

# Can Pluripotent Stem Cells Be Used in Cell-Based Therapy?

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## Abstract

Pluripotent stem cells, both embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), have the ability to differentiate into several cell types that can be used in drug testing and also in the study and treatment of diseases. These cells can be differentiated by *in vitro* systems, which may serve as models for human diseases and for cell transplantation. In this review, we address the pluripotent cell types, how to obtain and characterize these cells, and differentiation assays. We also focus on the potential of these cells in clinical trials, and we describe the clinical trials that are underway.

## Introduction

**P**LURIPOTENT STEM CELLS ARE a unique cells that are able to self-renew and differentiate into any adult tissue (epithelial, connective, muscle, neural, and others). This great differentiation capacity makes pluripotent stem cells very attractive to research studies with the hope of their being used in cell therapies in the future. We can divide pluripotent cells basically into two types. The first type, embryonic stem cells (ESCs), is physiological and is present in the blastocyst stage of embryonic development. These cells can be isolated from the inner cell mass (ICM) of the blastocyst (Bongso et al., 1994) during the stage of embryonic development when implantation occurs. The second type is an artificial or “induced” cell, called induced pluripotent stem cells (iPSCs); these cells were obtained for the first time in 2006 by the introduction of four genes able to reprogram somatic mouse cells into pluripotent stem cells (Takahashi and Yamanaka, 2006). One year later, it was demonstrated that human fibroblast cells also be reprogrammed (Takahashi et al., 2007). This new source of pluripotent cells has accelerated the number of studies in the pluripotent area. Figure 1 shows the evolution of publications in the field of ESCs and iPSCs since 2000 using data from PubMed.

The main objective of research with pluripotent stem cells is that these cells can be used in clinical trials. However, to use these cells in clinical applications, their efficiency and safety need to be proven scientifically. At the moment, there are still more questions than answers: What are the characteristics of a pluripotent cell? What is the best way to obtain and manipulate them? Are the differentiated cell lines derived from them really functional? Are iPSCs and ESCs equivalent? These questions still do not have answers. What

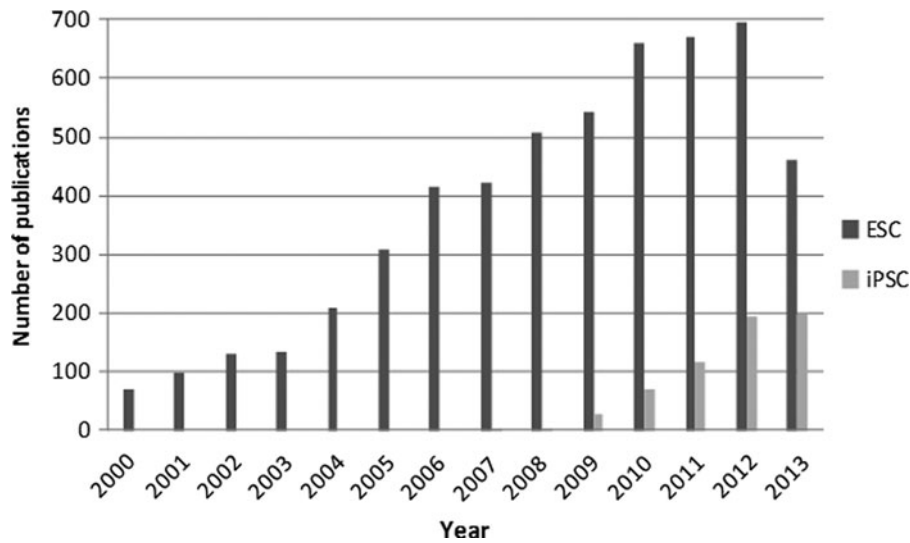
we have is the hope that stem cells may one day provide therapies for human diseases, a hope that seems more likely with the advancement of scientific research. In this review, we will discuss the types of pluripotent cells and their characterization, pluripotent pathways, differentiation processes, and the clinical trials using pluripotent stem cells.

## Pluripotent Cell Types

There are two types of pluripotent cells that occur in nature: (1) ESCs and (2) embryonic germ cells (EGCs). ESCs can be isolated from the ICM of the blastocyst 4–5 days postfertilization. Human (h) ESCs are isolated from frozen embryos that were not used in *in vitro* fertilization procedures. ESCs are isolated and cultured in specific culture media and expanded *in vitro*. These cells are fed daily and can be enzymatically or mechanically separated for expansion to be used in experiments (Thomson et al., 1998).

