Journal of Controlled Release xxx (2014) xxx–xxx

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Review

Induced pluripotent stem (iPS) cells: A new source for cell-based therapeutics?

Irene de Lázaro, Açelya Yilmazer [1], Kostas Kostarelos *

Nanomedicine Lab, Faculty of Medical and Human Sciences, University of Manchester, AV Hill Building, Manchester M13 9NT, United Kingdom
UCL School of Life & Medical Sciences, University College London, 29-39 Brunswick Square, WC1N 1AX London, United Kingdom

ARTICLE INFO

Article history:
Received 21 January 2014
Accepted 8 April 2014
Available online xxxx

Keywords:
Reprogramming
Pluripotency
Regenerative medicine
Cell replacement therapy

ABSTRACT

The generation of induced pluripotent stem (iPS) cells from somatic cells by the ectopic expression of defined transcription factors has provided the regenerative medicine field with a new tool for cell replacement strategies. The advantages that these pluripotent cells can offer in comparison to other sources of stem cells include the generation of patient-derived cells and the lack of embryonic tissue by maintaining a versatile differentiation potential. The promise of iPS cell derivatives for therapeutic applications is encouraging albeit very early in development, with the first clinical study currently ongoing in Japan. Many challenges are yet to be circumvented before this technology can be clinically translated widely though. The delivery and expression of the reprogramming factors, the genomic instability, epigenetic memory and impact of cell propagation in culture are only some of the concerns. This article aims to critically discuss the potential of iPS cells as a new source of cell therapeutics.

© 2014 Published by Elsevier B.V.

Contents

1. Introduction

The increased prevalence of degenerative diseases remains challenging to manage with the currently available small molecule therapeutics and surgical interventions. This has prompted the search for alternative strategies that pursue restoration of the damaged or degenerated tissue rather than just compensation of their impaired function [1]. The availability of adequate cell sources to populate injured or degenerated tissues is a central priority in regenerative medicine, and stem cells are invaluable candidates thanks to their capacity to self-renew and differentiate into several cell types [2]. Various stem cell types including embryonic, fetal, perinatal and adult stem cells have been investigated as sources for regenerative therapies [3,4]. Immune compatibility and differentiation potential of the stem cells are crucial features for their suitability. Unfortunately, these two parameters are not often both satisfactorily accomplished that limits their successful use [5,6]. In addition, propagation in culture can be challenging as in the case of fetal (isolated from aborted

* Corresponding author. Tel.: +44 1612751800.
E-mail address: kostas.kostarelos@manchester.ac.uk (K. Kostarelos).
[1] Present address: Department of Biology, Faculty of Science, Ankara University, 06100, Tandogan, Ankara, Turkey.

http://dx.doi.org/10.1016/j.jconrel.2014.04.011
0168-3659/© 2014 Published by Elsevier B.V.

Please cite this article as: I. de Lázaro, et al., Induced pluripotent stem (iPS) cells: A new source for cell-based therapeutics? J. Control. Release (2014), http://dx.doi.org/10.1016/j.jconrel.2014.04.011

UNCORRECTED PROOF

fetuses) and perinatal stem cells (from amniotic fluid, umbilical cord blood and placenta) [4]. Finally, the ethical issues surrounding the use of embryonic materials have prompted a variety of hurdles to research the clinical development of stem cells [7].

Despite the challenges, hundreds of clinical trials have explored the utilization of stem cells in regenerative medicine. Transplantation of bone marrow-derived stem cells for the treatment of hematopoietic diseases has already been safely and successfully used in the clinic for a number of years [8]. Nevertheless, the source, characterization and purity of any type of cell sourcing for transplantation purposes remain issues of intense controversy as recent cases reveal [9–11].

The generation of induced pluripotent stem (iPS) cells that can be derived from the adult cells of specific patients, has recently revolutionized the field posing hopes that some of the roadblocks traditionally associated with stem cell therapy could be overcome [12,13]. This article aims to offer an overview of the potential applications of iPS cells, and highlight their use in cell-based therapies for regenerative purposes.

2. Induced pluripotent stem (iPS) cells: a new source of stem cells

In 2006 Shinya Yamanaka and collaborators published a groundbreaking study demonstrating transcription factor-mediated cell reprogramming to pluripotency that was awarded the 2012 Nobel Prize in Medicine along with Sir John Gurdon's much earlier studies on reprogramming using somatic cell nuclear transfer [14]. In Yamanaka's work, mouse embryonic and adult fibroblasts were genetically reprogrammed to a pluripotent state by viral (retrovirus) gene transfer of four transcription factors (Oct3/4, Sox2, Klf4 and cMyc) that are involved in the maintenance of pluripotency in ESCs. The resulting cells, known as induced pluripotent stem (iPS) cells, grow indefinitely in culture forming colonies that are morphologically indistinguishable from those of embryonic stem (ES) cells [12]. The fully functional pluripotent character of iPS cells was confirmed one year after their initial description, when iPS cells selected for the expression of the pluripotency marker Nanog were found to contribute to the adult tissues of chimeric mice obtained by blastocyst injection, including the germline [15].

The generation of iPS cells from human fibroblasts has also been achieved by expression of human OCT3/4, SOX2, KLF4 and cMYC [13], replacement of cMYC and KLF4 by NANOG and LIN28 [16] and even elimination of the tumorigenic cMYC [17]. Recently, reprogramming of human fibroblasts has proven achievable by means of the overexpression of lineage specific genes and without SOX2 and OCT4, the original 'Yamanaka factors' that were thought to be indispensable for the induction of pluripotency. Such findings imply that the fully differentiated state of somatic cells inherently incorporates larger degrees of plasticity than what was thought until now [18].

iPS cells can be derived from a wide variety of starting cells, even though fibroblasts are the most common source for iPS cell generation today due to their accessibility (can be easily obtained with a skin biopsy) [19]. Other cell types from diverse developmental origins, such as hepatocytes (endoderm origin), circulating T cells (mesoderm) and keratinocytes (ectoderm) have also been successfully reprogrammed into iPS cells even though efficiencies vary [20]. Recently, umbilical cord blood and peripheral blood cells have been projected as advantageous candidate sources for the generation of iPS cells [21]. The main hurdles in the harvesting of dermal fibroblasts are the requirement for skin biopsy (accessible but invasive), the need to expand the collected cells for several passages in order to achieve enough cell numbers for iPS cell generation and the fact that these cells are directly exposed to the insults of the environment (e.g. mutations provoked by UV radiation). Mononuclear cells from peripheral blood on the contrary do not suffer from such drawbacks [22–24].

Along with different somatic cell types used to generate iPS cells, different methodologies have also been pursued to overexpress the reprogramming transcription factors and induce conversion to the pluripotent state [25]. The main goal has been to avoid use of integrating vectors and achieve safer yet efficient cell reprogramming. Table 1 summarizes the methodologies used today and classifies vectors according to their safety/efficiency balance. We determined 'safety' according to the reported levels of genomic integration and risk of immune reactions, while 'efficiency' according to the extent of reprogramming achieved (i.e. number of iPS colonies obtained from the starting somatic cells).

Much has been achieved since the initial report of iPS cell generation, not only in the optimization of the reprogramming protocols, but also in the elucidation of the mechanisms behind cellular reprogramming, as reviewed elsewhere [39]. Buganim et al. made use of single-cell analysis to show that cellular reprogramming can be divided into an early stochastic phase that has a higher degree of variability in the gene expression patterns among cells and a later phase that is more hierarchical [40]. Later, Polo et al. examined the course of reprogramming to pluripotency by genome-wide analyses and confirmed that the cells undergo two distinct waves of transcriptional changes to result in iPS cell generation. Also, genes that hinder the conversion of the partially reprogrammed intermediates to iPS cells were identified [41].

Many more studies are advancing our understanding of cellular plasticity and enable researchers to explore new alternative cellular reprogramming technologies most appropriate for each application. Recently, Rais et al. showed that the depletion of the levels of Mbd3 protein concomitantly with the overexpression of Oct3/4, Sox2, Klf4 and cMyc resulted in the deterministic reprogramming of cells to the pluripotent cell state with efficiencies very close to 100% [42].

3. The short- and long-term applications of iPS cell technology

The differentiation potential of iPS cells, considered practically equivalent to that of ES cells, along with the possibility to obtain them from individual patients has uncovered a wide range of potential utilizations [43] that are illustrated in Fig. 1.

In the short term, the ability to produce iPS cells that can then be differentiated *in vitro* from individuals suffering from a particular disease is thought to contribute towards development of better disease modeling for a diverse range of conditions [44]. Reliable disease models are generally difficult to obtain otherwise, since human primary cells are not easily maintained in culture for long periods of time and animal models inevitably involve inter-species variabilities [45]. The iPS-derived *in vitro* models can constitute an invaluable source of information to better understand the mechanism of diseases and to help recapitulate the features of the pathogenic phenotype [46].

