Neurosci Bull August 1, 2014, 30(4): 671–682. http://www.neurosci.cn
DOI: 10.1007/s12264-013-1438-4

·Review·

Current status of cell-mediated regenerative therapies for human spinal cord injury

Tongming Zhu*, Qisheng Tang*, Huasong Gao, Yiwen Shen, Luping Chen, Jianhong Zhu
Department of Neurosurgery, Fudan University Huashan Hospital, National Key Laboratory of Medical Neurobiology, Institutes of Brain Science, Shanghai Medical College, Fudan University, Shanghai 200040, China
*These authors contributed equally to this review.
Corresponding author: Jianhong Zhu. E-mail: jzhu@fudan.edu.cn

During the past decade, significant advances have been made in refinements for regenerative therapies following human spinal cord injury (SCI). Positive results have been achieved with different types of cells in various clinical studies of SCI. In this review, we summarize recently-completed clinical trials using cell-mediated regenerative therapies for human SCI, together with ongoing trials using neural stem cells. Specifically, clinical studies published in Chinese journals are included. These studies show that current transplantation therapies are relatively safe, and have provided varying degrees of neurological recovery. However, many obstacles exist, hindering the introduction of a specific clinical therapy, including complications and their causes, selection of the target population, and optimization of transplantation material. Despite these and other challenges, with the collaboration of research groups and strong support from various organizations, cell-mediated regenerative therapies will open new perspectives for SCI treatment.

Keywords: cell-mediated regenerative therapy; spinal cord injury; clinical trials; stem cell

Introduction

A recent literature survey on spinal cord injury (SCI) shows an incidence ranging from 10.4 to 83 cases per million per year (average, 29.5) and a prevalence of 223–755 per million (average, 485)[1]. After SCI, the release of inhibitory molecules, insufficient expression of growth factors, and formation of glial scar at the injury site are negative local consequences that lead to the formation of an impermeable barrier that prevents axons from regenerating across the site of injury[2, 3]. Meanwhile, the capacity of endogenous stem-cell regeneration is limited in the adult central nervous system (CNS). Treatment of SCI poses great challenges to any standard regenerative therapy. Over the past 20 years, great emphasis has been placed on cell-mediated regenerative therapies, and exogenous cell transplantation is thought to be an important means of treating SCI (Fig. 1). Neuronal function can be improved by applying different sources of cells to SCI, and these are not merely restricted to exogenous neural stem cells. Advances have been achieved albeit with considerable challenges. The safety of cell transplantation therapies *via* multiple routes has been widely confirmed. However, their therapeutic efficacy remains unsatisfactory, and the design of studies should be further considered (Fig. 2). In this review, we summarize clinical studies with cell-mediated transplantation for SCI and strategies for further clinical applications. Also, we provide a practical overview of independent clinical studies published in Chinese journals.

Clinical Outcomes of Transplantation Therapy for SCI

Mesenchymal Stem Cell Trials

Mesenchymal stem cells (MSCs) can be obtained from bone marrow, fat, umbilical cord, periosteum, and placenta. These tissues contain small numbers of adult stem cells, which can differentiate into various mesenchymal cells[4].

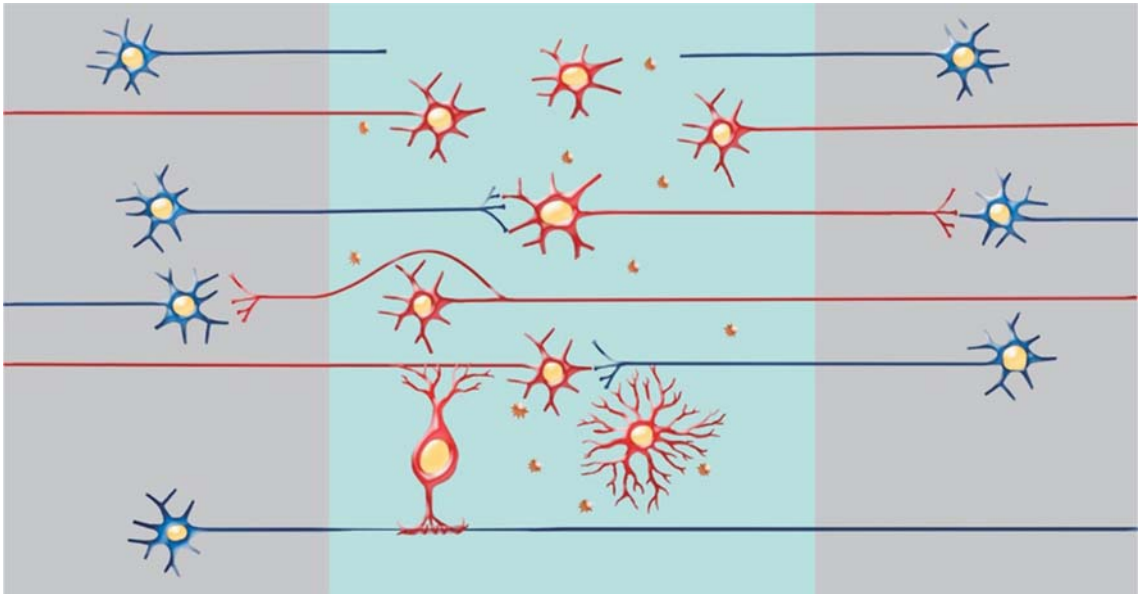

Fig. 1. Schematic diagram of the therapeutic mechanisms, including replacing neurons to reestablish axonal connections, providing a conducive microenviroment for axonal growth (including trophic factors secreted by grafted cells), and remyelinating axons. Red: grafted cells; blue: host cells; brown: trophic factors; aqua blue: supportive matrix.

