(Suppl. 1), 11911–11916.

42 Lin H, Lei JQ, Wininger D, Nguyen MT, Khanna R, Hartmann C *et al.* (2003) Multilineage potential of homozygous stem cells derived from metaphase II oocytes. *Stem Cells* **21**, 152–161.

43 Brevini TA, Gandolfi F (2008) Parthenotes as a source of embryonic stem cells. *Cell Prolif.* **41**(Suppl. 1), 20–30.

44 Lin G, OuYang Q, Zhou X, Gu Y, Yuan D, Li W *et al.* (2007) A highly homozygous and parthenogenetic human embryonic stem cell line derived from a one-pronuclear oocyte following in vitro fertilization procedure. *Cell Res.* **17**, 999–1007.

45 Mai Q, Yy Y, Li T, Wang L, Chen MJ, Huang SZ *et al.* (2007) Derivation of human embryonic stem cell lines from parthenogenic blastocysts. *Cell Res.* **17**, 1008–1019.

46 Revazova ES, Turovets NA, Kochetkova OD, Kindarova LB, Kuzmichev LN, Janus JD *et al.* (2007) Patient-specific stem cell lines derived from human parthenogenetic blastocysts. *Cloning Stem Cells* **9**, 432–449.

47 Revazova ES, Turovets NA, Kochetkova OD, Agapova Ls, Sebastian JL, Pryzhkova MV *et al.* (2008) HLA homozygous stem cell lines derived from human parthenogenetic blastocysts. *Cloning Stem Cells* **10**, 11–24.

48 Eckardt S, Dinger TC, Kurosaka S, Leu NA, Müller AM, McLaughlin KJ (2008) In vivo and in vitro differentiation of uniparental embryonic stem cells into hematopoietic and neural cell types. *Organogenesis* **4**, 33–41.

49 Koh CJ, Delo DM, Lee JW, Siddiqui MM, Lanza RP, Soker S *et al.* (2009) Parthenogenesis-derived multipotent stem cells adapted for tissue engineering applications. *Methods* **47**, 90–97.

50 Sanchez-Pernaute R, Lee H, Patterson M, Reske-Nielsen C, Yoshizaki T, Sonntag KC *et al.* (2008) Parthenogenetic dopamine neurons from primate embryonic stem cells restore function in experimental Parkinson's disease. *Brain* **131**, 2127–2139.

51 Gong SP, Kim H, Lee EJ, Lee ST, Moon S, Lee HJ *et al.* (2009) Change in gene expression of mouse embryonic stem cells derived from parthenogenetic activation. *Hum. Reprod.* **24**, 805–814.

52 Li C, Chen Z, Liu Z, Huang J, Zhang W, Zhou L *et al.* (2009a) Correlation of expression and methylation of imprinted genes with pluripotency of parthenogenetic embryonic stem cells. *Hum. Mol. Genet.* **18**, 2177–2187.

53 Jiang H, Sun B, Wang H, Zhang Z, Gao F, Shi G *et al.* (2007) Activation of paternally expressed imprinted genes in newly derived germline-competent mouse parthenogenetic embryonic stem cell lines. *Cell Res.* **17**, 792–803.

54 Latkovic MS (2002) The science and ethics of parthenogenesis. *Natl. Cathol. Bioeth. Q.* **2**, 245–255.

55 Hurlbut WB (2004) Altered Nuclear Transfer as a Morally Acceptable Means for the Procurement of Human Embryonic stem cells, commissioned working paper discussed at the Council's December 2004 meeting, The President's Council on Bioethics, Available at http://www.bioethics.gov/background/hurlbut.html reprinted in Perspectives in Biology and Medicine, vol. 48, number 2, spring 2005, pp.211-228.

56 Hurlbut WB (2005a) Altered nuclear transfer as a morally acceptable means for the procurement of human embryonic stem cell. *Natl. Cathol. Bioeth. Q.* **5**, 145–151.