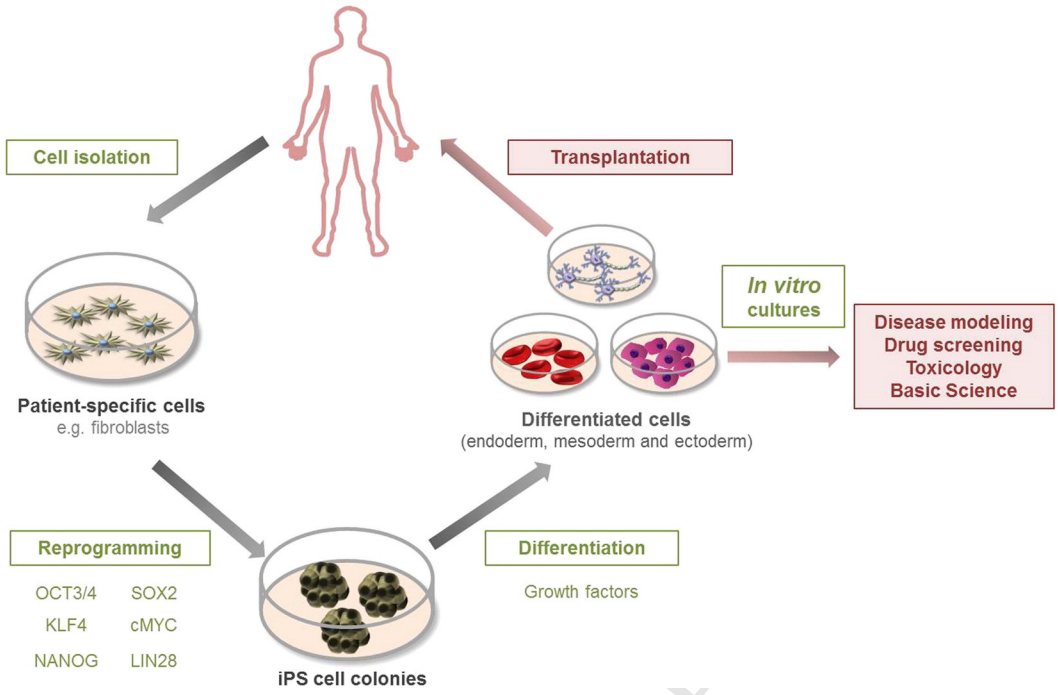
The majority of models developed to date focus on cardiovascular and neural or neuromuscular disorders [47], such as long QT syndrome (a disorder of the heart's electrical activity) [48], Alzheimer's disease [49], Friedreich ataxia [50] and myotonic dystrophy [51]. Other studies aim to achieve models for the elucidation of mechanisms involved in disorders associated with premature aging such as Hutchinson–Guilford progeria [52,53] and dyskeratosis congenita [54]. iPS cell

Table 1
Current vector technologies used for reprogramming transcription factor overexpression and the generation of iPS cells.

	Vector technology		Safety	Efficiency	Ref.
Viral vectors	Integrating	Retrovirus	−	++	[12]
		Lentivirus	−	++	[26]
		Inducible lentivirus	−	++	[27]
	Excisable	Excisable lentivirus	++	++	[28]
	Non-integrating	Adenovirus	++	−	[29]
	DNA free	Sendai virus	++	++	[30]
Naked DNA	PiggyBac transposon		++	+	[31]
	pDNA		+	−	[32,33]
	Episomal pDNA		+++	+++	[34,35]
	mRNA		+++	+++	[36]
DNA free	microRNA		+++	+	[37]
	Protein		+++	−	[38]

UNCORRECTED PROOF

Please cite this article as: I. de Lázaro, et al., Induced pluripotent stem (iPS) cells: A new source for cell-based therapeutics? J. Control. Release (2014), http://dx.doi.org/10.1016/j.jconrel.2014.04.011



Q14 **Fig. 1.** *Current and envisioned applications of iPS cells.* The short- and long-term potential applications of disease- and patient-specific iPS cell derivatives include the establishment of reliable disease models, drug and toxicology screening, basic biology research and their use for cell replacement interventions in regenerative medicine.

derivatives have also been used for the investigation of the course of retinal degeneration [55]. Modeling of other disease types, such as kidney pathologies, is also envisioned even if not yet achieved [56].

Consequently, the discovery of new effective treatments for certain diseases may benefit by such *in vitro* models. Disease-specific iPS cells can thus be useful in drug and toxicology screening. One such recent example is the discovery of a candidate chemical compound for the treatment of amyotrophic lateral sclerosis (ALS) using iPS-derived motor neurons generated from patients that suffered from the disease [57]. Small molecules able to restore the expression of the main gene involved in familial dysautonomia have also been identified following the derivation of patient-specific iPS cells [58].

iPS cells also gradually become an invaluable tool in fundamental biological research. In particular, the information and knowledge derived from their differentiation into cells of different lineages is of great interest for developmental biology studies. For example, many questions remain unanswered regarding the course of cortical development in the mammalian brain due to the lack of appropriate models that recapitulate the early events of this process. A recent study has reported that iPS cells can be differentiated to all the types of pyramidal neurons that populate this area of the brain, therefore enabling the study of cortex development [59]. In addition, the thorough characterization of the epigenetic changes that occur in the generation of iPS cells may also help inform about other biological processes mainly driven by epigenetic mechanisms, such as carcinogenesis [60].

Other than their applications in disease modeling, drug screening and basic biological research, iPS cells are regarded as perhaps the most promising source of personalized cells for regenerative therapies. The ultimate aim is that iPS cells could be generated from the patient that requires treatment (autologous iPS), differentiated ex vivo into the cells affected in the disease with or without the assistance of gene therapy to correct genetic defects, and finally transplanted back into that particular patient [61]. This application of iPS cells is considered to be of a long-term perspective due to many obstacles that have to be overcome for successful and safe cell transplantation therapy. However, the enormous benefits potentially offered to cell replacement strategies have mobilized great interest and investment to support research for the clinical development of this technology. The field is moving rapidly towards clinical investigations and the first clinical study involving the transplantation of iPS cell derivatives has recently started to recruit patients [62].

4. iPS cells as cell therapeutics

The opportunities offered by iPS cell technology could overcome most of the obstacles that surround the clinical utilization of other types of stem cells in regenerative medicine [63].

4.1. iPS versus ES cells as sources for cell therapeutics

Since their first derivation from a mouse in 1981 [64] and human blastocysts in 1998 [65] ES cells have been regarded as one of the most promising sources of cells for replacement therapies. This is mainly due to the feasibility to keep them in culture with their self-renewal capacity intact and their versatile differentiation potential. ES cells are pluripotent and can potentially differentiate into cells of any developmental lineage (ie endoderm, mesoderm and ectoderm).

While iPS cells possess a similar pluripotent character and can be maintained in this state in culture, they offer certain advantages over ES cells. First, the isolation of ES cells involves the destruction of the blastocysts, which has generated multiple discussions regarding the ethics of human ES cell derivation. Different regulations for human ES cell usage and research have been set across different countries. In the European Union, the Court of Justice established in 2011 that any process involving the destruction of human blastocysts cannot be patented, which has caused concern in the scientific community and fear for a possible loss of investor motivation for research using human ES cells [7]. The fact that iPS cells can be generated from a wide variety of different somatic cell types, eliminates the need to manipulate and destroy embryonic materials and therefore circumvents the ethical and legal concerns that surround ES cell isolation.

Immune rejection of heterologous cells (obtained from an individual other than the one receiving the transplant) generally complicates the clinical translation of cell-based therapies. This has been regarded as a limitation of the use of ES cell derivatives in cell therapies and hence

UNCORRECTED

Please cite this article as: I. de Lázaro, et al., Induced pluripotent stem (iPS) cells: A new source for cell-based therapeutics? J. Control. Release (2014), http://dx.doi.org/10.1016/j.jconrel.2014.04.011

has been the subject of numerous studies [6,66,67]. The goal of iPS cell technology is to generate autologous, functional and committed cells from patients, turning regenerative medicine into a personalized treatment approach by minimizing the risk of graft rejection [68].

An initial report by Zhao et al. alarmingly reported the occurrence of immune responses in mice transplanted with syngeneic (genetically identical) undifferentiated iPS cells [69]. This study made use of the inherent ability of undifferentiated cells, such as iPS cells, to form teratomas (tumors of undifferentiated origin) when implanted *in vivo* [70]. Immune responses against iPS cell-derived teratomas were reported along with aberrant expression of the *Hormad* and *Zg16* genes [69]. In contrast, two recent studies by Guha et al. and Araki et al. have not validated these findings [71,72]. These two genes are commonly overexpressed in tumor cells, so their aberrant expression might be more related to teratoma formation than to the immunogenicity of iPS cells themselves. The contradiction between those studies could be further attributed to the differences among iPS cell lines generated by different protocols [73]. Despite such observations, the suitability of iPS cell derivatives for regenerative medicine has been supported by the success of various preclinical models in which iPS cells have been transplanted with no signs of rejection [74–76]. However, more systematic studies on the immunogenicity of primate and human iPS cell derivatives are required to assure the safety of this approach.