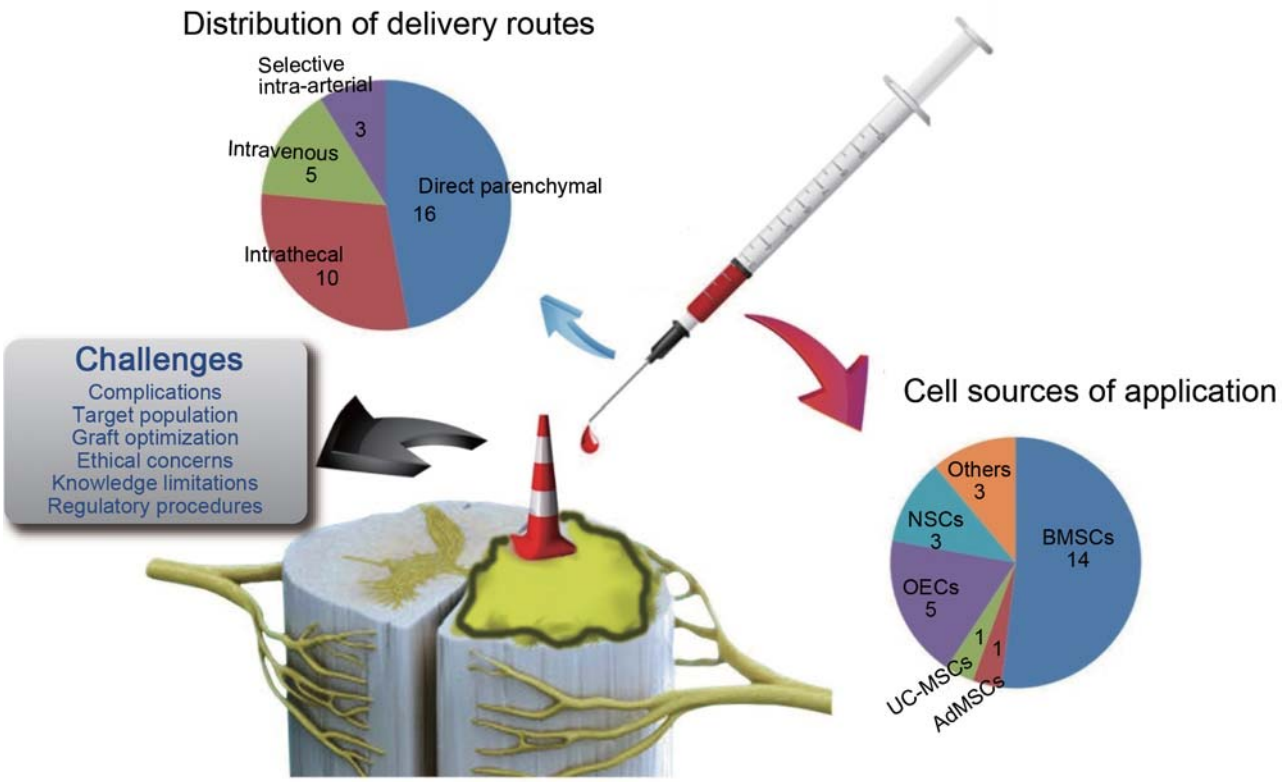

Fig. 2. Cell sources of application and the distribution of delivery routes in reported trials. Challenges of transplantation therapy face scientists and clinicians.

Table 1. Clinical trials for the treatment of SCI using mesenchymal stem cells (MSCs)

Reference	Transplanted cells	Patient group	Evaluation & outcome
Park *et al.*[12]	1.98×10^9 autologous MCPs. DI	6 patients, acute, AIS A, 5 cervical and 1 thoracic	Five patients showed improved neurological function (1 improved from AIS A to B and 4 improved from AIS A to C). No serious complications.
Yoon *et al.*[15]	2×10^8 MCPs. DI	35 patients, 17 acute, 6 subacute, 12 chronic, AIS A	Neurological improvement (29.5% of acute, 33.3% of subacute and 0% of chronic). Fever (62.9%), Neuropathic pain (20%). No tumor formation.
Callera *et al.*[13]	1×10^8 mononuclear cells, 1×10^6 $CD34^+$ cells. IT	10 patients, chronic, 7 paraplegia, 3 tetraplegia	No transplanted cells in CSF after 7 days. No neurological assessments and serious complications were reported.
Moviglia *et al.*[18]	BMMSCs (IA), effector T cells (IV), autologous NSCs (IA), unknown dose	8 patients, chronic, AIS A, 6 cervical and 2 thoracic	Five patients evolved from AIS A to D, 2 remained in the same condition, but exhibited motor and sensitive improvements. No serious complications.
Sykova *et al.*[14]	$104.0\pm55.3\times10^8$ mononuclear cells and $89.7\pm70.7\times10^6$ $CD34^+$ cells. 14 IV, 6 IA	20 patients, 8 subacute, 12 chronic. AIS A-C. 12 cervical and 8 thoracic	Four acute/subacute and 1 chronic patients (IA) showed improved neurological function. 1 acute/subacute patient (IV) showed AIS improvement. No serious complications.
Geffner *et al.*[17]	A total dose of 4×10^8 MCPs. DI, IT and IV	8 patients, 4 acute, 4 chronic. 5 AIS A, 1 AIS B and 2 AIS C. All thoracic	Three acute patients showed improved neurological function (AIS A to C) and 3 chronic patients also improved (1 improved from AIS A to C, 1 from AIS B to C and 1 from AIS C to D). No serious complications.
Mehta *et al.*[63]	AdMSCs, human ESCs-derived hematopoietic SCs, autologous BM–derived hematopoietic SCs, unknown dose. IT	163 patients, chronic, 156 paraplegia, 7 tetraplegia, all were HAI grade 9.	Function improved in 46 patients, 1 grade 4, 3 grade 5, 3 grade 6, and 17 grade 7. 22 patients had improvement in bowel sensations and sweating. 96 patients had postprocedural headache.
Kishk *et al.*[21]	5×10^6 to 10×10^6/kg of MCPs. IT	43 patients, chronic, AIS A	Eighteen patients increased motor scores by 1-2 points and changed from AIS A to B; 24 patients developed neuropathic pain.
Kumar *et al.*[19]	4×10^8 MCPs. IT	297 patients, chronic, AIS A-D	Ninety-seven patients exhibited neurological improvement. 63 patients developed postprocedural headache.
Pal *et al.*[25]	1×10^6 MSC cells/kg. IT	25 patients, 20 AIS A, 5 AIS C, 3 cervical and 22 thoracic	No neurological and electrophysiologic improvements. No serious complications. No tumor formation.
Cristante *et al.*[20]	2.5×10^6 $CD34^+$ MCPs/kg. IA	39 patients, chronic, AIS A	Improvement in SSEP (66.7%). No serious complications.
Chernykh *et al.*[62]	MSCs, Unknown dose. DI and IV	36 patients, chronic, 20 cervical and 16 thoracic	Neurological improvement (66.7%), no serious complications.
Karamouzian *et al.*[22]	7×10^5 to 1.2×10^6 MCPs. IT	11 patients (study group), 20 patients (control group), acute and sub- acute, AIS A thoracic.	Five (study group) and 3 patients (control group) showed marked recovery, but the result was statistically borderline (P=0.095). Eight patients (study group) developed neuropathic pain.
Deda *et al.*[16]	A total of $2.0\text{-}6.7\times10^7$ MCPs. DI, IT and IV	9 patients, AIS A. 6 cervical and 3 thoracic	Neurological and electrophysiological improvements in all patients. One patient improved from AIS A to B and 8 improved from AIS A to C. No serious complications, no tumor formation.
Ra *et al.*[27]	A total of 4×10^8 autologous AdMSCs. IV	8 patients, chronic, male, AIS A-B; 7 quadriplegia and 1 paraplegia	The latency of the left side in leg SSEP increased from 41.93 ± 4.39 to 48.27 ± 3.93 (P <0.05). In one patient, the AIS changed from A to C. No serious complications.
Liu *et al.*[30]	1×10^6 UC-MSCs/kg, once a week, four times as a course. IT	22 patients, subacute to chronic	Treatment was effective in 13 patients. 1 experienced lumbago, and 1 headache. No serious complications.

