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Review of operative considerations in spinal cord stem cell therapy

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Abstract

Spinal cord injury (SCI) can permanently impair motor and sensory function and has a devastating cost to patients and the United States healthcare system. Stem cell transplantation for treatment of SCI is a new technique aimed at creating biological functional recovery. Operative techniques in stem cell transplantation for SCI are varied. We review various clinical treatment paradigms, surgical techniques and technical considerations important in SCI treatment. The NCBI PubMed database was queried for "SCI" and "stem cell" with a filter placed for "clinical trials". Thirty-nine articles resulted from the search and 29 were included and evaluated by study authors. A total of 10 articles were excluded (9 not SCI focused or transplantation focused, 1 canine model). Key considerations for stem cell transplantation include method of delivery (intravenous, intrathecal, intramedullary, or excision and engraftment), time course of treatment, number of treatments and time from injury until treatment. There are no phase III clinical trials yet, but decreased time from injury to treatment and a greater number of stem cell injections both seem to increase the chance of functional recovery.

Key Words: Stem cell; Spinal cord injury; Operative techniques; Stem cell transplantation; Intramedullary

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Core Tip: Beyond the biological diversity of stem cell transplantation for spinal cord injury are the technical considerations in designing clinical treatment paradigms. The data suggest that time from injury to treatment, the duration and chronicity of treatment and the actual delivery method of cells are important considerations. This evidence seems to suggest that longer treatment paradigms soon after injury may be most beneficial.

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INTRODUCTION

Spinal cord injury (SCI) is an acute traumatic event that impairs patients' motor and sensory function; SCI is both debilitating for the individual patient as well as for the healthcare system as a whole. Although inconsistently reported, the global prevalence of SCI ranges between 236 to 1009 *per million*^[1]. In the United States this corresponds to an incidence of between 12000 and 20000 cases with an annual total cost of almost 10 billion dollars^[2]. The pathophysiology of SCI involves a primary traumatic insult followed by a secondary cascade characterized by immune activation, pro-inflammatory mediators, edema, ischemia, reactive oxygen species generation and loss of membrane integrity^[3]. The primary and secondary cascade combine to create profound neurological deficits that impair normal function.

Accordingly, SCI treatment is aimed at functional and neurological recovery. Given that the functional deficits stem from neuronal damage, a major focus in this field is the regeneration of nerve tissue. To achieve this end many preclinical and clinical trials using stem cell-based therapies have begun^[4]. All clinical trials to date have delivered stem cells *via* 3 routes: (1) Intrathecal/Intradural; (2) Intramedullary; or (3) Intravenous.

In this review we will highlight the operative considerations associated with SCI stem cell transplantation to identify common elements that may underlie the success of any given intervention. Special attention will be given to the injection site, method of delivery and treatment algorithms.

THE CURRENT LANDSCAPE OF CLINICAL INVESTIGATION INTO STEM CELL THERAPIES FOR SCI

Stem cell-based treatment for SCI is a topic of increasing clinical investigation. Currently, 18 clinical trials are registered as completed on clinicaltrials.gov with as many as 37 others recruiting patients. These span the gamut between Phase I and Phase III clinical trials. To date, most data show that stem cell injection into the spinal cord is safe with minimal side effects. While the first human trial in 2010 used human epithelial serous cystadenocarcinoma oligodendrocyte progenitor cells injected at the lesion site^[5], current clinical investigations use a host of different stem cell types. These types generally fall into three broad groups: Embryonic stem cells, mesenchymal stem cells, or neural-derived stem cells. The most common group, mesenchymal stem cells, can be harvested from many sites including bone marrow-mesenchymal stem cells (BM-MSC), umbilical cord-MSC or adipose tissue-MSC^[6]. The pros and cons of these different stem cells have been greatly debated^[4].

To provide an accurate summary, the database of clinical trials, clinicaltrials.gov was examined for any SCI studies using stem cells. The search criteria: "SCI and stem cell" was used and completed studies were examined. Furthermore, the NCBI PubMed database was searched for "SCI" and "stem cell" with a filter placed for "clinical trials". A total of 39 articles resulted, with 29 articles being relevant to the question at hand (9 articles were not focused on SCI/stem cell transplantation, 1 non-human model). The relevant data are summarized below with the articles summarized in [Table 1](#). Where motor improvement is reported, this references changes in key muscle group function based on a five point grading scale as standard in SCI literature. Follow-up across all clinical trials reporting functional outcomes, *vs* safety profile, was a minimum of 10 mo.

TECHNICAL CONSIDERATIONS IN STEM CELL DELIVERY

Stem cell transplant techniques

Across the many clinical trials examining the use of stem cells (SCs) in the treatment of SCI, there are a few key technical considerations. The first is the method of injection.