The fact that the generation of iPS cells avoids the use of embryonic material and can be achieved from patient-specific cells is hugely advantageous compared to ES cells. These differences are illustrated in Fig. 2. Whether the epigenetic state, genomic stability, mutational load and developmental potential of iPS cells are exactly equivalent to those of ES cells remains to be determined and should be thoroughly investigated [77,78].

4.2. iPS cells as sources for cell therapeutics: Pre-clinical studies

The first transplantation of iPS-derived cells for therapeutic purposes was carried out in a murine model of sickle cell anemia only one year after iPS cells were first described [79]. Since then, several other examples have highlighted the potential of iPS cell technology in regenerative medicine at the preclinical level. Table 2 summarizes the most important of these reports. Many of these studies describe successful engraftment of the transplanted cells and a degree of recovery of the diseased phenotype. For example, dopaminergic neurons re-differentiated from Parkinson patient-derived iPS cells successfully survived in the adult rodent brain and ameliorated motor asymmetry in Parkinsonian rats [80]. In a model of spinal cord injury, Nori et al. confirmed not only the engraftment of the cells but also their differentiation into mature neurons and axonal regrowth that led to functional recovery [81]. In another approach, reprogramming was accompanied with correction of the dystrophin gene in the iPS cells ex vivo to treat muscular dystrophy. The muscles of dystrophic animals engrafted with iPS-derived genetically corrected myogenic progenitors exhibited an improvement in their contractile capability [82,83].

Despite the positive results in these studies, others have encountered several challenges such as poor engraftment rates [84] or teratoma formation [85]. The latest has been attributed to undifferentiated cells among the transplanted cell population. This was especially notable in a mouse stroke model injected with undifferentiated iPS cells that were not able to offer any behavioral improvements, while tumor formation led to high death rates. Such observations highlight the importance of the differentiation stage in which the cells are injected as a determinant factor of successful outcome [86]. In another stroke model study undertaken by Jensen et al., the transplanted iPS derived cells were able to engraft in the host tissues at acceptable levels and differentiate into the appropriate cell type (i.e. neurons primarily) however with no functional improvement [87].

The field of cell transplantation is lacking clinically relevant technologies that can offer control of cell tracking and allow follow up (in terms of survival, location and functionality) of the transplanted cells. The studies available to date do not monitor the cells for longer than 4 months after transplantation that may not be enough to determine clinical outcome [88]. Overall, preclinical studies today highlight the

CORRECTED PROOF

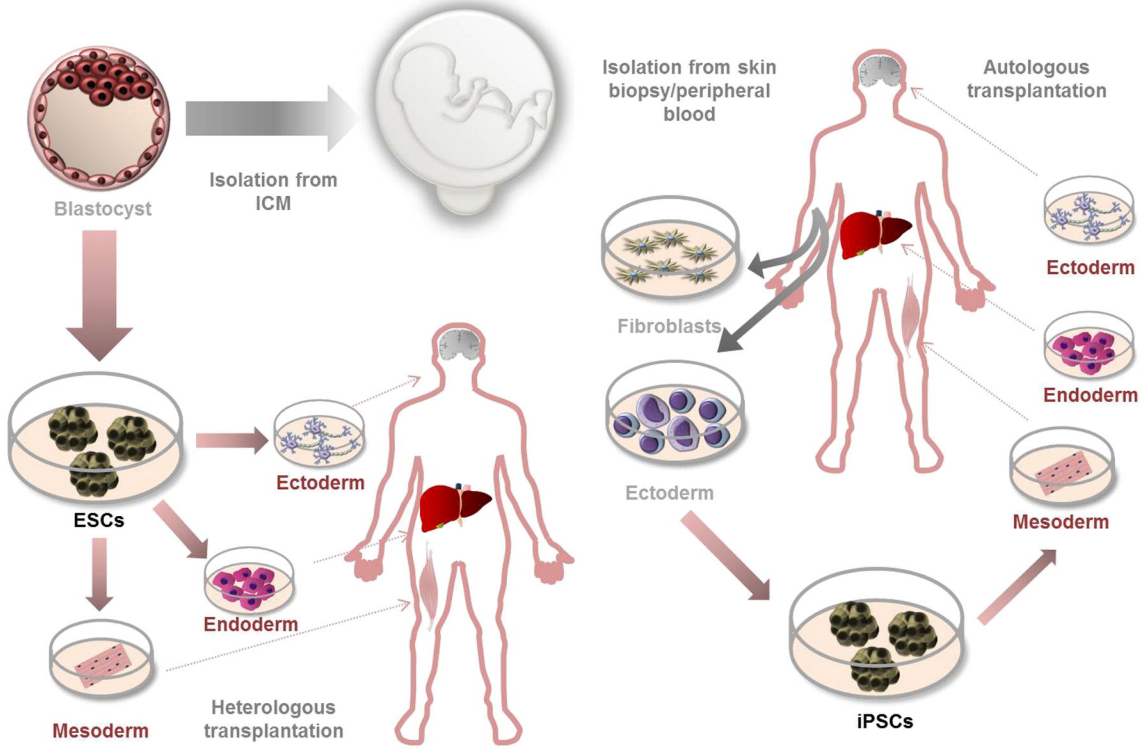


Fig. 2. *ES vs iPS cells in regenerative medicine applications.* The main advantages that iPS cells (right) offer as compared to ESCs (left) for cell replacement therapies are (1) the convenient harvesting of starting cells for their generation, avoiding the handling of embryonic material and (2) the fact that they can be generated from the same patient receiving the transplant.

Please cite this article as: I. de Lázaro, et al., Induced pluripotent stem (iPS) cells: A new source for cell-based therapeutics? J. Control. Release (2014), http://dx.doi.org/10.1016/j.jconrel.2014.04.011

ARTICLE IN PRESS

Table 2
Preclinical studies using iPS-derived cells in cell-based therapeutic approaches.

Disease model	iPS cell derivatives	Restorative effect	Ref.
Sickle cell anemia	Hematopoietic precursors (genetic defect corrected by gene therapy)	Normal erythrocyte phenotype restored	[79]
Parkinson's disease	Midbrain dopaminergic neurons	Recovery of Parkinsonian symptoms in behavioral tests	[74,80,85,89]
Muscular dystrophy	Myogenic progenitors (genetic defect corrected by gene therapy)	Improvement of muscle function	[83]
Spinal cord injury	Neurospheres	Enhanced recovery of motor function	[81]
Ischemic stroke	Neuroepithelial-like stem cells	Improved functional recovery of stroke-damaged brain	[88]
	Neural progenitor cells	Improvement of somatosensory and motor symptoms	[90]
	Neural progenitor cells	Graft survival and differentiation to neuronal phenotypes but no restorative effect	[87]
Intracerebral hemorrhage	Neuro-epithelial-like stem cells	Significant recuperation of neural function	[91]
Limb ischemia	Fetal liver kinase-1 positive cells	Revascularization of the ischemic limb accelerated via increased expression of VEGF	[92]
	Endothelial progenitors	Neovascularization	[76]
	Mesenchymal stem cells	Attenuation of severe ischemia	[93]
Myocardial infarction	iPS cells	Regeneration of infarcted tissue and improvement of contractile performance	[94]
	Endothelial progenitors	Neovascularization, reduction of fibrosis and infarction site	[76]
Cirrhotic liver	Hepatic progenitors	Liver regeneration	[84]
Retinitis pigmentosa	Retinal pigmented epithelial cells	Improved visual function	[75]
Age-related macular degeneration and retinitis pigmentosa	Developing rod photoreceptors	Neural activity similar to native photoreceptors	[95]

need to develop technologies for integration-free reprogramming protocols that lead to safe and efficient generation of iPS cells from patients, successful differentiation protocols and adequate cell tracking techniques as the key needs to enable future development of successful iPS cell-based therapies.

4.3. iPS cells as sources for cell therapeutics: Clinical studies

Considering the brief history of transcription factor induced cell reprogramming, the field is rapidly making its way towards clinical applications. An important factor behind this progress is the significant investment devoted to this technology. The Japanese government approved recently ¥21.4 billion in an ambitious program designed to bring these types of stem cells closer to the clinic [96]. Among the 8 programs that will be carried out in different research centers across the country, four of them involve the use of cells derived from iPS cells for tissue regenerative purposes [97].

The first human clinical study using iPS cell derivatives started recruiting patients in August 2013 and is expected to begin with cell transplantation procedures within 2014. Dr. Masayo Takahashi at the RIKEN Center for Developmental Biology in Kobe has been working on the generation of sheets of retinal pigment epithelium from patient-specific iPS cells to be implanted in the retina of patients suffering from age-related macular degeneration. This disease is the first cause of blindness in developed countries and affects approximately 1% of the population aged over 50. Dr Takahashi and her group have established a reproducible methodology to generate sheets of retinal pigment epithelium from human iPS cells [98]. In addition, the same group has confirmed that iPS derived retinal epithelium is functional upon transplantation in mice retina affected by the disease [95]. According to the clinical study design, the iPS-derived retinal cells will be implanted in the diseased retina of at least half a dozen patients. The expectation is that the transplanted cells derived from iPS cells will grow and repair the affected retinal epithelium, however the primary goal of this Phase I study will be to assess the safety of such intervention [99]. It is interesting to note that authorization by the Japanese Government has been granted for Takahashi's investigations on the basis of a clinical study, and not of a formal clinical trial. Therefore approval of iPS cell derivatives as a biological drug entity will not be conceded even if therapeutically positive results are obtained. However, assuming that the outcomes are positive, this could encourage the application for clinical trials and fuel the way of this technology towards the bedside [62].

