Treatment of Parkinson’s disease using human stem cells

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Abstract

The progressive loss of dopamine neurons (DAN) in the substantia nigra is the main characteristic of Parkinson’s disease (PD), the second most common neurodegenerative disorder causing several motor symptoms. Current treatment options for PD are available to help relieve primary motor symptoms, but their long-term effectiveness is limited and they do not stop neuronal degeneration. For this reason, alternative treatment options are being sought in the form of cell replacement therapies (CRT). Several open label clinical trials involving the intrastriatal transplantation of human fetal ventral mesencephalic tissue (hfVM) has provided proof of concept that CRT could be beneficial for some patients, providing relief of motor symptoms. However, the lack of availability of tissue and ethical issues limit the clinical use on a large scale of this strategy, therefore being sought alternative cell sources, based on the use of human stem cells.

In this review we provide an overview of the different types of human stem cells currently available, mainly multipotent and pluripotent stem cells, their advantages and disadvantages from an experimental and clinical point of view, and how they are being developed clinically for PD treatment.

Introduction

Parkinson’s disease (PD) is a progressive neurodegenerative disorder resulting from the loss of dopamine-producing neurons (DAN) in the substantia nigra pars compacta (SNpc) [1-3]. In addition, PD patients present with the deposition of α-synuclein-positive protein aggregates called Lewy bodies and neuro-inflammation in various brain regions, further contributing to the progression of the disease [4-6]. The loss of SNpc DAN triggers the recognizable primary motor symptoms, including tremor, rigidity and bradykinesia [7]. However, the pathophysiology of PD is now known to extend beyond the nigrostriatal dopaminergic pathway itself, leading to a number of secondary motor and non-motor symptoms that can be just as debilitating [8]. Although the precise etiology of PD is still unknown, a variety of pathogenic mechanisms have been proposed. These may include the loss of trophic support, excessive release of oxygen free radicals, a dysfunctional mechanism of protein degradation, abnormal kinase activity and impairment of mitochondrial function [4].

A variety of treatment options are available to help manage motor symptoms. These include medications in the form of the dopamine precursor levodopa (L-dopa), dopaminergic agonists, or inhibitors of dopamine breakdown (catechol-O-methyl transferase and monoamine oxidase inhibitors) or surgical procedures such as deep brain stimulation (DBS) [8,9]. With time, however, these treatments cease to be effective, and some of them are known to develop unpleasant side effects, such as dyskinesias. Most importantly, these treatments are not a cure. They are not reparative of basal ganglia circuitry, nor capable of stopping the disease from progressing [8,10,11].

For this reason, alternative treatment options are currently being investigated, among them is particularly interesting the cell replacement therapy (CRT). Here we summarize general approaches for experimental and clinical applications of stem cell therapy, discussing the common issues, different strategies and how they are being developed as a possible treatment option for PD.

Transplants of human fetal ventral mesencephalic tissue

Transplants of human fetal ventral mesencephalic (hfVM) tissue have been developed in the clinic for more than 30 years for PD treatment [12]. These grafts contain immature midbrain DAN and their progenitors, which are generally transplanted into the striatum (the target region of nigral DAN) where they are expected to release and replenish dopamine levels.

Preclinical studies performed in the 1970s and 1980s in animal models of PD demonstrated that DAN obtained from the fetal midbrain were able to survive transplantation in animal models of PD, integrate into host tissue, release dopamine and improve motor function. With this background, several groups were able to conduct open label clinical trials in PD patients, providing proof of principle that hfVM grafts can be an efficient and safe treatment option for PD. However, two double-blind, placebo-controlled clinical trials failed to meet their primary endpoint [13,14], and the overall results obtained from all trials were fairly inconsistent, both between and within trials. For this reason, several limiting challenges are still being faced in turning fetal VM grafts into a comparative treatment option for PD. The first challenge is to avoid further inconsistencies in the results, which can be achieved by establishing better standardization procedures and improved trial design [14-16]. The second challenge is the probability of the host developing an immune reaction, since all the transplants

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are allogenic [17-20]. The third, and arguably the biggest, is the ethical concerns with using fetal tissue, and the difficulty in obtaining enough tissue for a successful transplant.

