REVIEW

Cellular Treatments for Spinal Cord Injury: The Time is Right for Clinical Trials

Michael G. Fehlings · Reaz Vawda

Abstract More than 1 million people in the United States live with a spinal cord injury (SCI). Despite medical advances, many patients with SCIs still experience substantial neurological disability, with loss of motor, sensory, and autonomic function. Cell therapy is ideally suited to address the multifactorial nature of the secondary events following SCI. Remarkable advances in our understanding of the pathophysiology of SCI, structural and functional magnetic resonance imaging, image-guided micro-neurosurgical techniques, and transplantable cell biology have enabled the use of cell-based regenerative techniques in the clinic. It is important to note that there are more than a dozen recently completed, ongoing, or recruiting cell therapy clinical trials for SCI that reflect the views of many key stakeholders. The field of regenerative neuroscience has reached a stage in which the clinical trials are scientifically and ethically justified. Although experimental models and analysis methods and techniques continue to evolve, no model will completely replicate the human condition. It is recognized that more work with cervical models of contusive/compressive SCI are required in parallel with clinical trials. It is also important that the clinical translation of advances made through well-established and validated experimental approaches in animal models move forward to meet the compelling needs of individuals with SCI and to advance the field of regenerative neuroscience. However, it is imperative that such efforts at translation be done in the most rigorous and informed fashion to determine safety and possible efficacy, and to provide key information to clinicians and basic scientists, which will allow improvements in regenerative techniques and the validation and refinement of existing preclinical animal models and research approaches. The field of regenerative neuroscience should not be stalled at the animal model stage, but instead the clinical trials need to be focused, safe, and ethical, backed up by a robust, translationally relevant preclinical research strategy.

Keywords Spinal cord injury · Neural progenitor · Mesenchymal stem cell · Bone marrow · Clinical trial · Cell transplantation

Spinal Cord Injury

Introduction

Spinal cord injury (SCI), which results from sudden or sustained trauma or progressive neurodegeneration, is a devastating condition whereby sufferers experience significant functional and sensory deficits, as well as emotional, social, and financial burdens. They also have an increased risk of cardiovascular complications, deep vein thrombosis, osteoporosis, pressure ulcers, autonomic dysreflexia, and neuropathic pain. The estimated annual global incidence of SCI is 15 to 40 cases per million. In the United States (U.S.), approximately 1.275 million individuals are affected, with more than 12,000 new cases each year [1–3].

M. G. Fehlings (✉) · R. Vawda
University Health Network, Toronto Western Hospital,
Toronto, ON M5T 2S8, Canada
e-mail: Michael.Fehlings@uhn.on.ca

R. Vawda
e-mail: reaz.vawda@uhnresearch.ca

Despite advances in pre-hospital care, medical and surgical management, and rehabilitation approaches, many SCI sufferers still experience substantial neurological disability. Intensive efforts are underway to develop effective neuroprotective and neuroregenerative strategies. Given the debilitating consequences of SCI, and the significant advances in micro-neurosurgical techniques, image-guided surgery coupled with major progress in our understanding of the biology of cell-based therapeutic strategies for central nervous system (CNS) repair and regeneration through the large number of pre-clinical studies, as well as past clinical trials, we argue that the timing is optimal to commence and extend clinical trial investigations of cellular transplants for SCI. While it is acknowledged that much remains to be learned regarding the biology of transplantable cells, it is only with a balanced investigative approach, combining preclinical research with careful controlled clinical trials, that significant advances can be made in the field [4].

The significant advances that have been made on the basis of pre-clinical studies carried out in rodent models of SCI have enabled clinical trials demonstrating the safety of cell therapy for SCI to proceed, and have informed researchers of the knowledge gaps that remain to be addressed. Given that no experimental model, rodent or otherwise, will completely mimic the human condition, it is an unrealistic hurdle or criterion for all pre-clinical findings to be validated in larger animal models, such as pigs and primates. This would be financially and practically unfeasible for most laboratories and would place such a lengthy and unnecessary delay on the clinical translation of important developments already made, as to be detrimental to the field. Indeed, this has been recognized by the U.S. Food and Drug Administration (FDA) in allowing a clinical trial of embryonic stem cell-derived cells in SCI to proceed on the basis of pre-clinical safety demonstrated exclusively in rodent models (www.clinicaltrial.gov; clinical trial identifier: NCT01217008).

Moreover, it is important to note that rodent contusion/ compression models of SCI are generally "incomplete" with partial sparing of motor and sensory functions. These models closely mimic most patients with severe, partial lesions with an American Spinal Injury Association (ASIA) impairment (ASI) scale rating of AIS B or C. Given that most trials of cell therapy have been carried out in AIS A patients (the safest to inject, but also the least likely to show cell therapy-induced benefit), there is a need for future clinical trials to include patients actually modeled in the laboratory. In addition, there is a compelling need for preclinical researchers to develop valid models of compressive/contusive cervical SCI, given that approximately 50 to 60% of human SCIs involve the cervical region [2, 3, 5].

Epidemiology: Incidence and Impact on Sufferers and Healthcare

The estimated annual global incidence of SCI is 15 to 40 cases per million. In the U.S., approximately 1.275 million individuals are affected, with more than 12,000 new cases each year [1, 6, 7]. The most common causes of traumatic SCI are road traffic accidents, falls, occupational mishaps, and sports-related injuries, resulting in contusion and compression of the spinal cord [1]. Approximately 55% of SCIs occur at the cervical level (C1 to C7-T1) with a mortality of 10% in the first year following injury and an expected lifespan of only 10 to 15 years postinjury, and thoracic (T1 to T11), thoracolumbar (T11-T12 to L1-L2), and lumbosacral (L2 to S5) injuries each account for approximately 15% of SCI [1]. Depending on the age of the patient, the severity, and the level of SCI, the lifetime cost of healthcare and other injury-related expenses can reach $25 million [8]. Cell therapy can potentially enhance the quality of life of those affected by SCI, while reducing their financial and practical dependence on other individuals and ultimately on the society as a whole.