Table 1 Summary of clinical trials using stem cell therapy in spinal cord injury

Ref.	Patients	n	Technical description	Outcomes
Levi <i>et al</i> ^[17] , 2019	Chronic C-SCI patients	12	Perilesional intramedullary injection of human CNS-SCs using a two-hand stabilization technique	UEMS showed an increase in treatment group compared to control untreated SCI patients (2.8 points in 9 mo)
Curtis <i>et al</i> ^[11] , 2018	Chronic T-SCI	4	Instrumentation removal, laminectomy, durotomy and stereotactic injection-using a floating cannula of spinal cord-derived neural stem cells	ISNCSCI improvement in 2 subjects with no adverse events
Levi <i>et al</i> ^[18] , 2018	Chronic C/T-SCI	29	Free-hand intramedullary injection of human CNS-SCs	13/29 patients experienced adverse events, all resolved by 3 mo
Xiao <i>et al</i> ^[12] , 2018	Acute C/T-SCI	2	SCI injury site confirmed as complete and excised, collagen scaffold with hUC-MSCs transplanted as a bridge across injury site	Both patients improved from ASIA A → ASIA C
Vaquero <i>et al</i> ^[27] , 2018	Chronic SCI patients	9	Three intrathecal injections of 100×10^6 MSCs	44% patients increased voluntary muscle contraction and 66% improved in bladder compliance with no adverse effects
Anderson <i>et al</i> ^[25] , 2017	Subacute T-SCI	6	U/S + MRI used for navigation. Table mount (Geron Corp) and Hamilton syringe used for intramedullary microinjection of sural nerve-derived SCs	No major adverse events and no consistent improvement in ISNCSCI
Vaquero <i>et al</i> ^[18] , 2017	Chronic incomplete C/T/L SCI	12	Subarachnoid administration <i>via</i> lumbar puncture of autologous MSCs	Sexual function (2/8), spasticity (3/9) and bowel/bladder function improved (8/9) improvements were noted
Vaquero <i>et al</i> ^[28] , 2016	Chronic complete C/T/L SCI	12	Subarachnoid administration <i>via</i> lumbar puncture of autologous MSCs	All patients experienced improvement in sensation and sphincter control. Motor activity below the lesion obtained in 50% of patients
Satti <i>et al</i> ^[29] , 2016	Chronic and subacute T-SCI	6	Intrathecal injection of autologous MSCs	Evaluated safety only-no adverse events
Bansal <i>et al</i> ^[22] , 2016	SCI patients	8	Lumbar puncture at L1/L2 with autologous BMSCs injected 3 times every 4 wk	Patients with injury less than 6 mo improved-ASIA grade improvement in 6/10, walking with support restored in 8/10
Hur <i>et al</i> ^[21] , 2016	Subacute to chronic C/T/L-SCI	14	Intrathecal injection through lumbar tap of 9×10^7 ADMSC	ASIA motor improved in 5% patients. 4 adverse events included headache and UTI
Oh <i>et al</i> ^[10] , 2016	Chronic C-SCI	16	Laminectomy and durotomy with 1.6×10^7 BM-MSCs in 1 mL injected intramedullary with a 27 gauge needle. Fibrin glue used to prevent cell leakage. 3.2×10^7 BM-MSCs injected into the subdural space	12.5% of patients with significant motor improvement
Shin <i>et al</i> ^[20] , 2015	Acute/subacute C-SCI	15	Human fetal tissue-derived neural stem cell progenitor cells free hand injection 5 mm deep into lesion site	5/19 in the treatment group with improved ASIA grade, compared to 1/15 in the control group with ASIA improvement
Mendonça <i>et al</i> ^[19] , 2014	Chronic T/L-SCI	14	BM-MSCs injected based on lesion volume. Direct injection above and below level	8/14 developed lower limb functional gain in hip flexors. 7/14 improved ASIA grades to B/C 9/14 with improved urologic function
Cheng <i>et al</i> ^[30] , 2014	Chronic T/L-SCI	10	CT-guided intramedullary injection at the lesion site using purified UC-MSCs. Two transplantations separated by 10 d, each transplantation with 3 separate injections of 2×10^7 cells	7/10 patients had significant improvement in movement and muscle tension
Al-Zoubi <i>et al</i> ^[31] , 2014	Chronic T-SCI	19	Autologous purified CD34+/CD133+ SCs injected into cyst cavity or subarachnoid space	7/19 patients with segmental sensory improvement, 2/19 with motor improvement (ASIA-A → ASIA-C)
Yoon <i>et al</i> ^[23] , 2014	Acute/subacute/chronic C-SCI	25	Intramedullary perilesional injection of 2×10^8 BMCs in 6 locations + 5 cycles of GM-	ASIA grade increased in 30.4% of acute and subacute treated patients