Another group based in Kyoto University also intends to apply in the near future for the authorization of a clinical trial in which iPS cell-derived dopaminergic neurons would be used for the treatment of Parkinson's disease. This approach is supported by the encouraging results that have already been reported from studies in non-human primates [100], however previously failed clinical attempts using ES and fetal cells are illustrating how challenging such a therapeutic intervention can be.Elsewhere, the US-based biotechnology company Advanced Cell Technology has announced preparations to seek authorization of a clinical trial that would use iPS cell-derived platelets for the treatment of blood clotting disorders. In theory, such approaches can be less challenging compared to the clinical trials planned in Japan due to the fact that platelets lack a nucleus and hence would raise less safety concerns [99]. Overall, the rapid progress in clinical translation of iPS technology has been received with mixed reactions among experts. Some express excitement of the opportunities, while others believe that it might still be too early for iPS cell technology to have a role in the clinic and serious concerns have been expressed about the immaturity of the field and these trials [101].

4.4. Barriers to clinical translation of iPS cell technology

Despite the rapid and promising developments described above, several fundamental questions remain before iPS cells can be clinically used. One of the roadblocks that impacts on the safety of iPS cells is around the process of reprogramming itself. The fact that some of the reprogramming factors needed to achieve maximum efficiency (such as c-Myc) are known proto-oncogenes will need to be overcome [15]. Although the production of iPS cells without the expression of this proto-oncogene has been reported, a decrease in the efficiency of reprogramming has been concomitantly observed [17].

The vector technology used to overexpress the reprogramming factors in somatic cells is also critical. The most popular and efficient vectors to express the reprogramming factors today are retroviruses that contain the inherent risk of genomic aberrations in the transfected cells caused by insertional mutagenesis and can lead to tumorigenesis [102]. The gene therapy field has accumulated experience on the issues around insertional mutagenesis both at the preclinical and clinical level. Convergence of the two fields will significantly improve methodologies for iPS cell generation with the latest and safest vector technologies. Efforts towards use of safe, yet efficacious, cell reprogramming include the use of non-integrating vectors and even the use of DNA-free technologies to minimize or completely prevent the risk of insertional mutagenesis [103]. Episomal vectors currently offer the most optimum safety to efficiency ratio [34,35].

UNCORRECTED PROOF

Please cite this article as: I. de Lázaro, et al., Induced pluripotent stem (iPS) cells: A new source for cell-based therapeutics? J. Control. Release (2014), http://dx.doi.org/10.1016/j.jconrel.2014.04.011

ARTICLE IN PRESS

Another significant barrier is that of the complex culturing protocols used to generate, maintain and differentiate iPS cell colonies and the impact that these have on the cells. Epigenetic aberrations might appear if the process of cellular reprogramming is imperfect [104–106], while karyotypic abnormalities may be triggered as a result of long cell culture protocols [77]. Efforts have been made to improve the reprogramming methodologies and reduce the timeframe required to reach the pluripotent state. These could lower the number of stochastic steps that the cell needs to pass in order to achieve a pluripotent state, which would allow the generation of good-quality iPS colonies with lower aberrations [78,107].

Should iPS cell technology finally reach the realm of clinical regenerative medicine, the use of xeno-free media for their generation, maintenance and differentiation in culture will be imperative. While the generation of iPS cells in such xeno-free culture media has already been achieved by different laboratories, moving the field forward to meet Good Manufacturing Practice (GMP) and clinical-grade requirements [108–110], iPS differentiation protocols into different cell types still require the use of a wide variety of growth factors and culture conditions. The safety of the exposure to these molecular cues will also have to be thoroughly investigated prior to any clinical application [78,111].

Skepticism still prevails as to whether iPS cells can be considered identical or at least equivalent to ESCs with regards to their pluripotency and differentiation potential. Genome-wide analyses have found slight differences in gene expression profiles suggesting that epigenetic signatures from the tissue of origin remain in iPS cells after reprogramming [77]. The "epigenetic memory" of iPS cells can be an issue of concern as iPS cells from a particular origin may be prone or restricted to differentiate into cell types from the same lineage, thus complicating differentiation protocols to generate different cell types [112]. Indeed, epigenetic events in the early phases of the reprogramming process seem to be crucial in order to achieve full reprogramming to ground-state pluripotency, however these mechanisms have not yet been fully elucidated [113].

Given that undifferentiated stem cells are known to cause teratomas *in vivo*, it is imperative to guarantee that all iPS cells are successfully differentiated to the desired cell type before transplantation. This will need to be taken into consideration if production of GMP grade iPS cells for cell-based therapies is sought [114]. Inadequate engraftment of the iPS cell derivatives could also challenge their clinical application. In addition, if the cell identity is not stable after reprogramming and differentiation, the success of the therapy could also be limited [84].

Furthermore, generating clinical-grade iPS cells tailor-made to match every particular patient would be realistically very challenging, both in terms of economic resources and logistical (e.g. timing) requirements. With the technologies currently available, approximately 3 months are necessary to generate iPS cells from the somatic cells of a patient and subsequently differentiate the pluripotent population into the cell type needed [60]. Taking into account the necessary tests to assure the safety and quality of the cells, up to six months could be required. This timeframe constitutes a hurdle for the clinical relevance of iPS cells, especially in the treatment of lesions such as spinal cord injuries, in which the promptness of the intervention is very closely linked to the success of the therapy. Economic reasons could also be an issue if the demand of tailor-made iPS cells becomes widespread, since tens of thousands of dollars would be needed to derive each cell line.

In order to circumvent these obstacles, the establishment of banks of allogeneic iPS cell lines for their use not only in basic research but also in regenerative medicine has been proposed. Although at first glance this would act as a detriment of the notion of personalized iPS cell therapy, it has been calculated that a stock of 75 iPS cell lines derived from homozygous human leukocyte antigen (HLA) donors would be enough to match 80% of the population in Japan without triggering any immune response [34,115]. In a proposal that has been named the "*iPS cell Stock Project*", Shinya Yamanaka was authorized in September 2012 by the Japanese Health Ministry to generate iPS cells from samples stored in several cord blood banks established around the country [101]. Studies to follow a similar strategy have also highlighted that a pool of 150 cell lines from defined HLA donors would conveniently match 93% of the UK population [116].

4.5. Future Perspective: In vivo cell reprogramming to pluripotency for therapeutic applications

Induced transcriptional cell reprogramming to pluripotency and de-differentiation of liver tissue *in vivo* has been recently described. This was achieved by transiently forcing the overexpression of the original four reprogramming factors first described by Yamanaka and colleagues used for the generation of iPS cells by tail vein hydrodynamic injection of plasmid DNA [117,118]. This spatially (liver) and temporally (transient expression very soon after injection) targeted approach does not lead to the generation of teratomas. Subsequent studies further confirmed *in vivo* reprogramming to pluripotency by expression of the reprogramming factors that was "switched on" for long periods of time and in all tissues produced extensive teratomas [119,120]. Although the concept of *in vivo* reprogramming to pluripotency is still at its infancy, it could be of major interest in regenerative medicine applications and potentially help overcome some of the hurdles faced in the utilization of *in vitro* generated iPS cells. Provided that the *in vivo* tissue microenvironment will be able to drive the re-differentiation of the reprogrammed cells to normal functional phenotypes, the need for extraction of donor cells, iPS cell generation, culture, differentiation and transplantation could be by-passed. In that way the fields of gene therapy and the vector technologies developed in the last 20 years for a multitude of diseases and tissues could potentially merge with the field of cell reprogramming to achieve *in situ* tissue regeneration.

5. Conclusions

Great expectations are abundant for a variety of applications envisioned following the discovery of iPS cells. These include better and more pathologically-relevant models for pharmacological and toxicological screening, along with disease modeling and basic biological research in the short term. In the longer term cell replacement therapies and tissue regenerative medicines are also expected. iPS cells can offer advantages over ES cells, especially in terms of immune tolerance and design of personalized interventions. In addition, the minimally invasive nature of the procedure to generate such cells from human patients circumvents the difficulties of biopsies and the ethical controversies that surround the destruction of blastocysts to derive ES cells and have prompted different regulations around their use. The iPS technology is not exempt from challenges to be overcome and a lot more knowledge is needed before iPS cells can find their way in the clinic. However, promising results have already been obtained in pre-clinical studies involving different disease models. The immense interest and noteworthy investment in iPS cell technologies are fueling a rapid move towards the clinic only 8 years after its birth.