AdMSCs: adipose tissue-derived mesenchymal stem cells; AIS: American Spinal Injury Association impairment scale; BMMSCs: bone marrow mesenchymal stem cells; CSF: cerebrospinal fluid; DI: direct injection surrounding the lesion; ESCs: embryonic stem cells; HAI: Hauser Ambulation Index; IA: intra-arterial administration; IT: intrathecal administration; IV: intravenous administration; MCPs: mononuclear cells preparations; NSCs: neural stem cells; UC-MSCs: umbilical cord mesenchymal stem cells; SCs: stem cells; SSEP: somatosensory evoked potential.

The leading role of MSCs is believed to be neuroprotective by secreting neurotrophic factors, rather than inducing neural regeneration by transdifferentiation into neurons or glia[5, 6], while the exact mechanisms remain unknown[7]. The immunosuppressive effects of MSCs are considered to be benevolent, particularly as they are thought to ease the characteristic symptoms of SCI by settling the inflammatory response, which in due course reduces cavity formation and demyelination[8]. Under certain conditions, MSCs can be trans-differentiated into neurons and glial cells *in vitro* or *ex vitro*[9, 10], but only an extremely small proportion differentiate and the function of the trans-differentiated cells is not convincing[11] (Table 1).

Bone-marrow Mesenchymal Stem Cells

The majority of stem-cell-based clinical trials for SCI are based on the utilization of bone-marrow mesenchymal stem cells (BMMSCs). MSCs and hematopoietic stem cells (HSCs) are the known types of stem cells in bone marrow, and they are able to differentiate into mesenchymal and hematopoietic cell lineages, respectively. HSCs and MSCs are promising in clinical transplantation as autografts because they are easy to isolate from bone marrow and their effects are reproducible. Whole mononuclear cell preparations (MCPs), including almost all kinds of endothelial and hematopoietic cells, have been used in most clinical studies with bone marrow cells for SCI[12-22]. A comparison between culture-expanded MSCs and human MCPs was made by transplanting them into rodent SCI models, but no differences were found[23]. To date, no clinical study has been reported.

Granulocyte-macrophage colony-stimulating factor (GM-CSF) has the ability to guide MCPs to the injured site and improve functional recovery in rodent SCI[24]. In some studies, MCPs have been transplanted in combination with GM-CSF administration, and GM-CSF was found to guide MCP migration to the lesion site, enhance the survival of transplanted cells, and activate the secretion of neurotrophic factors[12, 15, 20]. Park *et al.* first reported combined therapy in acute patients (American Spinal Injury Association Impairment Scale (AIS) A) with direct injection of MCPs into the site of spinal cord damage within seven days post-injury[12]; this produced significant motor improvements, with no immediate worsening of neurological symptoms. The authors further conducted a phase I/II study applying the combined therapy to 35 patients with SCI (17 with acute treatment, 6 subacute, and 12 chronic)[15], with a control group of 13 participants receiving conventional surgery. They found that 5 acute, 2 subacute, and 1 control showed functional improvement during follow-up, while the chronic treatment group did not show any changes[15]. In other studies, only acute and subacute patients have shown functional improvements after intrathecal delivery[19, 21, 22, 25].

However, Deda *et al.* reported mild functional improvements in 9 chronic patients (AIS A) following direct MCP transplantation into multiple areas of the spinal cord[16]. It is noteworthy that these neurological improvements were reported in chronic patients, but a control group essential for evaluating the effectiveness of scar removal was absent[16, 17]. Importantly, after a freeze-thaw cycle, these cells are still able to promote functional recovery[16].

The reported rates of neurological improvement vary greatly. Furthermore, it is difficult to determine whether the small effect is a direct result of the cell-mediated therapy or the aggressive physical therapy program that was simultaneously performed[21]. Sykova *et al.* transplanted BMMSCs intravenously or intra-arterially into 13 chronic patients with complete SCI[14]. Without an aggressive surgical procedure, the improvements in neurologically stable chronic patients are mainly attributed to the effects of cell implantation.

Adipose Tissue-derived Mesenchymal Stem Cells and Umbilical Cord Mesenchymal Stem Cells

As each gram of adipose tissue contains 100,000 MSCs[26], and donor age has little influence on the differential capacity of adipose tissue-derived mesenchymal stem cells (AdMSCs)[27], adipose tissue is a suitable cell source for tissue engineering and regenerative therapy. The isolation of adult stem cells is accessible and reliable. Ra *et al.* applied AdMSCs intravenously to 8 chronic male patients (AIS A-B) suffering SCI for >12 months[27], and no serious adversity related to the transplantation was reported by any patient.

The human umbilical cord mesenchymal stem cell (UC-MSC) is another promising source of stem cells for its property of uniquely prodigious expansion *in vitro*, rapid proliferation, and low immunogenicity[28, 29]. In a clinical

trial by Liu *et al.*[30], UC-MSCs were injected intrathecally into 22 patients with SCI, for 1–3 courses (1×10^6 cells/kg body weight once a week for four weeks as a course), with an average time from injury to participation of 56 months (range, 2–204 months). The treatment was effective in 81.25% of patients with incomplete SCI, but ineffective in all 6 patients with complete SCI.

It is noteworthy that there is no detailed description of rehabilitation therapy in these reported MSCs trials other than "both the groups were given supervised physiotherapy, and it continued throughout the study period"[22]. Physical rehabilitation programs, which have proved their value in the functional recovery of SCI victims, should be described[31].

Neural Stem Cell Trials

Although embryonic stem cells (ESCs) have wide perspectives for clinical application in various kinds of diseases, only one single clinical trial of ESC-derived oligodendrocyte progenitor cell transplantation has been initiated; and that to determine safety and efficacy[32, 33]. In July 2010, the first trial of transplantation therapy for SCI patients finally received approval from the US Food and Drug Administration (FDA). Geron Inc. initiated a phase I trial for patients suffering from subacute complete thoracic spinal cord trauma (AIS A). In late 2011, the company announced cessation of this trial for lack of funding and discouragingly burdensome regulatory procedures. The company reported no serious adverse events.

The preclinical data of fetal human brain-derived stem cells promoted the phase I/II clinical trial of StemCells Inc. in July 2011[34, 35]. In that study, cell grafts were directly transplanted into the injury sites of 12 chronic thoracic SCI patients, with 12-month follow-up for safety and potential improvement. At the end of the study, an individual 4-year observational trial was initiated, and to date no complications have been reported.