Pathophysiology

SCIs involve a primary (the physical injury) and a secondary injury (the subsequent cascade of molecular and cellular events that amplify the original injury) [9]. The primary injury damages both upper and lower motor neurons and disrupts motor, sensory, and autonomic (including cardiac output, vascular tone, and respiration) functions. Pathophysiological processes occurring in the secondary injury phase, which are rapidly instigated in response to the primary injury in an attempt to homeostatically control and minimize the damage, are paradoxically, largely responsible for exacerbating the initial damage and creating an inhibitory milieu, which prevents endogenous efforts of repair, regeneration, and remyelination. These secondary processes include inflammation, ischemia, lipid peroxidation, production of free radicals, disruption of ion channels, axonal demyelination, glial scar formation (astrogliosis), necrosis, and programmed cell death. Nevertheless, endogenous repair and regenerative mechanisms are used during the secondary phase of injury to minimize the extent of the lesion (through astrogliosis), reorganize blood supply through angiogenesis, clear cellular debris, and reunite and remodel damaged neural circuits, and as such offer exploitable targets for therapeutic intervention. The spatial and temporal dynamics of these secondary mediators (for more detail see Figley et al. [10]) are fundamental to SCI pathophysiology.

A multitude of characteristics of cells tested both preclinically and clinically make them ideal for SCI repair and

form the basis of the possible mechanisms by which they can promote functional recovery, which are anti-inflammatory, immunomodulatory [11–13], anti-gliotic [14], pro-oligodendrogliogenic [15, 16], pro-neuronogenic [17], pro-axonogenic, and secrete various anti-apoptotic and pro-angiogenic neurotrophic factors. Given the pathophysiological targets of SCI [10], transplanted cells should: 1) enable regenerating axons to cross any cysts or cavities, 2) functionally replace dead or damaged cells, and/or 3) create an environment supportive of axonal regeneration and/or myelination. However, given the multifactorial nature of SCI and its dynamic pathophysiological consequences, the success of future clinical trials of cell therapy will likely depend on the informed co-administration of multiple strategies, including pharmacological and rehabilitation therapies [10].

Cell Therapy

Cell therapy is particularly well suited to addressing the multifactorial nature of the pathophysiology of secondary SCI, and as such its potential has been a focus in regenerative medicine for many years. Different sources and types of cells, including stem/progenitor cells (embryonic stem cells, neural progenitor cells, bone marrow mesenchymal cells) and non-stem cells (olfactory ensheathing cells [OECs] and Schwann cells) have been, and/or are being tested in clinical trials for SCI. Others are still in the pre-clinical stages of testing [18] (Table 1).

The advantages and disadvantages of each cell source and type being considered or already in clinical trials for SCI have been extensively described and compared elsewhere [10, 19–22], and reflect their potential in the clinic (Table 1). A recent systematic review [23] has highlighted the paucity of data on the transplantation of human cells into the most clinically relevant models of SCI (chronic, cervical) especially in larger-than-rodent animals and nonhuman primates. However, while the latter would be useful for optimizing surgical and cell delivery parameters and strategies, we argue that is it unreasonable to require that all pre-clinical data be verified in these models. The reason for this is that they do not replicate all the details of the human condition, in addition to being prohibitively costly and unfeasible for many, if not most, laboratories that would contribute substantially to the field through the study of well-established and better characterized rodent models.

A systematic review of pre-clinical investigations of cell therapies for SCI has recently been conducted by a consensus panel convened by the senior author (MGF) [23–25]. Although we acknowledge the urgent need for further investigations using cervical models to reflect the

Table 1 A Comparison of the Different Cell Types and Sources Currently In* Consideration or being Considered for Clinical Trials for SCI

	WJ/UCM	fMPC	BMSC*	ES*	iPS	fNPC	aNPC*	OEC*	SC*	SKP	AdipMPC
Isolation	Easy	Challenging	Challenging	Challenging	Challenging	Challenging	Challenging	Challenging	Challenging	Less challenging	Easy
Ethical considerations	None	Significant	Considerable	Significant	None	Significant	Considerable	Few	Considerable	Few	Few
Differentiation potential — Bone	✓	✓	✓	Pluripotent	Pluripotent	Neural	Neural	-	-	Peripheral myelin	✓
Fat	✓	✓	✓								✓
Cartilage	✓	✓	✓								✓
Storage — Pre-isolation	✓	✗	✗	✗	✗	✗	✗	✗	✗	✗	?
Post-isolation	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Immunogenicity	Low	Low	Low	Low	Low	Low	?	Low	Low	Low	
Immunosuppressant/ Anti-inflammatory	✓	✓	✓	?	?	✓	?	?	?	?	
Tumourigenicity	✗	✗	✗	✓	✓	✗	✗	✗	✗	?	✗
Transfection	✓	✓	✓	✓	✓	✓	✓	✓	✓	?	
Safety/Risk	✓	✓	✓	✓	?	✓	✓	✓	✓	?	✓
Pathotropism	✓	✓	✓	?	?	✓	?	?	?	?	
Autologous	Potential	No	✓	✗	Potential	✗	✗	✓	✓	✓	✓

adipMPC = Adipose tissue-derived mesenchymal cells; aNPC = Adult neural progenitor cells; BMSC = bone marrow mesenchymal/stromal cell; ES = embryonic stem cells; fMPC = Foetal mesenchymal progenitor cell; fNPC = fetal neural progenitor cells; iPS = induced pluripotent stem cells; OEC = olfactory ensheathing cells; SC = Schwann cells; SCI=spinal cord injury; SKP = skin-derived precursors; WJ/UCM = Wharton's Jelly cells/umbilical cord matrix cells

predominance of SCIs at this level, a considerable amount of information can be obtained from an examination of the published literature, especially in thoracic SCI models, particularly with regard to safety, even though the variation in the sources of each cell type, culture conditions, age of donor, and recipient can make comparisons between studies difficult. Indeed, so far, most patients being enrolled in the phase I safety trials (including the Geron trial on GRNOP1 cells [see below]), have been those who suffered thoracic SCIs with an AIS A rating for locomotor function.

Current Clinical Trials of Cell Therapy for SCI

There are currently more than a dozen recently completed, ongoing, or recruiting cell therapy clinical trials for SCI listed on clinicaltrials.gov [26], of which 2 do not involve stem cells per se (a phase I trial of OECs in thoracic AIS A subjects [clinical trial identifier: NCT01231893], and a phase II trial of activated macrophages in cervical and thoracic SCI [clinical trial identifier: NCT00073853], subsequently suspended due to financial constraints experienced by the sponsor). Most trials are phase I or I/II clinical safety and feasibility studies, indicating that cellular treatments for SCI developed in the laboratory are still in the very early stages of clinical translation.

These trials build on the development of micro-neurosurgical techniques and image-guided approaches that facilitate the safe targeting of the injured CNS with cell-based approaches. The safety of such techniques is borne out by the experience of the Florida group with fetal cell-based transplantation for SCI associated with syringomyelia in the 1990s [27], and the more recent efforts of MacKay-Sim and St. John [22] with OECs in patients with thoracic SCIs.