			CSF Subq	with no improvement in chronic treatment group
Dai <i>et al</i> ^[32] , 2013	Chronic complete C-SCI	20	BMMSC transplantation at site of injury with MIS-laminectomy, dural incision and injection at a depth of 3 mm at central dorsal aspect of the junction between the lesioned and normal spinal cord.	10/20 in BMMSC transplantation group had improvement in motor, light touch and pinprick sensory with 9/20 showing ASIA improvement. No improvement in any control patients (0/20)
Park <i>et al</i> ^[49] , 2012	Traumatic C-SCI	10	Laminectomy and durotomy with 8×10^6 MSCs in 1 mL injected intramedullary over 10 s with a 26.5 gauge needle. Fibrin glue used to prevent cell leakage. At 4-8 wks post-op additional 5×10^7 MSCs injected <i>via</i> lumbar tap	6/10 patients with motor power improvement of UE
Frolov <i>et al</i> ^[33] , 2012	Chronic C-SCI	20	Repeated intrathecal autologous HSCs (from leukapheresis) repeatedly injected over 1 yr	3-4 patients with improved SEP and MEP
Karamouzian <i>et al</i> ^[24] , 2012	Acute/subacute T-SCI	11	Purified BM-MS-C injected <i>via</i> standard lumbar puncture needle	5/11 in BM-MS-C treatment group had two grade improvement in ASIA score (<i>i.e.</i> A \rightarrow C) compared to 3/20 in control group ($P = 0.09$)
Ra <i>et al</i> ^[34] , 2011	Chronic SCI	8	IV administration of human ADMSCs	Safe with no adverse events related to transplantation at 3 mo
Lima <i>et al</i> ^[14] , 2009	Chronic C/T SCI	20	Laminectomy with partial scar excision and olfactory mucosal autograft placement. Rehabilitation focused on lower extremity stepping continued post-operatively	11/20 patients had ASIA improvement (6A \rightarrow C, 3B \rightarrow C, 2A \rightarrow B) with 1/20 having ASIA decline (B \rightarrow A). 15/20 with new voluntary EMG
Cristante <i>et al</i> ^[35] , 2009	Chronic C/T-SCI	39	Apheresis for isolation of CD34+ bone marrow mononuclear stem cells-injected endovascularly <i>via</i> intercostal arteries or vertebral arteries	26/39 patients showed recovery of SSEP to peripheral stimuli
Pal <i>et al</i> ^[36] , 2009	Subacute to Chronic C/T SCI	30	BM-MS-C expanded <i>ex-vivo</i> and injected <i>via</i> LP	Injection safe with no adverse events
Mackay-Sim <i>et al</i> ^[37] , 2008	Chronic complete T-SCI	6	Nasal biopsy for isolation of OESC, cultured for 4-10 wks. Laminectomy, durotomy and injection into damaged spinal cord and proximal/distal ends of lesion with a table mounted stereotactic injection apparatus	No adverse events, 1 of 6 patients with an improvement of 3 segments in LT/PP
Chernykh <i>et al</i> ^[38] , 2007	Chronic C/T/L SCI	18	Purified BM-MS-C injected into the cystic lesion cavity and given intravenously	Motor and sensory improvement was equivocal, spasticity was significantly improved by BM-MS-C injection
Lima <i>et al</i> ^[13] , 2006	Chronic C/T-SCI	7	Laminectomy, with scar excision with suturing graft loaded with olfactory tissue to meninges/superficial tissue layers	2 patients went from ASIA-A to ASIA-C (out of 7 total) with return of bladder sensation/VAC
Callera <i>et al</i> ^[39] , 2006	Chronic SCI	10	BM-MS-C injected <i>via</i> LP	Injection safe with no adverse events

C: Cervical; T: Thoracic; L: Lumbar; ISNCSCI: International Standards for the Neurological Classification of Spinal Cord Injury; ASIA: American Spinal Injury Association Impairment Scale; MSC: Mesenchymal stem cells; ADMSC: Adipose derived mesenchymal stem cells; BMC: Bone marrow cells; BM-MS-C: Bone marrow-derived mesenchymal stem cells; SEP: Somatosensory evoked potentials; MEP: Motor evoked potentials; HSC: Hematopoietic stem cell; GM-CSF: Granulocyte macrophage colony stimulating factor; Subq: Subcutaneous; LT/PP: Light touch/pinprick; VAC: Voluntary anal contraction; SCI: Spinal cord injury; UEMS: The European Union of Medical Specialists; CT: Computed tomography; EMG: Electromyography; OESC: Ovarian epithelial serous cystadenocarcinoma; UIT: U-shaped skin incision technique; CNS: Central nervous system; UC: Ulcerative colitis; UE: Upper-extremity; MRI: Magnetic resonance imaging.