In January 2013, Neuralstem Inc. announced that a phase I safety trial of NSCs (NSI-566RSC) in chronic SCI patients received approval from the FDA. NSI-566RSC, the lead cell therapy material of this company, is cultured human fetal spinal cord NSCs. Well-designed experimental studies have demonstrated the survival, migration, neuronal differentiation, and motor circuit integration of these promising cells in rat SCI models[36-38]. In addition to the preclinical data, the safety of cell administration has been demonstrated in an amyotrophic lateral sclerosis clinical trial[39]. This multicentre study recruited eight chronic thoracic SCI (T2-T12) patients (AIS A). To evaluate the safety of transplantation is the primary objective; while to assess survival of the grafts in the transplant site by magnetic resonance imaging (MRI) scan and the effectiveness of transient immunosuppression are the secondary objectives.

Trials of Other Cells

Schwann cells (SCs) are the main supportive glia in the peripheral nervous system. They were the first cells to be used in SCI animals for the potential of promoting axon regeneration in the CNS[40]. Transplantation of SCs has been extensively investigated as a therapeutic intervention in preclinical SCI studies[41]. In December 2012, the University of Miami announced that a phase I safety trial of autologous human SCs in subacute SCI patients received approval from the FDA. In a completed clinical study of SC transplantation, Saberi *et al.*[42] injected SCs harvested from the sural nerve into multiple locations of the traumatized spinal cord in 33 patients with complete chronic SCI (AIS A-B). During a follow-up of 2 years, considerable improvements were observed in motor function and light touch sensation, especially in the cervical injury group.

Olfactory ensheathing cells (OECs) are specialized glia surrounding olfactory nerve fascicles. OECs can be obtained from either biopsy of the olfactory mucosa or cultured from aborted fetal olfactory bulbs. Mackay-Sim *et al.* treated six chronic SCI patients with cultured autologous OECs obtained by biopsy 4–10 weeks before treatment[43]. Safety was demonstrated, but no significant functional benefit was found after transplantation. Lima *et al.* transplanted small pieces of olfactory mucosa into 20 patients with chronic traumatic SCI (AIS A-B)[44], and found that the lesion site was filled in all patients, with no neoplastic growth or syringomyelia on MRI. Huang *et al.* implanted fetal olfactory bulbs (3–4 months gestation) above and below the injured spinal cord site in 656 patients with chronic SCI[45]. The follow-up MRI did not reveal any new changes in the spinal cord parenchyma.

Macrophages can generate neurotrophic factors and block inhibitors in the peripheral nervous system. Knoller *et al.* initiated a phase I study with eight participants

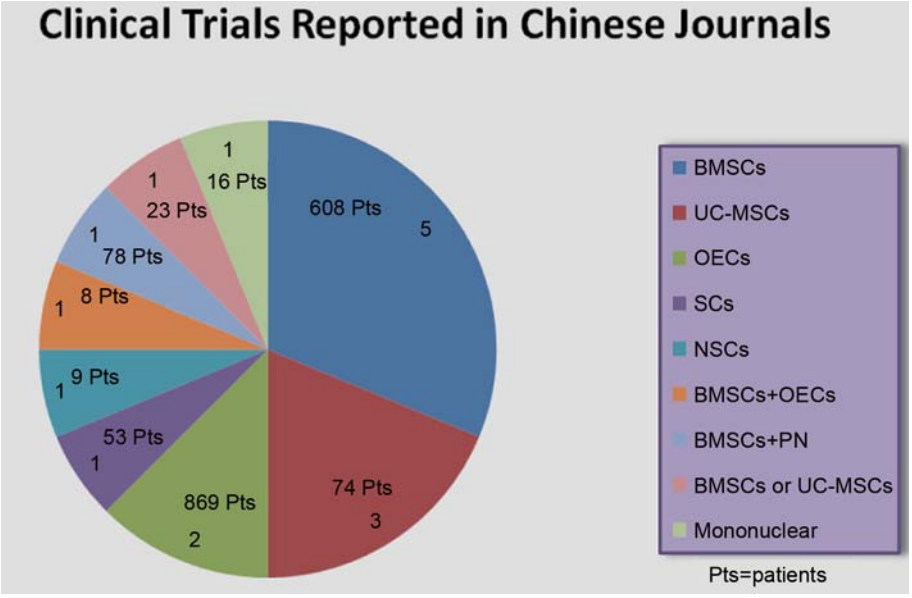

Fig. 3. Clinical trials of various grafts reported in Chinese journals. Number of trials and treated patients.

using direct injection of autologous macrophages into the spinal cord within 14 days after SCI[46]. Mild functional improvements without any critical adverse effects were found in three patients.

Current Status of Clinical Studies in China

Sixteen independent clinical trials have been reported in China, using BMMSCs, UC-MSCs, OECs, bone marrow-derived NSCs, SCs and mononuclear cells[45, 47-61]. More than 1700 SCI patients have received cell-mediated transplantation therapy. However, almost all studies were reported in Chinese journals (Fig. 3).

Wang *et al.* initiated the earliest trial in 420 chronic SCI patients (42 complete and 378 incomplete) in 2003[50], which was also the largest of the eight BMMSC studies[47-54]. The cells were transplanted into all patients through multiple routes including direct parenchymal, intrathecal, and intravenous. The dosage of a single injection was 2×10^2–3×10^2/kg body weight. Incomplete SCI patients exhibited significant functional recovery, but no improvement was observed in the complete group; and no severe adverse effects were reported but several patients developed temporary headache and low fever.

Dai *et al.* treated 23 chronic SCI patients with either 1×10^7 BMMSCs (n = 15) or UC-MSCs (n = 8) *via* lumbar puncture. Incomplete injury patients benefited more from the therapy[49]. The BMMSC group showed more motor function improvement than the UC-MSC group 3 months after transplantation.

Transplantation of autologous BMMSCs combined with peripheral nerve was initiated by Li *et al.* in 2003[52]. Autologous sural nerve was cut into cauda equina-like tissues, which were longitudinally transplanted into the spinal cord or intramedullary cysts. All of the 78 patients were discharged smoothly except for 1 with serious combined injury death with no autopsy. All 77 patients were improved, and no obvious adverse event was found.

Huang *et al.* initiated the first and largest trial of OECs in 2001[45]. They injected 1×10^6 cells directly into the injury site in each of the 656 chronic SCI patients. Two patients died of hypertension and cerebral hemorrhage, and severe pulmonary infection (1.5 and 1 month after operation). There were no postmortem examinations. Cerebrospinal fluid leakage occurred in 38 patients, and 8 suffered varying degrees of functional decline.

Cui *et al.* assessed the short-term curative effect and safety of autologous bone marrow mononuclear cell transplantation in 16 patients with SCI using intravenous or intrathecal delivery[60]. There was no significant functional

improvement. The adverse effects, including headache, abdominal distension, and meningeal irritation, were found in the intrathecal group.

