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Stem cells and regenerative medicine for neural repair

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Clinical trials of cell-based therapies that use pluripotent stem cells (PSC) have already started for several neurological diseases including spinal cord injury and age-related macular degeneration. Regarding future PSC-based clinical trials for other neurological diseases, these trials have been instrumental at recognizing first, the difference between research cell lines and clinical cell lines of a stem cell product, second, the selection of an appropriate animal model for preclinical study, third, criteria and the quality control of donor cells, and fourth, the mode of action of the grafts.

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Introduction

Advances in research on neural stem cells (NSCs) and pluripotent stem cells (PSCs) is expected to achieve wide clinical application in the field of neural regenerative medicine. Clinical trials have already started for several diseases including age-related macular degeneration (AMD) and spinal cord injury (SCI), and both encouraging and discouraging results have been reported. In 2017, a NSC product for clinical use (HuCNS-SC) supplied by StemCells Inc. failed to recover motor dysfunction in murine models of SCI [1] or show cognitive benefit in murine models of Alzheimer's disease [2]. The same year, extreme results for the transplantation of retinal pigment epithelium (RPE) cells derived from stem cells to treat AMD patients were reported [3••,4•]. Finally, the launch of a clinical trial in China for Parkinson's disease (PD) using an embryonic stem cells (ESC) product was reported [5]. In this review article, I consider the translational research of stem cell-based therapies based on these reports (Table 1).

Spinal cord injury (SCI)

Patients suffering from SCI generally show little spontaneous recovery and have no promising treatment options for SCI sequela. Several early phase clinical trials of stem cell-based therapies to treat SCI have investigated efficacy and long-term safety.

As mentioned above, a clinical cell line (CCL) failed to improve motor dysfunction in a murine model of SCI [1]. In contrast, research cell lines (RCLs) provided by the same company demonstrated behavioral improvement in a murine model of contusion SCI [6–8]. Based on these results, StemCells Inc. began a clinical trial in 2014 (ClinicalTrials.gov Identifier: NCT02163876). The trial was categorized as a single-blind, randomized, parallel arm, phase II Proof-of-Concept study for the safety and efficacy of HuCNS-SC transplantation in cervical SCI. Although interim 6-month data showed improvements in motor strength in 4/5 subjects, subsequent observation (up to one year) could not find a trend for the improvement over time, and the company terminated the study in 2016.

The lack of efficacy in the CCL study might explain the lack of efficacy in the clinical trial and raises questions about differences in CCLs and RCLs originating from the same NSCs and manufactured from the same company. CCLs are established under a current good manufacturing practice (cGMP) in order to produce a reliably consistent product. Therefore, it is likely that different reagents or culture dishes were used, which could have caused the differences between CCLs and RCLs. Alternatively, the discrepancy could suggest a significant difference between product batches. In this case, more strict regulation on the manufacturing process and release criteria of the final products will be needed. Either way, better criteria for the evaluation of the efficacy and safety of the CCL-derived products are necessary before going to the clinic.

Another issue raised by this case is the limitation of rodent models for predicting efficacy in human neurological diseases. Results from rodent studies are not always reflected in clinical trials, in large part because of differences in organ size and anatomy. For example, cortical motor neurons in rodents travel in the dorsal columns of the spinal cord and are not directly connected to cervical motor neurons [9]. In contrast, in human and old-world monkeys, direct connections between cortical and spinal motor neurons are well developed, and the fibers are located mostly in the lateral column. Therefore, one should exercise caution when selecting an animal model

Table 1

Topics of stem cell-based therapy for neural repair in 2017

Name of disease	Cell source	Cell product	Proposed mode of action	Topic in 2017	ClinicalTrials.gov identifier	Started/(will be) completed	Sponsor or Institute	Reference
Spinal cord injury (SCI)	NSC	NSC	Neuroprotection, axon regeneration	Failure of CCLs in animal SCI models	NCT02163876	October 2014/May 2016	StemCells, Inc.	[1,6–8]
	ESC	OPC	Re-myelination, neuroprotection		NCT02302157	March 2015/ December 2018	Asterias Biotherapeutics, Inc.	[12–14]
Age-related macular degeneration (AMD)	iPSC	RPE sheet	Maintenance of overlying photoreceptors and underlying choroidal vessels	First-in-human study using iPSCs	UMIN000011929 (non-GMP clinical research)	October 2013/ November 2017	RIKEN	[3••,23,24]
	Adipose tissue-derived stem cell	Adipose tissue-derived stem cell	Unknown	Severe adverse effects in unproven stem cell therapy	NCT02024269 (withdrawn before enrollment)	December 2013/ September 2015	A stem-cell clinic. A case of unproven stem cell therapies.	[4•]
Parkinson's disease (PD) Therapeutics Pty Limited	[34–37]	Parthenogenetic stem cell	NSC	Neuroprotection, secretion of dopamine		NCT02452723	March 2016/March 2018	Cyto
	ESC	NSC	Secretion of dopamine	First-in-human trial using ESCs for PD	NCT03119636	May 2017/November 2018	Chinese Academy of Sciences	[5]

and consider the mode of action (MOA) of the cell products.