ESCs hold out the promise of being used in different cell therapies, and the demand continues for more hESC lines that can be used in clinical trials. Twenty-five countries are investing in the development of hESC lines (Fig. 2). There are a total of 695 validated hESC human cell lines (data from European Human Embryonic Stem Cell Registry, [www.hescreg.eu/](http://www.hescreg.eu/)), of which 213 are in the United States and 119 in the United Kingdom.

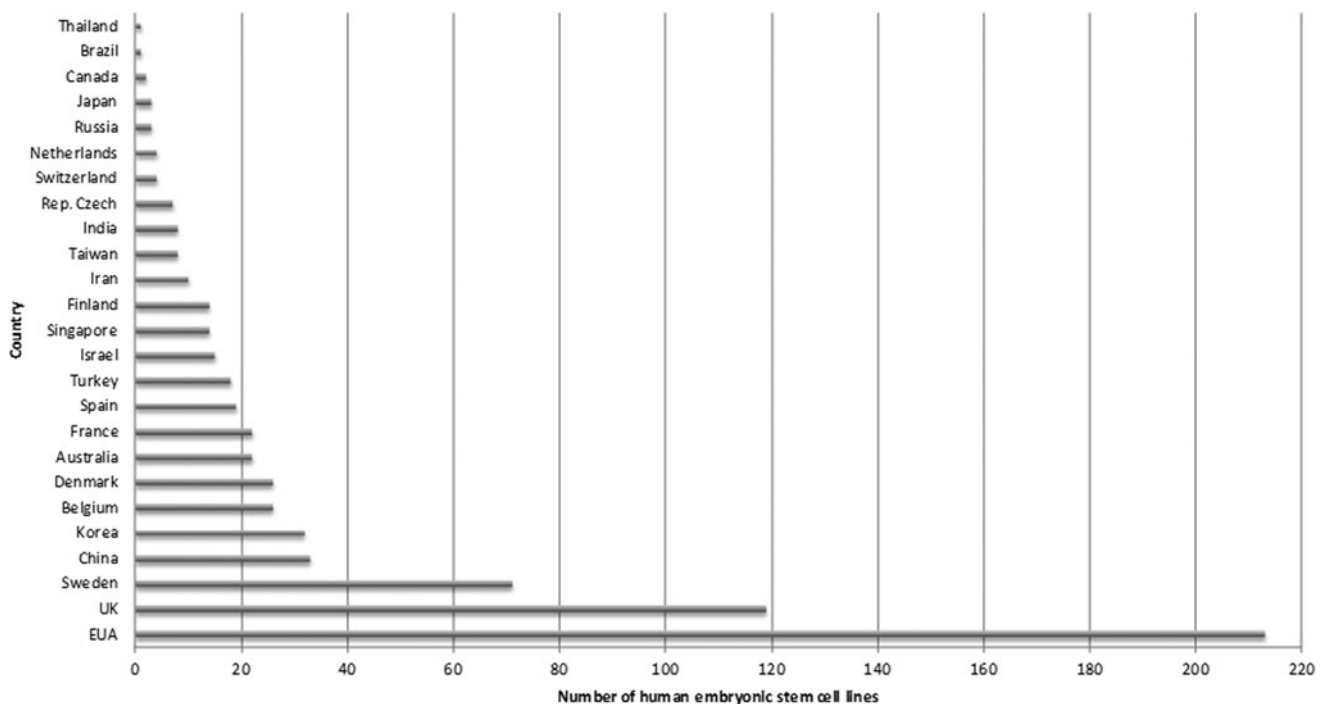
The second type of pluripotent stem cells, EGCs, can be isolated from fetal gonads over 6–8 weeks postconception (Liu et al., 2004). In general, germ cells originate near the gut of an embryo and migrate to the developing gonads. EGCs can also be isolated from teratocarcinomas; these cells are usually aneuploid and cannot be used in therapeutic approaches.