The following section discusses the different cell types and sources used in past and current clinical trials, and critically evaluates the scientific evidence justifying their use in the clinic.

Bone Marrow Mesenchymal/Stromal Cells

Bone marrow mesenchymal/stromal cells (BMSCs) are isolated from the stromal compartment of bone marrow, which also comprises hematopoietic stem cells by virtue of their adherence to tissue culture plastic and/or their expression of distinct cell surface antigenic markers and nonexpression of hematopoietic stem cells markers. They have anti-inflammatory and immunomodulatory effects, mesodermal differentiation potential, and secrete several neurotrophic factors, making them attractive candidates in CNS cell rescue and as autologous transplanted cellular sources of trophic support for endogenous and co-implanted cells. Contrary to recurring claims of their

neurogenic differentiation potential *in vitro* or *in vivo*, there is no conclusive evidence to support these claims [28].

Most current and recently completed trials involve autologous BMSC transplantation, given the well-established safety record of using these cells as part of bone marrow transplants for leukemia, amyotrophic lateral sclerosis, and multiple sclerosis (MS) sufferers (see as follows). Furthermore, most studies of BMSCs have been carried out in rats (likely due to practical considerations due to size differences from mice and also due to closer modeling of the clinical situation), which were found to have a beneficial effect of BMSC administration after thoracic SCI, largely as a result of neurotrophic factor secretion [29, 30] and possibly also anti-inflammatory cytokine secretion. Intra-spinal (within or adjacent to the injury site), as well and intrathecal and systemic (intravenous) delivery have been successful in most studies [31–33]. Porcine and nonhuman primate studies have been carried out to further support their clinical use [34, 35], and as in rodent studies, found that BMSCs promote a certain degree of axonal regrowth and sprouting, at least in transection models [36]. This strongly supports their use in trials of thoracic SCI.

However, the use of BMSCs in SCI does present certain issues, their migration beyond the injection site (for intra-spinally delivered cells) is limited, and inter-donor variability in efficacy and immunomodulatory potency might be reflected in variable clinical outcome [37], making their evaluation as therapy for SCI difficult. Studies of BMSCs in cervical contusion-compression models have yet to be carried out. BMSCs have, in all but 2 studies by the same group, been used in subacute and acute models [38–41]. Based on the limited number of pre-clinical studies in chronic models, it is not yet possible to evaluate their efficacy in the latter. It is also not known whether BMSCs provide functional preservation of axons or *de novo* axonal regrowth across the lesion site in contusion-compression models, as these are more difficult to distinguish in these models, unlike in transection models [42–46].

Ongoing clinical studies and those carried out to date have enrolled small patient numbers and have used autologous BM-derived cells rather than purified stromal cells (understandable from a practical perspective) [47–50]. A recently published dose escalation trial examined autologous BMSCs in patients with chronic SCI [51]. Although BMSCs were safe, they were not found to be beneficial in this cohort of patients.

Having clearly established the safety and feasibility of the clinical use of BM-derived cells specifically for SCI in these trials, and as suggested by Tetzlaff et al. [23] in 2011 (coauthored by MGF) based on the pre-clinical literature, we now urge the immediate testing of BMSCs in properly designed and executed clinical trials using International
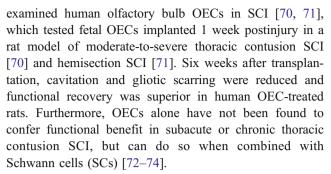
Campaign for Cures of Spinal Cord Injury Paralysis (ICCP) guidelines in SCI patients carefully selected as the most likely to benefit (acute and subacute thoracic SCI with a rating of AISA A or B on the impairment scale).

It is worth mentioning the Canadian Bone Marrow Transplant (BMT) clinical trials for MS led by Freedman et al. [51]. These have shown that immunomodulation through an intravenous infusion of autologous bone marrow-derived cells can not only reduce, but also halt relapses and halt the formation of new MS lesions, reduce brain atrophy, and overall reduce disease burden [52]. The inflammatory component of SCI and subsequent demyelination of surviving axons are serious limiting factors in the efficacy of early cell therapy for SCI, as implanted cells are more likely to be eliminated by the host. It is therefore worth considering interventions to modify the inflammatory milieu to enhance donor cell survival and efficacy, as in the newly initiated clinical trial of autologous BMSCs in children suffering from SCI (Table 2) (clinical trial identifier: NCT01328860). On the other hand, and considering the 1 case of neoplasms in a recipient of fetal neural progenitor cells (NPCs) reported by Amariglio et al. [53], the question of whether this strategy may increase the oncogenic risk of cell therapy must be considered. It is noteworthy that no BMT-related tumors have been reported by the Canadian MS BMT study.

OECs

OECs can be isolated autologously from the nerve fiber layer of the olfactory bulb, as well as the lamina propria of the olfactory epithelium. They support the constant regeneration of olfactory axons from the PNS (the olfactory mucosa) into the CNS (the olfactory bulb) [54], and, as such, represent cells potentially capable of creating a microenvironment permissive for axonogenesis across the inhibitory lesion site into levels of the spinal cord caudal to the injury site.

OECs have also been used in a few clinical trials [22, 55, 56] and smaller studies that do not necessarily conform to the strict criteria and protocols of formal clinical trials [ICCP guidelines]), with some measure of success, at least from the safety and feasibility perspective [22]. These trials were initiated on the basis of promising results reported in transection SCI models [57–61], which have been variably replicated [62–67]. There are several possible reasons for this, including known differences between sources of OECs [68], differences in culture conditions, and their changing phenotype in prolonged culture [69]. Studies are ongoing to address these differences. Nonetheless, beneficial effects have been reported in 2 separate clinical trials for chronic SCI, admittedly undertaken by the same group [60, 61]. Of note, these trials were carried out despite the fact that only 2 studies (by the same group) have

examined human olfactory bulb OECs in SCI [70, 71], which tested fetal OECs implanted 1 week postinjury in a rat model of moderate-to-severe thoracic contusion SCI [70] and hemisection SCI [71]. Six weeks after transplantation, cavitation and gliotic scarring were reduced and functional recovery was superior in human OEC-treated rats. Furthermore, OECs alone have not been found to confer functional benefit in subacute or chronic thoracic contusion SCI, but can do so when combined with Schwann cells (SCs) [72–74].