To help overcome these challenges, the European consortium TRANSEURO (www.transeuro.org.uk), a multicenter clinical trial that is currently working to analyze the feasibility and effectiveness of transplantation of human fetal cell suspensions in PD patients, in hopes of providing more consistent results for a better understanding of the potential therapeutic benefit [21-23] (Table 1).

Interestingly, in a recently published study it has shown that grafts of hVM can survive for at least 24 years inside the derenervated putamen of a Parkinson's patient, with no signs of inflammation [24]. The patient had clinically improved during the first decade post-implantation, although eventually this improvement began decreasing, indicating that the transplant was no longer functional. The histopathological analysis showed that approximately 12% of the neurons of the graft were positive for α-synuclein, reflecting the transfer of the host brain pathology to the implanted neurons [24].

**Human stem cell-based therapies for PD treatment**

Due to the above limitations of using fetal tissue, important research efforts are currently underway to find alternative types of cells for transplantation in PD. Several sources have been explored, and the most promising so far have been stem cells. Stem cells are undifferentiated cells that have the ability to differentiate to more specialized cell types. Because of these properties, they are currently considered the best option for developing a uniform source of DAn to be used for cell replacement therapy.

In recent years it has made enormous progress in this field, which has made it possible to obtain human dopaminergic precursors from different types of stem cells such as: human Embryonic Stem Cells (hESCs) [25-28], human induced Pluripotent Stem Cells (hiPSCs) [25-28], human Mesenchymal Stem Cells (hMSCs) [36], human Embryonic Neural Stem Cells (hNSCs) of different origin. The company Celavie Bioscences, LLC, is conducting a clinical trial consisting in the intraputaminal injection in PD patients of undifferentiated human fetal stem cells (OK99 cell line) obtained from a fetal brain tissue and growth in a bioreactor. This is a phase 1, open-label and safety study which aims to analyze the potential efficacy of this type of grafting for PD treatment (Table 1).

### Table 1. Human stem cells used currently in clinical trials (www.clinicaltrials.gov) for treatment of Parkinson’s disease.

<table>
<thead>
<tr>
<th>Stem cell type</th>
<th>Transplant type</th>
<th>Method and target</th>
<th>Status and identifier</th>
<th>Sponsor</th>
</tr>
</thead>
<tbody>
<tr>
<td>hNSCs from fetal ventral mesencephalic tissue</td>
<td>Allogenic</td>
<td>Intracerebral implantation</td>
<td>Phase 1 NCT01898390</td>
<td>TRANSEURO Project University of Cambridge</td>
</tr>
<tr>
<td></td>
<td>Allogenic</td>
<td>Not provided</td>
<td>Phase 1/2 NCT01860794</td>
<td>Bundang CHA Hospital</td>
</tr>
<tr>
<td></td>
<td>Allogenic</td>
<td>Intracerebral implantation</td>
<td>Phase 1/2 NCT02538315</td>
<td>University of Saskatchewan</td>
</tr>
<tr>
<td>hNSCs from fetal brain</td>
<td>Allogenic</td>
<td>Intraputaminal implantation</td>
<td>Phase 1 NCT02780895</td>
<td>Celavie Bioscences LLC</td>
</tr>
<tr>
<td>hNSCs from adult cerebral cortex</td>
<td>Autologous</td>
<td>Intracerebral implantation to the left putamen</td>
<td>Phase 0 NCT01329926</td>
<td>NeuroGeneration [35]</td>
</tr>
<tr>
<td>ISC-hpNSCs</td>
<td>Allogenic</td>
<td>Intracerebral implantation to striatum and substantia nigra</td>
<td>Phase 1 NCT02427273</td>
<td>Cyto Therapeutics Pty Limited</td>
</tr>
<tr>
<td>hMSCs from bone marrow</td>
<td>Allogenic</td>
<td>Intravenous administration</td>
<td>Phase 1/2 NCT02611167</td>
<td>The University of Texas Health Science Center</td>
</tr>
<tr>
<td></td>
<td>Autologous</td>
<td>Intravenous administration</td>
<td>Phase 1/2 NCT01446614</td>
<td>Guangzhou General Hospital of Guangzhou Military Command</td>
</tr>
<tr>
<td>hMSCs from adipose tissue</td>
<td>Autologous</td>
<td>Delivered to vertebral artery and intravenous administration</td>
<td>Phase 1/2 NCT01453803</td>
<td>Ageless Regenerative Institute</td>
</tr>
<tr>
<td></td>
<td>Autologous</td>
<td>Not provided</td>
<td>Recruiting NCT02184546</td>
<td>StemGenex</td>
</tr>
</tbody>
</table>