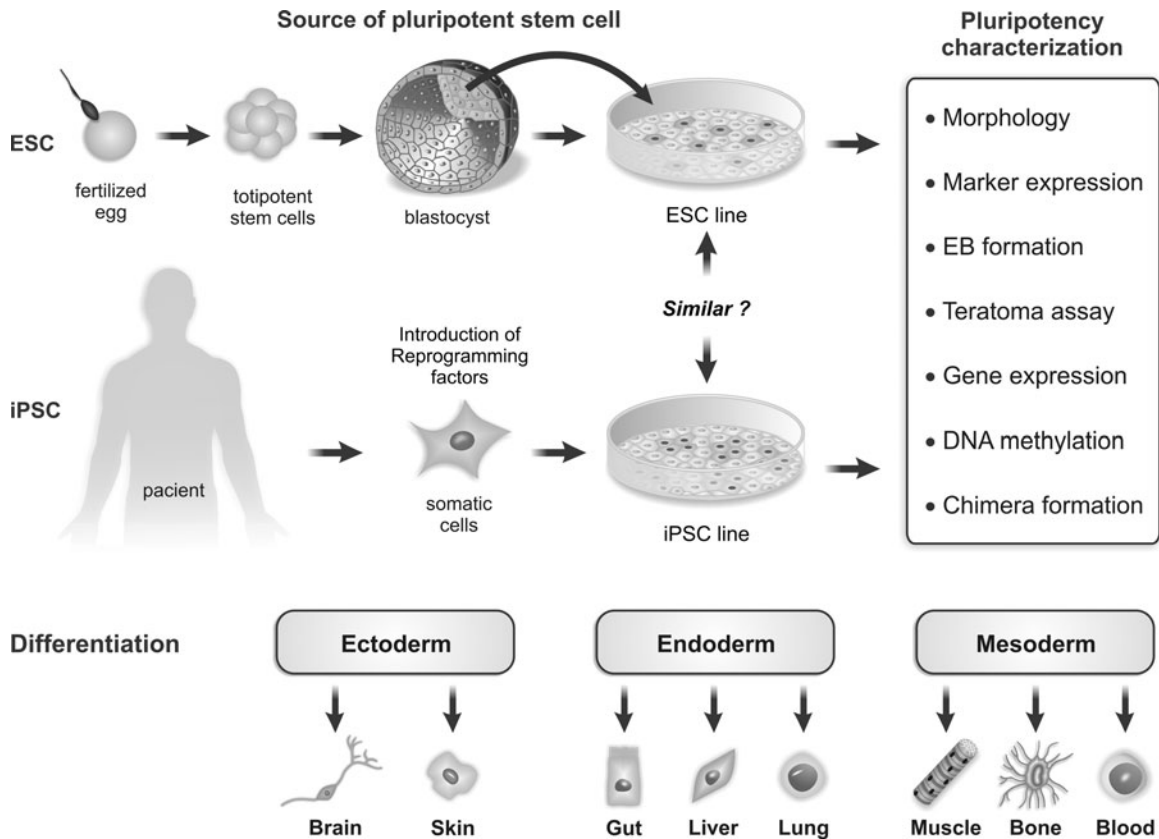
**FIG. 1.** Articles on pluripotent stem cells published from 2000–2014. (Data from Pubmed [www.ncbi.nlm.nih.gov/pubmed](http://www.ncbi.nlm.nih.gov/pubmed); accessed 10/12/2013.)

Human EGCs cells express the transcription factor Oct4, a marker of pluripotency, at a high and steady level. These cells also can be differentiated *in vitro* into embryoid bodies (EBs) (Liu et al., 2004). Despite several similarities with ESCs, EGCs display some differences, such as transient self-renewal capability and distinct lineage-specific characteristics. In fact, under normal conditions, EGCs are believed to differentiate into germ cells only—oogonia/oocytes in the female and prospermatogonia in the male—that will produce eggs and sperm, respectively (De Felici et al., 2009).

In addition to these two natural types of pluripotent stem cells, there is another type, the artificial or “induced” cells, or iPSCs. This type of pluripotent stem cell is artificially derived from a nonpluripotent cell—typically an adult somatic cell—by inducing a “forced” expression of specific genes. The first human iPSCs were derived in 2007 from human fibroblasts in a series of experiments by Shinya Yamanaka’s team at Kyoto University, Japan, and by James Thomson’s team at the University of Wisconsin–Madison (Takahashi et al., 2007). Yamanaka had transformed human fibroblasts into pluripotent stem cells using four transcription factors—OCT3/4, SOX2,



**FIG. 2.** Numbers of hESC lines per country deposited in European Human Embryonic Stem Cell Registry. (Data from [www.hescreg.eu/](http://www.hescreg.eu/); accessed 10/12/2013.)