Acknowledgments

Irene de Lázaro would like to thank Obra Social LaCaixa and UCL School of Pharmacy for jointly funding part of this PhD Studentship. The funding sources had no involvement in the writing and submission of this manuscript. Q2

References

[1] K.K. Mallick, S.C. Cox, Biomaterial scaffolds for tissue engineering, Front. Biosci. (Elite Ed) 5 (2013) 341–360.
[2] D. Ilic, J.M. Polak, Stem cells in regenerative medicine: introduction, Br. Med. Bull. 98 (2011) 117–126.
[3] L.A. Fortier, Stem cells: classifications, controversies, and clinical applications, Vet. Surg. 34 (2005) 415–423.

UNCORRECTED PROOF

Please cite this article as: I. de Lázaro, et al., Induced pluripotent stem (iPS) cells: A new source for cell-based therapeutics? J. Control. Release (2014), http://dx.doi.org/10.1016/j.jconrel.2014.04.011

ARTICLE IN PRESS

[4] D.G. Phinney, D.J. Prockop, Concise review: mesenchymal stem/multipotent stromal cells: the state of transdifferentiation and modes of tissue repair—current views, Stem Cells 25 (2007) 2896–2902.
[5] C.V. Alvarez, M. Garcia-Lavandeira, M.E. Garcia-Rendueles, E. Diaz-Rodriguez, A.R. Garcia-Rendueles, S. Perez-Romero, T.V. Vila, J.S. Rodrigues, P.V. Lear, S.B. Bravo, Defining stem cell types: understanding the therapeutic potential of ESCs, ASCs, and iPS cells, J. Mol. Endocrinol. 49 (2012) R89–R111.
[6] K. English, K.J. Wood, Immunogenicity of embryonic stem cell-derived progenitors after transplantation, Curr. Opin. Organ Transplant 16 (2011) 90–95.
[7] I. Wilmut, Consternation and confusion following EU patent judgment, Cell Stem Cell 9 (2011) 498–499.
[8] V. Volarevic, B. Ljujic, P. Stojkovic, A. Lukic, N. Arsenijevic, M. Stojkovic, Human stem cell research and regenerative medicine—present and future, Br. Med. Bull. 99 (2011) 155–168.
Q3 [9] A. Abbott, Italian stem-cell trial based on flawed data, Nature News (2013).
Q4 [10] A. Abbott, Italian court rules science advisers unlawful, Nature News (2013).
[11] A. Abbott, Leaked files slam stem-cell therapy, Nature 505 (2014) 139–140.
[12] K. Takahashi, S. Yamanaka, Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors, Cell 126 (2006) 663–676.
[13] K. Takahashi, K. Tanabe, M. Ohnuki, M. Narita, T. Ichisaka, K. Tomoda, S. Yamanaka, Induction of pluripotent stem cells from adult human fibroblasts by defined factors, Cell 131 (2007) 861–872.
[14] A. Abbott, Cell rewind wins medicine Nobel, Nature 490 (2012) 151–152.
[15] K. Okita, T. Ichisaka, S. Yamanaka, Generation of germline-competent induced pluripotent stem cells, Nature 448 (2007) 313–317.
[16] J. Yu, M.A. Vodyanik, K. Smuga-Otto, J. Antosiewicz-Bourget, J.L. Frane, S. Tian, J. Nie, G.A. Jonsdottir, V. Ruotti, R. Stewart, I.I. Slukvin, J.A. Thomson, Induced pluripotent stem cell lines derived from human somatic cells, Science 318 (2007) 1917–1920.
[17] M. Nakagawa, M. Koyanagi, K. Tanabe, K. Takahashi, T. Ichisaka, T. Aoi, K. Okita, Y. Mochiduki, N. Takizawa, S. Yamanaka, Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts, Nat. Biotechnol. 26 (2008) 101–106.
[18] N. Montserrat, E. Nivet, I. Sancho-Martinez, T. Hishida, S. Kumar, L. Miquel, C. Cortina, Y. Hishida, Y. Xia, C.R. Esteban, J.C. Belmonte, Reprogramming of human
Q5 fibroblasts to pluripotency with lineage specifiers, Cell Stem Cell (2013).
[19] W.E. Lowry, L. Richter, R. Yachechko, A.D. Pyle, J. Tchieu, R. Sridharan, A.T. Clark, K. Plath, Generation of human induced pluripotent stem cells from dermal fibroblasts, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 2883–2888.
[20] Y.S. Chun, P. Chaudhari, Y.Y. Jang, Applications of patient-specific induced pluripotent stem cells; focused on disease modeling, drug screening and therapeutic potentials for liver disease, Int. J. Biol. Sci. 6 (2010) 796–805.
[21] K. Okita, T. Yamakawa, Y. Matsumura, Y. Sato, N. Amano, A. Watanabe, N. Goshima, S. Yamanaka, An efficient nonviral method to generate integration-free human-induced pluripotent stem cells from cord blood and peripheral blood cells, Stem Cells 31 (2013) 458–466.
[22] Y.H. Loh, S. Agarwal, I.H. Park, A. Urbach, H. Huo, G.C. Heffner, K. Kim, J.D. Miller, K. Ng, G.Q. Daley, Generation of induced pluripotent stem cells from human blood, Blood 113 (2009) 5476–5479.
[23] J. Staerk, M.M. Dawlaty, Q. Gao, D. Maetzel, J. Hanna, C.A. Sommer, G. Mostoslavsky, R. Jaenisch, Reprogramming of human peripheral blood cells to induced pluripotent stem cells, Cell Stem Cell 7 (2010) 20–24.
[24] X.B. Zhang, Cellular reprogramming of human peripheral blood cells, Genomics
Q6 Proteomics Bioinformatics (2013).
[25] F. Gonzalez, S. Boue, J.C. Izpisua Belmonte, Methods for making induced pluripotent stem cells: reprogramming a la carte, Nat. Rev. Genet. 12 (2011) 231–242.
[26] C.A. Sommer, M. Stadtfeld, G.J. Murphy, K. Hochedlinger, D.N. Kotton, G. Mostoslavsky, Induced pluripotent stem cell generation using a single lentiviral stem cell cassette, Stem Cells 27 (2009) 543–549.
[27] B. Hamilton, Q. Feng, M. Ye, G.G. Welstead, Generation of induced pluripotent stem cells by reprogramming mouse embryonic fibroblasts with a four transcription factor, doxycycline inducible lentiviral transduction system, J. Vis. Exp. (2009).
Q7 [28] C.A. Sommer, A.G. Sommer, T.A. Longmire, C. Christodoulou, D.D. Thomas, M. Gostissa, F.W. Alt, G.J. Murphy, D.N. Kotton, G. Mostoslavsky, Excision of reprogramming transgenes improves the differentiation potential of iPS cells generated with a single excisable vector, Stem Cells 28 (2010) 64–74.
[29] K. Tashiro, Optimization of adenovirus vectors for transduction in embryonic stem cells and induced pluripotent stem cells, Yakugaku Zasshi 131 (2011) 1333–1338.
[30] N. Fusaki, H. Ban, A. Nishiyama, K. Saeki, M. Hasegawa, Efficient induction of transgene-free human pluripotent stem cells using a vector based on Sendai virus, an RNA virus that does not integrate into the host genome, Proc. Jpn. Acad. Ser. B Phys. Biol. Sci. 85 (2009) 348–362.
[31] K. Woltjen, I.P. Michael, P. Mohseni, R. Desai, M. Mileikovsky, R. Hamalainen, R. Cowling, W. Wang, P. Liu, M. Gertsenstein, K. Kaji, H.K. Sung, A. Nagy, piggyBac transposition reprograms fibroblasts to induced pluripotent stem cells, Nature 458 (2009) 766–770.
[32] K. Okita, M. Nakagawa, H. Hyenjong, T. Ichisaka, S. Yamanaka, Generation of mouse induced pluripotent stem cells without viral vectors, Science 322 (2008) 949–953.
[33] K. Okita, H. Hong, K. Takahashi, S. Yamanaka, Generation of mouse-induced pluripotent stem cells with plasmid vectors, Nat. Protoc. 5 (2010) 418–428.
[34] K. Okita, Y. Matsumura, Y. Sato, A. Okada, A. Morizane, S. Okamoto, H. Hong, M. Nakagawa, K. Tanabe, K. Tezuka, T. Shibata, T. Kunisada, M. Takahashi, J. Takahashi, H. Saji, S. Yamanaka, A more efficient method to generate integration-free human iPS cells, Nat. Methods 8 (2011) 409–412.