57 Hurlbut WB (2005b) Altered nuclear transfer as a morally acceptable means for the procurement of human embryonic stem cells. *Perspect. Biol. Med.* **48**, 211–228.

58 Check E (2005a) Altered embryos offered as solution to stem-cell rift. *Nature* **436**, 309.

59 Deb K, Sivaguru M, Yong HY, Roberts RM (2006) *Cdx2* gene expression and trophectoderm lineage specification in mouse embryos. *Science* **311**, 992–996.

60 Sritanaudomchal H, Sparman M, Tachibana M, Clepper L, Woodward J, Gokhale S *et al.* (2009) CDX2 in the formation of the trophectoderm lineage in primate embryos. *Dev. Biol.* **335**, 179–187.

61 Hurlbut WB (2005c) Altered nuclear transfer. *N. Engl. J. Med.* **352**, 1153–1154.

62 Meissner A, Jaenisch R (2006) Generation of nuclear transfer-derived pluripotent ES cells from cloned *cdx2*-deficient blastocysts. *Nature* **439**, 212–215.

63 Melton DA, Daley GQ, Jennings CG (2004) Altered nuclear transfer in stem-cell research – a flawed proposal. *N. Engl. J. Med.* **351**, 2791.

64 Beck F (2002) Homeobox genes in gut development. *Gut* **51**, 450–454.

65 Turnpenny L (2005) Embryo's moral status is unaffected by alteration. *Nature* **437**, 26.

66 Vogel G (2005) Embryo-free techniques gain momentum. *Science* **309**, 240–241.

67 Pan G, Pei D (2005) The stem cell pluripotency factor NANOG activates transcription with two unusual point subdomains at its C terminus. *J. Biol. Chem.* **280**, 1401–1407.

68 Boyer LA, Lee TI, Cole MF, Johnstone SE, Levine SS, Zucker JP *et al.* (2005) Core transcriptional regulatory circuitry in human embryonic stem cells. *Cell*, **122**, 1–10.

69 Byrnes WM (2005) Why human "altered nuclear transfer" is unethical. a holistic systems view. *Natl. Cathol. Bioeth. Q.* **5**, 271–279.

70 Schamblott MJ, Axelman J, Wang S, Bugg EM, Littlefield JW, Donovan PJ *et al.* (1998) Derivation of pluripotent stem cells from cultured human primordial germ cells. *Proc. Natl. Acad. Sci. USA* **95**, 13726–13731.

71 Turnpenny L, Brickwood S, Spalluto CM, Piper K, Cameron IT, Wilson DI *et al.* (2003) Derivation of human embryonic germ cells: an alternative source of pluripotent cells. *Stem Cells* **21**, 598–609.