Intravenous and intrathecal injection have relatively few technical considerations since IV injection and lumbar puncture are routine procedures. Intramedullary injection of stem cells, however, poses a greater challenge. The spinal cord generally oscillates with respiration and cardiac pulsation. Studies in animals show that the pulsatile nature of the cord is increased following increases in blood pressure and that it is not due to cerebrospinal fluid based wave transmission. While respiratory oscillation causes greatest spinal cord movement in the thoracic spine, the genuine spinal cord pulsation is thought to be driven by the blood flow through radicular arteries^[7]. This spinal cord pulsation may pose an issue with intramedullary injection techniques. In general there are two canonical ways of achieving intramedullary spinal cord injections: The free hand technique, and a stereotactic technique utilizing a bed

mounted frame. It is important to note that the stereotactic technique, as it is mounted to the bed, does not necessarily improve stability in relation to spinal cord pulsation. A recent study by Levi *et al*^[8] described the use of a free hand technique. Specifically, to achieve an injection depth of 3-5 mm they marked the injection needle using a rongeur or silicone tip and had the surgeon stabilize the needle using two hands anchored at the edge of the surgical field. The injection time, which was a maximum of 3 min and 30 s *per* injection, required the use of two hands for stabilization^[8].

Although no serious complications occurred in this trial or others that used a free-hand technique^[9,10], the damage to the spinal cord may be obfuscated by the presence of preexisting pathology. Moreover, the need for stabilization may limit the maximum injection time and thereby the amount of stem cells able to be transplanted. Curtis *et al*^[11] described a novel technique using a floating cannula that is able to address these theoretical issues. This cannula is attached to an XYZ manipulator mounted directly on a patient's vertebral column. The cannula is able to move with pulsation of the spinal cord making long term injections feasible with minimal damage to the existing spinal cord tissue^[11].

Whether the preservation of existing spinal cord tissue is technically necessary is up for debate. While the previously described studies focused on chronic SCI, a study by Xiao *et al*^[12] in acute SCI patients completely excised the necrotic spinal cord around the SCI lesion. The authors placed a collagen scaffold impregnated with human umbilical cord MSCs in this area and showed nerve conduction across the SCI lesion plus functional improvement from American Spinal Injury Association Impairment Scale (ASIA) A to ASIA C in two patients^[12]. This study was built on previous work by Lima *et al*^[13], where necrotic scar tissue was excised and an olfactory mucosal autograft was placed. Importantly, this radical technique of laminectomy, scar excision and mucosal autograft replacement in chronic cervical and thoracic SCI led to marked functional improvement. In the earlier study by Lima *et al*^[13], 28% of patients had recovery of bladder sensation or voluntary anal contraction^[13], while in the more recent study 55% of patients had ASIA improvement of at least 1 grade^[14]. Importantly, this second study involved intensive rehabilitation following autograft transplantation. It is important to note that olfactory mucosa grafts have been associated with spinal masses pointing to their increased regenerative potential but also to their increased side effect profile^[15,16]. As a technical consideration, excision of scar tissue may allow for increased regeneration of neural white matter tracts and may be an important technical consideration even with other injection techniques that do not use a graft substrate or scaffold.

Number of treatments

Although some studies of SCI show improvement following a single stem cell injection^[11,17], studies with multiple injections spaced out over time seem to have greater improvements in outcomes^[8,18]. A comparison of two trials conducted by the same group, Oh *et al*^[10] and Park *et al*^[9], further illustrated this point. The original study, by Park *et al*^[9], was a Phase I single-arm study and included the injection of BM-MSCs derived from iliac crest grafts into both the intramedullary and subdural space with additional injections into the thecal space using lumbar puncture at 4 wk and 8 wk following the initial operation. Six of 10 patients showed motor improvement and 3 showed gradual improvement in activities of daily living^[9]. The follow-up study, by Oh *et al*^[10], was a Phase III clinical trial and the study authors used the same initial injection treatment paradigm with no follow-up injections. The one time injection yielded poor functional improvement (12.5% with improved motor outcomes) compared to the multiple injection protocol (60% with motor improvement or improvement on Activities of Daily Living). Although limited, these data point to the importance of optimizing chronicity of SC injection in SCI patients. Importantly, while 5/16 patients had diffusion tensor imaging magnetic resonance imaging changes showing tracts spanning the SCI level in the single injection Phase III clinical trial compared to 7/10 showing such changes in the pilot study^[10]. It is difficult to associate the differences solely due to the presence of multiple treatments. Multiple other studies have shown improvements in ASIA scores, motor function and urodynamics with only a single stem-cell treatment^[19,20]. Furthermore, understanding the number of cells transplanted and its effect on outcomes is more difficult. Various trials have transplanted cell numbers ranging from 1×10^5 to 40×10^7 . There was no consistent relationship between cell number and outcome over the trials reported. It remains to be seen whether a multiple treatment *vs* single treatment protocol could improve functional recovery in a large-scale clinical trial.

