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Progress in the Use of Induced Pluripotent Stem Cell-Derived Neural Cells for Traumatic Spinal Cord Injuries in Animal Populations: Meta-Analysis and Review

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Key Words. Regenerative medicine • Induced pluripotent stem cells • Embryonic stem cells • Spinal cord injury • Meta-analysis

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ABSTRACT

Induced pluripotent stem cells (iPSCs) are cells genetically reprogrammed from somatic cells, which can be differentiated into neurological lineages with the aim to replace or assist damaged neurons in the treatment of spinal cord injuries (SCIs) caused by physical trauma. Here, we review studies addressing the functional use of iPSC-derived neural cells in SCIs and perform a meta-analysis to determine if significant motor improvement is restored after treatment with iPSC-derived neural cells compared with treatments using embryonic stem cell (ESC)-derived counterpart cells and control treatments. Overall, based on locomotion scales in rodents and monkeys, our meta-analysis indicates a therapeutic benefit for SCI treatment using neural cells derived from either iPSCs or ESCs, being this of importance due to existing ethical and immunological complications using ESCs. Results from these studies are evidence of the successes and limitations of iPSC-derived neural cells in the recovery of motor capacity. STEM CELLS TRANSLATIONAL MEDICINE *2019;8:681–693*

SIGNIFICANCE STATEMENT

The present review and meta-analysis evaluates the efficacy of using induced pluripotent stem cells (iPSCs)-derived neural cells in restoring motor functionality in experimental animal models sustaining traumatic spinal cord injuries. The study also addresses existing concerns with the use of iPSC-derived neural cells and whether this provides similar results as treatment with cells derived from embryonic stem cells, which have already been successfully used to treat injuries to the central nervous system.

INTRODUCTION

Traumatic spinal cord injuries (SCIs) are injuries caused by contusion or compression of the spinal cord and can lead to impairment of muscle movement depending on the severity of the injury (Fig. 1). In worst cases, permanent dysfunction can lead to paraplegia or quadriplegia depending on the site of injury. SCIs in the cervical and thoracic regions of the spinal cord are the most prevalent. According to the U.S. National Spinal Cord Statistical Center [1], the incidence of traumatic SCIs is 17,500 cases each year and globally it is between 250,000 and 500,000. Traumatic SCIs occur mostly in people younger than 30 years old; however, the average age is 42 years old. Males account for 81% of SCIs and the ratio of men to women is 3:1 [1]. Vehicular accidents are the leading causes, followed by falls, violence, and sports. Most SCIs at the cervical level are from practicing high physical activity sports such as hockey, skiing, diving, and American football, whereas over half of SCIs from horseback riding and snowboarding occur at the thoracic or lumbosacral region [2]. Likewise, geriatric patients with osteoporosis or degenerative spondylolisthesis are at an increased risk of suffering an SCI [3]. SCI is an expensive traumatic condition, with an estimated cost of around $200,000 in the United States in the first 2 years [4]. Therefore, there is a need to optimize treatment for SCI.

Pathophysiology Behind an SCI

Myelopathies in SCI cause damage to the white matter that contains nerve axons and tracts to and from the brain and to the gray matter, which results in a loss of motor neurons [5].

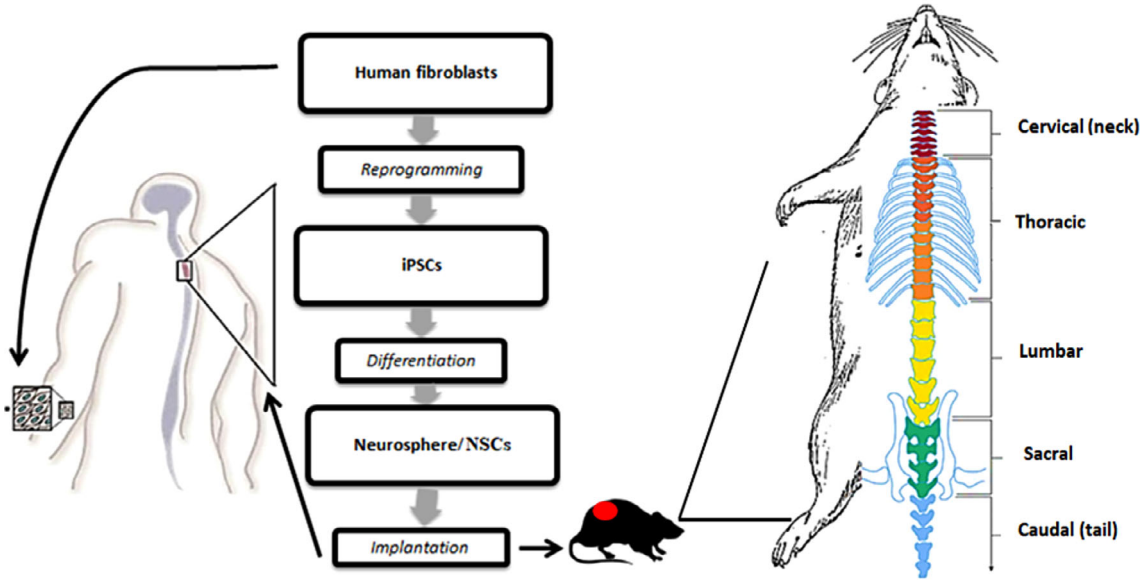


Figure 1. Schematic illustration of the process to treat spinal cord injuries with cells derived from iPSCs. The procedures by which a spinal cord injury (SCI) can be treated using autologous cells derived from iPSCs are illustrated in the left side. Fibroblasts will be obtained from a patient suffering from an SCI. After genetic reprogramming, the fibroblasts will be converted into iPSCs. The iPSCs will then be differentiated into neurospheres or NSCs, which will be used for cell transplantation in the site of injury. Currently, experimental procedures have been done in rodents to demonstrate the feasibility of this cell therapy. On the right, the different areas of spinal column of rodents are illustrated. Abbreviations: iPSCs, induced pluripotent stem cells; NSCs, neuronal stem cells.