In general, in the research field it is assumed that in order for the stem cells to become a clinically competitive treatment option, these cells need to be equivalent to those of hVM tissue. And after grafting they must be able to survive, re-innervate the striatum and integrate into the neural circuitry of the host. They also have to significantly improve motor symptoms, cause no side effects and meet a number of safety requirements, such as eliminating the risk of tumor formation, immune response and the development of dyskinesias [40,41].

**Human multipotent stem cells**

**Human neural stem cells**

Human Neural Stem Cells (hNSCs) are multipotent stem cells with the ability to generate all neural cells of the CNS. They can be obtained from fetal, neonatal and adult brains or from the directed differentiation of pluripotent stem cells. In theory, the human VM neural precursors are considered the ideal candidates for cell therapies in PD, but as mentioned above, their use is very limited. Furthermore, they present poor growth potential, unstable phenotypes (especially upon repeat passage), and survive poorly in the brain after grafting [34,42,43].

Different techniques have been developed to optimize the expansion of these cells, including the formation of neurospheres in the presence of growth factors such as basic Fibroblast Growth Factor (bFGF) and Epidermal Growth Factor (EGF) [44,45] or the transduction with immortalizing genes such as v-Myc, c-Myc or TERT [34,46]. Furthermore, in a different approach an efficient method involving the addition of Wnt5a showed a 6-fold increase in the amount of midbrain DAn obtained, as compared to the starting VM preparation [47].

However, currently there are several clinical trials involving the use of hNSCs of different origin. The company Celavie Bioscences, LLC, is conducting a clinical trial consisting in the intraputaminal injection in PD patients of undifferentiated human fetal stem cells (OK99 cell line) obtained from a fetal brain tissue and growth in a bioreactor. This is a phase 1, open-label and safety study which aims to analyze the potential efficacy of this type of grafting for PD treatment (Table 1).
In a different approach, the company NeuroGeneration Inc have obtained hNSCs from biopsies from the own patient, which after culturing and expansion were differentiated to neurons (approximately 14% of these neurons were DA\(\text{N}\)) and transplanted into PD patients. These patients showed some clinical improvements, but clinical trials are ongoing to determine the safety and efficacy of this strategy (Table 1 and Figure 1) [35].

**Human mesenchymal stem cells**

Human Mesenchymal Stem Cells (hMSCs) are non-hematopoietic and multipotent self-renewing cells arising easily from adult bone marrow [48], adipose tissue [49], umbilical cord blood [50], dental pulp [51], placenta and brain [52,53]. In the last few years, these cells have emerged as a promising approach to regenerative medicine due to their great proliferative potential and widespread availability in the body [54]. hMSCs are stromal cells that exhibit a multi-lineage differentiation potential of mesodermal origin (generally into adipocytes, osteocytes and chondrocytes) [55-57]. Besides regulating differentiation subclasses, the potential for trans-differentiation to a neural lineage (ectodermal origin), and even to a DA\(\text{N}\) phenotype, have been demonstrated [39,53,55,58-61]. However, this remains somewhat controversial, as inconsistent results are unable to confirm the ability of these cells to correctly integrate into the host-neural circuitry to form synaptic connections [36,57,62].

An alternative approach is utilizing the ability of hMSCs to secrete protective neurotrophic factors, growth factors and cytokines (including VEGF, HGF, IGF-1, BDNF, \(\beta\)-NGF, TGF-\(\beta\), FGF2 and GDNF) that are known to promote protection, repair and exhibit immunomodulatory effects, by contributing to immunosuppression in the brain and inhibiting the release of pro-inflammatory cytokines (TNF-\(\alpha\), IL-1 \(\beta\) and INF-\(\gamma\)) detected in brains of PD patient [63-65]. Additionally, hMSCs is attractive clinically because, if isolated from an autologous source, they would circumvent the need for immunological regimes and eliminate any ethical concerns [54,66-68]. Further more if infused systemically, they have shown the ability to migrate to sites of injury in animals, suggesting migratory capacity towards damaged areas where they can then promote repair processes [54].