Time from injury to treatment

Generally, SCI is characterized based on chronicity into acute, subacute and chronic phases. While the time course for each period is highly variable, the acute phase generally spans days post-injury, the subacute phase weeks post-injury and the chronic phase months post-injury. Although some studies on chronic SCI show improvement following stem cell injection^[8,11], studies in the acute or subacute SCI population seem to show a more dramatic return of function^[10,21,22]. A study by Yoon *et al*^[23] stratified patients into acute (< 13 d), subacute (14 d to 8 wk) and chronic (> 8 wk) groups with all patients receiving intramedullary injection of BM-MSCs with systemic granulocyte macrophage colony stimulating factor treatment. Notably, 30.4% of the acute and subacute treated patients had improved ASIA grade (ASIA A to Asia B/C), while none of the chronic patients showed any improvement^[23]. This was not limited to intramedullary cell transplantation. Bansal *et al*^[22] demonstrated that lumbar puncture for delivery of BM-MSCs led to ASIA grade improvement in 6/10 patients who were less than 6 mo from injury with no patients over 6 mo from injury achieving functional improvement^[22].

A study by Shin *et al*^[20], used human fetal tissue-derived neural stem cell progenitor cells and included both a control group and an intramedullary cell transplantation group. Notably, while 26% of patients in the transplantation group had ASIA grade improvement, only 6.6% had improvement in the control group^[20]. This disparity between transplantation and control group patients was also seen by Karamouzian *et al*^[24]. These authors injected purified BM-MSCs *via* lumbar puncture and noted that 45 patients in the cell transplantation group had ASIA improvement compared to 15% in the control group ($P = 0.09$)^[24]. This helps to answer the major critique that some degree of functional improvement occurs in the acute/subacute period naturally and may explain the functional improvements seen in the acute to subacute cell transplantation studies. Also, the fact that both studies with control groups used different stem cell types and different methods of injection (lumbar puncture *vs* intramedullary injection) also highlights the importance of the treatment window *vs* mechanism of treatment. This is further supported by the studies carried out by Bansal *et al*^[22] and Yoon *et al*^[23]. These studies had patients in multiple treatment windows, with intramedullary or lumbar puncture delivered BM-MSCs and noted that patients in the acute to subacute period from injury achieved greater functional improvement^[22,23]. In fact, of the studies that focused on the acute/subacute SCI population, only one out of seven studies showed no motor or functional improvement with the rest having a subset of patients that had ASIA grade improvement. The single study with no functional improvement notably used a novel form of stem cells derived from peripheral Schwann cells and included subjects from 4-7 wk following injury-generally on the upper limit as compared to other acute/subacute studies^[25].

FUTURE DIRECTIONS

In summary, clinical studies to date have highlighted a few key findings. First, that stem cell injection is generally well tolerated with a minimal side effect profile when appropriately dosed. The use of stem cell treatment in SCI may lead to functional improvement when intervention is performed in the acute to subacute treatment window and when multiple treatment injections are utilized. A few pre-clinical studies are examining devices that could facilitate intramedullary stem cell injection. One such device tested in rodents and pigs by Kutikov *et al*^[26] injects cells in a trail creating longitudinal tracts of neural stem cells *vs* isolated injection sites. In doing so they demonstrated a novel technique for stem cell injections able to create new tracts that span multiple spinal cord levels^[26]. Continued technological development could help facilitate intramedullary stem cell injection over longer periods of time, thereby obviating the greatest risk to this treatment, the need for surgical delivery. The aim of this manuscript is to help optimize clinical trial parameters, patient selection and cell transplantation techniques. Comparative clinical trials using different types of stem cells are necessary to determine what type of cells are most efficacious in improving functional outcomes in patients.

CONCLUSION

Stem cell treatment for SCI is a burgeoning field. While numerous studies have focused on the biological aspect of this treatment, technical challenges remain. Time from injury to treatment, the duration and chronicity of treatment and the actual delivery of cells are important considerations. Currently, the lack of phase III clinical trials directly studying these factors makes it difficult to draw conclusions. Early evidence seems to suggest that longer treatment paradigms soon after injury may be most beneficial. Finally, operative techniques and devices that can effectively target the intramedullary space could help with stem cell delivery and functional recovery following treatment.