Challenges

Negative Outcomes of Clinical Trials

In the reported completed trials and those in progress, overall complications were rare, with no incidence of death due to transplantation therapy. The reports of specific complication details were variable throughout the published literature, and no adverse events were noted in those studies[14, 25, 62].

The complications seem to be related to the aggressive procedure and application routes of cell-mediated therapy[14, 20]. For example, the most common complications of intrathecal injection, the most frequently used method of transplantation, are headache and neuropathic pain[21, 63].

An additional concern by Kishk *et al.* is the development of neuropathic pain after this therapy, perhaps due to the recovery and formation of neuronal circuitry[21]. Neuroplasticity is the foundation of recovery after SCI, but this contributes to neuropathology at the same time[64]. The negative effects of neuroplasticity vary, depending on conditions. Treatment strategies aiming at increasing neurotrophins in the spinal cord powerfully promote axon growth. However, this effect appears to be most highly related to the negative aspects of neuroplasticity. Treatments or conditions with the objective of mitigating growth inhibition, lead to less incidence of pain due to the moderate side effects. Finally, because of the least pain incidence, neuroprotection focusing on sparing tracts of spinal cord and limiting stimulation by the deafferentation may be the best strategy. Neuroprotection *via* the secretion of various neurotrophic factors is thought to be the major role of MSCs in SCI transplantation therapy. Thus, compared to NSCs and other pluripotent stem cells, MSCs seem to contribute less to complications and adverse effects.

Overall, these cell-mediated therapies are well-tolerated. However, the incidence of adverse events has been shown to correlate with the utilization of independent auditors and predefined definitions of complications[65-68], neither of which was noted in any of the reported series. As a result, it is suspected that the published studies under-report the true incidence of adverse events with these procedures. On the other hand, to mitigate the potential influence of these variables and to understand the incidence with which they occur, further basic investigations and randomized controlled studies are necessary.

Selection of Target Population

To ensure proper conduct of clinical trials in SCI, guidelines from the International Campaign for Cures of SCI Paralysis were published in 2007[69-72]. Nevertheless, the reported clinical trials only partially meet or totally ignore the guidelines even after 2007. The inclusion criteria of staging, severity, and segmenting of SCIs are variable and disputable among the clinical trials reviewed here.

It is unclear whether transplantation should be restricted to a certain stage of injury in future SCI treatment, but there is a suggestion of an optimal temporal window and novel reasonable staging for cell-mediated therapy. Briefly, the acute stage, during which patients are at a high risk of developing complications, would be expected to last until the end-point of spinal shock. The definition of the subacute stage would be the stationary phase of physical status, during which the bodily functions impaired by serious trauma will have been well managed. This period could be prolonged to half a year or even longer. The stability of neurological function should be confirmed by another 6-month observation. It might then be presumed that the SCI patient has entered the chronic stage while there is no confirmed indication of dysfunctional change.

The cell transplantation therapies for SCI patients mainly focus on the cervical, thoracic, and cervicothoracic segments. The neurological recovery potential varies after an acute traumatic SCI; patients with cervical injuries tend to have a greater likelihood of motor improvement than those with thoracic injuries[62, 73]. Currently, there are still no persuasive data to compare the outcomes between cervical and thoracic SCI, but attention should be paid to this lacuna while establishing the inclusion criteria.

Patients from the chronic AIS-A population have limited risk of losing potential neurological function if transplantation therapy has any adverse or unforeseen complications. This is why they are the chief target of reported SCI clinical trials. The stable neurological status allows assessment of the clinical outcomes after transplantation. At the same

time, because of repeated operations and long-term evaluation, the patients without enough will to assume this responsibility should not be recruited into clinical trials[74]. A chronic complete SCI population with stable dysfunction and better tolerance would be suitable for a phase I clinical trial. It is important to ensure that scientific knowledge, not unreasonable expectations of treatment, drives the study[75]. In a phase II clinical trial, the group of subacute incomplete SCI participants is considered to be a better choice. The goal of this phase is to achieve the greatest benefit with the least harm.

Optimization of Transplantation Material

Many sources of cells have already been used in clinical trials of SCI, with an emphasis on stem cells. Selection of the most suitable transplantation material for therapeutic application is a great challenge of clinical design.

Different kinds of cells possess specific properties, so one cell type may be more suitable than others in a specific condition or disease. For example, MSCs seem to be suitable for multiple sclerosis, and large quantities of stem cells are required for the multi-site pathological changes. Application of relatively few cells with restricted differentiation to a specific site is valuable for SCI treatment. The characteristics of NSCs meet the selection criteria. Highly-characterized stem-cell populations, like NSI-566RSC, of which the safety and efficacy have been well defined, would be considered first for SCI therapy.

The majority of transplanted cells differentiate into certain types such as oligodendrocytes or motor neurons, while others have the capacity of unplanned differentiation, even tumorigenesis. Indeed, tumorigenesis is rarely reported in animal studies. However, the length of follow-up in these studies is short, and humans with SCI may survive much longer after cell-mediated transplantation.

The third fundamental issue for the development of cell-mediated therapies is the inherently cumbersome process. These transplantation materials have to be obtained by experienced clinicians, cultured, prepared under Good Manufacturing Practices conditions, and then further prepared immediately before the initiation of therapy. These highly time- and labor-intensive steps lead to risks of failure and expense. This sequence should be modified and the standard of manufacture conditions developed before extensive practice of any transplantation therapy begins.

Practical Issues

Several authors have stated that proof of safety and efficacy through the use of large-animal models is indispensable in the development of stem-cell transplantation therapies, an opinion shared by others engaged in similar research[75-79]. However, the requirement for large-animal models is unsettled, and some authors argue that rodent models provide sufficient preclinical evidence of treatment[80, 81]. The fact that the first pluripotent stem-cell trial approved by the FDA was based on rodent models alone, suggests that this level of preclinical evidence is acceptable. Several articles have highlighted the need for independent replication of promising discoveries before clinical research commences[76, 77]. However, lack of funding is a significant obstacle. It is hoped that collaboration between government and industry will further such projects, in which partners share the risk, burden, and opportunities of transmuting cell-mediated therapy from bench to clinic.

Difficult regulatory procedures are frequently-cited obstacles to the manipulation of stem cells; procedures believed to be needlessly cumbersome inhibit research innovation and product development. In the first pluripotent stem-cell trial for SCI, the company announced its discontinuance partly due to the cumbersome regulatory procedures[82]. Governing bodies should streamline procedures and make necessary adjustments to keep pace with scientific progress.

Perspectives

There are multiple challenges for the efficient and routine practice of transplantation therapy for neurological diseases. Identifying suitable cell populations is the most important step forward; these would be commercially available, well characterized, and ethically free for clinical use. For induced pluripotent stem cells (iPSCs), studies are moving forward very rapidly, and they are thought to be a great optional source for clinical applications in the future. More work should be done to better understand the nature of iPSCs.