Despite this evidence, their use in clinical trials has been limited due to the heterogeneous nature of populations isolated from different tissues [69]. However, hMSCs from bone marrow or adipose tissue are currently being used to investigate the efficacy of autologous and allogenic treatments in PD patients with the idea of taking advantage of their immune-modulatory and trophic properties, and anti-inflammatory effects [70] (Table 1, Table 2, Figure 1).

The University of Texas Health Science Center is conducting a clinical trial consisting in the intravenous administration of allogenic bone marrow-derived hMSCs into PD patients. Apart from of safety, feasibility and efficacy studies, they are assessing some changes in immunologic response but so far it has not disclosed any data or result of this strategy. In other different trial, the Ageless Regenerative Institute has obtained adipose tissue-derived hMSCs from the abdomen of PD patients to study safety and efficacy of this type of transplant for PD treatment. This is a phase 1/2, but study results have not posted still (Table 1 and Figure 1).

**Human pluripotent stem cells**

**Human embryonic stem cells**

Human Embryonic Stem Cells (hESCs) were isolated for the first time in 1998, from the inner cell mass of the blastocyst stage embryos.
show typical hESCs morphology, express appropriate pluripotency on chemical stimuli that mimic sperm penetration. Thus (hpESCs). Parthenogenesis in mammalian oocytes can be induced by parthenogenesis named human parthenogenetic Embryonic Stem Cells Human parthenogenetic embryonic stem cells begin at the end of 2017 [77].

Least one of them is awaiting approval by the FDA and it is expected can begin at the end of 2017 [77].

As such, numerous different protocols have been developed, the most efficient to date are those involving the formation of embryoid bodies, dual SMAD inhibition and the use of Shh and Wnt signaling agonists that efficiently convert floor plate precursors into DA neurons [25,27,72-74].

However, several challenges persist, such as high ethical concerns, the risk of tumor formation, phenotypic instability and the risk of host-graft rejection due to the difficulty of HLA typing [21,26,75,76]. Furthermore, it is crucial to eliminate risk of contamination with animal products by following GLP/GMP (Good Laboratory and Manufacture Procedures) to optimize their use for clinical application (Figure 1).

Despite the high expectations generated, there are still no clinical trails using ESCs to treat PD. However clinical trails are planned and at least one of them is awaiting approval by the FDA and it is expected can begin at the end of 2017 [77].

**Human parthenogenetic embryonic stem cells**

Another type of pluripotent stem cells are those derived by parthenogenesis named human parthenogenetic Embryonic Stem Cells (hpESCs). Parthenogenesis in mammalian oocytes can be induced by electrical on chemical stimuli that mimic sperm penetration. Thus hpESCs are pluripotent cells derived from unfertilized oocytes that show typical hESCs morphology, express appropriate pluripotency markers, possess high alkaline phosphatase levels, high telomerase activity, generate embryoid bodies in culture and form teratomas after injection to immunodeficient animals [78,79].

However, the lack of paternal contribution could make their clinical use problematic, as normal cell cycle progression and proper differentiation could be affected (Table 2). At the end of 2015, the very first clinical trial with pluripotent stem cells for treating PD was approved, and involves the use of hpESCs [78]. This is a phase 1 study to evaluate the safety and therapeutic benefit of transplantation of hNSCs derived from differentiation of hpESCs and injected into the striatum and substantia nigra of PD patients. The trial will be conducted by the company Cyto Therapeutics Pty Limited at the Royal Melbourne Hospital. (Table 1 and Figure 1).

**Human induced pluripotent stem cells**

Human induced Pluripotent Stem Cells (hiPSCs) are obtained from adult somatic cells by reprogramming and they share many similarities with those of hESCs, including cell morphology, expression of pluripotent markers, epigenetic changes, potential to differentiate into cells of the three germ layers in vitro and in vivo (by teratoma formation) and the ability to generate viable chimeras [2,80].

hiPSCs were described for the first time in 2006 by Takahashi and Yamanaka; this discovery was a remarkable breakthrough in the stem cell research- and regenerative medicine fields. They achieved this goal by the introduction of four main transcription factors Oct3/4, Sox2, Klf4 and c-Myc to re-induce a state of pluripotency [81]. iPSC cell technology offers exciting possibilities for biomedical research in PD. These cells can be used as in vitro cellular models of the disease and be a source of autologous cells that would not raise ethical concerns. For PD in particular, human DA precursors have been efficiently derived