For balance, clinical trials have been carried out on the basis of a small, but compelling, body of scientific evidence [75, 76] for functional benefit in SCI, even though the patients selected for these trials have not always matched the pre-clinical models used. At least 2 of these trials [60, 61] have reported functional improvement in small patient cohorts, which may be confirmed by future pre-clinical studies on human OECs of various anatomical derivations to support future clinical trials. The safe use of OECs in clinical trials for other conditions, such as ischemic stroke, further strengthens the case for their use in SCI (clinical trial identifier: NCT01327768).

NPCs

NPCs can be generated from embryonic stem (ES) cells (ESCs), which are derived from the inner cell mass of the embryo. The latter have indefinite self-renewal capacity and are pluripotent, with the potential to generate all cell types of the body, making them a potentially limitless source of cells for therapy. However, they are not without their problems (Table 1), including and especially the moral dilemmas and the practical constraints of their embryonic derivation, their karyotypic instability with repeated freeze-thaw cycles [77, 78] and their teratogenicity in the host.

Pre-clinical studies have shown that animals transplanted with human ESC-derived oligodendrocytic progenitors (OPCs) show a marked improvement in functional recovery following SCI [79–84]. After observing such promising pre-clinical data, extensive pre-clinical studies were conducted to characterize the safety and efficacy of these human ESCs exclusively in rodent models [85].[1] The Geron trial, which was originally approved by the FDA, but then halted due to concerns of abnormal cyst formation, was reinitiated and approved for phase I clinical trials in the U.S. in October 2010, using human ESC-derived OPCs implanted within 2 weeks into patients with thoracic SCI, after the FDA was satisfied with pre-clinical safety data

---

[1] There is a precedent for the approval for a clinical trial being given on the basis of rodent-only pre-clinical data (i.e., the ProCord Phase I and II trials of incubated macrophages) [85, 180].

Table 2 Clinical Trials of Cell Therapy for SCI Listed on www.clinicaltrials.gov

Status	Study	NCT	Cells	Administration route	Phase	Country	Sponsor/Investigator	Duration	Numbers enrolled
Recruiting	Transfer of bone marrow-derived stem cells for the treatment of SCI	NCT 01162915	Autologous BMSCs, expanded ex vivo	Intrathecal infusion, single dose	I, single centre	USA	TCA Cellular Therapy, LLC; Gabriel P. Lasala	July 2010-June 2012	10
Completed	Cell transplant in SCI patients Condition: Chronic SCI Procedure: physical therapy	NCT 00816803	Autologous bone marrow	?	I/II	Egypt	Cairo University, Cancer Institute of New Jersey, UMDNJ/ RWJMS; Hatem E. Sabaawy	May 2005-Dec 2008	80
Recruiting	Transplantation of autologous OECs in complete human SCI Other: rehabilitation	NCT 01231893	Autologous olfactory mucosa ensheathing cells (OECs) and fibroblasts	Intra-spinal	I	Poland	Wroclaw Medical University; Wlodzimierz Jarmundowicz, Pawel Tabakow	May 2008	10
Terminated	Treatment for acute SCI	NCT 00695149	BMSC	Into cerebrospinal fluid	I/II	Japan	Translational Research Informatics Center	July 2005-Mar 2010	23
Completed	Autologous adipose-derived MSCs transplantation in patients with SCI	NCT 01274975	Autologous adipose-derived MSCs	Intravenous infusion, 4×10^8 cells	I	Korea	RNL Bio Company Ltd, SangHan Kim	July 2009-Feb 2010	8
Recruiting	Safety and feasibility of umbilical cord blood cell transplant into injured spinal cord Drug: +/- methylprednisolone drug: +/- lithium	NCT 01046786	Umbilical cord blood mononuclear cell, dose comparison	Intra-spinal	I/II	China	China Spinal Cord Injury Network	Jan 2010-June 2012	20
Completed	Safety and Efficacy of Autologous Bone Marrow Stem Cells in Treating SCI Condition: Acute, subacute and chronic SCI Procedure: laminectomy	NCT 01186679	Autologous bone marrow	Intrathecal	I/II	India	International Stemcell Services Limited, Arvind Bhateja	Jan 2008-Aug 2010	12
Enrolling by invitation	Umbilical cord blood mononuclear cell transplant to treat chronic SCI Other : methylprednisolone, sodium succinate or lithium carbonate plus rehabilitation	NCT 01354483	HLA-matched umbilical cord blood mononuclear cells		I/II	China	Treating Center of Spinal Cord Injury Chengdu Army Kunming General Hospital (Dr Hui Zhu)	Sep 2010-Dec 2012	20
Suspended	Autologous incubated macrophages for patients with complete SCIs condition: Acute SCI	NCT 00073853	Autologous incubated macrophages	Intra-spinal	II	USA, Israel	Proneuron Biotechnologies, Marcus Foundation B.I.R.D. (Israel-U.S. Binational Industrial Research and Development); Daniel Lammertse, Nachshon Knoller, Marca Sipski, Edward Benzel	Sep 2003	61
Recruiting	Safety study of GRNOPC1 in SCI Condition: Complete T3-T9 level sub-acute (7–14 days post-injury) SCI	NCT 01217008	GRNOPC1 (ES cell-derived oligodendrocytic progenitors)	Intra-spinal, single dose of 2 million cells	I	USA	Geron, Gary K. Steinberg, David Apple, Richard G Fessler, James S Harrop, Shekar Kurpad	Oct2010-Oct 2012	10
Recruiting	Autologous stem cells for SCI in children Condition: Primary SCI, to minimize secondary SCI	NCT 01328860	Autologous BMSCs	Intravenous infusion	I	USA	Memorial Hermann Healthcare System, James E. Baumgartner	Apr 2011-Oct 2014	10

Table 2 (continued)

Status	Study	NCT	Cells	Administration route	Phase	Country	Sponsor/Investigator	Duration	Numbers enrolled
Recruiting	Autologous bone marrow stem cell transplantation in patients with SCI	NCT 01325103	Autologous BMSC	Intra-spinal	I	Brazil	Hospital Sao Rafael; Ricardo R. dos Santos	July 2010-Jan 2013	20
Recruiting	Study of human CNS stem cells (HuCNS-SC) in patients with thoracic SCI Condition: subacute thoracic (T2-T11) SCI	NCT 01321333	HuCNS-SC cells	Single dose, intramedullary	I/II	Switzerland	StemCells, Inc.; Armin Curt	Mar 2011-Mar 2016	12

Phase I trials are generally small studies to ensure that the new drug or procedure is safe and well tolerated. Phase II trials are to test efficacy in a small group of well defined, treated and control patients. Phase III trials are large multi-centre studies to test the efficacy and safety of the treatment in a large number of patients as a prelude to introducing the drug or procedure to clinical practice.