[35] J. Yu, K. Hu, K. Smuga-Otto, S. Tian, R. Stewart, I.I. Slukvin, J.A. Thomson, Human induced pluripotent stem cells free of vector and transgene sequences, Science 324 (2009) 797–801.
[36] M. Li, I. Sancho-Martinez, J.C. Izpisua Belmonte, Cell fate conversion by mRNA, Stem Cell Res. Ther. 2 (2011) 5.
[37] F. Anokye-Danso, C.M. Trivedi, D. Juhr, M. Gupta, Z. Cui, Y. Tian, Y. Zhang, W. Yang, P.J. Gruber, J.A. Epstein, E.E. Morrisey, Highly efficient miRNA-mediated reprogramming of mouse and human somatic cells to pluripotency, Cell Stem Cell 8 (2011) 376–388.
[38] D. Kim, C.H. Kim, J.I. Moon, Y.G. Chung, M.Y. Chang, B.S. Han, S. Ko, E. Yang, K.Y. Cha, R. Lanza, K.S. Kim, Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins, Cell Stem Cell 4 (2009) 472–476.
[39] K. Plath, W.E. Lowry, Progress in understanding reprogramming to the induced pluripotent state, Nat. Rev. Genet. 12 (2011) 253–265.
[40] Y. Buganim, Dina A. Faddah, Albert W. Cheng, E. Itskovich, S. Markoulaki, K. Ganz, Sandy L. Klemm, A. van Oudenaarden, R. Jaenisch, Single-cell expression analyses during cellular reprogramming reveal an early stochastic and a late hierarchic phase, Cell 150 (2012) 1209–1222.
[41] Jose M. Polo, E. Anderssen, Ryan M. Walsh, Benjamin A. Schwarz, Christian M. Nefzger, Sue M. Lim, M. Borkent, E. Apostolou, S. Alaei, J. Cloutier, O. Bar-Nur, S. Cheloufi, M. Stadtfeld, Maria E. Figueroa, D. Robinton, S. Natesan, A. Melnick, J. Zhu, S. Ramaswamy, K. Hochedlinger, A molecular roadmap of reprogramming somatic cells into iPS cells, Cell 151 (2012) 1617–1632.
[42] Y. Rais, A. Zviran, S. Geula, O. Gafni, E. Chomsky, S. Viukov, A.A. Mansour, I. Caspi, V. Krupalnik, M. Zerbib, I. Maza, N. Mor, D. Baran, L. Weinberger, D.A. Jaitin, D. Lara-Astiaso, R. Blecher-Gonen, Z. Shipony, Z. Mukamel, T. Hagai, S. Gilad, D. Amann-Zalcenstein, A. Tanay, I. Amit, N. Novershtern, J.H. Hanna, Deterministic direct reprogramming of somatic cells to pluripotency, Nature (2013). Q8
[43] R. Jaenisch, R. Young, Stem cells, the molecular circuitry of pluripotency and nuclear reprogramming, Cell 132 (2008) 567–582.
[44] I.H. Park, N. Arora, H. Huo, N. Maherali, T. Ahfeldt, A. Shimamura, M.W. Lensch, C. Cowan, K. Hochedlinger, G.Q. Daley, Disease-specific induced pluripotent stem cells, Cell 134 (2008) 877–886.
[45] Y. Maury, M. Gauthier, M. Peschanski, C. Martinat, Human pluripotent stem cells for disease modelling and drug screening, Bioessays 34 (2011) 61–71.
[46] F.T. Merkle, K. Eggan, Modeling human disease with pluripotent stem cells: from genome association to function, Cell Stem Cell 12 (2013) 656–668.
[47] M. Bellin, M.C. Marchetto, F.H. Gage, C.L. Mummery, Induced pluripotent stem cells: the new patient? Nat. Rev. Mol. Cell Biol. 13 (2012) 713–726.
[48] D. Ma, H. Wei, Y. Zhao, J. Lu, G. Li, N.B. Sahib, T.H. Tan, K.Y. Wong, W. Shim, P. Wong, S.A. Cook, R. Liew, Modeling type 3 long QT syndrome with cardiomyocytes derived from patient-specific induced pluripotent stem cells, Int. J. Cardiol. (2013). Q9
[49] M.A. Israel, S.H. Yuan, C. Bardy, S.M. Reyna, Y. Mu, C. Herrera, M.P. Hefferan, S. Van Gorp, K.L. Nazor, F.S. Boscolo, C.T. Carson, L.C. Laurent, M. Marsala, F.H. Gage, A.M. Remes, E.H. Koo, L.S. Goldstein, Probing sporadic and familial Alzheimer's disease using induced pluripotent stem cells, Nature 482 (2012) 216–220.
[50] A. Eigentler, S. Boesch, R. Schneider, G. Dechant, R. Nat, Induced pluripotent stem cells from Friedreich ataxia patients fail to upregulate frataxin during *in vitro* differentiation to peripheral sensory neurons, Stem Cells Dev. (2013). Q10
[51] J. Du, E. Campau, E. Soragni, C. Jespersen, J.M. Gottesfeld, Length-dependent CTG. CAG triplet-repeat expansion in myotonic dystrophy patient-derived induced pluripotent stem cells, Hum. Mol. Genet. (2013). Q11
[52] G.H. Liu, B.Z. Barkho, S. Ruiz, D. Diep, J. Qu, S.L. Yang, A.D. Panopoulos, K. Suzuki, L. Kurian, C. Walsh, J. Thompson, S. Boue, H.L. Fung, I. Sancho-Martinez, K. Zhang, J. Yates 3rd, J.C. Izpisua Belmonte, Recapitulation of premature ageing with iPSCs from Hutchinson–Gilford progeria syndrome, Nature 472 (2011) 221–225.
[53] J. Zhang, Q. Lian, G. Zhu, F. Zhou, L. Sui, C. Tan, R.A. Mutalif, R. Navasankari, Y. Zhang, H.F. Tse, C.L. Stewart, A. Colman, A human iPSC model of Hutchinson Gilford Progeria reveals vascular smooth muscle and mesenchymal stem cell defects, Cell Stem Cell 8 (2011) 31–45.
[54] S. Agarwal, Y.H. Loh, E.M. McLoughlin, J. Huang, I.H. Park, J.D. Miller, H. Huo, M. Okuka, R.M. Dos Reis, S. Loewer, H.H. Ng, D.L. Keefe, F.D. Goldman, A.J. Klingelhutz, L. Liu, G.Q. Daley, Telomere elongation in induced pluripotent stem cells from dyskeratosis congenita patients, Nature 464 (2010) 292–296.
[55] D.M. Gamm, M.J. Phillips, R. Singh, Modeling retinal degenerative diseases with human iPS-derived cells: current status and future implications, Expert Rev. Ophthalmol. 8 (2013) 213–216.
[56] A.C. O'Neill, S.D. Ricardo, Human kidney cell reprogramming: applications for disease modeling and personalized medicine, J. Am. Soc. Nephrol. 24 (2013) 1347–1356.
[57] N. Egawa, S. Kitaoka, K. Tsukita, M. Naitoh, K. Takahashi, T. Yamamoto, F. Adachi, T. Kondo, K. Okita, I. Asaka, T. Aoi, A. Watanabe, Y. Yamada, A. Morizane, J. Takahashi, T. Ayaki, H. Ito, K. Yoshikawa, S. Yamawaki, S. Suzuki, D. Watanabe, H. Hioki, T. Kaneko, K. Makioka, K. Okamoto, H. Takuma, A. Tamaoka, K. Hasegawa, T. Nonaka, M. Hasegawa, A. Kawata, M. Yoshida, T. Nakahata, R. Takahashi, M.C.N. Marchetto, F.H. Gage, S. Yamanaka, H. Inoue, Drug screening for ALS using patient-specific induced pluripotent stem cells, Sci. Transl. Med. 4 (2012) (145ra104).
[58] G. Lee, C.N. Ramirez, H. Kim, N. Zeltner, B. Liu, C. Radu, B. Bhinder, Y.J. Kim, I.Y. Choi, B. Mukherjee-Clavin, H. Djaballah, L. Studer, Large-scale screening using familial dysautonomia induced pluripotent stem cells identifies compounds that rescue IKBKAP expression, Nat. Biotechnol. 30 (2012) 1244–1248.
[59] I. Espuny-Camacho, K.A. Michelsen, D. Gall, D. Linaro, A. Hasche, J. Bonnefont, C. Bali, D. Orduz, A. Bilheu, A. Herpoel, N. Lambert, N. Gaspard, S. Peron, S.N. Schiffmann, M. Giugliano, A. Gaillard, P. Vanderhaeghen, Pyramidal neurons derived from human pluripotent stem cells integrate efficiently into mouse brain circuits *in vivo*, Neuron 77 (2013) 440–456.
[60] K. Takahashi, S. Yamanaka, Induced pluripotent stem cells in medicine and biology, Development 140 (2013) 2457–2461.
[61] J. Sng, T. Lufkin, Emerging stem cell therapies: treatment, safety, and biology, Stem Cells Int. 2012 (2012) 521343.