### Table 2. Advantages and disadvantages of using human stem cells to the treatment for Parkinson's disease.

<table>
<thead>
<tr>
<th>Stem cell type</th>
<th>Transplant type</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>hNSCs Allogenic</td>
<td>Differentiation into specific neural lineages (including DA)</td>
<td>Limited source tissue</td>
<td>Ethical concerns</td>
</tr>
<tr>
<td>hMSCs Autologous and allogenic</td>
<td>Widespread availability by the body and easily accessible</td>
<td>Inconsistent results on integration in host-neural circuitry</td>
<td></td>
</tr>
<tr>
<td>hESCs Allogenic</td>
<td>Differentiation into specialized cells from all three germ layers</td>
<td>Risk of tumor formation</td>
<td>Ethical concerns</td>
</tr>
<tr>
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<td>Differentiation into specialized cells from all three germ layers</td>
<td>Risk of tumor formation</td>
<td>Ethical concerns</td>
</tr>
<tr>
<td>hiPSCs Autologous and allogenic</td>
<td>Differentiation into specialized cells from all three germ layers</td>
<td>Risk of tumor formation</td>
<td>Epigenetic memory</td>
</tr>
<tr>
<td>hpESCs Allogenic</td>
<td>Differentiation into specialized cells from all three germ layers</td>
<td>Risk of tumor formation</td>
<td>Mutations occurring during reprogramming</td>
</tr>
<tr>
<td>hpESCs Autologous and allogenic</td>
<td>Differentiation into specialized cells from all three germ layers</td>
<td>Risk of tumor formation</td>
<td>Phenotypic instability</td>
</tr>
<tr>
<td></td>
<td>Potential for production limitless of DA</td>
<td>Ethical concerns</td>
<td>Safety concerns</td>
</tr>
<tr>
<td></td>
<td>Survive transplantation and some degree of functional recovery in animal models</td>
<td>Histocompatibility concerns</td>
<td>Legislation of most countries limit their use in clinical trials</td>
</tr>
<tr>
<td></td>
<td>Potential for production limitless of DA</td>
<td>Ethical concerns</td>
<td>Lack of paternal contribution</td>
</tr>
<tr>
<td></td>
<td>No histocompatibility concerns (in autologous transplant)</td>
<td>No ethical concerns</td>
<td></td>
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<tr>
<td></td>
<td>No histocompatibility concerns (in autologous transplant)</td>
<td>No ethical concerns</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No histocompatibility concerns (in autologous transplant)</td>
<td>No ethical concerns</td>
<td>Low hiPSCs yield</td>
</tr>
<tr>
<td></td>
<td>No histocompatibility concerns (in autologous transplant)</td>
<td>No ethical concerns</td>
<td>Elevated production cost</td>
</tr>
</tbody>
</table>

of fertilized oocytes [71], and at that time they were considered the optimal source for cell replacement therapies due to their pluripotent state without the need for reprogramming (Table 2).

In order to make hESCs applicable for clinical use in treating PD, they must meet a number of strict requirements concerning efficient differentiation to functional neurons with the correct DA phenotype. As such, numerous different protocols have been developed, the most efficient to date are those involving the formation of embryoid bodies, dual SMAD inhibition and the use of Shh and Wnt signaling agonists that efficiently convert floor plate precursors into DA neurons [25,27,72-74].

However, several challenges persist, such as high ethical concerns, the risk of tumor formation, phenotypic instability and the risk of host-graft rejection due to the difficulty of HLA typing [21,26,75,76]. Furthermore, it is crucial to eliminate risk of contamination with animal products by following GLP/GMP (Good Laboratory and Manufacture Procedures) to optimize their use for clinical application (Figure 1).

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from hiPSCs following similar protocols as those used to differentiate hESCs [25,29-32].

Despite their promise, several challenges continue to limit the use of hiPSCs to large-scale clinical level. It is expected that these cells generate similar problems as hESCs, as well as problems of epigenetic memory of autologous tissue, genomic instability, problems with the reprogramming process itself, especially when integrating viral vectors are used and the risk of teratoma formation [82].