**FIG. 3.** Isolation, characterization, and differentiation potential of pluripotent stem cells. ESCs and iPSCs are obtained from different sources: hESCs are isolated from ICM cells of the blastocyst postfertilization. iPSCs can be generated from adult somatic cells and reprogrammed into pluripotent stem cells by the “forced” expression of specific genes. In culture, both pluripotent stem cells grow colonies, but the question is: Are ESCs and iPSCs equivalents? These cells can be characterized as “pluripotent” using different methodologies and the ability to differentiate into cells derived from ectoderm, mesoderm, and endoderm.

KLF4, and c-MYC—cloned in retroviral vectors, whereas Thomson and colleagues used OCT4, SOX2, NANOG, and LIN28 using a lentiviral system (Yu et al., 2007).

iPSCs emerged as a potential cell type to be used in cell therapy approaches. They represented a source of autologous cells that can avoid immune rejection frequently associated with allogeneic source such as ESCs or donated cells (Nishikawa et al., 2008; Yamanaka, 2008; Zhao and Daley, 2008). Only recently has the possibility that these cells have some immunogenic potential been discussed (Fairchild, 2010; Kadereit and Trounson, 2011). This idea was shown more clearly by Zhao et al. (2011), who demonstrated that there was rejection of syngeneic undifferentiated iPSCs when they were transplanted into mice. The authors showed that iPSCs were frequently rejected and showed T cell infiltration in the teratomas that originated from these cells. The same results were not observed in syngeneic ESC transplantation.

In 2013, four important studies tried to solve this issue (Araki et al., 2013; Guha et al., 2013; Morizane et al., 2013; Thanasegaran et al., 2013). Araki and Zhao’s groups demonstrated that syngeneic integration-free iPSCs were rejected when injected into syngeneic mice (Araki et al., 2013; Zhao et al., 2011). However, unlike the results by Zhao’s group, syngeneic ESCs also showed a similar frequency of rejection.

These data suggest that rejection must be connected to the expression of pluripotency genes and not to the specific features of iPSCs. Araki and colleagues also tested the rejection of differentiated cells. When ESCs or iPSCs were differentiated *in vivo* (matured in chimera mice), they were rarely rejected in syngeneic mice. However, when iPSCs were differentiated *in vitro*, rejection occurs frequently and infiltration of T cells was observed in the transplant. Guha and colleagues showed that autologous iPSCs could be used for cell replacement therapy without eliciting immune rejection. They tested viral iPSCs, episomal-generated iPSCs, and ESCs and observed no evidence of increased T cell proliferation *in vitro*, rejection of syngeneic cells after transplantation, or an antigen-specific secondary immune response. They concluded that undifferentiated or differentiated iPSCs were not rejected after transplantation. Likewise, Thanasegaran and colleagues analyzed the immunogenicity of iPSC clones derived from different ages of mice and, similarly to Guha, they did not find immunogenicity of undifferentiated iPSCs in syngeneic host mice (Thanasegaran et al., 2013; Guha et al., 2013). A similar result was founded by Morizane’s group, which compared autologous and allogeneic transplantation of iPSCs differentiated into neural cells (Morizane et al., 2013). Pluripotent cells were differentiated *in vitro* in dopaminergic neurons and transplanted into the primate brain. They

observed that the autologous transplantation of iPSC-derived neurons elicited only a minimal immune response in the brain, in contrast to allogeneic transplants that showed a great number of T cells infiltrated in the transplant.

The factors that lead to immune response when iPSCs are injected into syngeneic animals can be many. There can be many changes in gene expression and epigenetic pattern during the reprogramming process; injected cells are not differentiated into mature cells or are not identical to adult cells. Chemical reagents can be present in the culture medium, nonhuman sialic acid Neu5Gc can be secreted by mice feeder cells (Martin et al., 2005), and gene correction of a protein that is not expressed can occur (for example, iPSCs derived from hemophilia A and B patients) (Wang et al., 2008). All of these factors can potentially trigger an immune response that results in the rejection of the transplanted cells. It is not possible to conclude that iPSCs are more immunogenic than ESCs. The main difference among these studies is the site of injection of the cells (subcapsular renal space, brain, or subcutaneous), which certainly influences the rejection of the implanted cells. Despite the efforts that are being made in this area, studies need to continue to try to clarify whether iPSCs can trigger immune reactions before so they can be used in clinical trials.