UNCORRECTED PROOF

Please cite this article as: I. de Lázaro, et al., Induced pluripotent stem (iPS) cells: A new source for cell-based therapeutics? J. Control. Release (2014), http://dx.doi.org/10.1016/j.jconrel.2014.04.011

[62] D. Cyranoski, iPS cells in humans, Nat. Biotechnol. 31 (2013) 775.
[63] S. Yamanaka, A fresh look at iPS cells, Cell 137 (2009) 13–17.
[64] M.J. Evans, M.H. Kaufman, Establishment in culture of pluripotential cells from mouse embryos, Nature 292 (1981) 154–156.
[65] J.A. Thomson, J. Itskovitz-Eldor, S.S. Shapiro, M.A. Waknitz, J.J. Swiergiel, V.S. Marshall, J.M. Jones, Embryonic stem cell lines derived from human blastocysts, Science 282 (1998) 1145–1147.
[66] D.C. Wu, A.S. Boyd, K.J. Wood, Embryonic stem cells and their differentiated derivatives have a fragile immune privilege but still represent novel targets of immune attack, Stem Cells 26 (2008) 1939–1950.
[67] R.J. Swijnenburg, S. Schrepfer, F. Cao, J.I. Pearl, X. Xie, A.J. Connolly, R.C. Robbins, J.C. Wu, *In vivo* imaging of embryonic stem cells reveals patterns of survival and immune rejection following transplantation, Stem Cells Dev. 17 (2008) 1023–1029.
[68] J.D. Ebben, M. Zorniak, P.A. Clark, J.S. Kuo, Introduction to induced pluripotent stem cells: advancing the potential for personalized medicine, World Neurosurg. 76 (2011) 270–275.
[69] T. Zhao, Z.N. Zhang, Z. Rong, Y. Xu, Immunogenicity of induced pluripotent stem cells, Nature 474 (2011) 212–215.
[70] F.J. Muller, J. Goldmann, P. Loser, J.F. Loring, A call to standardize teratoma assays used to define human pluripotent cell lines, Cell Stem Cell 6 (2010) 412–414.
[71] P. Guha, J.W. Morgan, G. Mostoslavsky, N.P. Rodrigues, A.S. Boyd, Lack of immune response to differentiated cells derived from syngeneic induced pluripotent stem cells, Cell Stem Cell 12 (2013) 407–412.
[72] R. Araki, M. Uda, Y. Hoki, M. Sunayama, M. Nakamura, S. Ando, M. Sugiura, H. Ideno, A. Shimada, A. Nifuji, M. Abe, Negligible immunogenicity of terminally differentiated cells derived from induced pluripotent or embryonic stem cells, Nature 494 (2013) 100–104.
[73] S. Kaneko, S. Yamanaka, To be immunogenic, or not to be: that's the iPSC question, Cell Stem Cell 12 (2013) 385–386.
[74] M. Wernig, J.P. Zhao, J. Pruszak, E. Hedlund, D. Fu, F. Soldner, V. Broccoli, M. Constantine-Paton, O. Isacson, R. Jaenisch, Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease, Proc. Natl. Acad. Sci. U. S. A. 105 (2008) 5856–5861.
[75] Y. Li, Y.T. Tsai, C.W. Hsu, D. Erol, J. Yang, W.H. Wu, R.J. Davis, D. Egli, S.H. Tsang, Long-term safety and efficacy of human induced pluripotent stem cell (iPS) grafts in a preclinical model of retinitis pigmentosa, Mol. Med. (2012). Q12
[76] C.H. Yoo, H.J. Na, D.S. Lee, S.C. Heo, Y. An, J. Cha, C. Choi, J.H. Kim, J.C. Park, Y.S. Cho, Endothelial progenitor cells from human dental pulp-derived iPS cells as a therapeutic target for ischemic vascular diseases, Biomaterials 34 (2013) 8149–8160.
[77] M.C. Puri, A. Nagy, Concise review: embryonic stem cells versus induced pluripotent stem cells: the game is on, Stem Cells 30 (2012) 10–14.
[78] J. Bilic, J.C. Izpisua Belmonte, Concise review: induced pluripotent stem cells versus embryonic stem cells: close enough or yet too far apart? Stem Cells 30 (2012) 33–41.
[79] J. Hanna, M. Wernig, S. Markoulaki, C.W. Sun, A. Meissner, J.P. Cassady, C. Beard, T. Brambrink, L.C. Wu, T.M. Townes, R. Jaenisch, Treatment of sickle cell anemia mouse model with iPS cells generated from autologous skin, Science 318 (2007) 1920–1923.
[80] G. Hargus, O. Cooper, M. Deleidi, A. Levy, K. Lee, E. Marlow, A. Yow, F. Soldner, D. Hockemeyer, P.J. Hallett, T. Osborn, R. Jaenisch, O. Isacson, Differentiated Parkinson patient-derived induced pluripotent stem cells grow in the adult rodent brain and reduce motor asymmetry in Parkinsonian rats, Proc. Natl. Acad. Sci. U. S. A. 107 (2010) 15921–15926.
[81] S. Nori, Y. Okada, A. Yasuda, O. Tsuji, Y. Takahashi, Y. Kobayashi, K. Fujiyoshi, M. Koike, Y. Uchiyama, E. Ikeda, Y. Toyama, S. Yamanaka, M. Nakamura, H. Okano, Grafted human-induced pluripotent stem-cell-derived neurospheres promote motor functional recovery after spinal cord injury in mice, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 16825–16830.
[82] I.H. Park, DYS-HAC-iPS cells: the combination of gene and cell therapy to treat duchenne muscular dystrophy, Mol. Ther. 18 (2010) 238–240.
[83] A. Filareto, S. Parker, R. Darabi, L. Borges, M. Iacovino, T. Schaaf, T. Mayerhofer, J.S. Chamberlain, J.M. Ervasti, R.S. McIvor, M. Kyba, R.C. Perlingeiro, An ex vivo gene therapy approach to treat muscular dystrophy using inducible pluripotent stem cells, Nat. Commun. 4 (2013) 1549.
[84] H. Liu, Y. Kim, S. Sharkis, L. Marchionni, Y.Y. Jang, *In vivo* liver regeneration potential of human induced pluripotent stem cells from diverse origins, Sci. Transl. Med. 3 (2011) (82ra39).
[85] Y.H. Rhee, J.Y. Ko, M.Y. Chang, S.H. Yi, D. Kim, C.H. Kim, J.W. Shim, A.Y. Jo, B.W. Kim, H. Lee, S.H. Lee, W. Suh, C.H. Park, H.C. Koh, Y.S. Lee, R. Lanza, K.S. Kim, Protein-based human iPS cells efficiently generate functional dopamine neurons and can treat a rat model of Parkinson disease, J. Clin. Invest. 121 (2011) 2326–2335.
[86] H. Kawai, T. Yamashita, Y. Ohta, K. Deguchi, S. Nagotani, X. Zhang, Y. Ikeda, T. Matsuura, K. Abe, Tridermal tumorigenesis of induced pluripotent stem cells transplanted in ischemic brain, J. Cereb. Blood Flow Metab. 30 (2010) 1487–1493.
[87] M.B. Jensen, H. Yan, R. Krishnaney-Davison, A. Al Sawaf, S.C. Zhang, Survival and differentiation of transplanted neural stem cells derived from human induced pluripotent stem cells in a rat stroke model, J. Stroke Cerebrovasc. Dis. 22 (2013) 304–308.
[88] K. Oki, J. Tatarishvili, J. Wood, P. Koch, S. Wattananit, Y. Mine, E. Monni, D. Tornero, H. Ahlenius, J. Ladewig, O. Brustle, O. Lindvall, Z. Kokaia, Human-induced pluripotent stem cells form functional neurons and improve recovery after grafting in stroke-damaged brain, Stem Cells 30 (2012) 1120–1133.
[89] A. Swistowski, J. Peng, Q. Liu, P. Mali, M.S. Rao, L. Cheng, X. Zeng, Efficient generation of functional dopaminergic neurons from human induced pluripotent stem cells under defined conditions, Stem Cells 28 (2010) 1893–1904.
[90] J. Polentes, P. Jendelova, M. Cailleret, H. Braun, N. Romanyuk, P. Tropel, M. Brenot, V. Itier, C. Seminatore, K. Baldauf, K. Turnovcova, D. Jirak, M. Teletin, J. Come, J. Tournois, K. Reymann, E. Sykova, S. Viville, B. Onteniente, Human induced pluripotent stem cells improve stroke outcome and reduce secondary degeneration in the recipient brain, Cell Transplant. 21 (2012) 2587–2602.
[91] J. Qin, B. Song, H. Zhang, Y. Wang, N. Wang, Y. Ji, J. Qi, A. Chandra, B. Yang, Y. Zhang, G. Gong, Y. Xu, Transplantation of human neuro-epithelial-like stem cells derived from induced pluripotent stem cells improves neurological function in rats with experimental intracerebral hemorrhage, Neurosci. Lett. 548 (2013) 95–100.
[92] H. Suzuki, R. Shibata, T. Kito, M. Ishii, P. Li, T. Yoshikai, N. Nishio, S. Ito, Y. Numaguchi, J.K. Yamashita, T. Murohara, K. Isobe, Therapeutic angiogenesis by transplantation of induced pluripotent stem cell-derived Flk-1 positive cells, BMC Cell Biol. 11 (2010) 72.
[93] Q. Lian, Y. Zhang, J. Zhang, H.K. Zhang, X. Wu, F.F. Lam, S. Kang, J.C. Xia, W.H. Lai, K. W. Au, Y.Y. Chow, C.W. Siu, C.N. Lee, H.F. Tse, Functional mesenchymal stem cells derived from human induced pluripotent stem cells attenuate limb ischemia in mice, Circulation 121 (2010) 1113–1123.
[94] T.J. Nelson, A. Martinez-Fernandez, S. Yamada, C. Perez-Terzic, Y. Ikeda, A. Terzic, Repair of acute myocardial infarction by human stemness factors induced pluripotent stem cells, Circulation 120 (2009) 408–416.
[95] K. Homma, S. Okamoto, M. Mandai, N. Gotoh, H.K. Rajasimha, Y.S. Chang, S. Chen, W. Li, T. Cogliati, A. Swaroop, M. Takahashi, Developing rods transplanted into the degenerating retina of Crx-knockout mice exhibit neural activity similar to native photoreceptors, Stem Cells 31 (2013) 1149–1159.
[96] D. Cyranoski, Japan's stimulus package showers science with cash, Nature 493 (2013) 465.
[97] P.P. Song, Y. Inagaki, Y. Sugawara, N. Kokudo, Perspectives on human clinical trials of therapies using iPS cells in Japan: Reaching the forefront of stem-cell therapies, Biosci. Trends 7 (2013) 157–158.
[98] Y. Hirami, F. Osakada, K. Takahashi, K. Okita, S. Yamanaka, H. Ikeda, N. Yoshimura, M. Takahashi, Generation of retinal cells from mouse and human induced pluripotent stem cells, Neurosci. Lett. 458 (2009) 126–131.
[99] D. Cyranoski, Stem cells cruise to clinic, Nature 494 (2013) 413.
[100] A.M. Tetsuhiro Kikuchi, Daisuke Doi, Hirotaka Onoe, Hayashi Takuya, Toshiyuki Kawasaki, Hidemoto Saiki, Susumu Miyamoto, Jun Takahashi, Survival of human induced pluripotent stem cell-derived midbrain dopaminergic neurons in the brain of a primate model of Parkinson's disease, J. Park. Dis. 1 (2011) 395–412.
[101] D. Cyranoski, Stem-cell pioneer banks on future therapies, Nature 488 (2012) 139.
[102] G. Tavernier, B. Mlody, J. Demeester, J. Adjaye, S.C. De Smedt, Current methods for inducing pluripotency in somatic cells, Adv. Mater. (2013). Q13
[103] D.A. Robinton, G.Q. Daley, The promise of induced pluripotent stem cells in research and therapy, Nature 481 (2012) 295–305.
[104] S.M. Hussein, N.N. Batada, S. Vuoristo, R.W. Ching, R. Autio, E. Narva, S. Ng, M. Sourour, R. Hamalainen, C. Olsson, K. Lundin, M. Mikkola, R. Trokovic, M. Peitz, O. Brustle, D.P. Bazett-Jones, K. Alitalo, R. Lahesmaa, A. Nagy, T. Otonkoski, Copy number variation and selection during reprogramming to pluripotency, Nature 471 (2011) 58–62.
[105] R. Lister, M. Pelizzola, Y.S. Kida, R.D. Hawkins, J.R. Nery, G. Hon, J. Antosiewicz-Bourget, R. O'Malley, R. Castanon, S. Klugman, M. Downes, R. Yu, R. Stewart, B. Ren, J.A. Thomson, R.M. Evans, J.R. Ecker, Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells, Nature 471 (2011) 68–73.
[106] A. Gore, Z. Li, H.L. Fung, J.E. Young, S. Agarwal, J. Antosiewicz-Bourget, I. Canto, A. Giorgetti, M.A. Israel, E. Kiskinis, J.H. Lee, Y.H. Loh, P.D. Manos, N. Montserrat, A.D. Panopoulos, S. Ruiz, M.L. Wilbert, J. Yu, E.F. Kirkness, J.C. Izpisua Belmonte, D.J. Rossi, J.A. Thomson, K. Eggan, G.Q. Daley, L.S. Goldstein, K. Zhang, Somatic coding mutations in human induced pluripotent stem cells, Nature 471 (2011) 63–67.
[107] K. Miura, Y. Okada, T. Aoi, A. Okada, K. Takahashi, K. Okita, M. Nakagawa, M. Koyanagi, K. Tanabe, M. Ohnuki, D. Ogawa, E. Ikeda, H. Okano, S. Yamanaka, Variation in the safety of induced pluripotent stem cell lines, Nat. Biotechnol. 27 (2009) 743–745.
[108] B.A. Tucker, K.R. Anfinson, R.F. Mullins, E.M. Stone, M.J. Young, Use of a synthetic xeno-free culture substrate for induced pluripotent stem cell induction and retinal differentiation, Stem Cells Transl. Med. 2 (2013) 16–24.
[109] G. Chen, D.R. Gulbranson, Z. Hou, J.M. Bolin, V. Ruotti, M.D. Probasco, K. Smuga-Otto, S.E. Howden, N.R. Diol, N.E. Propson, R. Wagner, G.O. Lee, J. Antosiewicz-Bourget, J.M. Teng, J.A. Thomson, Chemically defined conditions for human iPSC derivation and culture, Nat. Methods 8 (2011) 424–429.
[110] K. Rajala, B. Lindroos, S.M. Hussein, R.S. Lappalainen, M. Pekkanen-Mattila, J. Inzunza, B. Rozell, S. Miettinen, S. Narkilahti, E. Kerkela, K. Aalto-Setala, T. Otonkoski, R. Suuronen, O. Hovatta, H. Skottman, A defined and xeno-free culture method enabling the establishment of clinical-grade human embryonic, induced pluripotent and adipose stem cells, PLoS One 5 (2010) e10246.
[111] S. Asgari, M. Moslem, K. Bagheri-Lankarani, B. Pournasr, M. Miryounesi, H. Baharvand, Differentiation and transplantation of human induced pluripotent stem cell-derived hepatocyte-like cells, Stem Cell Rev. 9 (2013) 493–504.
[112] G.J. Sullivan, Y. Bai, J. Fletcher, I. Wilmut, Induced pluripotent stem cells: epigenetic memories and practical implications, Mol. Hum. Reprod. 16 (2010) 880–885.
[113] B. Papp, K. Plath, Epigenetics of reprogramming to induced pluripotency, Cell 152 (2013) 1324–1343.
[114] W.E. Lowry, W.L. Quan, Roadblocks en route to the clinical application of induced pluripotent stem cells, J. Cell Sci. 123 (2010) 643–651.
[115] F. Nakajima, K. Tokunaga, N. Nakatsuji, Human leukocyte antigen matching estimations in a hypothetical bank of human embryonic stem cell lines in the Japanese population for use in cell transplantation therapy, Stem Cells 25 (2007) 983–985.
[116] C.J. Taylor, S. Peacock, A.N. Chaudhry, J.A. Bradley, E.M. Bolton, Generating an iPSC bank for HLA-matched tissue transplantation based on known donor and recipient HLA types, Cell Stem Cell 11 (2012) 147–152.