Regarding a cell-based therapy for SCI, several MOA have been suggested including neuroprotection, immunomodulation, axon sprouting and/or regeneration, neuronal relay formation, and myelin regeneration [10•]. In the preclinical studies using HuCNS-SCs, the authors concluded that the grafted cells differentiated into both neurons and oligodendrocytes in the spinal cord, and contributed to synapse formation with host neurons and remyelination [6,7]. Remyelination by the grafted NSCs in murine SCI models can be interpreted as a MOA for human patients. On the other hand, murine models may not be appropriate to predict the repair of corticospinal tract in humans.

Another strategy to treat SCI is the transplantation of human ESC-derived oligodendrocyte progenitor cells (OPCs), which are expected to promote remyelination [11]. A clinical trial using OPCs sponsored by Asterias Biotherapeutics Inc. has been ongoing since 2015 (ClinicalTrials.gov Identifier: NCT02302157), which is an open-label, single-arm, phase I/IIa study for severe cervical SCI. Importantly, in this case, CCL-derived OPCs (AST-OPC1) had been proved to be safe and effective in preclinical studies using rodent SCI models [12,13]. The update for this trial reported that 83% (15/18) and 100% (6/6) of the patients showed motor recovery at six and 12 months, respectively [14]. In addition, there have been no serious, unexpected, adverse events related to AST-OPC1, the surgical procedure, or the drug used for immunosuppression in any of the total of 30 patients. The MOA of this strategy is more straightforward compared to transplantation of NSCs, which suggests an optimal window of the injury for treatment. It would be expected that the clinical trial gives us an important insight as to what kind of patients are most suitable for this treatment.

Age-related macular degeneration (AMD)

AMD impairs visual acuity and primarily afflicts older populations. AMD has been divided into neovascular (or wet) and atrophic (or dry) types. Physical disruption and functional impairment of the RPE, a monolayer sheet of cells that supports overlying photoreceptors and underlying choroidal vasculature, is the main cause of the disease, and it is the reason why cell replacement therapy with stem cell-derived RPE is expected.

The first clinical application of PSCs for AMD patients used ESCs and was done by Astellas Pharma Inc. (ClinicalTrials.gov Identifier: NCT01344993). After one year's observation, the grafted ESC-derived RPE cells survived in 13 (72%) of 18 patients. In each patient, only one eye was treated. Visual acuity improved in 10 eyes, remained stable in seven eyes, and continued to degenerate in one

eye, whereas visual acuity in the untreated eyes did not show improvements [15]. In addition, there was no evidence of adverse proliferation, rejection, or serious ocular or systemic safety issues related to the grafts. These AMD results suggest that stem cell-derived cells could provide a new cell source for the treatment of neurological diseases.

The first-in-human trial using iPSCs started in 2014, and the results of one-year-follow up were reported in 2017 [3••]. In the original method used to produce iPSCs, four transcriptional factors (c-Myc, Oct4, Sox2, Klf4) were introduced into dermal fibroblasts by retroviral vectors [16,17]. This method cannot be used in clinical therapies, because the proto-oncogene c-Myc risks tumorigenesis and the retroviral integration may cause genomic mutations. Accordingly, iPSC derivation methods toward clinical application have been reported including those that replaced the c-Myc expression with L-Myc [18] and further include LIN28 [19] and the inhibition of p53 [20,21] in the derivation protocol. Additionally, integration-free introduction of the six genes was achieved using plasmid vectors [22]. Taking advantage, the authors of the first-in-human trial incorporated these modifications to derive iPSCs from the patient's fibroblasts and induced differentiation to produce RPE cell sheets [23]. The follow-up study reported that the RPE cells survived for one year in the eye, and the patient's visual acuity had stabilized without the injection of an anti-VEGF drug, which the patient had received regularly before the surgery [3••]. More importantly, the patient suffered from no serious complications and no unexpected proliferation of the grafted cells during the observation period.

At the same time as this successful iPSC study, a disastrous clinical treatment for AMD patients was reported [4•]. In this trial, autologous adipose tissue-derived stem cells were injected intravitreally into both eyes of three AMD patients. Blindness occurred in one patient, and marked visual loss in the other two.