Many studies have been showing that iPSCs are very similar or equivalent to ESCs in many aspects, including the expression of certain stem cell genes and proteins, chromatin methylation patterns, doubling time, EB formation, teratoma formation, viable chimera formation, and potency and differentiation potential. However, the full extent of their relation to natural pluripotent stem cells is still being assessed. There is a hypothesis that this variation results from residual transgene expression (Sommer et al., 2012). However, preliminary data suggest that even transgene-free iPSCs are epigenetically distinct from ESCs. Thus, the most acceptable hypothesis is that iPSCs contain a residual epigenetic signature of the origin tissue (“epigenetic memory”), and that the reprogramming process confers unique molecular features on iPSCs (Kim et al., 2010).

### Pluripotent Pathways: Mechanisms That Induce and Support Pluripotency

Pluripotency and self-renewal are two important characteristics of pluripotent cells. These features enable these cells to be maintained indefinitely in culture and to differentiate into several cell types. Some signaling pathways regulate pluripotency and self-renewal. In the case of hESCs, the process of self-renewal is regulated mainly by transforming growth factor- $\beta$  (TGF- $\beta$ ). The TGF- $\beta$  pathway is activated by the signal transducer Smad2/3, and the fibroblast growth factor receptor (FGFR) activates mitogen-activated protein kinase (MAPK) and Akt (James et al., 2005; Ichida et al., 2009; Maherali and Hochedlinger 2009). The Wnt pathway also is involved in maintaining pluripotency in ESCs by a mechanism involving the inactivation of TCF3 (Sokol, 2011). These pathways result in the expression and activation of three main factors—Oct-4, Sox2, and Nanog—that will activate other genes related to pluripotency.

In the case of iPSCs, after the classic combination used by Takahashi and Yamanaka (2006), other combinations of transcription factors have been used successfully to derive the pluripotent state from different human somatic cell sources

(for review, see Stadtfeld and Hochedlinger, 2010). These discoveries indicate that distinct gene combinations can also result in iPSC generation. Other genes, such as Esrrb (Feng et al., 2009), Nr5a2 (Heng et al., 2010), UTF1 (Zhao et al., 2008), TCL-1 (Picanço-Castro et al., 2011), Glis 1 (Maekawa et al., 2011), E-cadherin (Samavarchi-Tehrani et al., 2010), and selected microRNAs (mir 34, Choi et al., 2011), can also improve the reprogramming efficiency.

In addition to different combinations of transcription factors tested, other efforts have been made to improve the efficiency of iPSC generation and also the quality of these cells. This includes different types of vectors, such as polycistronic lentiviruses, adenoviruses, PiggyBac transposons, Sendai viruses, episomal DNA, mRNA transfections of the pluripotent genes, plasmid DNA, and also proteins of the pluripotent factors (Carey et al., 2009; Fusaki et al., 2009; Kim et al., 2009; Okita et al., 2008; Sommer et al., 2009; Stadtfeld et al., 2008; Warren et al., 2010; Woltjen et al., 2009; Yu et al., 2009; Yusa et al., 2009; Zhou and Freed 2009). Nonintegration approaches generate iPSCs free from exogenous genetic material, representing in the future, a source of cells that can be used in cell therapy trials.

### Pluripotency Characterization

The pluripotency of ESCs and iPSCs can be assessed mainly by morphologic characteristics, marker and gene expression, and *in vitro* and *in vivo* differentiation. Figure 3 shows the generation of these cells and the main laboratory methods to prove the pluripotency.

#### Morphology

The pluripotent stem cells cultured on inactivated mouse embryonic feeder cells (MEFs) monolayer or extracellular matrix (Matrigel) grow in colonies formed by small round cells. During reprogramming, iPSCs change their fibroblastoid morphology and begin to grow in colonies, similarly to ESCs.

#### Marker expression

There are several surfaces or intracellular antigens used to identify pluripotent stem cells, such as SOX2, Nanog, Oct3/4, TRA-1-60, and SSEA-1 in hESCs. These markers are widely used for characterization of hESCs and iPSCs.

#### Gene expression

The gene expression profile of pluripotent stem cells can be usually evaluated by real-time PCR and microarrays assay. These methodologies are frequently used for identifying genes that are differentially expressed in ESCs and iPSCs.