UNCORRECTED PROOF

Please cite this article as: I. de Lázaro, et al., Induced pluripotent stem (iPS) cells: A new source for cell-based therapeutics? J. Control. Release (2014), http://dx.doi.org/10.1016/j.jconrel.2014.04.011

ARTICLE IN PRESS

[117] A. Yilmazer, I. de Lazaro, C. Bussy, K. Kostarelos, *In vivo* cell reprogramming towards pluripotency by virus-free overexpression of defined factors, PLoS One 8 (2013) e54754.
[118] A. Yilmazer, I. de Lazaro, C. Bussy, K. Kostarelos, *In vivo* reprogramming of adult somatic cells to pluripotency by overexpression of Yamanaka factors, JoVE 17 (2013).
[119] M. Abad, L. Mosteiro, C. Pantoja, M. Canamero, T. Rayon, I. Ors, O. Grana, D. Megias, O. Dominguez, D. Martinez, M. Manzanares, S. Ortega, M. Serrano, Reprogramming *in vivo* produces teratomas and iPS cells with totipotency features, Nature 502 (2013) 340–345.
[120] K. Ohnishi, K. Semi, T. Yamamoto, M. Shimizu, A. Tanaka, K. Mitsunaga, K. Okita, K. Osafune, Y. Arioka, T. Maeda, H. Soejima, H. Moriwaki, S. Yamanaka, K. Woltjen, Y. Yamada, Premature termination of reprogramming *in vivo* leads to cancer development through altered epigenetic regulation, Cell 156 (2014) 663–677.

UNCORRECTED PROOF

Please cite this article as: I. de Lázaro, et al., Induced pluripotent stem (iPS) cells: A new source for cell-based therapeutics? J. Control. Release (2014), http://dx.doi.org/10.1016/j.jconrel.2014.04.011