BMSCs = bone marrow mesenchymal/stromal cells; CNS = cerebrospinal fluid; ES = embryonic stem cell; HLA = human leukocyte antigen; HuCNS-SC cells = xxxxxxx cells; MSCs = xxxx; NCT = xxxxxx; OECs = olfactory ensheathing cells; SCI = spinal cord injury;

generated by Geron, but not without considerable objection and controversy [86–88]. In addition to the data from Keirstead's group (which has not been independently verified), and the unpublished data generated by Geron for the FDA, the initiation of this clinical trial is further supported by behavioral and histological data from studies implanting glial restricted progenitors (GRPs) [89] and OPCs [90, 91] isolated from embryonic and postnatal rodents in SCI models, albeit indirectly. Although these predominantly show astroglial differentiation of GRP implanted within the blunt contusion-induced thoracic lesion site, there is a shift toward oligodendrocytic specification beyond the injury site correlated with the degree of functional improvement [92–94], both of which can be enhanced by transduction of factors, such as D15A, BDNF, and/or NT-3 [95]. GRP implantation was also shown to be neuroprotective and inhibited neuropathic pain.

Although more studies are required on the effects of GRPs and OPCs of various derivations in subacute and chronic cervical models, the overall picture emerging from the current literature and reports of undisclosed safety studies by Geron on ESC-derived OPCs is one that favors the continuation of this clinical trial primarily on the grounds of verification of safety, and secondarily on the grounds of efficacy in humans.

Neural progenitor cells can also be derived from several regions of the fetal, postnatal, and adult CNS, including the subventricular zone of the brain, the central canal of the spinal cord, the hippocampus, and the cortex. They can be expanded in culture as nonadherent neurospheres, which have the potential to generate all 3 neural cell types with the appropriate conditions. The key advantage of this NPC source is the amenability to *in vitro* manipulation (including immortalization; see below) prior to implantation, as well as the lack of tumorigenicity. However, autologous derivation of the CNS NPCs would be unfeasible for cell therapy purposes.

On the basis of promising results in the highly and clinically relevant primate models [96–98]), the canine cervical contusion models of SCI, and cell number-dependent locomotor recovery in acute, subacute, and chronic thoracic rodent models (stemcellsinc.com) [99–102], a recently listed clinical trial in Switzerland sponsored by the biotechnology firm StemCells Inc. is the only one currently recruiting to treat SCI sufferers with nonimmortalized fetal human CNS stem cells (HuCNS-SC) (clinical trial identifier: NCT01321333). Despite an origin of the fetal brain, they are regulated and often referred to as adult NPCs, because they are nonembryonic (stemcellsinc.com). These have been previously tested for the fatal infantile demyelination disorder Pelizaeus-Merzbacher disease (clinical trial identifier: NCT01005004) and neuronal ceroid lipofuscinosis (also referred to as Batten disease) (clinical trial identifier:

NCT00337636). In the latter, they have recently been shown to persist without causing harm for as long as 2.5 years after implantation into immunosuppressed patients (http://www.internationaldrugdiscovery.com/ViewArticle.aspx?ContentID=2474).

There is a lack of trials of NPCs in SCI, in spite of the bulk of pre-clinical findings to date in support of the potential of fetal and adult neural stem/progenitor cells (particularly the former) in experimental SCI models [23], which is likely to reflect ethical concerns as to their origins and practical issues hindering their isolation and directed differentiation. Another possible explanation for the absence of clinical trials of NPCs for SCI is that the mechanisms through which NPCs provide functional benefit (including immuno-modulation and angiogenesis) are only now beginning to be understood, dismissing the absolute requirement in all cases for cellular replacement by highly purified populations of pre-differentiated NPC-derived neural cells. Also, aims of axonal regeneration through the injury site have been replaced pre-clinically by more realistic objectives of remyelination [23] and provision of trophic support for endogenous precursors and axons. This makes NPCs much more promising candidates for cell therapy for SCI and probably heralds their increased use in clinical trials.

In spite of the gaps in this field remaining to be filled by further pre-clinical research, there is a compelling amount and strength of evidence along with extensive safety and efficacy testing that has been undertaken by the biotech-nology firms sponsoring such trials (including Geron and Stem Cells Inc.) to gain FDA approval. These support clinical trials of fetal, adult, and ESC-derived NPCs for SCI repair, although the considerable ethical and practical challenges and concerns imposed by their origins and derivation should not be ignored.

SCs

SCs myelinate axons of the peripheral nervous system and can also remyelinate spared CNS axons as occurs after SCI. They are promising in that they are obtainable autologously and are not tumorigenic. SCs are the longest studied cell type in the cell field of transplantation research [103, 104]. It has been shown that transplantation of SCs, but also OECs and BMSCs promote the invasion of host SCs into the lesion site after SCI [105, 106], and although this might be interpreted as endogenous SCs playing a role in repair, it might paradoxically hinder the endogenous repair process [107]. The consequences of schwannosis, referring to the invasion of host SCs into the post-SCI lesion site, need further study to elucidate the possible undesirable side effects of SC transplantation.

SC transplants have been studied the most in thoracic models of contusion-compression and transection SCI.

Although they are demonstrably effective at promoting sensory axonal sprouting, when transplanted alone they are not capable of stimulating corticospinal tract regeneration or of allowing axons that have penetrated the graft site to grow across and re-enter the CNS parenchymal microenvironment.

Although neonatal SCs have not been shown to be effective in cervical crush SCI [108], subarachnoid neonatal rodent SC transplantation after clip compression-induced thoracic SCI has demonstrated dramatic functional improvement after injecting only 50,000 cells into young rats (100–140 g; 45–60 days old) [109].

Of the studies on adult nerve-derived SCs, only approximately one quarter have carried out BBB assess-ment of functional outcome. Of these, only 2 of 5 studies in subacute and chronic thoracic contusion SCI have reported improved functional motor outcome (BBB scores) with SC transplantation alone [73, 74]. The remaining studies, which were performed by the same group, combined SCs and either OECs or other molecules [72, 110–113]. In studies of SCs in transection models of SCI, all the required controls often have not been included [114], making interpretation of their results difficult.