#### *In vitro* and *in vivo* differentiation

In culture (*in vitro* assay), pluripotent stem cells spontaneously differentiate into structures called EBs. These structures are composed of cells of three germ layers (ectoderm, mesoderm, and endoderm). To verify the pluripotency of ESCs and iPSCs *in vivo*, these cells are injected into immune-compromised mice, and their capacity to form teratomas, benign tumors composed of cell types from three germ layers, is evaluated.

### The Differentiation Process of Pluripotent Stem Cells

Pluripotent stem cells have the ability to differentiate into all 216 cell types found in an adult organism. The first

TABLE 1. CLINICAL STUDIES REGISTRY IN CLINICALTRIALS.GOV WEBSITE USING SEARCH TERM “EMBRYONIC STEM CELLS”

Rank	Study	Cell type	Disease	Sponsor	Status
1	The Derivation of Human Embryonic Stem Cell Lines From PGD Embryos	Human embryonic stem cell lines from PGD embryos	Not applied	Hadassah Medical Organization	Recruiting
2	Derivation of New Human Embryonic Stem Cell Lines for Clinical Use	Human embryonic stem cell	Not applied	Hadassah Medical Organization	Recruiting
3	A Study Of Implantation Of Human Embryonic Stem Cell Derived Retinal Pigment Epithelium In Subjects With Acute Wet Age Related Macular Degeneration And Recent Rapid Vision Decline	Human embryonic stem cell–derived retinal pigment epithelium (RPE)	Age-related macular degeneration	Pfizer	Not yet recruiting
4	Safety and Tolerability of Sub-retinal Transplantation of Human Embryonic Stem Cells Derived Retinal Pigmented Epithelial (hESC-RPE) Cells in Patients With Stargardt’s Macular Dystrophy (SMD)	Human embryonic stem cell–derived retinal pigmented epithelial (hESC-RPE) cells	Stargardt’s macular dystrophy (SMD)	Advanced Cell Technology	Recruiting
5	A Phase I/IIa, Open-Label, Single-Center, Prospective Study to Determine the Safety and Tolerability of Sub-retinal Transplantation of Human Embryonic Stem Cell Derived Retinal Pigmented Epithelial (MA09-hRPE) Cells in Patients With Advanced Dry Age-related Macular Degeneration(AMD)	Human embryonic stem cell–derived retinal pigmented epithelial (MA09-hRPE) cells	Age-related macular degeneration	CHA Bio & Diostech	Recruiting
6	Derivation of New Human Embryonic Stem Cell Lines: Identification of Instructive Factors for Germ Cells Development	Human embryonic stem cells	Not applied	Soroka University Medical Center	Unknown
7	The Role of TBX3 in Human ES Cell Differentiation	Human embryonic stem cells	Not applied	University of California, Irvine	Unknown
8	Safety and Tolerability of Sub-retinal Transplantation of Human Embryonic Stem Cells Derived Retinal Pigmented Epithelial (hESC-RPE) Cells in Patients With Stargardt’s Macular Dystrophy (SMD)	Human embryonic stem cell–derived retinal pigmented epithelial (MA09-hRPE) cells	Stargardt’s macular dystrophy (SMD)	CHA Bio & Diostech	Recruiting
9	Sub-retinal Transplantation of hESC Derived RPE (MA09-hRPE) Cells in Patients With Stargardt’s Macular Dystrophy	Human embryonic stem cell–derived retinal pigmented epithelial (MA09-hRPE) cells	Stargardt’s macular dystrophy (SMD)	Advanced Cell Technology	Recruiting
10	Safety and Tolerability of Sub-retinal Transplantation of hESC Derived RPE (MA09-hRPE) Cells in Patients With Advanced Dry Age Related Macular Degeneration (Dry AMD)	Human embryonic stem cell–derived retinal pigmented epithelial (MA09-hRPE) cells	Advanced dry age-related macular degeneration (dry AMD)	Advanced Cell Technology	Recruiting
11	Isolation and Characterization of Mammary Stem Cells	Tumor stem cells	Not applied	National Cancer Institute (NCI)	Completed

(continued)

TABLE 1. (CONTINUED)