The expectations placed on hiPSCs remain huge. Several clinical trials involving their use to treat PD are already planned and are currently awaiting government approval. One is expected to begin in Japan soon, led by the group of Jun Takahashi [83-85] (Figure 1). One of the great advantages of iPSCs in relation to ESCs is the ability to generate patient-specific cells, which, in theory, would avoid the use of immunosuppressants. However, so far, the process itself is too expensive to be done on a large scale [86].

Conclusions and future perspectives

The current treatment options available for most neurological disorders, including PD, only provide temporary symptomatic relief but do not prevent the disease from progressing. Therefore, the development of cell-replacement therapies could provide substantial benefits for PD patients. The rationale of CRT in PD is to replace the lost DAn in PD with immature and healthy DAn to restore neuronal circuits and dopamine levels.

Previous open label clinical trials in PD patients with hfVM cell suspensions have shown that these grafts can be an efficient and safe treatment option for at least some PD patients. However, ethical and tissue availability problems, limit their widespread clinical use.

In theory, the human VM neural precursors are considered the ideal candidates for cell therapies in PD, but their use is very limited. Furthermore, they present poor growth potential, unstable phenotypes (especially upon repeated passage), and survive poorly in the brain after grafting; therefore must seek new cell sources (Table 2).

The enormous progress made in recent years in the stem cell research field has made it possible to obtain human dopaminergic precursors from different types of stem cells including hMSCs by transdifferentiation and specially from human pluripotent stem cells (hESCs and hiPSCs) (Figure 1). hMSCs are clinically attractive because they can be isolated from the own patient, so they would circumvent the need for immunological regimes and eliminate any ethical concerns. The potential for transdifferentiation into DAn phenotype, have been shown in different studies [60-62]. However, this process remains somewhat controversial, as inconsistent results are unable to circumvent the need for immunological regimes and eliminate any ethical concerns. The potential for transdifferentiation into DAn phenotype, have been shown in different studies [60-62]. However, this process remains somewhat controversial, as inconsistent results are unable to circumvent the need for immunological regimes and eliminate any ethical concerns.

An alternative approach is utilizing the ability of hMSCs to secrete protective neurotrophic factors, growth factors and cytokines that exhibit immunomodulatory effects [63-65]. We look forward to having the first results obtained in the clinical trials are currently underway.

Human pluripotent stem cells (hESCs, hpESCs, hiPSCs) have the ability to undergo unlimited proliferation and can differentiate into any specialized cell of the body. There are currently very efficient protocols for the generation of DAn from pluripotent stem cells. These DAn present molecular and functional properties very similar to those of VM DAn. However, the possibility of tumor formation, histocompatibility problems or ethical concerns are some of the challenges that lead to strict regulation of pluripotent stem cell therapy in most countries.

Human pluripotent stem cells possess the worrying potential of teratoma generation in the transplant area due to undifferentiated stem cell populations remaining in the grafts. Some possible strategies to reduce the risk of tumor formation after transplantation could be further improving the differentiation protocols to DAn (in order to obtain cultures phenotypically more homogeneous), to select efficiently the correct type of cells to be grafted or remove undifferentiated cells by using for example suicide genes. It will be also essential improve the isolation and differentiation of human pluripotent stem cells in GMP conditions, it means using animal products (feeder cells or sera) in order to obtain clinically applicable DAn. One of the great advantages of hiPSCs over hESCs is the ability to generate patient-specific cells, which would avoid the use of immunosuppressant. Unfortunately for the moment, the process seems to be too expensive. Several cell lines have to be generated for each patient, and these cell lines have to be characterized and properly analyzed, with particular attention to biosafety. It is estimated that the approximate cost of treating one single patient with autologous cells could be around one million dollars [86,87]. A possible solution proposed by Yamanaka is the creation of human stem cell banks for therapeutic use, where a standard matrix of different cell lines containing the main HLA types, are available and can be tolerated by 80% of the population. Thus it is intended to reduce the deriving and testing time in each patient, alleviate histocompatibility problems and reduce therapy costs [88] (Table 2). Another important problem to be solved in hiPS cells is the observation of mutations in some of the cell lines, which were not present in the original patient fibroblasts; probably due to the reprogramming process itself [89].

Thereby improving the reprogramming process is essential for the future therapeutic application of these cells.

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Author disclosure statement

The authors declare that they have no competing interests.

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