These two extreme reports emphasize the importance of criteria and quality control of the donor cells. Importantly, the MOA of the grafted cells should be scientifically established before going to the clinic. In the first trial, the induction protocol from iPSCs to RPE cells was rigorously confirmed, and the characteristics and function of the induced RPE cells were confirmed by the expression of RPE markers and physiological experiments [23]. Furthermore, safety of the grafted cells was extensively examined using immunodeficient mice [3••,24]. The RPE sheet was placed under the retina and was expected to maintain overlying photoreceptors and underlying choroidal vessels as the MOA. In the second trial, however, there is no evidence that the grafted cells were differentiated into RPE cells, and the MOA of the cells has not been reported. Despite these uncertainties, the adipose tissue-derived stem cells were injected intravitreally into both eyes. Worse is that the related clinical trial (ClinicalTrials.gov Identifier: NCT02024269) had been withdrawn before enrollment and it was not informed to the patients. This case exemplifies the danger of unproven stem cell therapies.

It is noteworthy that the iPSC trial had originally been planned for two patients, but the RPE sheet transplantation for the second patient was cancelled because of three aberrations in DNA copy number (deletions). Genomic analysis of the iPSC-derived cells remains inconclusive for clinical use. Although there is no evidence in the published literature that the alterations are related to tumorigenesis, the researchers decided to use standard anti-VEGF treatment on the patient. Regarding genetic changes in PSCs, there is an international survey of over 100 human PSC lines (125 ESCs and 11 iPSCs) that concluded most lines have remained karyotypically normal, but that there is a progressive tendency to acquire changes with prolonged culture, which commonly affect chromosomes 1, 12, 17 and 20 [25]. Especially, a gain of *BCL2L1* on chromosome 20 provides a strong growth advantage to PSCs. Another study also reported that a genetic change on chromosome 20 leads to the overexpression of *BCL2L1* and escape from apoptosis [26]. More recently, *TP53* mutations, which are common in human cancers, were detected by the exome sequencing of 140 human ESC lines, including 26 lines prepared for potential clinical use [27••]. This mutant allelic fraction increased with passage number too, suggesting an additional selective advantage for favored expansion [28]. These two mutations are considered dangerous and emphasize the importance of referencing cancer-related genes listed in the COSMIC (Catalogue of Somatic Mutations in Cancer) database when evaluating the safety of iPSCs and their derivatives [29]. There is a chance of *de novo* point mutations for iPSCs not only during cell culture but also in the process of reprogramming, but these latter mutations preferentially occur in lamina-associated domains and are underrepresented in open chromatin regions [30•]. Epigenetic changes have also been observed in PSCs and even in their somatic cells of origin, which might affect phenotype, stability and growth of the PSCs and their derivatives [31]. The relationship between genetic and epigenetic changes and tumor formation by the grafts needs to be examined not only in preclinical studies but also in clinical trials.

Parkinson's disease (PD)

PD is caused by the progressive loss of nigrostriatal dopaminergic (DA) neurons, and the main symptoms are motor dysfunctions such as tremor, rigidity and akinesia. Since 1987, fetal cells from the ventral mesencephalon have been grafted, and the results of clinical trials showed that the grafted cells survived and functioned as DA neurons over 20 years in some patients [32,33].

However, ethical issues regarding the use of fetal tissues and the limited amounts of accessible donor tissues have prevented fetal cell transplantation from becoming a standard therapy to treat PD patients. Stem cells, especially ESCs and iPSCs, are expected as alternative donor cells.

In 2016, a phase I clinical trial using parthenogenetic stem cells was started in Australia (ClinicalTrials.gov Identifier: NCT02452723). Parthenogenetic cells are unique because they are derived from unfertilized oocytes through the suppression of the second meiotic division, leading to a pluripotent diploid cell line that contains exclusively maternal chromosomes. The trial has been approved base on previous preclinical studies [34–37], in which the authors induced and grafted NSCs and then confirmed the efficacy and safety of the grafts in rat and monkey models.

In 2017, another clinical trial using ESCs was launched in China (ClinicalTrials.gov Identifier: NCT03119636) [5], but the preclinical data have not yet been reported in peer-reviewed journals.

As discussed above, the criteria of the donor cells are critical for the efficacy and safety of any cell-based therapy. In the trial using parthenogenetic stem cells, the grafted NSCs were still immature and expressed nestin and PAX6 [34]. DA neurons of the nigro-striatal pathway are derived from the floor plate in the midbrain [38] and never express PAX6 even in their progenitors. Therefore, it is unlikely that the PAX6-positive NSCs differentiate into authentic midbrain DA neurons after transplantation. Even if some of the transplanted NSCs become dopaminergic, they will likely be frontal DA neurons. An alternative possible MOA for the positive effects involves the secretion of neurotrophic factors such as BDNF and GDNF, but this MOA is only speculative.