72 Baha A, Takeuchi T, Tanaka N, Neri QV, Rosenwaks Z, Palermo GD (2005) Examination of individual blastomeres as a source of stem cells, Session 04, *Stem Cells*, Abstract O-018, p.i7, Abstracts of the 21st Annual Meeting of the European Society of Human Reproduction and Embryology, Copenhagen, Denmark, 19-22 June 2005. *Hum. Reprod.*, **20**(Suppl. 1), i7.
73 Sills ES, Takeuchi T, Tanaka N, Neri QV, Palermo GD (2005) Identification and isolation of embryonic stem cells in reproductive endocrinology: theoretical protocols for conservation of human embryos derived from in vitro fertilization. *Theor. Biol. Med. Model.* **2**, 25 (1-8).
74 Wakayama S, Hikichi T, Suetsugu R, Sakaide Y, Bul HT, Mizutani E *et al.* (2007) Efficient establishment of mouse embryonic stem cell lines from single blastomeres and polar bodies. *Stem Cells* **25**, 986–993.
75 Klimanskaya I, Chung Y, Becker S, Lu SJ, Lanza R (2006) Human embryonic stem cell lines derived from single blastomeres. *Nature* **444**, 481–485.
76 Chung Y, Klimanskaya I, Becker S, Li T, Maserati M, Lu SJ *et al.* (2008) Human embryonic stem cell lines generated without embryo destruction. *Cell Stem Cell* **2**, 113–117.
77 Geens M, Mateizel I, Sermon K, de Rycke M, Spits C, Cauffman G *et al.* (2009) Human embryonic stem cell lines derived from single blastomeres of two 4-cell stage embryos. *Hum. Reprod.* **24**, 2709–2717.
78 Feki A, Bosman A, Dubuisson JB, Irion O, Dahoun S, Pelte MF *et al.* (2008) Derivation of the first Swiss human embryonic stem cell line from a single blastomere of an arrested four-cell stage embryo. *Swiss Med. Wkly.* **138**, 540–550.
79 Van de Velde H, Geens M, Mateizei I, Spits C, Sermon K, de Rycke M *et al.* (2008) A human embryonic stem cell line derived from a single blastomere of a 4-cell stage embryo, Abstract of the 24th Meeting of the ESHRE, Barcelona, Spain, 7-9 July 2008. *Hum. Reprod.* **23**(Suppl. 1), i110. Abstract 0⁄274.
80 Chung Y, Klimanskaya I, Becker S, Marh J, Lu SJ, Johnson J *et al.* (2006) Embryonic and extraembryonic stem cell lines derived from single mouse blastomeres. *Nature* **439**, 216–219.
81 Abbott A (2006) ‘‘Ethical’’ stem-cell paper under attack. *Nature* **443**, 12.
82 Surani MA (2001) Reprogramming of genome function through epigenetic inheritance. *Nature* **414**, 122–128.
83 Cowan CA, Atienza J, Melton DA, Eggan K (2005) Nuclear reprogramming of somatic cells after fusion with human embryonic stem cells. *Science* **309**, 1369–1373.
84 Strelchenko N, Kukharenko V, Shkumatov A, Verlinsky O, Kuliev A, Verlinsky Y (2006) Reprogramming of human somatic cells by embryonic stem cell cytoplast. *Reprod. BioMed. Online* **12**, 107–111.
85 Check E (2005b) Rebooted cells tackle ethical concerns. Fusion technique resets adult skin to embryonic state. *Nat. News*, published online 27 June 2005; doi: 10.1038/news050627-1.
86 Abuljadayel IS (2003) Induction of stem cell-like plasticity in mononuclear cells derived from immobilized adult human peripheral blood. *Curr. Med. Res. Opin.* **19**, 355–375.
87 Odelberg SJ (2004) Unraveling the molecular basis for regenerative cellular plasticity. *PLoS Biol.* **2**, E232.
88 McGann CJ, Odelberg SJ, Keating MT (2001) Mammalian myotube dedifferentiation induced by newt regeneration extract. *Proc. Natl. Acad. Sci. USA* **98**, 13699–13704.
89 Odelberg SJ, Kollhoff A, Keating MT (2000) Dedifferentiation of mammalian myotubes induced by msx1. *Cell* **103**, 1099–1109.