This manifests in deficiencies in motor and sensory skills. In the primary injury phase, SCI results from contusion of shattered cervical/thoracic vertebral bones or compression that leads to an increase in pressure because of the blood or bone on the spinal cord. At the secondary injury phase, cell apoptosis and necrosis, oxidative damage, glutamate excitotoxicity, and axon tracts are destroyed by autoimmune responses [5]. The spinal cord is immune privileged, as it is isolated from the rest of the body through the blood-brain barrier and by secretion of immunosuppressive cytokines [6]. In addition, the central nervous system (CNS) contains low levels of major histocompatibility complex molecules because of the absences of lymphatic vessels that carry white blood cells. This indicates that only certain immune responses occur in the spinal cord [6]. However, once the blood-brain barrier in the spinal cord is broken, permeability of cells carried out into the blood invade the injured tissue increases and causes inflammation. This leads to platelet and fibrin clots aimed to reduce local bleeding. Furthermore, astrocytes become eosinophilic and get involved in immune responses. Their migration increases permeability to leukocytes, also causing inflammation [7]. After a SCI, astrocytes proliferate and express glial fibrillary acid protein and congregate to form glial scars during the chronic stage. The neural scar tissue expresses semaphorin 3A, an inhibitor of axonal regeneration [3], which affects CNS recovery by creating a physical barrier. These scars secrete chondroitin sulfate proteoglycans that inhibit axonal growth [3]. Oligodendrocytes and neurons at the SCI site die because of the disruption of the cell membrane, triggering axon demyelination and affecting signal transduction and generation of action potentials.

Neural Cells Derived from Induced Pluripotent Stem Cells as an Alternative Source for Cell Therapy

Considering the complexity of the trauma sustained in a SCI and its prevalence in society, this medical condition has prompted research for therapeutic treatments. Cell therapy using cells derived from human embryonic stem cells (ESCs) has been used in clinical trials in SCI patients and can potentially improve their quality of life [8, 9]. However, because of the ethical concerns and immunocompatibility issues, the use of these cells is under debate. Induced pluripotent stem cells (iPSCs) can be used as an alternative, as they are functionally equivalent to ESCs and have same potential to differentiate into any cell type of the body, which make them suitable for tissue regeneration [5, 8, 10, 11]. iPSCs are derived from somatic cells (i.e., dermal fibroblasts and blood cells) after genetic reprogramming by overexpression of Oct4, Sox2, Kl4, and c-Myc [12] or the combination of other transcription factors [13] and reprogramming molecules [14, 15] that promote the expression of core transcription factors related to pluripotency: Oct4, Sox2, and Nanog [10]. Therefore, autologous iPSCs from patients with an SCI can be obtained in a noninvasive way. iPSCs can then be differentiated into neuronal stem cells (NSCs) and neuronal progenitor cells (NPCs) in vitro [16] and can be further differentiated into neural cells specific to the spinal cord (i.e., oligodendrocytes, astrocytes, and neurons) [17]. All these cell populations can be transplanted into the SCI site of the recipient/patient and have the potential to contribute in regeneration of the damaged spinal cord (Fig. 1) [18].

Here, we review literature that reports the use of iPSC-derived neural cells such as NSCs, neuronal progenitors, oligodendrocytes, and astrocytes to treat SCIs in animal models. A meta-analysis was performed on the data reported in these studies with the goal to determine whether treatment with iPSC-derived neural cells is as effective as the use of human ESC counterpart cells, and therefore a good alternative. [18]. Although, this manuscript was in preparation, a meta-analysis reported effective outcomes in the use of iPSC-derived neural cells for SCI treatments in rat models [19]. Our meta-analysis

Table 1. Quality assessment of studies using induced pluripotent stem cells

Study	1	2	3	4	5	6	7	8	9	10	11
All (2015) [21]	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Amemori et al. (2015) [22]	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓
Fujimoto et al. (2012) [23]	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓
Hayashi et al. (2011) [24]	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓
Kawabata et al. (2016) [25]	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓
Kobayashi et al. (2012) [26]	✓	✓	✓	✓	✓	✓		✓		✓	✓
Liu et al. (2017) [27]	✓	✓	✓	✓		✓		✓		✓	✓
Lopez-Serrano et al. (2016) [28]	✓	✓	✓	✓	✓	✓		✓		✓	✓
Lu et al. (2014) [29]	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓
Nori et al. (2011) [30]	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓
Nori et al. (2015) [6]	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓
Nutt et al. (2013) [31]	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓
Oh et al. (2015) [32]	✓	✓	✓	✓	✓	✓		✓		✓	✓
Okubo et al. (2016) [33]	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓
Pomeshchik et al. (2015) [34]	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓
Romanyuk et al. (2015) [35]	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓
Ruzicka (2017) [36]	✓	✓	✓	✓	✓	✓		✓	✓	✓	✓
Salewski et al. (2015) [37]	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Suzuki et al. (2017) [38]	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Tang et al. (2013) [39]	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Tsuji et al. (2010) [11]	✓	✓	✓	✓	✓	✓		✓		✓	✓
Yang et al. (2018) [40]	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

Note: Studies in bold were included in the meta-analysis.
The following criteria are based on previous reviews [18, 19]. 1, Compliance with animal welfare regulations; 2, Publication in a peer-reviewed journal; 3, description of animals used; 4, designation of strain; 5, number of animals per group; 6, description of level of injury; 7, randomly assigning animals to a specific group; 8, description of the control groups; 9, blindness of assessor; 10, description of statistical analysis; 11, statement of any potential conflict of interest.

complemented this study, and our review included additional studies using mouse and nonhuman primates and included results from studies using NSCs from iPSCs, ESC, and wild-type lines. Furthermore, previous meta-analyses performed subgroup analyses predominantly using a random effects model (REM), considering a broad range of factors including gender, recipient species, use of immunosuppressive agents, donor age, graft type, donor species, etc., to analyze motor functional recovery, allodynia, and hyperalgesia [20]. Our meta-analysis instead used a fixed effects model (FEM). Based on our analysis and like previous studies, we concluded that there is significant evidence indicating that motor function can be preserved after sustaining a debilitating injury to the spinal cord by using iPSC-derived neural cell treatment.

Materials and Methods

Recollection of Literature and Inclusion and Exclusion Criteria for Meta-Analysis

The studies used in the review were collected from online scholarly, peer-reviewed journals, or books from 2000 to 2018 through databases, including PubMed and Web of Science, using the following key words: *behavior assessment, ESCs, iPSCs, motor recovery/functionality, SCIs,* and *stem cell therapy.* Studies that included the use of iPSCs or ESCs in treating neurodegenerative diseases rather than neurological trauma sustained to the spinal cord were discarded. Initially, studies reviewed needed to include iPSCs for treatment of SCIs and assess functional recovery before and after implantation of iPSC-derived neural cells, so all animal models and locations were considered (Table 1). For the meta-analysis, studies were narrowed to thoracic SCI models using rats or mice, to the use of the locomotor scales Basso mouse scale (BMS) and Basso, Beattie, and Bresnahan scale (BBB), to intervention at the subacute phase, to studies in which injury was induced by either the balloon compression method or contusion, and to those that reported means, SD, and *n* values (Table 2). Studies were excluded if they did not report data assessing motor functionality, if a different animal model was used, because of the differences in the scales, especially given that greater detailed motor skills can be evaluated in monkeys, and if they used cervical SCI models, because the BMS is not the most appropriate method to detect motor improvement or deficit for this anatomical region [38]. Lastly, the overall procedure for implantation of iPSC-derived cells should be similar among studies with minor variations (Table 3).