In the field of neuroscience, research on cell-mediated regenerative therapy for human diseases is still at the preliminary stage. Although the desire to promote clinical trials with multiple types of stem cells for various

diseases is extremely strong, current knowledge about the mechanisms of cell-mediated regenerative therapy is poor, so the situation once the cells are introduced into the patients remains unclear. Despite all this, it is inspiring that many research groups are pooling their efforts, the consensus of which should open new perspectives for cell-mediated regenerative therapy. Strong support and adequate funding from various organizations worldwide are needed to rapidly develop new clinical trials and make remarkable achievements in the next few years.

ACKNOWLEDGEMENTS

This review was supported by grants from the National Natural Science Foundation of China (81271003), and the National Basic Research Development Program (973 Program) of China (2010CB945500, 2012CB966300, 2009CB941100).

Received date: 2013-08-07; Accepted date: 2013-09-28

REFERENCES

[1] Wyndaele M, Wyndaele JJ. Incidence, prevalence and epidemiology of spinal cord injury: what learns a worldwide literature survey? Spinal Cord 2006, 44: 523–529.

[2] Ronaghi M, Erceg S, Moreno-Manzano V, Stojkovic M. Challenges of stem cell therapy for spinal cord injury: human embryonic stem cells, endogenous neural stem cells, or induced pluripotent stem cells? Stem Cells 2010, 28: 93–99.

[3] Yuan YM, He C. The glial scar in spinal cord injury and repair. Neurosci Bull 2013, 29: 421–435.

[4] Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini F, Krause D*, et al.* Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 2006, 8: 315–317.

[5] Kim HJ, Lee JH, Kim SH. Therapeutic effects of human mesenchymal stem cells on traumatic brain injury in rats: secretion of neurotrophic factors and inhibition of apoptosis. J Neurotrauma 2010, 27: 131–138.

[6] Sasaki M, Radtke C, Tan AM, Zhao P, Hamada H, Houkin K*, et al.* BDNF-hypersecreting human mesenchymal stem cells promote functional recovery, axonal sprouting, and protection of corticospinal neurons after spinal cord injury. J Neurosci 2009, 29: 14932–14941.

[7] Tetzlaff W, Okon EB, Karimi-Abdolrezaee S, Hill CE, Sparling JS, Plemel JR*, et al.* A systematic review of cellular transplantation therapies for spinal cord injury. J Neurotrauma 2011, 28: 1611–1682.

[8] Uccelli A, Benvenuto F, Laroni A, Giunti D. Neuroprotective features of mesenchymal stem cells. Best Pract Res Clin Haematol 2011, 24: 59–64.

[9] Levy YS, Bahat-Stroomza M, Barzilay R, Burshtein A, Bulvik S, Barhum Y*, et al.* Regenerative effect of neural-induced human mesenchymal stromal cells in rat models of Parkinson's disease. Cytotherapy 2008, 10: 340–352.

[10] Jiang J, Lv Z, Gu Y, Li J, Xu L, Xu W*, et al*. Adult rat mesenchymal stem cells differentiate into neuronal-like phenotype and express a variety of neuro-regulatory molecules *in vitro*. Neurosci Res 2010, 66: 46–52.

[11] Barnabe GF, Schwindt TT, Calcagnotto ME, Motta FL, Martinez G, Jr., de Oliveira AC*, et al.* Chemically-induced RAT mesenchymal stem cells adopt molecular properties of neuronal-like cells but do not have basic neuronal functional properties. PLoS One 2009, 4: e5222.

[12] Park HC, Shim YS, Ha Y, Yoon SH, Park SR, Choi BH*, et al.* Treatment of complete spinal cord injury patients by autologous bone marrow cell transplantation and administration of granulocyte-macrophage colony stimulating factor. Tissue Eng 2005, 11: 913–922.

[13] Callera F, do Nascimento RX. Delivery of autologous bone marrow precursor cells into the spinal cord via lumbar puncture technique in patients with spinal cord injury: a preliminary safety study. Exp Hematol 2006, 34: 130–131.

[14] Sykova E, Homola A, Mazanec R, Lachmann H, Konradova SL, Kobylka P*, et al.* Autologous bone marrow transplantation in patients with subacute and chronic spinal cord injury. Cell Transplant 2006, 15: 675–687.

[15] Yoon SH, Shim YS, Park YH, Chung JK, Nam JH, Kim MO*, et al.* Complete spinal cord injury treatment using autologous bone marrow cell transplantation and bone marrow stimulation with granulocyte macrophage-colony stimulating factor: Phase I/II clinical trial. Stem Cells 2007, 25: 2066–2073.

[16] Deda H, Inci MC, Kurekci AE, Kayihan K, Ozgun E, Ustunsoy GE*, et al.* Treatment of chronic spinal cord injured patients with autologous bone marrow-derived hematopoietic stem cell transplantation: 1-year follow-up. Cytotherapy 2008, 10: 565–574.

[17] Geffner LF, Santacruz P, Izurieta M, Flor L, Maldonado B, Auad AH*, et al.* Administration of autologous bone marrow stem cells into spinal cord injury patients via multiple routes is safe and improves their quality of life: comprehensive case studies. Cell Transplant 2008, 17: 1277–1293.

[18] Moviglia GA, Varela G, Brizuela JA, Moviglia Brandolino MT, Farina P, Etchegaray G*, et al.* Case report on the clinical results of a combined cellular therapy for chronic spinal cord injured patients. Spinal Cord 2009, 47: 499–503.

[19] Kumar AA, Kumar SR, Narayanan R, Arul K, Baskaran M.

Autologous bone marrow derived mononuclear cell therapy for spinal cord injury: A phase I/II clinical safety and primary efficacy data. Exp Clin Transplant 2009, 7: 241–248.

[20] Cristante AF, Barros-Filho TE, Tatsui N, Mendrone A, Caldas JG, Camargo A*, et al.* Stem cells in the treatment of chronic spinal cord injury: evaluation of somatosensitive evoked potentials in 39 patients. Spinal Cord 2009, 47: 733–738.

[21] Kishk NA, Gabr H, Hamdy S, Afifi L, Abokresha N, Mahmoud H*, et al.* Case control series of intrathecal autologous bone marrow mesenchymal stem cell therapy for chronic spinal cord injury. Neurorehabil Neural Repair 2010, 24: 702–708.

[22] Karamouzian S, Nematollahi-Mahani SN, Nakhaee N, Eskandary H. Clinical safety and primary efficacy of bone marrow mesenchymal cell transplantation in subacute spinal cord injured patients. Clin Neurol Neurosurg 2012, 114: 935–939.

[23] Samdani AF, Paul C, Betz RR, Fischer I, Neuhuber B. Transplantation of human marrow stromal cells and mononuclear bone marrow cells into the injured spinal cord: a comparative study. Spine (Phila Pa 1976) 2009, 34: 2605–2612.