90 Chen S, Zhang Q, Wu X, Schultz PG, Ding S (2004) Dedifferentiation of lineage-committed cells by a small molecule. *J. Am. Chem. Soc.* **126**, 410–411.
91 Kim S, Rosania GR, Chang YT (2004) Dedifferentiation? What’s next? *Mol. Interv.* **4**, 83–85.
92 Wu X, Ding S, Ding Q, Gray NS, Schultz PG (2002) A small molecule with osteogenesis-inducing activity in multipotent mesenchymal progenitor cells. *J. Am. Chem. Soc.*, **124**, 14520–14521.
93 Chen X, Mao Z, Liu S, Liu H, Wang X, Wu H *et al.* (2005b) Dedifferentiation of adult human myoblasts induced by ciliary neurotrophic factor in vitro. *Mol. Biol. Cell* **16**, 3140–3151.
94 Håkelien AM, Landsverk HB, Robl JM, Skålhegg BS, Collas P (2002) Reprogramming fibroblasts to express T-cell functions using cell extracts. *Nat. Biotechnol.* **20**, 460–466.
95 Eminli S, Jaenisch R, Hochedlinger K (2006) Strategies to induce nuclear reprogramming. *Ernst Schering Found Symp. Proc.* **5**, 83–98.
96 Hochedlinger K, Jaenisch R (2006) Nuclear reprogramming and pluripotency. *Nature* **441**, 1061–1067.
97 Check E (2006) Simple recipe gives adult cells embryonic powers. *Nature* **442**, 11.
98 Vogel G (2006) Stem cell research. Four genes confer embryonic potential. *Science* **313**, 27.
99 Yamanaka S (2006), Identification of factors that generate Es-like pluripotent cells from fibroblast culture. Plenary Session IV. *International Society for Stem Cell Research*, 4th Annual Meeting, 29 June-1 July, Toronto, Canada. Available at http://www.abstractonline.com/viewer/viewsession.asp?
100 Takahashi K, Yamanaka S (2006) Induction of pluripotent stem cells from mouse embryonic and adult fibroblasts cultures by defined factors. *Cell* **126**, 663–676.
101 Takahashi K, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K *et al.* (2007) Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* **131**, 861–872.
102 Okita K, Ichisaka T, Yamanaka S (2007) Generation of germline-competent induced pluripotent stem cells. *Nature* **448**, 313–317.
103 Maherali N, Sridharan R, Xie W, Utikal J, Eminli S, Arnold K *et al.* (2007) Directly reprogrammed fibroblasts show global epigenetic remodeling and widespread tissue contribution. *Cell Stem Cell* **1**, 55–70.
104 Wernig M, Meissner A, Foreman R, Brambrink T, Ku M, Hochedlinger K *et al.* (2007) In vitro reprogramming of fibroblasts into a pluripotent ES-cell-like state. *Nature* **448**, 318–324.
105 Yu J, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S *et al.* (2007) Induced pluripotent stem cell lines derived from human somatic cells. *Science* **318**, 1917–1920.
106 Vogel G (2008) Breakthrough of the year. Reprogramming cells. *Science* **322**, 1766–1767.
107 Park IH, Zhao R, West JA, Yabuuchi A, Huo H, Ince TA *et al.* (2008) Reprogramming of human somatic cells to pluripotency with defined factors. *Nature* **451**, 141–146.
108 Lowry WE, Richter L, Yachechko R, Pyle AD, Tchieu J, Sridharan R *et al.* (2008) Generation of human induced pluripotent stem cells from dermal fibroblasts. *Proc. Natl. Acad. Sci. USA* **105**, 2883–2888.
109 Marion RM, Strati K, Li H, Tejera A, Schoeftner S, Ortega S *et al.* (2009) Telomeres acquire embryonic stem cell characteristics in induced pluripotent stem cells. *Cell Stem Cell* **4**, 141–154.
110 Prelle K, Zink N, Wolf E (2002) Pluripotemt stem cells – model of embryonic development, tool for gene targeting and basis of cell therapy. *Anat. Histol. Embryol.* **31**, 169–186.