Table 2. Selection criteria for studies included in meta-analysis

Study	1. Motor assessed	2. BMS/ BBB scale	3. Thoracic	4. Mean, SD, *n* given	5. Intervention phase: subacute	6. Rat or mouse recipient	7. Control	8. Injury type: balloon compression or contused	9. Immunosuppressed or SCID
All (2015) [21]	✓	✓	✓			✓	✓	✓	✓
Amemori et al. (2015) [22]	✓	✓	✓	✓	✓	✓	✓	✓	✓
Fujimoto et al. (2012) [23]	✓	✓	✓		✓	✓	✓	✓	✓
Hayashi et al. (2011) [24]	✓	✓	✓		✓	✓	✓	✓	✓
Kawabata et al. (2016) [25]	✓	✓	✓		✓	✓	✓	✓	✓
Kobayashi et al. (2012) [26]	✓				✓		✓	✓	✓
Liu et al. (2017) [27]			✓		✓	✓	✓	✓	✓
Lopez-Serrano et al. (2016) [28]	✓	✓	✓	✓	✓	✓	✓	✓	✓
Lu et al. (2014) [29]	✓				✓	✓	✓		✓
Nori et al. (2011) [30]	✓	✓	✓		✓	✓	✓	✓	✓
Nori et al. (2015) [6]	✓	✓	✓	✓	✓	✓	✓	✓	✓
Nutt et al. (2013) [31]	✓				✓	✓	✓	✓	✓
Oh et al. (2015) [32]	✓	✓	✓		✓	✓	✓		✓
Okubo et al. (2016) [33]	✓	✓	✓	✓	✓	✓	✓	✓	✓
Pomeshchik et al. (2015) [34]	✓	✓	✓	✓	✓	✓	✓	✓	✓
Romanyuk et al. (2015) [35]	✓	✓	✓	✓	✓	✓	✓	✓	✓
Ruzicka et al. (2017) [36]	✓	✓	✓		✓	✓	✓	✓	✓
Salewski et al. (2015) [37]	✓	✓	✓	✓	✓	✓	✓	✓	✓
Suzuki et al. (2017) [38]	✓	✓				✓	✓	✓	✓
Tang et al. (2013) [39]	✓		✓		✓		✓	✓	✓
Tsuji et al. (2010) [11]	✓	✓	✓	✓	✓	✓	✓	✓	?
Yang et al. (2018) [40]	✓	✓	✓		✓	✓	✓	✓	✓

Note: if all the above criteria were fulfilled, then those studies were included into the meta-analysis, with the exception of Tsuji: No immunosuppressant was administered nor was SCID mice used; however, iPSCs were cultured from own fibroblasts. Previous subgroup analyses indicate motor recovery outcome was significantly affected by the injury model (compression, contusion, and hemisection), intervention phase (subacute, acute, and chronic), and immunosuppression; thus, these criteria were included in order to screen out studies for the meta-analysis [19].
Abbreviations: BBB, Bresnahan scale; BMS, Basso mouse scale; SCID, severe combined immunodeficiency.

This strategy identified 22 studies; however, 14 were excluded because insufficient information was reported to calculate a *t*-statistic and effect size. Instead, those studies were included for qualitative synthesis. According to previous subgroup analyses, motor recovery was significantly impacted by the type of injury model examined (compression, contusion, and hemisection), the intervention phase (subacute, acute, and chronic), location (cervical or thoracic), and the use of immunosuppression; thus, these criteria were included in order to screen out for the most homogeneous studies for the meta-analysis (Table 2) [19, 20]. Thus, only eight studies qualified for our meta-analysis.

Comparative and Statistical Analysis Within Studies

Before the meta-analysis, a *t*-statistic was used to determine which studies showed significant results. Means were estimated from data (e.g., graphs) provided for all studies that used the BMS or BBB scales to measure motor improvement; however, if neither a SD nor SEM was given, the *t*-statistic could not be performed. In those cases, because significance could not be verified, it was stated that the study itself reported significance at a certain *p* value (e.g., .05 and .01). A right-tailed *t* test was performed at α level of 0.05, corresponding to the null hypothesis $\mu_{iPSCs} = \mu_{control.}$ The alternative hypothesis assumed that the mean BMS score post-transplantation in the iPSCs group should be higher than the control group, thus $\mu_{iPSCs} > \mu_{control.}$ Given high variability (8 weeks vs. 90 days) reported in recorded repeated measures of BMS scores, means were derived for each study at the 42 day mark for the *t*-statistic, as this was the lowest duration observed. The values of *t*-statistics were verified for significant difference based on critical values from a Student's *t* distribution.

Comparative and Statistical Analysis Between Studies

After performing the *t* test and determining significance, a weighted mean and SD was calculated for eight studies. Their weights were allocated in order to detect the overall effect of the usage of iPSCs as a regimen for a SCI, which can be then generalized to a larger population of studies focusing on this topic. The meta-analysis was performed using the BioStat Comprehensive Meta-Analysis 2.0 Software, using guidance for the general procedure as reported previously [41]. A right-tailed *t* test was conducted under an α level of 0.05 corresponding to

Table 3. General information about studies using iPSCs in rodent and monkey populations with SCIs