[24] Koda M, Nishio Y, Kamada T, Someya Y, Okawa A, Mori C*, et al*. Granulocyte colony-stimulating factor (G-CSF) mobilizes bone marrow-derived cells into injured spinal cord and promotes functional recovery after compression-induced spinal cord injury in mice. Brain Res 2007, 1149: 223–231.

[25] Pal R, Venkataramana NK, Bansal A, Balaraju S, Jan M, Chandra R*, et al. Ex vivo*-expanded autologous bone marrow-derived mesenchymal stromal cells in human spinal cord injury/paraplegia: a pilot clinical study. Cytotherapy 2009, 11: 897–911.

[26] Sen A, Lea-Currie YR, Sujkowska D, Franklin DM, Wilkison WO, Halvorsen YD*, et al*. Adipogenic potential of human adipose derived stromal cells from multiple donors is heterogeneous. J Cell Biochem 2001, 81: 312–319.

[27] Ra JC, Shin IS, Kim SH, Kang SK, Kang BC, Lee HY*, et al.* Safety of intravenous infusion of human adipose tissue-derived mesenchymal stem cells in animals and humans. Stem Cells Dev 2011, 20: 1297–1308.

[28] Troyer DL, Weiss ML. Wharton's jelly-derived cells are a primitive stromal cell population. Stem Cells 2008, 26: 591–599.

[29] Weiss ML, Anderson C, Medicetty S, Seshareddy KB, Weiss RJ, VanderWerff I*, et al.* Immune properties of human umbilical cord Wharton's jelly-derived cells. Stem Cells 2008, 26: 2865–2874.

[30] Liu J, Han D, Wang Z, Xue M, Zhu L, Yan H*, et al.* Clinical analysis of the treatment of spinal cord injury with umbilical cord mesenchymal stem cells. Cytotherapy 2013, 15: 185–191.

[31] Mehrholz J, Kugler J, Pohl M. Locomotor training for walking after spinal cord injury. Spine (Phila Pa 1976) 2008, 33: E768–777.

[32] Keirstead HS, Nistor G, Bernal G, Totoiu M, Cloutier F, Sharp K*, et al.* Human embryonic stem cell-derived oligodendrocyte progenitor cell transplants remyelinate and restore locomotion after spinal cord injury. J Neurosci 2005, 25: 4694–4705.

[33] Sharp J, Frame J, Siegenthaler M, Nistor G, Keirstead HS. Human embryonic stem cell-derived oligodendrocyte progenitor cell transplants improve recovery after cervical spinal cord injury. Stem Cells 2010, 28: 152–163.

[34] Hooshmand MJ, Sontag CJ, Uchida N, Tamaki S, Anderson AJ, Cummings BJ. Analysis of host-mediated repair mechanisms after human CNS-stem cell transplantation for spinal cord injury: correlation of engraftment with recovery. PLoS One 2009, 4: e5871.

[35] Salazar DL, Uchida N, Hamers FP, Cummings BJ, Anderson AJ. Human neural stem cells differentiate and promote locomotor recovery in an early chronic spinal cord injury NOD-scid mouse model. PLoS One 2010, 5: e12272.

[36] Xu L, Ryugo DK, Pongstaporn T, Johe K, Koliatsos VE. Human neural stem cell grafts in the spinal cord of SOD1 transgenic rats: differentiation and structural integration into the segmental motor circuitry. J Comp Neurol 2009, 514: 297–309.

[37] Boulis NM, Federici T, Glass JD, Lunn JS, Sakowski SA, Feldman EL. Translational stem cell therapy for amyotrophic lateral sclerosis. Nat Rev Neurol 2011, 8: 172–176.

[38] Lu P, Wang Y, Graham L, McHale K, Gao M, Wu D*, et al.* Long-distance growth and connectivity of neural stem cells after severe spinal cord injury. Cell 2012, 150: 1264–1273.

[39] Riley J, Federici T, Polak M, Kelly C, Glass J, Raore B*, et al*. Intraspinal stem cell transplantation in amyotrophic lateral sclerosis: a phase I safety trial, technical note, and lumbar safety outcomes. Neurosurgery 2012, 71: 405–416; discussion 416.

[40] Duncan ID, Aguayo AJ, Bunge RP, Wood PM. Transplantation of rat Schwann cells grown in tissue culture into the mouse spinal cord. J Neurol Sci 1981, 49: 241–252.

[41] Bunge MB, Wood PM. Realizing the maximum potential of Schwann cells to promote recovery from spinal cord injury. Handb Clin Neurol 2012, 109: 523–540.

[42] Saberi H, Firouzi M, Habibi Z, Moshayedi P, Aghayan HR, Arjmand B*, et al.* Safety of intramedullary Schwann cell transplantation for postrehabilitation spinal cord injuries: 2-year follow-up of 33 cases. J Neurosurg Spine 2011, 15: 515–525.

[43] Mackay-Sim A, Feron F, Cochrane J, Bassingthwaighte L, Bayliss C, Davies W*, et al.* Autologous olfactory ensheathing cell transplantation in human paraplegia: a 3-year clinical

trial. Brain 2008, 131: 2376–2386.

[44] Lima C, Escada P, Pratas-Vital J, Branco C, Arcangeli CA, Lazzeri G*, et al.* Olfactory mucosal autografts and rehabilitation for chronic traumatic spinal cord injury. Neurorehabil Neural Repair 2010, 24: 10–22.

[45] Huang H, Chen L, Xi H, Wang Q, Zhang J, Liu Y*, et al.* Olfactory ensheathing cells transplantation for central nervous system diseases in 1,255 patients. Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi 2009, 23: 14–20.

[46] Knoller N, Auerbach G, Fulga V, Zelig G, Attias J, Bakimer R*, et al.* Clinical experience using incubated autologous macrophages as a treatment for complete spinal cord injury: phase I study results. J Neurosurg Spine 2005, 3: 173–181.

[47] Cheng H, Zhang Z, Li M. CT guided bone marrowl stem cells transplantation therapy for adult spinal cord injury. The 3rd Beijing International Forum on Rehabilitation 2008.

[48] Zhao T, Lu Z, Zhao L. Preliminary observation of stem cells and olfactory ensheathing cells graft for treatment of spinal cord injury. Orthopedic J Chin 2006, 14: 3.

[49] Dai X, Feng M, Lu A. Treatment effect of mesenchymal stem cell transplantation on spinal cord injury:23-case curative effect analysis. Chin J Rehabil Med 2012, 27: 5.

[50] Wang Y, Wang L, Yin Z. Autologous bone marrow stem cell transplantation for spinal cord injury In 420 cases. J Clin Rehabil Tissue Eng Res 2009, 13: 4.

[51] Fang M, Wang M, Wang Y. Clinical study of autologous bonemarrow mesenchymal stem cell transplantation for spinal cord injury. J Qiqihar Univ Med 2011, 32: 3.