A phase I clinical safety and feasibility trial of purified autologous sural nerve-derived has already been conducted according to ICCP guidelines[2] in 33 stable chronic mid-thoracic (T6-9) SCI patients with AIS motor scores A to C [115], despite a lack of studies on adult human SCs without co-administered therapies in cervical and thoracic contusion spinal cord lesion models [116–118]. All patients received intensive rehabilitation pre-surgery and post-surgery. Four patients aged 22 to 43 were followed up for as long as 1 year post-transplant and so far have been reported. This trial found autologous adult SC transplantation to be safe (no pathological findings were reported), although a beneficial outcome was found (as rated by using the ASIA motor scale) in only 1 patient with incomplete SCI that could not be attributed to cell treatment since the donor cells could not be localized by magnetic resonance imaging.

Similarly, human SCs have been examined pre-clinically in spinal cord demyelination models [119, 120], and they have been implanted into MS patients, demonstrating safety and feasibility, as well as remyelination [121]. These trials offer partial support for clinical trials of SCs for SCI, from the safety aspect, as well as from the point of view of remyelinating the injured spinal cord. However, the deleterious consequences of contaminating fibroblasts demonstrated by pre-clinical studies [120] will greatly

---

[2] A series of guidelines has recently been published and the principles for ethical implementation of clinical trials in patients with SCI have been established by the ICCP panel [163–166].

impact future clinical trials of SCs for SCI. It is clear that more clinically relevant studies are required to examine the potential of human SCs in SCI, even in conjunction with other cells and therapies. Nonetheless, preliminary data from the first trials are encouraging by helping to establish the safety of SC transplantation for SCI.

Promising Cell Sources Evaluated in Pre-Clinical Studies but Not Yet in Clinical Trials

In addition to the cell sources and types mentioned so far, there are other promising cell sources with the potential to be used in cell therapy clinical trials for SCI. However, apart possibly from fetal and immortalized NPCs, their safety and efficacy in clinically relevant experimental SCI models still remain to be more extensively studied.

Human Adult and Immortalized NPCs

As previously mentioned, NPCs can be derived from the adult, as well as the fetal CNS. It is generally recognized that adult-derived progenitor cells tend to fare less well than their fetal and embryonic counterparts in terms not only of proliferative and differentiation potentials, but also in regard to postimplantation survival, migration, and integration within the recipient CNS.

However, the serious and well-founded ethical and practical concerns of using material of fetal and embryonic derivation have limited their study at the clinical level, at least to date. From our own work and that of other groups, the predominating oligodendrogenic differentiation potential of adult rodent NPCs has been demonstrated following intra-spinal [122, 123] or intravenous [124] implantation into a range of rat and murine models (i.e., thoracic contusion), compression, and cervical transection SCI. The efficacy of NPCs can be enhanced by co-administered chondroitinase treatment [122, 123]. As with some other cell types, however, there have been indications of increased neuropathic pain, which remain to be confirmed [125].

The conditional immortalization of cells might provide a partial solution to limiting the use of fetal and embryonic tissue, overcoming the limited supply of cells irrespective of donor age, removing a source of variability between clinical trials and enabling extensive characterization of cell lines prior to clinical application. Clinically approved, conditionally immortalized fetal cortical NPCs (ReN001 or CTX0E03) of human origin are already in a phase I clinical trial for stroke in Scotland, UK (Pilot Investigation of Stem Cells in Stroke; clinical trial identifier: NCT01151124). These cells have been developed by the biotechnology firm ReNeuron using a karyotypically stable immortalization platform to enable cells to proliferate in

culture and differentiate when induced without oncogenic transformation. Although this clinical trial will not include SCI patients, the results of this trial will be very interesting and relevant because stroke and SCI share many common pathophysiological mechanisms.

The large differences in cells (including age of donor, immortalization strategy, culture conditions) and the models used make a direct comparison between studies challenging. For instance, no study has yet compared the efficacy of adult versus fetal NPCs lines using the same immortalization strategy and in the same SCI model; in fact, no study has yet been published on immortalized adult NPCs in SCI, an approach which would limit the use of an ethically objectionable and difficult to procure posthumous source of cells in limited supply.

There are also no direct comparisons of functional outcome between adult and fetal NPCs in SCI, and between different times of implantations following SCI. It has been shown that implantation of fetal NPCs into the acutely compressed or contused (and microenvironment of the highly inflammatory) thoracic spinal cord is unsuccessful compared to a subacute (7–9 days post-SCI) implantation time point [126]. This suggests that implanting in the subacute stages of SCI would be more likely to provide benefit. On the other hand, immortalized fetal human NPCs (ReN001; ReNeuron) have been shown to have anti-inflammatory properties (to the extent that stroke patients in the Pilot Investigation of Stem Cells in Stroke trial are not being immunosuppressed following implantation of these cells). This suggests that NPCs could potentially be implanted in the acute stages of SCI. Clearly, more pre-clinical studies are required to investigate this to determine the likely outcomes of cell implantation at different times following SCI.

Finally, NPCs are a heterogenous population of cells, and differences in culture methods and durations are likely to create and amplify differences in efficacy, depending on the particular models used, as well as the design specifics of each study examining them. However, there has been extensive pre-clinical safety testing (reneuron.com; similar to Geron and its GRNOPC1 cell line, and Stem Cells Inc. and its HuCNS-SC adult NPC line) of at least 1 line of immortalized fetal NPCs carried out by ReNeuron prior to implantation into stroke patients. This addresses important questions regarding the safety and tolerability of their clinical use.

Human Wharton's Jelly Cells/Umbilical Cord Matrix Cells

Nonembryonic tissues, such as bone marrow, peripheral and umbilical cord blood, and umbilical cord matrix [127, 128] represent plentiful, ethical, and easily accessible sources of mesenchymal stem cells (MSCs) for neural

repair [129]. BMSCs, currently the main source of autologous stem cells (apart from adipose stem cells, incubated macrophages, olfactory mucosal cells and OECs, of which the latter three are not stem cells per se), are already in several clinical trials as mentioned.

The human umbilical cord consists of an outer layer of amniotic epithelial cells enclosing a gelatinous matrix known as Wharton's jelly cells (WJCs) [130], which harbors a stem cell population of WJCs [15]. Although WJCs are similar to MSCs from other sources (umbilical cord blood, amniotic fluid, bone marrow, and fetal blood), there are several clinically important advantages to using WJCs for cell transplantation (for more detail see Vawda et al. [131, 132]). Most importantly, WJCs are more highly proliferative and can thus be more rapidly and extensively propagated than adult BMSCs [129, 133], but unlike the latter, WJCs can undergo repeated freeze–thaw cycles without a significant loss of viability, mesodermal differentiation potential, and without accumulating karyotypic abnormalities [129, 133]. WJCs are readily obtainable without ethical constraints after normal and Caesarean births. They are thought to be nonimmunogenic, and may even have the capacity to suppress the immune response [134], potentially making them suitable for allogeneic transplantation [125]. WJCs are highly pathotropic following transplantation [135]. Of greatest relevance to SCI, they secrete a wide range of trophic factors known to promote neural cell survival (including FGF2 and SDF-1a) [136], which would make them useful for cell rescue and as support cells. Unlike ES and induced pluripotent stem (iPS) cells [137], WJCs are nontumorigenic following transplantation [133, 135, 136], and they even exhibit anti-tumor properties [138, 139].