Study	Injury site	Type of injury	Cell implanted and time post-SCI	iPSCs (species, parental cell line, and reprogramming method)	Animal recipient	Immunosuppressant
Rat models						
All (2015) [21]	T8–T10	Contusion	OPs 2 days	1. Human: derived from adult bone marrow (blood monocytes; episomal vector) 2. Human: derived from 16-week fetal lung fibroblasts (retrovirus) 3. Human: derived from adult bone marrow (MSCs; retrovirus)	10-week-old adult female Lewis rats	Cyclosporine A
Amemori et al. (2015) [22]	T8	Balloon compressed	NPs 7 days	Human: derived from 16-week-gestation female fetal lung fibroblasts (lentivirus)	10-week-old adult male Wistar rats	Cyclosporine A, azathioprine sodium, methyl-prednisolone
Hayashi et al. (2011) [24]	T9–T10	Contusion	Astrocytes 3 or 7 days	Mouse: derived from male embryonic fibroblast (retrovirus)	8-week-old female Sprague Dawley rats	Cyclosporine A
Lopez-Serrano et al. (2016) [28]	T8–T9	Contused	NSCs 0 or 7 days	Human: derived from 48-year-old male dermal fibroblasts (retrovirus)	9-week-old male rat	Tacrolimus
Nutt et al. (2013) [31]	C4	Contusion	NPCs 4 weeks	Human: derived from 16-week fetal lung fibroblasts (lentivirus)	8-week-old adult female Long Evans rats	Cyclosporine A
Romanyuk et al. (2015) [35]	T8 or T9	Balloon compressed	NPs 7 days	Human: derived from 16-week fetal lung fibroblasts (lentivirus)	10-week-old male Wistar rats	Cyclosporine A, azathioprine sodium, methyl-prednisolone
Ruzicka et al. (2017) [36]	T8	Balloon compressed	NPs 7 days	Human: derived from 16-week female fetal lung fibroblasts (lentivirus)	10-week-old male Wistar rat	Cyclosporine A, azathioprine sodium
Yang et al. (2018) [40]	T10	Contused	OPC 7 days	Mice: derived from dermal fibroblasts of pregnant females (retrovirus)	7-week-old Sprague Dawley rat	Cyclosporine A
Mouse models						
Fujimoto et al. (2012) [23]	T9	Contusion	NESs 7 days	Human: Derived from 36-year-old female dermal fibroblasts (lentivirus)	8–10-week-old female NOD-SCID mice	None given
Kawabata et al. (2016) [25]	T10	Contusion	OPC-enriched NSC/PC 9 days	Human: derived from 36-year-old female dermal fibroblasts (lentivirus)	Adult female NOD-SCID mice	None given
Liu et al. (2017) [27]	T9	Contusion	NPCs 9 days	1. Human: derived from male USCs (Sendai viral vector) 2. Human: derived from male or female skin fibroblasts (Sendai viral vector)	Adult SCID mice	None given

(Continues)

Table 3. (*Continued*)

Study	Injury site	Type of injury	Cell implanted and time post-SCI	iPSCs (species, parental cell line, and reprogramming method)	Animal recipient	Immunosuppressant
Lu et al. (2014) [29]	C5	Lateral hemisection	NSCs 2 weeks	Human: derived from adult 86-year-old male dermal fibroblast (retrovirus)	Adult female athymic nude rats and SCID mice	None given
Nori et al. (2011) [30]	T10	Contusion	NS 9 days	Human: derived from 36-year-old female dermal fibroblasts (lentivirus)	Adult female NOD-SCID mice	None given
Nori et al. (2015) [6]	T10	Contusion	NS 9 days	Human: derived from 36-year-old female dermal fibroblasts (lentivirus)	Adult female NOD-SCID mice	None given
Oh et al. (2015) [32]	T11	Compressed	NPCs 9 days	Human: derived from intervertebral disc tissue (retrovirus)	Adult male ICR mice	Cyclosporine A
Okubo et al. (2016) [33]	T10	Contusion	NSC/PC 9 days	1. Human: derived from 36-year-old female dermal fibroblasts (retrovirus) 2. Human: derived from cornea fibroblasts (episomal plasmid vector) 3. Human: derived from 36-year-old female dermal fibroblasts (retrovirus)	Adult female NOD-SCID mice	None given
Pomeshchik et al. (2015) [34]	T10	Contusion	NPCs 7 days	Human: derived from female skin fibroblasts (lentivirus)	8–10-week-old adult female C57BL/6J mice	Tacrolimus
Salewski et al. (2015) [37]	T6	Clip Compressed	NSC 6 days	Mouse: derived from embryonic fibroblasts (PiggyBac transposon)	Wild-type female mice and *Shiverer* female mice	Cyclosporine A
Suzuki et al. (2017) [38]	C6 or C7	Clip contusion	NSC 7 weeks.	Mouse: derived from embryonic fibroblasts (PiggyBac transposon)	8–10-week-old adult female wild-type mice	Cyclosporine A
Tsuji et al. (2010) [11]	T10	Contusion	PNS and SNS 9 days	1. Mouse: derived from embryonic fibroblasts (retrovirus) 2. Mouse: derived from adult TTF (lentivirus) 3. Mouse: derived from adult TTF (lentivirus)	Adult female mice	Not given
Nonhuman primate models						
Kobayashi et al. (2012) [26]	C5	Contusion	NSC/PC 9 days	Human: derived from adult female dermal fibroblasts (lentivirus)	>2-year-old adult female marmosets	Cyclosporine A
Tang et al. (2013) [39]	T9	Contusion	NSCs 7 days	Human: derived from female/male scalp tissue fibroblasts (retrovirus)	Rhesus monkeys	Cyclosporine A

Note: Studies in bold were included in the meta-analysis.

Abbreviations: C, cervical; ICR, imprinting control region; iPSC, induced pluripotent stem cell; MSCs, mesenchymal stem cells; NESs, neural epithelial cells; NOD-SCID, nonobese diabetic severe combined immunodeficiency; NP, neural precursor; NPC, neural progenitor cell; NSC, neuronal stem cell; OP, oligodendrocyte progenitor; OPC, oligodendrocyte precursor cell; PC, progenitor cell; PNS, primary neurosphere; SCI, spinal cord injury; SNS, secondary neurosphere; TTF, tail tip fibroblasts; USC, urinary stem cell.

the null hypothesis $\mu_{iPSCs} = \mu_{control}$ and the alternative hypothesis $\mu_{iPSCs} > \mu_{control}$, which assumes that the mean BMS score post-transplantation in the iPSCs group should be higher than the control group.

Objectives of the Meta-Analysis

Our meta-analysis addresses three questions about recovery in motor functionality: (a) whether there is a difference in BMS scores between rodent populations treated with iPSCs-derived cells compared with the control groups, (b) whether the iPSC-derived neural cells treated group showed advantageous results compared with the control groups, and (c) to what degree motor functionality was restored in comparison to a noninjured rat or mouse. Sensation was not analyzed given that it is not as frequently assessed as motor.

Methods to Evaluate Locomotor Behavior

Rats and mice may be physiologically distinct in certain regards but are classified under the terrestrial locomotion category [42], and previous comparative studies have demonstrated no significant variation in motor functional recovery when comparing studies using both animal models for a SCI cell transplantation treatment [20]. Therefore, for our meta-analysis, the results from locomotion measurements of rats and mice were pulled together. To assess locomotor recovery after a SCI in mice and rats, the BMS/BBB scales are used. The BBB test is based on a 21-point system, giving a score of 0 to no movement and a score of 21 to indicate complete normal limb movement [43]. The BMS was derived from the BBB scale [43], and it is based on a nine-point scale described in Supporting Information Table S1 [37]. For instances in which the BBB scale was used, scores were converted into compatible BMS scores. This was necessary because if the BBB scores (0 to 21) were averaged with the BMS scores (0 to 9), the weighted mean would be skewed.