[52] Li Z, Bu X, Zhang S, Liang Q, Li T, Chen S, *et al.* Autologous bone marrow mesenchymal stem cells in combination with peripheral nerve transplantation for treating spinal cord injury. J Clin Rehabil Tissue Eng Res 2008, 12: 3041–3044.

[53] Cui G, Li Y, Gao H. Autologous Mesenchymal StemCell Transplantation in Patients with Diseases of Nervous System. Chin J Rehabil Theory Pract 2006, 12: 4.

[54] Bu X, Zhao H, Qian B. Treatment of injured spinal cord by mesenchymal stem cells transplantation in combination with neurotrophic factors and comprehensive rehabilitation. J Chin Pract Diagnosis Therapy 2009, 23: 3.

[55] Yang H, Zhang R, Du L. Clinical Study of Umbilical Cord Mesenchymal Stem Cell Transplantation Therapy for Spinal Cord Injury. Prog Biomed 2012, 12: 5.

[56] Liu J, Han D, Wang Z. Clinical analysis of umbilical cord mesenchymal stem cells in treatment of spinal cord injury. Chin J Inj Repair Wound Heal 2011, 6: 8.

[57] Guo G, Shen L, Li Z. Clinical study of umbilical cord blood mesenchymal stem cell for spinal cord injury. Chinese J Pract Med 2012, 39: 3.

[58] Zhang Z, Dai G, Wang X. Observation on Clinical Effect of Neural Stem Cells Transplantation on Spinal cord Injury. J Med Forum 2010, 31: 4.

[59] Zheng Z, Wei K, Liu F. Clinical verification of olfactory ensheathing cell transplantation in treatment of spinal cord injury. J Clin Rehabil Tissue Eng Res 2010, 14: 4.

[60] Cui G, Song C, Li Y. Clinical study of autologous marrow mononuclear cells transplantation in patients with spinal cord injury. Chin J Rehabil Med 2009, 24.

[61] Zhu H, Feng Y, You S. Schwann cell transplantation for the treatment of chronic spinal cord injury. Chin J Neurosurg Dis Res 2007, 6: 4.

[62] Chernykh ER, Stupak VV, Muradov GM, Sizikov MY, Shevela EY, Leplina OY*, et al.* Application of autologous bone marrow stem cells in the therapy of spinal cord injury patients. Bull Exp Biol Med 2007, 143: 543–547.

[63] Mehta T, Feroz A, Thakkar U, Vanikar A, Shah V, Trivedi H. Subarachnoid placement of stem cells in neurological disorders. Transplant Proc 2008, 40: 1145–1147.

[64] Brown A, Weaver LC. The dark side of neuroplasticity. Exp Neurol 2012, 235: 133–141.

[65] Campbell PG, Yadla S, Malone J, Maltenfort MG, Harrop JS, Sharan AD*, et al.* Complications related to instrumentation in spine surgery: a prospective analysis. Neurosurg Focus 2011, 31: E10.

[66] Lebude B, Yadla S, Albert T, Anderson DG, Harrop JS, Hilibrand A*, et al.* Defining "complications" in spine surgery: neurosurgery and orthopedic spine surgeons' survey. J Spinal Disord Tech 2010, 23: 493–500.

[67] Nasser R, Yadla S, Maltenfort MG, Harrop JS, Anderson DG, Vaccaro AR*, et al.* Complications in spine surgery. J Neurosurg Spine 2010, 13: 144–157.

[68] Yadla S, Malone J, Campbell PG, Maltenfort MG, Sharan AD, Harrop JS*, et al.* Preoperative diagnosis and early complications in thoracolumbar spine surgery: a single center prospective study. J Spinal Disord Tech 2011, 24: E16–20.

[69] Fawcett JW, Curt A, Steeves JD, Coleman WP, Tuszynski MH, Lammertse D*, et al.* Guidelines for the conduct of clinical trials for spinal cord injury as developed by the ICCP panel: spontaneous recovery after spinal cord injury and statistical power needed for therapeutic clinical trials. Spinal Cord 2007, 45: 190–205.

[70] Lammertse D, Tuszynski MH, Steeves JD, Curt A, Fawcett JW, Rask C*, et al.* Guidelines for the conduct of clinical trials for spinal cord injury as developed by the ICCP panel: clinical trial design. Spinal Cord 2007, 45: 232–242.

[71] Steeves JD, Lammertse D, Curt A, Fawcett JW, Tuszynski MH, Ditunno JF*, et al.* Guidelines for the conduct of clinical trials for spinal cord injury (SCI) as developed by the ICCP panel: clinical trial outcome measures. Spinal Cord 2007, 45: 206–221.

[72] Tuszynski MH, Steeves JD, Fawcett JW, Lammertse D,

Kalichman M, Rask C*, et al*. Guidelines for the conduct of clinical trials for spinal cord injury as developed by the ICCP Panel: clinical trial inclusion/exclusion criteria and ethics. Spinal Cord 2007, 45: 222–231.

[73] Steeves JD, Kramer JK, Fawcett JW, Cragg J, Lammertse DP, Blight AR*, et al*. Extent of spontaneous motor recovery after traumatic cervical sensorimotor complete spinal cord injury. Spinal Cord 2011, 49: 257–265.

[74] Emanuel EJ, Wendler D, Grady C. What makes clinical research ethical? JAMA 2000, 283: 2701–2711.

[75] Kimmelman J, London AJ. Predicting harms and benefits in translational trials: ethics, evidence, and uncertainty. PLoS Med 2011, 8: e1001010.

[76] Chien KR. Stem cells: lost in translation. Nature 2004, 428: 607–608.

[77] Hyun I, Lindvall O, Ahrlund-Richter L, Cattaneo E, Cavazzana-Calvo M, Cossu G*, et al*. New ISSCR guidelines underscore major principles for responsible translational stem cell research. Cell Stem Cell 2008, 3: 607–609.

[78] Kwon BK, Hillyer J, Tetzlaff W. Translational research in spinal cord injury: a survey of opinion from the SCI community. J Neurotrauma 2010, 27: 21–33.

[79] Lee DH, Lee JK. Animal models of axon regeneration after spinal cord injury. Neurosci Bull 2013, 29: 436–444.

[80] Fehlings MG, Vawda R. Cellular treatments for spinal cord injury: the time is right for clinical trials. Neurotherapeutics 2011, 8: 704–720.

[81] Mathews DJ, Sugarman J, Bok H, Blass DM, Coyle JT, Duggan P*, et al*. Cell-based interventions for neurologic conditions: ethical challenges for early human trials. Neurology 2008, 71: 288–293.

[82] Sandner B, Prang P, Rivera FJ, Aigner L, Blesch A, Weidner N. Neural stem cells for spinal cord repair. Cell Tissue Res 2012, 349: 349–362.