REFERENCES

- 1 **Singh A**, Tetreault L, Kalsi-Ryan S, Nouri A, Fehlings MG. Global prevalence and incidence of traumatic spinal cord injury. *Clin Epidemiol* 2014; **6**: 309-331 [PMID: [25278785](#) DOI: [10.2147/CLEP.S68889](#)]
- 2 **Ma VY**, Chan L, Carruthers KJ. Incidence, prevalence, costs, and impact on disability of common conditions requiring rehabilitation in the United States: stroke, spinal cord injury, traumatic brain injury, multiple sclerosis, osteoarthritis, rheumatoid arthritis, limb loss, and back pain. *Arch Phys Med Rehabil* 2014; **95**: 986-995. e1 [PMID: [24462839](#) DOI: [10.1016/j.apmr.2013.10.032](#)]
- 3 **Alizadeh A**, Dyck SM, Karimi-Abdolrezaee S. Traumatic Spinal Cord Injury: An Overview of Pathophysiology, Models and Acute Injury Mechanisms. *Front Neurol* 2019; **10**: 282 [PMID: [30967837](#) DOI: [10.3389/fneur.2019.00282](#)]
- 4 **Jin MC**, Medress ZA, Azad TD, Doulames VM, Veeravagu A. Stem cell therapies for acute spinal cord injury in humans: a review. *Neurosurg Focus* 2019; **46**: E10 [PMID: [30835679](#) DOI: [10.3171/2018.12.FOCUS18602](#)]
- 5 **Frantz S**. Embryonic stem cell pioneer Geron exits field, cuts losses. *Nat Biotechnol* 2012; **30**: 12-13 [PMID: [22231081](#) DOI: [10.1038/nbt0112-12](#)]
- 6 **Kibat P**, Stricker H. [Factors of elimination of liposome-encapsulated drug model substances after intravenous administration]. *Arzneimittelforschung* 1988; **38**: 1472-1478 [PMID: [3196388](#) DOI: [10.3390/ijms21020659](#)]
- 7 **Matsuzaki H**, Wakabayashi K, Ishihara K, Ishikawa H, Kawabata H, Onomura T. The origin and significance of spinal cord pulsation. *Spinal Cord* 1996; **34**: 422-426 [PMID: [8963998](#) DOI: [10.1038/sc.1996.75](#)]
- 8 **Levi AD**, Okonkwo DO, Park P, Jenkins AL 3rd, Kurpad SN, Parr AM, Ganju A, Aarabi B, Kim D, Casha S, Fehlings MG, Harrop JS, Anderson KD, Gage A, Hsieh J, Huhn S, Curt A, Guzman R. Emerging Safety of Intramedullary Transplantation of Human Neural Stem Cells in Chronic Cervical and Thoracic Spinal Cord Injury. *Neurosurgery* 2018; **82**: 562-575 [PMID: [28541431](#) DOI: [10.1093/neuros/nyx250](#)]
- 9 **Park JH**, Kim DY, Sung IY, Choi GH, Jeon MH, Kim KK, Jeon SR. Long-term results of spinal cord injury therapy using mesenchymal stem cells derived from bone marrow in humans. *Neurosurgery* 2012; **70**: 1238-47; discussion 1247 [PMID: [22127044](#) DOI: [10.1227/NEU.0b013e31824387f9](#)]
- 10 **Oh SK**, Choi KH, Yoo JY, Kim DY, Kim SJ, Jeon SR. A Phase III Clinical Trial Showing Limited Efficacy of Autologous Mesenchymal Stem Cell Therapy for Spinal Cord Injury. *Neurosurgery* 2016; **78**: 436-47; discussion 447 [PMID: [26891377](#) DOI: [10.1227/NEU.0000000000001056](#)]
- 11 **Curtis E**, Martin JR, Gabel B, Sidhu N, Rzesiewicz TK, Mandeville R, Van Gorp S, Leerink M, Tadokoro T, Marsala S, Jamieson C, Marsala M, Ciacci JD. A First-in-Human, Phase I Study of Neural Stem Cell Transplantation for Chronic Spinal Cord Injury. *Cell Stem Cell* 2018; **22**: 941-950. e6 [PMID: [29859175](#) DOI: [10.1016/j.stem.2018.05.014](#)]
- 12 **Xiao Z**, Tang F, Zhao Y, Han G, Yin N, Li X, Chen B, Han S, Jiang X, Yun C, Zhao C, Cheng S, Zhang S, Dai J. Significant Improvement of Acute Complete Spinal Cord Injury Patients Diagnosed by a Combined Criteria Implanted with NeuroRegen Scaffolds and Mesenchymal Stem Cells. *Cell Transplant* 2018; **27**: 907-915 [PMID: [29871514](#) DOI: [10.1177/0963689718766279](#)]
- 13 **Lima C**, Pratas-Vital J, Escada P, Hasse-Ferreira A, Capucho C, Peduzzi JD. Olfactory mucosa autografts in human spinal cord injury: a pilot clinical study. *J Spinal Cord Med* 2006; **29**: 191-203; discussion 204 [PMID: [16859223](#) DOI: [10.1080/10790268.2006.11753874](#)]
- 14 **Lima C**, Escada P, Pratas-Vital J, Branco C, Arcangeli CA, Lazzeri G, Maia CA, Capucho C, Hasse-Ferreira A, Peduzzi JD. Olfactory mucosal autografts and rehabilitation for chronic traumatic spinal cord injury. *Neurorehabil Neural Repair* 2010; **24**: 10-22 [PMID: [19794133](#) DOI: [10.1177/1545968309347685](#)]
- 15 **Woodworth CF**, Jenkins G, Barron J, Hache N. Intramedullary cervical spinal mass after stem cell transplantation using an olfactory mucosal cell autograft. *CMAJ* 2019; **191**: E761-E764 [PMID: [31285379](#) DOI: [10.1503/cmaj.181696](#)]
- 16 **Dlouhy BJ**, Awe O, Rao RC, Kirby PA, Hitchon PW. Autograft-derived spinal cord mass following olfactory mucosal cell transplantation in a spinal cord injury patient: Case report. *J Neurosurg Spine* 2014; **21**: 618-622 [PMID: [25002238](#) DOI: [10.3171/2014.5.SPINE13992](#)]