RESULTS

Out of a total of 22 reports that assessed iPSC-derived neural cells for SCI treatment, the following studies were incorporated into the meta-analysis [6, 11, 22, 28, 33–35, 37]. A few studies [21, 23–25, 27, 29–32, 36, 38, 40] were excluded, because statistical significance could not be verified, motor recovery was not assessed, used a different scale to assess motor recovery, or focused on a cervical SCI model. Other studies [26, 39] were conducted in primates using iPSCs, and insufficient data were provided to perform a separate *t*-statistic.

t-Statistic Analysis

Data from reports that used the BMS/BBB scale for motor evaluation post cell transplantation were incorporated into the *t*-statistic analysis and were rearranged in alphabetical order, not by the level of significance (Supporting Information). If the *t*-statistic could not be calculated, the *p* value at which the study expressed significance at was acknowledged. Eight studies provided sufficient data to perform *t* tests and six of eight refuted the null hypothesis, showing significant evidence of a statistical difference in BMS scores and better outcome in the iPSC group. Two studies indicated that the control did better than the treatment [28, 34].

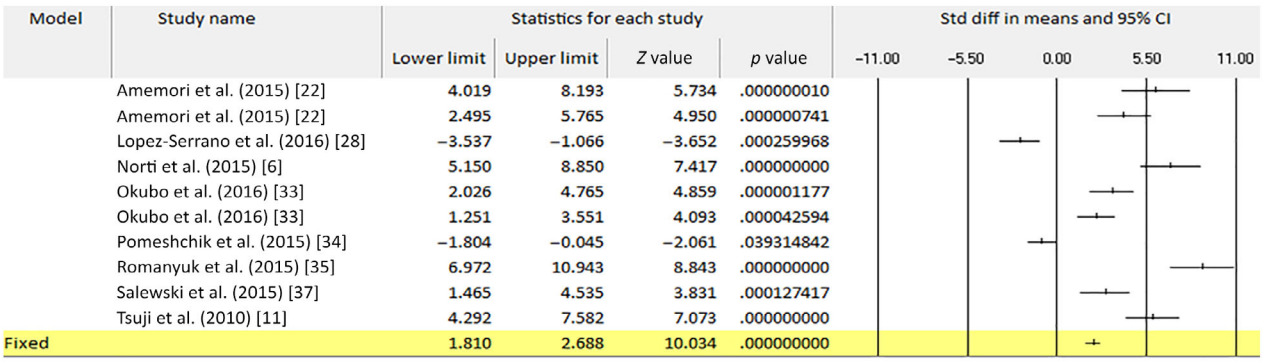
Weighted Mean Analysis

A weighted mean and SD were computed for both control and treated iPSC-derived neural cells groups' BMS scores in order to determine how the weight of each study reflects its relative importance on assessing the overall effect of motor recovery. The weighted mean and SD consist of studies included in the meta-analysis.The iPSC-treatment group has a higher mean than the control group (Supporting Information).

Meta-Analysis Using the Fixed Effect Size Model

Given that the scale used to assess locomotor functional recovery was the same across studies and the overall procedure was homogenous, a FEM was used in the meta-analysis [44] (Fig. 2). The effect size was based on means and was calculated using the standard difference of means. A total of 238 rodents were included in the selected studies, 128 of which were in the iPSC-derived cells group and 110 in the control (Supporting Information Fig. S1). On the forest plot, the scale used was from −11 to 11 for a 95% confidence interval. Based on the forest plot, zero was not included in the confidence interval for any of the studies except for two, indicating that the *p* values were less than .05, and in fact, the *p* value was close to zero and the *Z* value was 10.034 (Fig. 2). Additionally, all the intervals except for two studies [28, 34] were to the right of zero, indicating that the treatment has a larger mean than the control. More importantly, the overall standard difference of mean was 2.249, which indicates there is a significant difference in the means between the BMS score of the iPSC-derived neural cells group and the control, which answers the first objective, so the null hypothesis is rejected by the data. This indicates that the data support the claim that the iPSC-treated group performed better on the locomotion scales at the 42-day mark than the control group, which addresses the second objective of the meta-analysis. For comparison, under the REM, the *p* value is <.05, with a *Z* score of 3.319 and standard difference of mean of 3.705, indicating that under both models, there is significant evidence that the population pooled experienced a positive common effect on improving motor function postimplantation of iPSC-derived NPCs at the SCI site (Table 4).

In terms of heterogeneity, as the *Q* score was 212.577 and the I^2 score was 95.76, this suggests that there is some heterogeneity (Table 4). This may be because of the within study error, because dispersion was wider than expected given that $Q - \mathrm{df} > 0$. The null hypothesis for heterogeneity assumed that all studies share the same common effect size, whereas the alternative hypothesis stated that studies do not share a common effect size. The *p* value was close to zero, indicating that there is some evidence to reject the null, suggesting that either there is some statistical heterogeneity of effect size because of the observed dispersion or minor observed dispersion with precise studies. However, because of the small number of studies, a conclusion could not be adequately reached, nor are the heterogeneity tests reliable in this case and may not reflect the true between study variance. Additionally, once two studies [28, 34] showing no significant improvement with use of iPSC-derived neural cells were removed, heterogeneity dropped to 50.714 (Table 4). Even though these studies met the criteria for the meta-analysis, another aspect of their experimental procedure (e.g., incomplete differentiation) could have been impacted heterogeneity, especially, because a



Model	Study name	Statistics for each study						
		Std diff in means	Standard	Variance	Lower limit	Upper limit	Z value	p value
	Amemori et al. (2015) [22]	6.106	1.065	1.134	4.019	8.193	5.734	.000000010
	Amemori et al. (2015) [22]	4.130	0.834	0.696	2.495	5.765	4.950	.000000741
	Lopez-Serrano et al. (2016) [28]	−2.302	0.630	0.397	−3.537	−1.066	−3.652	.000259968
	Norti et al. (2015) [6]	7.000	0.944	0.891	5.150	8.850	7.417	.000000000
	Okubo et al. (2016) [33]	3.395	0.699	0.488	2.026	4.765	4.859	.000001177
	Okubo et al. (2016) [33]	2.401	0.587	0.344	1.251	3.551	4.093	.000042594
	Pomeshchik et al. (2015) [34]	−0.925	0.449	0.201	−1.804	−0.045	−2.061	.039314842
	Romanyuk et al. (2015) [35]	8.958	1.013	1.026	6.972	10.943	8.843	.000000000
	Salewski et al. (2015) [37]	3.000	0.783	0.613	1.465	4.535	3.831	.000127417
	Tsuji et al. (2010) [11]	5.937	0.839	0.704	4.292	7.582	7.073	.000000000
Fixed		2.249	0.224	0.050	1.810	2.688	10.034	.000000000

Figure 2. CI and forest plot for locomotion scores post-transplantation with induced pluripotent stem cell (iPSC)-derived cells (above). This indicates functionary recovery after transplanting iPSCs-derived neuronal stem cell/neural precursors. Effect size based on standard difference in means of implanted iPSC-derived cells compared with the control for fixed effects model (below). There is significant evidence of a difference between scores of locomotion. The row highlighted in yellow indicates overall outcomes. Note: Amemori et al. is represented twice because both cases used iPSCs except one was implanted intra-spinally (is) and the other intra-thecally (it), Okubo et al. is represented twice because the study analyzed two iPSC cell lines: 201B7 and 253G1. Abbreviation: CI, confidence interval.