<i>Rank</i>	<i>Study</i>	<i>Cell type</i>	<i>Disease</i>	<i>Sponsor</i>	<i>Status</i>
12	Patient Specific Induced Pluripotency Stem Cells (PSiPS)	Pluripotent stem cells from skin	Not applied	Royan Institute	Completed
13	Skin and Blood Research Samples From Healthy Volunteers and Patients With Hematologic Diseases	Not applied	Hematologic diseases	Washington University School of Medicine	Terminated
14	Development of IPS From Donated Somatic Cells of Patients With Neurological Diseases	iPSCs from donated somatic cells of patients with neurological diseases	Neurological disease	Hadassah Medical Organization	Active, not recruiting
15	Evaluation of Circulating Levels of Adult Stem Cells in the Peripheral Blood of Patients With Acute Decompensated Heart Failure and Following Stabilization, in Comparison With Healthy Volunteers (CIRCSTEM-HF)	Examine the levels of a number of different types of stem cells in patients with heart failure	Acute decompensated heart failure	Monash University	Active, not recruiting
16	The Transendocardial Autologous Cells (hMSC or hBMC) in Ischemic Heart Failure Trial (TAC-HFT)	Bone marrow–derived cells	Ischemic heart failure trial (TAC-HFT)	University of Miami	Completed
17	The Percutaneous Stem Cell Injection Delivery Effects on Neomyogenesis Pilot Study (The POSEIDON-Pilot Study)	Bone marrow–derived cells	Not applied	University of Miami	Completed
18	Percutaneous Stem Cell Injection Delivery Effects On Neomyogenesis in Dilated CardioMyopathy (The POSEIDON-DCM Study) (PoseidonDCM)	Bone marrow–derived cells	Dilated cardiomyopathy	University of Miami	Recruiting

Data are from [www.clinicaltrials.gov](http://www.clinicaltrials.gov); accessed on October 24, 2013. The search was for studies: “embryonic stem cells.”

embryonic stem cell was isolated from mice in 1981 by Martin and colleagues (Martin, 1981). Since the late 1990s, when the first human line of ESCs was established in culture by Thomson and colleagues (Thomson et al., 1998), several cell types could be obtained from these cells, such as neurons, cardiomyocytes, smooth muscle cells, osteocytes, hepatocytes, keratinocytes, insulin-producing cells, hematopoietic cells, and endothelial cells (Kaufman et al., 2001; Kehat et al., 2001; Levenberg et al., 2002; Rambhatla et al., 2003; Reubinoff et al., 2001; Segev et al., 2004; Zhang et al., 2001). Moreover, the *in vitro* differentiation system allows the study of mechanisms involved in lineage specification of pluripotent stem cells to distinct cell types.

Currently, two methods are widely used to differentiate ESCs and iPSCs: (1) By EB formation; and (2) by co-culture of pluripotent stem cells with a mesenchymal stromal cell (MSC) monolayer. In the first method, pluripotent cells differentiate spontaneously and then form three-dimensional (3D) aggregates called EBs. This method has been used to obtain several cellular lineages *in vitro*. When sectioned, these structures contain cells of endodermal, mesodermal, and ectodermal tissues. The second method uses mainly mouse stromal cell lines. Pluripotent stem cells are cultivated with these stromal cells, which secrete growth factors and cytokines that induce differentiation. The choice of the differentiation method depends on the cellular type desired, with variations in the efficiency of differentiation.

The major cell types used from the endoderm include hepatic cells (Agarwal et al., 2008; Lavon and Benvenisty, 2005), lung epithelium (Van Vranken et al., 2005; Wang et al., 2007), and insulin-producing cells (Assady et al., 2001; Brolen et al., 2005). Studies have demonstrated that ESCs can be differentiated into functional hepatocytes both *in vitro* and *in vivo* (Agarwal et al., 2008; Duan et al., 2010; Yi et al., 2012). More recently, it was demonstrated that human iPSCs could also be differentiated into functional hepatocytes (Chen et al., 2012).

The mesodermal progenitors obtained from ESCs and iPSCs includes cardiomyocytes, endothelial cells, and hematopoietic cells. These cell types could be used for treatment of ischemic heart disease, repair of ischemic tissue, and to obtain all types of blood cells, respectively. However, the generation of these cell types is still inefficient, and thus the establishment of a refined protocol applicable for regenerative medicine is necessary.

The cells differentiated into the ectoderm lineage include cells of the epidermis, external sense organs, and central and peripheral nervous system (Gilbert, 2006). The generation of functional neurons has been shown by several groups employing the EB method (Reubinoff et al., 2000; Reubinoff et al., 2001) or mouse stromal cell line (Kawasaki et al., 2000; Zeng et al., 2004), and these cells can be used for the treatment of neurodegenerative diseases, such as acute spinal cord injury.