111 Zhao XY, Li W, Lv Z, Liu L, Tong M, Hai T *et al.* (2009) iPS cells produce viable mice through tetraploid complementation. *Nature* **461**, 86–90.

112 Meissner A, Wernig M, Jaenisch R (2007) Direct reprogramming of genetically unmodified fibroblasts into pluripotent stem cells. *Nat. Biotechnol.* **25**, 1177–1181.

113 Feng B, Ng JH, Heng JC, Ng HH (2009) Molecules that promote or enhance reprogramming of somatic cells to induced pluripotent stem cells. *Cell Stem Cell* **4**, 301–312.

114 Mali P, Ye Z, Hommond HH, Yu X, Lin J, Chen G *et al.* (2008) Improved efficacy and pace of generating induced pluripotent cells from human and fetal fibroblasts. *Stem Cells* **26**, 1998–2005.

115 Mikkelsen TS, Hanna J, Zhang K, Ku M, Wernig M, Schorderet P *et al.* (2008) Dissecting direct reprogramming through integrative genome analysis. *Nature* **454**, 49–55.

116 Zhao Y, Yin X, Qin H, Zhu F, Liu H, Ysang W *et al.* (2008) Two supporting factors greatly improve the efficiency of human iPSC generation. *Cell Stem Cells* **3**, 475–479.

117 Judson RL, Babiarz JE, Venere M, Blelloch R (2009) Embryonic stem-cell-specific microRNAs promote induced pluripotency. *Nat. Biotechnol.* **27**, 459–461.

118 Blelloch R, Venere M, Yen J, Ramalho-Santos M (2007) Generation of induced pluripotent stem cells in the absence of drug selection. *Cell Stem Cell* **1**, 245–247.

119 Nakagawa M, Koyanagi M, Tanabe K, Takahashi K, Ichisaka T, Aoi T *et al.* (2008) Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. *Nat. Biotechnol.* **26**, 101–106.

120 Wernig M, Meissner A, Cassidy JP, Jaenisch R (2008) c-Myc is dispensable for direct reprogramming of mouse fibroblasts. *Cell Stem Cell* **2**, 10–12.

121 Lehrman S (1999) Virus treatment questioned after gene therapy death. *Nature* **401**, 517–518.

122 Hacein-Bey-Abina S, von Kalle C, Schmidt M, Le Deist F, Wulfraat N, McIntyre E *et al.* (2003) A serious adverse event after successful gene therapy for X-linked severe combined immunodeficiency. *N. Engl. J. Med.* **348**, 255–258.

123 Stadtfeld M, Nagaya M, Utikal J, Weir G, Hochedlinger K (2008) Induced pluripotent stem cells generated without viral integration. *Science* **322**, 945–949.

124 Carey BW, Markoulaki S, Hanna J, Saha K, Gao Q, Mitalipova M *et al.* (2009) Reprogramming of murine and human somatic cells using a single polycistronic vector. *Proc. Natl. Acad. Sci. USA* **106**, 157–162.

125 Sommer CA, Stadtfeld M, Murphy GJ, Hochedlinger K, Kotton DN, Mostoslavsky G (2009) Induced pluripotent stem cell generation using a single lentiviral stem cell cassette. *Stem Cells* **27**, 543–549.

126 Gonzalez F, Barragan Monasterio M, Tiscornia G, Montserrat Pulido N, Vassena R, Batlle Morera LB *et al.* (2009) Generation of mouse-induced pluripotent stem cells by transient expression of a single nonviral polycistronic vector. *Proc. Natl. Acad. Sci. USA* **106**, 8918–8922.

127 Okita K, Nakagawa M, Hyenjong H, Ichisaka T, Yamanaka S (2008) Generation of mouse induced pluripotent stem cells without viral vectors. *Science* **322**, 949–953.

128 Woltjen K, Michael IP, Mohseni P, Desai R, Mileikovsky M, Hämäläinen R *et al.* (2009) piggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. *Nature* **458**, 766–770.

129 Kaji K, Norrby K, Paca A, Mileikovsky M, Mohseni P, Woltjen K (2009) Virus-free induction of pluripotency and subsequent excision of reprogramming factors. *Nature* **458**, 771–775.

130 Yu J, Hu K, Smuga-Otto K, Tian S, Stewart R, Slukvin II *et al.* (2009) Human induced pluripotent stem cells free of vector and transgene sequences. *Science* **324**, 797–801.

131 Huangfu D, Maehr R, Guo W, Eijkelenboom A, Sniyow M, Chen AE *et al.* (2008) Induction of pluripotent stem cells by defined factors is greatly improved by small-molecules compounds. *Nat. Biotechnol.* **26**, 795–797.

132 Li W, Wei W, Zhu S, Zhu J, Shi Y, Lin T *et al.* (2009) Generation of rat and human induced pluripotent stem cells by combining genetic reprogramming and chemical inhibitors. *Cell Stem Cell* **4**, 16–19.