Table 4. Hypothesis and heterogeneity testing

		Effect size and 95% confidence interval					Test of null (2 tail)		Heterogeneity			
Model	Number studies	Point estimate	SE	Variance	Lower limit	Upper limit	*Z* value	*p* value	*Q* value	df (*Q*)	*p* value	I^2
Fixed	10	2.249	0.224	0.050	1.810	2.688	10.034	.000	212.577	9	.000	95.766
Random	10	3.705	1.116	1.246	1.517	5.893	3.319	.001				
Excluding Lopez-Serrano et al. [28] and Pomeshchik et al. [34] studies												
Fixed	8	4.441	0.284	0.081	3.885	4.997	15.651	.000	50.714	7	.000	86.197
Random	8	5.024	0.778	0.605	3.499	6.548	6.458	.000				

tumor was detected in one study [28], whereas the other had limited donor cells survival [34].

Likewise, according to the funnel plot (Supporting Information Fig. 2), it seems that there is some publication bias, although only a small number of studies were included in the meta-analysis. This assertion was not definitive. Just because the heterogeneity is statistically significant, it does not indicate that the REM would be more appropriate in this case.

Discussion

From our meta-analysis of eight independent studies, we conclude that there is a significant improvement in locomotion in animals treated with iPSC-derived neural cells compared with nontreated animals. These conclusions were based on studies performed in mice and rats as experimental animal models. However, it should be noted that most animals in the control groups showed a slight increase in BMS/BBB scores over time because incomplete SCIs caused by contusion or compression resulted in mild neuroplasticity and partial regeneration. In addition, preimplantation scores were 0 to 1, indicating paralysis except for one study [38]. Our conclusion is supported by the recent meta-analysis from studies that used rats as experimental animal model reaching similar conclusions [19].

The meta-analysis was performed in only 8 studies out of 22 studies found by our search because of our inclusion/exclusion criteria; however, we took into consideration information

of all studies to form a concise review in the current use of iPSC-derived neural cells for SCI treatment (Table 5). Regarding the meta-analysis of the eight studies, six of them (75%) used NSC/progenitor cell (PC) groups obtained after differentiation of human iPSCs derived from either adult male and female dermal or fetal lung fibroblasts; whereas the remaining two studies (25%) used NSC/PCs obtained from mouse iPSCs derived from embryonic fibroblasts. All eight studies assessed motor recovery using the BMS/BBB scale for a thoracic SCI model caused from balloon compression or contusion. In all the studies used for the meta-analysis, cells were transplanted during the subacute stage post-SCI, and either immunosuppression or SCID mice was used. Regarding the animal recipient model, five out of the eight studies used mice, whereas the remaining three studies used rat models. Both rats and mice have been classified under the terrestrial locomotion category [42], and additionally, it has been reported that there are no statistical differences in functional motor recovery between rat and mouse animal models after cellular treatment for SCI [20]. Therefore, our meta-analysis demonstrated that even though there is heterogeneity present, the use of NSC/PCs from iPSCs has a significant benefit in the motor recovery.

From the data that were provided in all 22 studies, we extracted that a total of 29 different iPSC lines and 3 ESC lines were used to derive the transplanted cells for SCI treatment. Thirteen of these cell lines were female, whereas five were of male origin. In terms of recipient animals, these studies were done using both female (∼266) and male (∼120) animals. A previous subgroup meta-analysis indicates no difference in relation to sex of donors and recipients, whether the recipient was a rat or mouse, or whether observers were blinded when rating motor outcomes [20].

Comparative studies between cells originating from iPSC or ESCs concluded that the BMS scores at 42 and 56 days post-SCI were similar [11, 23]. In one study, treatment with NPCs derived from iPSCs was compared with treatments with cells originating from other sources such the bone marrow stromal stem cells and neural progenitors from spinal fetal cells, showing higher locomotor recovery, in addition to more white matter ($p < .05$) and gray matter ($p < .001$) [36]. Additionally, robust effects were found when the transplanted cells were secondary neurospheres derived from iPSCs compared with primary neurospheres ($p < .01$) [11].

The following studies experimented with the same iPSC lines. Nori and collaborators used NSCs derived from the 253G1-human iPSCs [30]. Their results showed deterioration in motor skills after the 47-day mark and tumor formation from undifferentiated NSCs based on detecting an increase in $Nestin^+$ cells [6]. The same line was used by Okubo and collaborators [33], who reported no tumor formation when the cellular treatment was accompanied with gamma secreting inhibitor (GSI) and resulted in significant motor recovery ($p < .01$). Treatment with GSI ensures that NSCs become fully differentiated into appropriate subtypes and prevents tumor formation [33]. This indicates that even though this cell line may be tumorigenic, it can still provide therapeutic benefit if GSI is administered. Three independent studies used NSCs derived from the 201B7 human iPSC line and found significant locomotion recovery in the treated group [25, 30, 33].

The role of exogenous myelination on functional recovery was evaluated by comparing treatments with NSCs derived from wild-type iPSCs and from *shiverer* mutants (*Shi)*-iPSCs. In this mutation, oligodendrocytes lack capacity to produce myelin protein. This defect in myelination reflected negatively in the functional recovery for the *shiverer* group, and results were significantly lower compared with the wild-type iPSCs group ($p = .0008$). Meanwhile, the wild-iPSC-derived neural cell group achieved significant motor recovery compared with the nontreated control group ($p = .0001$) [37]. Similarly, it has been reported that transplantation of oligodendrocytes differentiated from iPSCs during the subacute stage promoted functional motor recovery in SCI thoracic animal model [40]. The effect of astrocytes transplantation has been done to determine whether the acute (3 days) or subacute stage (7 days) is an optimal timeframe for implantation and recovery in the treatment of a thoracic SCI. Results indicate no significant improvement in BBB scores [24]; however, it should not be interpreted as astrocytes are not useful in treating SCIs, because astrocytes have been found to preserve respiratory function [45] and to promote functional recovery in cervical SCI models [46].