- 17 **Levi AD**, Anderson KD, Okonkwo DO, Park P, Bryce TN, Kurpad SN, Aarabi B, Hsieh J, Gant K. Clinical Outcomes from a Multi-Center Study of Human Neural Stem Cell Transplantation in Chronic Cervical Spinal Cord Injury. *J Neurotrauma* 2019; **36**: 891-902 [PMID: 30180779 DOI: 10.1089/neu.2018.5843]
- 18 **Vaquero J**, Zurita M, Rico MA, Bonilla C, Aguayo C, Fernández C, Tapiador N, Sevilla M, Morejón C, Montilla J, Martínez F, Marín E, Bustamante S, Vázquez D, Carballido J, Rodríguez A, Martínez P, García C, Ovejero M, Fernández MV; Neurological Cell Therapy Group. Repeated subarachnoid administrations of autologous mesenchymal stromal cells supported in autologous plasma improve quality of life in patients suffering incomplete spinal cord injury. *Cytotherapy* 2017; **19**: 349-359 [PMID: 28089079 DOI: 10.1016/j.jcyt.2016.12.002]
- 19 **Mendonça MV**, Larocca TF, de Freitas Souza BS, Villarreal CF, Silva LF, Matos AC, Novaes MA, Bahia CM, de Oliveira Melo Martinez AC, Kaneto CM, Furtado SB, Sampaio GP, Soares MB, dos Santos RR. Safety and neurological assessments after autologous transplantation of bone marrow mesenchymal stem cells in subjects with chronic spinal cord injury. *Stem Cell Res Ther* 2014; **5**: 126 [PMID: 25406723 DOI: 10.1186/scrt516]
- 20 **Shin JC**, Kim KN, Yoo J, Kim IS, Yun S, Lee H, Jung K, Hwang K, Kim M, Lee IS, Shin JE, Park KI. Clinical Trial of Human Fetal Brain-Derived Neural Stem/Progenitor Cell Transplantation in Patients with Traumatic Cervical Spinal Cord Injury. *Neural Plast* 2015; **2015**: 630932 [PMID: 26568892 DOI: 10.1155/2015/630932]
- 21 **Hur JW**, Cho TH, Park DH, Lee JB, Park JY, Chung YG. Intrathecal transplantation of autologous adipose-derived mesenchymal stem cells for treating spinal cord injury: A human trial. *J Spinal Cord Med* 2016; **39**: 655-664 [PMID: 26208177 DOI: 10.1179/2045772315Y.0000000048]
- 22 **Bansal H**, Verma P, Agrawal A, Leon J, Sundell IB, Koka PS. Autologous Bone Marrow-Derived Stem Cells in Spinal Cord Injury. *J Stem Cells* 2016; **11**: 51-61 [PMID: 28296864]
- 23 **Yoon SH**, Shim YS, Park YH, Chung JK, Nam JH, Kim MO, Park HC, Park SR, Min BH, Kim EY, Choi BH, Park H, Ha Y. 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 [PMID: 17464087 DOI: 10.1634/stemcells.2006-0807]
- 24 **Karamouzian S**, Nematollahi-Mahani SN, Nakhiae 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 [PMID: 22464434 DOI: 10.1016/j.clineuro.2012.02.003]
- 25 **Anderson KD**, Guest JD, Dietrich WD, Bartlett Bunge M, Curiel R, Dididze M, Green BA, Khan A, Pearse DD, Saraf-Lavi E, Widerström-Noga E, Wood P, Levi AD. Safety of Autologous Human Schwann Cell Transplantation in Subacute Thoracic Spinal Cord Injury. *J Neurotrauma* 2017; **34**: 2950-2963 [PMID: 28225648 DOI: 10.1089/neu.2016.4895]
- 26 **Kutikov AB**, Moore SW, Layer RT, Podell PE, Sridhar N, Santamaria AJ, Aimetti AA, Hofstetter CP, Ulich TR, Guest JD. Method and Apparatus for the Automated Delivery of Continuous Neural Stem Cell Trails Into the Spinal Cord of Small and Large Animals. *Neurosurgery* 2019; **85**: 560-573 [PMID: 30169668 DOI: 10.1093/neuros/nyy379]
- 27 **Vaquero J**, Zurita M, Rico MA, Aguayo C, Bonilla C, Marín E, Tapiador N, Sevilla M, Vazquez D, Carballido J, Fernandez C, Rodriguez-Boto G, Ovejero M; Neurological Cell Therapy Group from Puerta de Hierro-Majadahonda Hospital. Intrathecal administration of autologous mesenchymal stromal cells for spinal cord injury: Safety and efficacy of the 100/3 guideline. *Cytotherapy* 2018; **20**: 806-819 [PMID: 29853256 DOI: 10.1016/j.jcyt.2018.03.032]
- 28 **Vaquero J**, Zurita M, Rico MA, Bonilla C, Aguayo C, Montilla J, Bustamante S, Carballido J, Marín E, Martínez F, Parajon A, Fernandez C, Reina L; Neurological Cell Therapy Group. An approach to personalized cell therapy in chronic complete paraplegia: The Puerta de Hierro phase I/II clinical trial. *Cytotherapy* 2016; **18**: 1025-1036 [PMID: 27311799 DOI: 10.1016/j.jcyt.2016.05.003]
- 29 **Satti HS**, Waheed A, Ahmed P, Ahmed K, Akram Z, Aziz T, Satti TM, Shahbaz N, Khan MA, Malik SA. Autologous mesenchymal stromal cell transplantation for spinal cord injury: A Phase I pilot study. *Cytotherapy* 2016; **18**: 518-522 [PMID: 26971680 DOI: 10.1016/j.jcyt.2016.01.004]
- 30 **Cheng H**, Liu X, Hua R, Dai G, Wang X, Gao J, An Y. Clinical observation of umbilical cord mesenchymal stem cell transplantation in treatment for sequelae of thoracolumbar spinal cord injury. *J Transl Med* 2014; **12**: 253 [PMID: 25209445 DOI: 10.1186/s12967-014-0253-7]
- 31 **Al-Zoubi A**, Jafar E, Jamous M, Al-Twal F, Al-Bakheet S, Zalloum M, Khalifeh F, Radi SA, El-Khateeb M, Al-Zoubi Z. Transplantation of purified autologous leukapheresis-derived CD34+ and CD133+ stem cells for patients with chronic spinal cord injuries: long-term evaluation of safety and efficacy. *Cell Transplant* 2014; **23** Suppl 1: S25-S34 [PMID: 25372344 DOI: 10.3727/096368914X684899]
- 32 **Dai G**, Liu X, Zhang Z, Yang Z, Dai Y, Xu R. Transplantation of autologous bone marrow mesenchymal stem cells in the treatment of complete and chronic cervical spinal cord injury. *Brain Res* 2013; **1533**: 73-79 [PMID: 23948102 DOI: 10.1016/j.brainres.2013.08.016]
- 33 **Frolov AA**, Bryukhovetskiy AS. Effects of hematopoietic autologous stem cell transplantation to the chronically injured human spinal cord evaluated by motor and somatosensory evoked potentials methods. *Cell Transplant* 2012; **21** Suppl 1: S49-S55 [PMID: 22507680 DOI: 10.3727/096368912x633761]
- 34 **Ra JC**, Shin IS, Kim SH, Kang SK, Kang BC, Lee HY, Kim YJ, Jo JY, Yoon EJ, Choi HJ, Kwon E. Safety of intravenous infusion of human adipose tissue-derived mesenchymal stem cells in animals