The induction of ESC and iPSC differentiation to produce different cell types requires complex differentiation steps with specific culture medium and growth factors, addition of cytokines, and supplements, representing a challenge in differentiation control. The high differentiation potential of ESCs into specific cell lineages through *in vitro* systems, EB formation, or co-culture with stromal cells represents a source of cells that can be used for testing new drugs and also for cell

therapy clinical trials. For this purpose, it is essential that the protocols for maintenance or differentiation of these cells are carried out in the absence of animal components.

### Clinical Trials

The most frequent question asked today is whether hESCs can be used in clinical trials. In 2009, the company Geron received by the Food and Drug Administration (FDA) approval to begin a Phase I clinical trial using hESCs. This clinical trial aimed at treating patients with acute spinal cord injury using hESCs differentiated into oligodendrocytes. Initial studies had shown significant restoration of mobility in animals with spinal injuries that received these cells (Keirstead et al., 2005). However, this study was discontinued in November, 2011, and in July, 2012, Geron abandoned its studies using stem cells due to financial constraints (Walsh, 2012).

In 2010, two new clinical trials were approved. Advanced Cell Technology (ACT), located in Marlborough, Massachusetts, leads studies to improve the vision of patients with Stargardt's macular dystrophy (SMD) and dry age-related macular degeneration (dry AMD), studies 4, 9, and 10. Originally, 12 patients participated in this clinical trial in three hospitals in the United States. In this clinical trial, patients were injected with retinal pigment epithelial cells (RPE) derived from hESCs (Vergano, 2010). In January, 2012, an article with preliminary results was published and showed that the hESC-derived RPE cells had no signs of hyperproliferation, tumorigenicity, ectopic tissue formation, or apparent rejection after 4 months (Schwartz et al., 2012). This study is still ongoing. In February, 2013, ACT received approval to initiate a Phase I/III clinical trial with RPE cells derived from hESCs for the treatment of severe myopia. CHA Bio & Diostech has two clinical trials very similar to the ACT studies (Table 1).

There are currently 18 registered clinical trials ([www.clinicaltrials.gov](http://www.clinicaltrials.gov)) related to ESCs. However, three studies (1, 2, and 6 in Table 1) are related to the generation of ESC lines and not related to their use in clinical trials, and other studies (16, 17, and 18) use adult stem cells, such as mesenchymal stem cells. Moreover, there are two studies with iPSCs (12 and 14). Studies 7, 11, 13, and 15 are related to differentiation of hESCs, isolation and characterization of mammary stem cells, hematologic disease, and heart failure. In summary, only six studies (3, 4, 5, 8, 9, and 10) actually use ESCs in clinical trials, all of them to treat SMD or dry AMD.

There is no doubt that many advances have been made since the isolation of the first human embryonic lineage in 1994 (Bongso et al., 1994), and the possibilities for stem cell therapy seem limitless. However, there is still a great lack of knowledge about the processes of cell differentiation and how to control cell differentiation. The use of differentiated cells requires the development of well-controlled and safe differentiation protocols. One major issue is the risk of teratoma formation. Studies aimed at understanding the basic biology of these cells will determine whether these cells can be used effectively in cellular therapies. In relation to iPSCs, several studies on the derivation of these cells from adult cells belonging to affected tissues have been enrolled, but there is no study based on differentiation of these cells and their use in patients.

## Conclusions

There are currently six clinical trials using hESCs to treat diseases. However, many basic questions about the efficiency and safety of the use of these cells still remain unanswered. In recent years, great progress has been made in the characterization of pluripotent cells. Differentiation protocols have been optimized; however, it is still necessary to ensure full differentiation, so that any remaining pluripotent cells are capable of generating teratomas. In the field of iPSCs, great advancement has been made in the generation of these cells from a variety of cell types and different combinations of transcription factors, and a huge variety of vectors have been tested. However, iPSCs still have large genetic and epigenetic variability in comparison with ESCs and do not represent a suitable source for clinical use. If the clinical application is the main goal of stem cell research, there are still many molecular mechanisms that need to be understood before these cells can be efficiently used in clinical trials in the future.

## Author Disclosure Statement

The authors declare that no conflicting financial interests exist.

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