The induction of midbrain DA neurons from PSCs is performed by a combination of dual inhibition of BMP and TGF/Activin/Nodal signals, midbrain specification by Wnt signal activation, and ventralization by Sonic hedgehog [39–41]. In addition, to enrich midbrain DA progenitors and exclude immature NSCs, fluorescence-activated cell sorting using antibodies for CORIN, a floor plate marker [42], or ALCAM, a central nervous system microvascular endothelium marker [43], has been developed. When grafted into the striatum of 6-OHDA-lesioned rats [40,44] or MPTP-treated monkeys [45,46••], DA progenitors induced this way showed robust survival and function that improved behavioral impairments. In addition, human ESC-derived DA neurons showed equal potency and efficacy to fetal midbrain DA neurons those improved the neurological symptoms of PD patients [47]. These results support the idea that ESCs or iPSCs can be a cell source for a cell-based therapy against PD.

A PET study using [^{18}F]DOPA in monkeys showed dopamine synthesis by the grafts [45,46••], and optogenetic alteration of the function of the grafted cells resulted in behavioral change of rat PD models [44]. These results suggest that the MOA of the grafted DA neurons is reinnervation and dopamine secretion in the striatum.

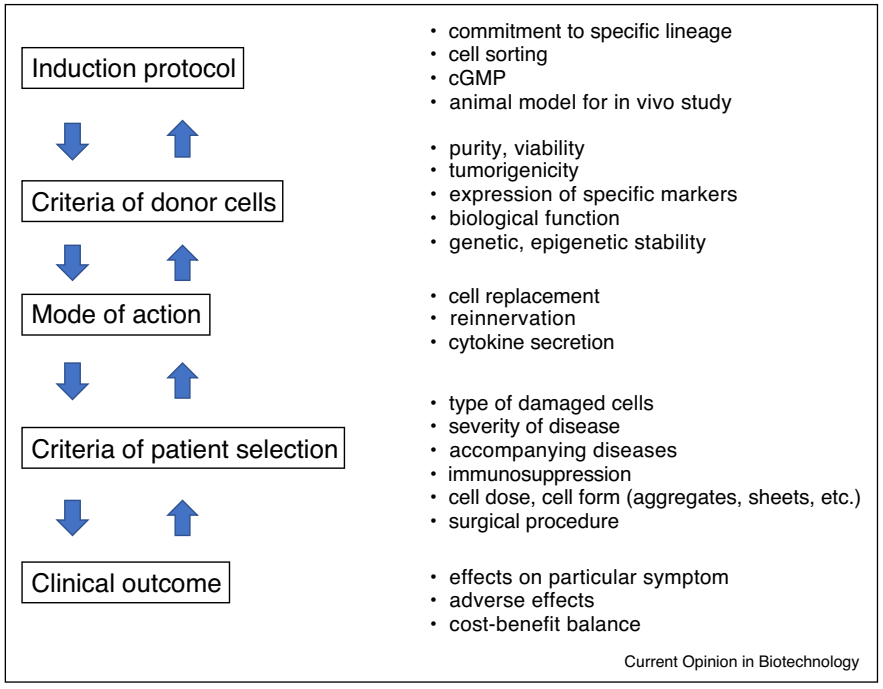
In the pursuit of better transplantation results, more studies are being performed. A single-cell RNA sequencing revealed molecular diversity in developing mouse and human midbrain and PSC-derived cells [48••]. A combination of RNA-seq analysis of the donor cells and *in vivo* studies of PD model rats following transplantation will help identify predictive markers of the donor cells for better outcomes [49,50••]. A monkey allogeneic transplantation study demonstrated that major histocompatibility complex-matched transplantation reduced the immune response by the host brain and promoted the survival of the grafted DA neurons [51•].

Conclusion

2017 was year in which extremely positive and negative findings regarding stem cell-based therapies for neural repair were reported. These polarized findings emphasize the importance of multiple considerations when preparing preclinical or clinical studies: first, the difference between the RCLs and CCLs of the cell product, second, the selection of an appropriate animal model, third, the criteria and quality control of the donor cells, and fourth, the MOA of the grafts. These four items are not independent. The criteria of the donor cells define the MOA of the grafts, and the MOA defines the inclusion and exclusion criteria of the patients in a clinical study. In reality, however, it might be impossible to finalize each item before a clinical study. For this reason, the results of clinical outcomes should be used to optimize the above considerations (Figure 1).

For example, in the transplantation of fetal midbrain (ventral mesencephalon) for PD patients, it became apparent that some patients suffer from graft-induced dyskinesia [52,53], possibly due to the contamination of serotonergic neurons in the grafts [54]. Based on these clinical outcomes, a new European trial using fetal midbrain tissue, called TRANSEURO (www.transeuro.org.uk), was initiated. In this study, the protocol design was modified to minimize the risk of graft-induced dyskinesia [55]. The success of stem cell-based therapies will depend on feedback from clinical outcomes to optimize criteria for the donor cells, patient selection, observation period and evaluation of neurological symptoms.

Figure 1



A feedback loop for the better cell-based therapies.

Conflict of interest

There is no conflict of interest relating to this article.

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