The location for implantation of transplanted cells has also been investigated to determine its effect on BBB scores and recovery. Analysis 2 months post-transplantation shows that iPSC-NPCs injected intraspinal increased gray and white matter and axonal sprouting and reduced astrogliosis, promoting enhanced long-term spinal cord regeneration. Transplantation intrathecal also showed improvement in white matter and axonal sprouting compared with control groups, indicating that transplantation in both locations has a therapeutic benefit [22]. Our meta-analysis did not include studies treating SCIs at the cervical level; however, future studies should consider them, as injuries at this site account for more than 60% of SCI cases in humans [47]. Few reports describe the use of implanted iPSC-NSCs at cervical level at the chronic stage (7 weeks post-SCI) and show motor improvement on the CatWalk scale [38]. This cellular treatment was done in combination with chondroitinase ABC (C-ABC), as cell treatment alone was not able to restore motor capacity. Degradation of chondroitin sulfate proteoglycans by C-ABC treatment resulted in improved axonal regeneration [38]. This study also indicates that although grafted cells can survive at the chronic stage, they do so at a lower rate. Furthermore, it shows that neurons derived from the implanted NSCs have a hindered capacity to integrate a synapse. Other scales to assess behavioral recovery for cervical models are the limb-use asymmetry test, forelimb-reaching task, scales vertical exploration, and grid walking [29, 31, 38]. The BMS is not as sensitive to detect changes in the cervical region as it was designed to assess motor deficits at the thoracic/lumbar level. When used in a cervical model, before sustaining a SCI, mice had a score of 9 and then dropped to 3 to 4 [38]. In comparison, injuries at the thoracic region caused the BMS score to drop lower than 0 to 1 immediately postinjury.

The implantation time after an SCI is initiated is an important determinant in functional outcome. The subacute period seems to be the optimal phase for implantation, because the microenvironment is most conducive for grafted cell survival and for re-establishment of neural connections since inflammation has decreased and glial scars have not been formed [3]. Motor recovery in BMS scores after transplantation of neural sphere-PCs during the subacute stage reached a score of 4.8 in 7 weeks, which was significantly different ($p < .01$) to

Table 5. Outcomes for studies using iPSCs in rodent and monkey populations with SCIs

Study	Length of evaluation	Tumor formed in iPSCs-treated group(s)	Motor recovery, scale(s) used
All (2015) [21]	8 weeks	Not detected	Yes, BBB
Amemori et al. (2015) [22]	9 weeks	Not detected	Yes BBB, plantar test, beam walking
Fujimoto et al. (2012) [23]	10 weeks	Not detected	Yes BMS, MEP
Hayashi et al. (2011) [24]	8 weeks	Not detected	No BBB, inclined plane
Kawabata et al. (2016) [25]	12 weeks	Not detected 22.6% $\pm$ 2.5% were Nestin$^+$/HNA$^+$ cells	Yes BMS, rotarod test
Kobayashi et al. (2012) [26]	12 weeks	Not detected 23.9% $\pm$ 2.8% Nestin$^+$	Yes Open field, bar grip, cage climbing test
Liu et al. (2017) [27]	8 weeks	Not assessed	Not assessed
Lopez-Serrano et al. (2016) [28]	60 days	Yes	No BBB, treadmill speed
Lu et al. (2014) [29]	12 weeks	Not assessed	No Vertical exploration, grid walking
Nori et al. (2011) [30]	112 days	Not detected, Nestin$^+$ decreased from 10.7% $\pm$ 2.2% at 47 days to 7.5 $\pm$ 1.0 at 103 post-transplant	Yes BMS, rotarod test, DigiGait system (treadmill gait)
Nori et al. (2015) [6]	103 days	Yes (253G1) cell line Nestin$^+$ increased from 19.6% $\pm$ 0.5% at 47 days to 33.1% $\pm$ 7.4% at 103 days	No, deterioration BMS, rotarod test, stride length
Nutt et al. (2013) [31]	8 weeks	Not detected	Limited LUAT
Oh et al. (2015) [32]	6 weeks	Not detected	Yes BMS, stride length, stance length, sway length
Okubo et al. (2016) [33]	89 days	Not detected for 201B7 cell line Yes for 253G1 cell line control (iPSCs only) Nestin$^+$ cells increased to 30.3% $\pm$ 1.6% at 89 days, not detected for (iPSCs + GSI)	Yes BMS, rotarod test, treadmill gait
Pomeshchik et al. (2015) [34]	42 days	Not detected	No BMS, CatWalk
Romanyuk et al. (2015) [35]	9, 17 weeks (only iPSC group)	Not detected	Yes BBB, flat beam walking test, rotarod test
Ruzicka et al. (2017) [36]	9 weeks	Not detected	Yes BBB, flat beam, rotarod, plantar test
Salewski et al. (2015) [37]	8 weeks	Not detected	Yes BMS, CatWalk, hind limb intensity, stride length
Suzuki et al. (2017) [38]	16 weeks	Not detected	Limited, (for iPSC-NSC + C-ABC) BMS, CatWalk, forelimb grip strength, inclined plane test
Tang et al. (2013) [39]	30 days	Not detected	Yes, Tarlov criteria
Tsuji et al. (2010) [11]	42 days	Not detected in 38C2 iPSC line or 335DI iPSC line Not detected in 256H18 cell line	Yes in 38C2 iPSC-SNS Yes in 335D1 iPSC-SNS Not in other cell lines or in 38C2 iPSC-PNS, BMS
Yang et al. (2018) [40]	28 days	Not detected	Yes BBB, paw withdrawal threshold

Note: Studies in bold were included in the meta-analysis.
Abbreviations: BBB, Basso, Beattie, and Bresnahan locomotor scale; BMS, Basso mouse scale; C-ABC, chondroitinase ABC; iPSC, induced pluripotent stem cell; iPSC-PNS, induced pluripotent stem cell primary neurosphere; iPSC-SNS, induced pluripotent stem cell secondary neurosphere; LUAT, limb use asymmetry task; MEP, motor evoke potential.

the control and to the group with cells transplanted at the chronic stage, which reached a score of 3 [48]. Like the chronic stage, the acute stage is not suitable for implantation because of upregulation of inflammatory cytokines and free radicals. However, even though the subacute stage is the best option to implant iPSC-neurosphere (NS), this might not be feasible in autologous treatments. The time frame for the subacute phase in humans is 2–4 months postinjury [49], whereas the time frame for an autologous derivation of iPSCs and differentiation into NSCs/PCs might take approximately 6 months. By this time, the patient would have advanced to the chronic stage post-SCI.

Most research supports that motor improvement is limited or not evident when iPSCs-derived neural cells are implanted during the chronic stage. However, it has been reported that the use of the C-ABC enzyme with exercise and cell transplantation treatment of iPSC-NSCs induces motor recovery and extension of serotonergic neuronal fibers [50]. This suggests that the injured spinal cord even at the chronic stage retains the capacity to regenerate if axonal growth inhibitors are suppressed [50]. Similarly, it has been reported that mice under physical therapy, such as treadmill training after transplantation experienced greater locomotor recovery compared to control groups [3]. Although combined therapy of treadmill training and NSC/PC transplanted during this stage indicates improvement in BMS scores ($p = .035$), the recovery is significantly lower than when cells are implanted during the subacute stage [51]. This indicates that physical therapy is still not as optimal as transplantation during the subacute stage.