133 Lyssiotis CA, Foreman RK, Staerk J, Garcia M, Mathur D, Markoulaki S *et al.* (2009) Reprogramming of murine fibroblasts to induced pluripotent stem cells with chemical complementation of KLf4. *Proc. Natl. Acad. Sci. USA* **106**, 8912–8917.

134 Shi Y, Do JT, Desponts C, Hahm HS, Schöler HR, Ding S (2008) A combined chemical and genetic approach for the generation of induced pluripotent stem cells. *Cell Stem Cell* **2**, 525–528.

135 Kim D, Kim CH, Moon JI, Chung YG, Chang MY, Han BS *et al.* (2009) Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins. *Cell Stem Cell* **4**, 472–476.

136 Zhou H, Wu S, Joo JY, Zhu S, Han DW, Lin T *et al.* (2009) Generation of induced pluripotent stem cells using recombinant proteins. *Cell Stem Cell* **4**, 381–384.

137 Yamanaka S (2009) A fresh Look at iPS Cells. *Cell* **137**, 13–17.

138 Vogel G (2010) Stem cells. Reprogrammed cells come up short, for now. *Science* **327**, 1191.

139 Hu BY, Weick JP, Yu J, Ma LXC, Zhang XQ, Thomson JA *et al.* (2010) Neural differentiation of human induced pluripotent stem cells follows developmental principles but with variable potency. *Proc. Natl. Acad. Sci. USA* **107**, 4335–4340.

140 Feng Q, Lu SJ, Klimanskaya I, Gomes I, Kim D, Chung Y *et al.* (2010) Hemangioblastic derivatives from human induced pluripotent stem cells exhibit limited expansion and early senescence. *Stem Cells* **28**, 704–712.

141 Dolgin E (2010) Straight talk with George Daley. *Nat. Med.* **16**, 624.

142 Leibel C (2009) New ethical questions are being raised in stem cell research. *Medical News Today*, Available at http://www.medicalnewstoday.com/artilce/173785.php (accessed 12 December 2009).

143 Linda Geddes, No embryos were harmed. *New Scientist*. 15 December 2007, pp.44–45

144 Hübner K, Fuhrmann G, Christenson LK, Kehler J, Reinbold R, De la Fuente R *et al.* (2003) Derivation of oocytes from mouse embryonic stem cells. *Science* **300**, 1251–1256.

145 Toyooka Y, Tsunekawa N, Akasu R, Noce T (2003) Embryonic stem cells can form germ cells in vitro. *Proc. Natl. Acad. Sci. USA* **100**, 11457–11462.

146 Nayernia K, Nolte J, Michelmann HW, Lee JH, Rathsack K, Drusenheimer N (2006) In vitro-differentiated embryonic stem cells give rise to male gametes that can generate offspring mice. *Dev. Cell* **11**, 125–132.

147 Kee K, Angeles VT, Flores M, Nguyen HN, Reijo Pera RA (2009) Human DAZL DAZ and BOULE genes modulate primordial germ-cell and haploid gamete formation. *Nature* **462**, 222–225.

148 Mathews DJ, Donovan PJ, Harris J, Lovell-Badge R, Savulescu J, Faden R (2009) Pluripotential stem cell-derived gametes: truth and (potential) consequences. *Cell Stem Cell* **5**, 11–14.

149 Hyun I (2010) The bioethics of stem cell research and therapy. *J. Clin. Invest.* **120**, 71–75.

150 Sun N, Longaker MT, Wu JC (2010) Human iPS cell-based therapy: considerations before clinical applications. *Cell Cycle* **9**, 880–885.
151 Caulfield T, Scott C, Hyun I, Lovell-Badge R, Kato K, Zarzeczny A (2010) Stem cell research policy and iPS cells. *Nat. Methods* **7**, 28–33.
152 Editorial (2010) Regulators must step up stem cell oversight. *Nat. Med.* **16**, 492.
153 Zarzeczny A, Scott C, Hyun I, Bennett J, Chandler J, Chgarge S *et al.* (2009) iPS cells: mapping the policy issues. *Cell* **139**, 1032–1037.