Two studies so far have examined the use of WJCs in SCI models, but were poorly conceived and designed. Nonetheless, they did indicate that WJC administration into SCI models can potentially promote repair and recovery through the release of trophic factors [140, 141].

Fetal Human Mesenchymal Progenitor Cells

Fetal human mesenchymal progenitor cells can be isolated by cardiocentesis from first trimester fetal blood and have been characterized by Campagnoli et al.'s group [142]. Fetal human mesenchymal progenitor cells are antigenically and morphologically similar to adult BMSCs and virtually indistinguishable from WJCs, and they have already been clinically used in Sweden for osteogenesis imperfecta [143]. Given the source of fetal human mesenchymal progenitor cells from elective abortions, there are serious ethical and practical issues constraining more widespread clinical use, but their potential in SCI cannot be ignored.

Immunosuppression might not be necessary for successful donor fetal MPC survival and engraftment, not only without rejection but leading to functional benefit. In 2005, Le Blanc et al.'s [143] team in Sweden transplanted allogeneic human leukocyte antigen-mismatched male fetal liver MSCs into an immunocompetent patient with osteogenesis imperfecta (a genetic bone defect caused by dysfunctional collagen) in utero at 32 weeks of gestation. This study found successful engraftment and osteogenic differentiation of donor cells, leading to functional recovery.

Skin-Derived Progenitors

Nearly a decade ago, Miller's group described a population of multipotent progenitor cells residing within the adult dermis (termed skin-derived precursors [SKPs]) [144–146] with the potential to generate myelinating cells [105, 147–152] and enable axons to grow across the lesion site (unlike SCs) when injected intraspinally after SCI [105]. On the basis of convincing histological and behavioral data in the latter study, the potential for autologous transplantation of treated SKPs for SCI is obvious. It is interesting to note the similarity between this study and one on BMSCs, which were found to further promote axonal sprouting when treated preimplantation with growth factors [29]. However, more studies of the effects of SKP injection in direct comparison with SCs into more clinically relevant models of SCI, including cervical and chronic models, would be required to characterize the safety parameters of this cell source and to determine the optimal implantation criteria to maximize functional benefit post-SCI.

Induced Pluripotent Stem Cells

Recently, the discovery of iPS cells has opened a new potential therapeutic approach for regenerative neuroscience, although iPS cells have not yet been used clinically in SCI cell therapy [153–155].

We recently reviewed the potential of iPS cells in SCI [154]. Given the novel nature of the technology and the safety and reliability of recent variations on the induction technique, these cells are not yet ready for use in clinical trials. As with ES cells, there are well-founded concerns in regard to the teratomatous potential of iPS cells *in vivo*. In addition, their controlled differentiation remains to be thoroughly compared to ES cells and conditions optimized for the subsequent derivation of each neural lineage. Of note, Tsuji et al. [156] recently derived "safe" mouse iPS cells and observed trilineage neural differentiation and functional recovery in a contusion model of SCI without teratoma formation [156].

A further concern is the inherent variability of the differentiation and proliferative potential of different iPS cell lines, an issue also raised by different ES cell lines and by the low transduction rates of current transfection strategies, and the requirement for extensive retrospective characterization to ensure ES cell-like phenotype. This needs to be resolved to be able to allow the "individualization" of iPS technology to derive patient-specific lines for autologous transplantation.
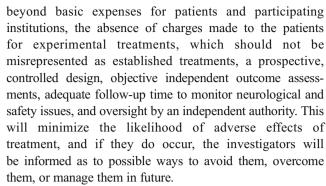
Even then, the time required to generate and characterize (and if required genetically manipulate) iPSC lines would render individualized iPSC-based treatment impractical. More likely is that a set of well-characterized iPSC lines will be generated and tested pre-clinically for subsequent administration into SCI patients. In this situation, the immunogenicity of iPSCs is a major obstacle that will need to be addressed [157]. As such, although clinical trials for these cells may be a possibility in time, the current state of the field would not warrant them.

Endogenous Progenitors

Stem/progenitor cells have been identified in the central canal adult of the mammalian spinal cord [158, 159]. They proliferate extensively following SCI [160] or in response to the infusion of exogenous growth factors into the fourth ventricle of the adult brain [161], which can also reduce inflammation, and generate astrocytes and oligodendrocytes. Although the potential of stimulating the proliferation and subsequent differentiation of endogenous NPCs to effect repair is clear, it needs to be seen alongside the possible risks that supra-physiological levels of neurotrophic factors within the cerebrospinal fluid might carry, including epileptogenesis and oncogenesis.

Recommendations for the Conduct and Evaluation of Pre-Clinical Studies and Clinical Trials of Cell Therapies for SCI

The absolute requirement for international peer-reviewed assessment, regulation, independent monitoring and duplication, complete transparency, and accurate record keeping of every step of the process of designing, initiating, and executing clinical trials cannot be over-emphasized [162]. In this regard, a series of guidelines and criteria has recently been published, and the principles for ethical implementation of clinical trials in patients with SCI have been established by the ICCP panel [163–166]. These include a sound evidentiary basis and compelling clinical rationale for conducting a clinical trial, registration with clinicaltrials.gov, protection of the rights of participants and volunteers through informed consent, the absence of renumeration

beyond basic expenses for patients and participating institutions, the absence of charges made to the patients for experimental treatments, which should not be misrepresented as established treatments, a prospective, controlled design, objective independent outcome assessments, adequate follow-up time to monitor neurological and safety issues, and oversight by an independent authority. This will minimize the likelihood of adverse effects of treatment, and if they do occur, the investigators will be informed as to possible ways to avoid them, overcome them, or manage them in future.