It has been shown that immunization with neural-derived peptides (INDP) and glial scar excision can be beneficial to overcome the negative effects of cell transplantation at the chronic stage [52]. Experimental groups treated with INDP and scar removal resulted in the greatest increase ($p < .05$) in motor recovery, and 55.5% of the animals achieved a BBB score of 9 or higher [52]. In contrast, INDP or scar removal alone was not as effective in achieving the therapeutic benefit of restoring locomotion. By modifying the immune system with INDP and scar removal, a better microenvironment is created for the spinal cord. INDP activates T lymphocytes and induces an anti-inflammatory response that reduces the number of free radicals. An alternative to this will be to prolong the subacute stage by using a glial scar inhibitor such as olomucine or rolipram [5], with the aim to promote remyelination by oligodendrocytes, axon elongation, and repair of neuron circuits [8]. During remyelination, key neurotrophins and angiogenesis are upregulated, and during adaptive immune responses, T cells use neurotrophins to reduce further degeneration of the spinal cord [37].

The studies presented here use xenograft or allograft transplantation in combination with immunosuppressive regimens to prevent immune rejection of iPSC-derivative cells. Most studies used the immunosuppressant cyclosporine A, whereas others used tacrolimus, which has been shown to be less nephrotoxic [28, 34]. Three studies [22, 35, 36] administered a combination of immunosuppressive drugs, for which findings indicate better outcomes compared with monotherapy [53]. All immunosuppressive regimens started at least 1 day before transplantation and administered daily for the duration of experiment, showing the importance of these agents for graft survival. The long-term effect of the immune suppressive regimens in these studies was not reported; however, follow-up studies would be required, as an increased risk of cancer and infection are known as side effects of persistent systemic immune suppression. Similarly, autologous transplantation studies will be required to determine the need of immune suppressive treatment, as one of the objectives of using autologous iPSC-derivative cells for SCI treatment is to reduce immune rejection of transplanted cells. However, it is known that iPSCs are rejected by allogeneic and autologous natural killer cells [54]. Conversely, it is known that cell derivatives from iPSCs elicit different degrees of autoimmunogenicity. For example, smooth muscle cells derived from iPSCs are highly immunogenic to autologous immune system, whereas retinal pigmented epithelial (RPE) cells derived from iPSCs are immune tolerated [55], as observed in a patient treated with autologous iPSC-derived RPE cells during a clinical trial to treat macular degeneration [56]. The immunogenicity of NSCs, neuronal progenitors, oligodendrocytes, and astrocytes derived from iPSCs remain to be determined. Diverse strategies have been developed to improve the feasibility of allograft or autologous iPSC-derivative cell transplantation, and readers are referred to existing literature reviews concerning them [57].

Conclusion

This meta-analysis and review allowed us to identify commonalities and variations within the approaches to use iPSC-derived neural cells and reinforces the value of these cells for SCI treatment. Based on the meta-analysis of these independent studies conducted in animal models with thoracic SCI, there is a significant benefit to their use, as evident of the BMS scores in the iPSC-NS/neural precursor group compared with control groups. The transplanted cells play an important role in restoring vital neurological structures at the spinal cord. However, a caveat is that to achieve the most therapeutic benefit using these cells, cell transplantation should be intraspinally during the subacute phase. If transplantation will be done during the chronic stage, the microenvironment needs to be altered to optimize the survival rate of the transplanted cells. Additionally, physical therapy should be encouraged as data suggest that it can stimulate neuronal regeneration. For future analysis, it will be ideal to standardize the use of a common scale to measure locomotion in rodents and nonhuman primates in order to compare effect sizes between studies. Similarly, certain parameters and data should be required to be reported to perform quantitative assessments. This is important because knowledge obtained through these studies in experimental animal models will be translated to human studies.

Future Perspectives

Before transplantation of cells generated from iPSCs can reach SCI clinical trials in humans, cell transplantation studies need to be performed in nonhuman primates, who have closer proximity to human anatomy, size, physiology, and finer motor skills, in relation to rodents. The white matter in the spinal cord contains axons, tracts of nerve fibers, and oligodendrocytes, whereas the gray matter contains neuronal cell bodies. Unlike rats, humans possess twice the amount of white matter because of the elevated encephalization quotient of brain size to body weight, as well as greater detail and capacity of sensations and motor skills in limbs [7]. Furthermore, complications

might emerge in studies using rodents as experimental models with implanted human iPSC-derived neural cells, as human NSCs retain the intrinsic human rate of maturation in the rodent's spinal cord. Normal maturation of NSCs in rats occurs faster than in humans, and this will reflect in how fast the recovery will be evident in rodent versus human populations [29]. Whereas in humans, neurological recovery occurs mostly during the first year, with the first 3 months having the steepest curve of locomotion and follow-up between 1 and 5 years post the SCI treatment [2]. Therefore studies done in rats may not reflect the length of time that it will take to detect functional recovery in a human.

At the moment, two studies have reported the SCI treatment with iPSC generated cells in nonhuman primates, indicating promising results. Kobayashi and collaborators observed that the iPSC-NS-treated group significantly outperformed the control group in the open field rating scale, bar grip test, and cage climbing test ($p < .05$) [26]. Similarly, Tang and collaborators observed that treated animals were able to climb and experienced almost full recovery 30 days post cell transplantation [39].

However, these findings alone do not support the conclusion that the use of cells derived from iPSCs can restore full motor functionality to what it was once before sustaining an SCI. There is still a significant difference between a normal noninjured rat or mouse, which has a score of 9 and a rat or mouse with an SCI treated with iPSC-derived neural cells with a weighted mean between 4 and 5. Another aspect to consider is the optimization and use of autologous iPSC-derived neural cells in order to avoid suppressing the immune system, as well as the use of transgene-free iPSCs [58–60], and methods to eliminate undifferentiated cells from the transplanted cells with the goal to minimize the risk of tumor formation [61, 62]. If an immunosuppressant treatment would be required, the most effective treatment is still to be determined. Given that the optimal window for implantation is narrow, the grafted cells will be required to be produced in a faster way and the subacute phase will need to be extended to allow for optimal time for transplantation.

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Author Contributions

C.R.: conception/design, collected and/or assembly of data, data analysis and interpretation, statistical analyses, manuscript writing; X.Q.: data analysis and interpretation; L.G.V.-D.: provision of study material, manuscript writing. C.R., X.Q., L.G.V.-D.: final approval of manuscript.

Disclosure of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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