- and humans. *Stem Cells Dev* 2011; **20**: 1297-1308 [PMID: 21303266 DOI: 10.1089/scd.2010.0466]
- 35 **Cristante AF**, Barros-Filho TE, Tatsui N, Mendrone A, Caldas JG, Camargo A, Alexandre A, Teixeira WG, Oliveira RP, Marcon RM. Stem cells in the treatment of chronic spinal cord injury: evaluation of somatosensitive evoked potentials in 39 patients. *Spinal Cord* 2009; **47**: 733-738 [PMID: 19333245 DOI: 10.1038/sc.2009.24]
- 36 **Pal R**, Venkataramana NK, Bansal A, Balaraju S, Jan M, Chandra R, Dixit A, Rauthan A, Murgod U, Totey S. 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 [PMID: 19903102 DOI: 10.3109/14653240903253857]
- 37 **Mackay-Sim A**, Féron F, Cochrane J, Bassingthwaite L, Bayliss C, Davies W, Fronck P, Gray C, Kerr G, Licina P, Nowitzke A, Perry C, Silburn PA, Urquhart S, Geraghty T. Autologous olfactory ensheathing cell transplantation in human paraplegia: a 3-year clinical trial. *Brain* 2008; **131**: 2376-2386 [PMID: 18689435 DOI: 10.1093/brain/awn173]
- 38 **Chernykh ER**, Stupak VV, Muradov GM, Sizikov MY, Shevela EY, Leplina OY, Tikhonova MA, Kulagin AD, Lisukov IA, Ostanin AA, Kozlov VA. Application of autologous bone marrow stem cells in the therapy of spinal cord injury patients. *Bull Exp Biol Med* 2007; **143**: 543-547 [PMID: 18214319 DOI: 10.1007/s10517-007-0175-y]
- 39 **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 [PMID: 16459180 DOI: 10.1016/j.exphem.2005.11.006]



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