Amariglio et al. [53] published a report of a young boy with ataxia telangiectasia who had received a multi-donor-derived intrathecal injection of fetal human NPCs and developed a benign multi-focal brain and spinal cord glioneuronal neoplasm. It has been suggested that the dysfunctional immune system of ataxia telangiectasia patients increased the risk of this particular recipient to develop donor cell-derived neoplasms. There are serious doubts remaining in regard to the details of this clinical case, especially relating to the karyotypic status of the implanted cells after culture and immediately prior to implantation. This is a genuine concern, which deserves further investigation, as highlighted by the oncogenic potential of ES cell-derived neural cells reported by Roy et al. [167]. However, unfortunately, it is unlikely that this particular case will yield any useful information for future studies and trials, given the lack of details on the cells, their origin, their isolation, and the implantation procedure, and any co-administered treatments.

Although we advocate a combined strategy of careful exploration of cell-based therapies in phase I and II trials in humans, and intensive pre-clinical evaluation of these approaches in cervical models of SCI, it is important to emphasize that clinical investigation needs to follow sound ethical and scientific principles. One of the key principles involves establishing a sound scientific basis for the preclinical research [23, 24, 162, 168, 169].

Concluding Remarks

The National Institutes of Health translational roadmap (http://commonfund.nih.gov/clinicalresearch/overview-translational.aspx; http://CTSAweb.org/) was drafted to validate animal models and protocols developed in the laboratory, and to drive the implementation of pre-clinical studies and basic research, thus enabling scientists to begin addressing and overcoming the issues and challenges emanating from the clinic. In this regard, immediate, further, and larger clinical trials are justified for cells already proven safe and even effective through extensive laboratory testing, and/or through past and ongoing clinical

trials, such as autologous BMSCs, OECs, olfactory mucosal cells, NPCs, and ESC-derived OPCs.

Given the paucity of studies carried out in more clinically relevant models of SCI, such as chronic cervical SCI [23], current clinical trials are justified primarily on the basis of pre-clinical studies conducted predominantly in thoracic models to determine the relative safety and feasibility of the cellular interventions currently at our disposal in thoracic SCI, and to iron out neurosurgical technical and logistical issues, and also to objectively evaluate functional outcome to determine efficacy using internationally recognized and established standards.

It is acknowledged that animal models are continuing to evolve and improve so as to more closely reflect the clinical situation [6–8, 170–177], and that better and more accurate standardized analysis techniques (including the CatWalk system [178]) will enable more objective assessment of functional outcome. However, we argue that there is a real and urgent need to press ahead with human trials on the basis of relevant solid scientific pre-clinical evidence gathered from well-established models analyzed using thoroughly validated techniques. The feasibility of this approach is amply demonstrated by the ongoing trials of ESC-derived OPCs (GRNOPC1) by Geron.

Although the case in support of clinical trials of cell therapies for SCI generates vigorous debate and divergent views, it is our strong view that the field is now at a stage in which advancement into phase I and early phase II trials is justified and essential to move the field forward. Certainly, an important principle in considering the clinical translation of a therapeutic strategy is the need for replication of results. However, this principle may be more complex when a biotechnology company is driving the translational process, given the considerations of protecting intellectual property. Moreover, the need for data from 1 laboratory to be replicated using identical conditions and cells may be an unrealistic and unreasonable challenge to meet, given the pressure on researchers to publish novel rather than confirmatory data. Therefore, there will always be differences between studies, even on the same cell type (or types) or the same model (or models) that might make a direct comparison of results difficult. On the other hand, it is precisely those differences that are the most informative and pave the way ahead toward new developments and findings preceding clinical trials.

Taking into account the various issues at hand, it is our view that, while this is by no means a clear-cut situation, there are nonetheless sufficient pre-clinical and earlier clinical safety studies of satisfactory quality and reliability carried out so far to justify the immediate translation into the clinic of a greater number of pre-clinical findings, such as the clinical trial of OECs by Mackay-Sim et al.'s [55]

group, and Mackay-Sim and St. John's [179], the work on fetal cells by Giovanini et al.'s [27] group and the phase I Procord trial on macrophages [85].

Finally, no clinical intervention is 100% risk-free. The decision to forge ahead with clinical trials of cell therapy for SCI relies on striking a balance between the current and anticipated burden of SCI and the potential risks of cell therapy. Setbacks are an expected feature of novel therapies (as in the case of bone marrow transplantation, development of the polio vaccine, and initial gene therapy trials), and as difficult as setbacks can be to contemplate and overcome, they have been overcome, and they have even provided vital clinical and scientific information that has enabled subsequent therapies to be made safe and effective. Without the clinical translation of the firmly established basic scientific findings, however, we will be stuck at the preclinical level. Advances in the application of regenerative neuroscience to SCI can only be made with an investigative approach that balances excellent preclinical research with rigorous, ethical clinical trials.

References

1. Sekhon LH, Fehlings MG. Epidemiology, demographics, and pathophysiology of acute spinal cord injury. Spine (Phila Pa 1976) 2001;26:(24 suppl):S2-S12.
2. ASpinal cord injury facts and figures at a glance. Birmingham, AL: National Spinal Cord Injury Statistical Center, 2010.
3. One Degree of Separation: Paralysis and Spinal Cord Injury in the United States. Christopher and Dana Reeve Foundation, 2010.
4. Fehlings MG, Tighe A. Spinal cord injury: the promise of translational research. Neurosurg Focus 2008;25:E1.
5. Spinal cord injury facts and statistics: Rick Hansen Spinal Cord Injury Registry, 2006.
6. Dunham KA, Siriphorn A, Chompoopong S, Floyd CL. Characterization of a graded cervical hemicontusion spinal cord injury model in adult male rats. J Neurotrauma 2010;27:2091–2106.
7. Usvald D, Vodicka P, Hlucilova J, et al. Analysis of dosing regimen and reproducibility of intraspinal grafting of human spinal stem cells in immunosuppressed minipigs. Cell Transplant 2010;19:1103–1122.
8. Vodicka P, Smetana K Jr., Dvorankova B, et al. The miniature pig as an animal model in biomedical research. Ann N Y Acad Sci 2005;1049:161–171.
9. Tator CH, Fehlings MG. Review of the secondary injury theory of acute spinal cord trauma with emphasis on vascular mechanisms. J Neurosurg 1991;75:15–26.
10. Figley SA, Austin JW, Rowland JW, Fehlings MG. Pathophysiology of spinal cord injury. The Cervical Spine, 5th ed. Lippincott, Williams and Wilkins, 2011.

11. Wang L, Shi J, van Ginkel FW, et al. Neural stem/progenitor cells modulate immune responses by suppressing T lymphocytes with nitric oxide and prostaglandin E2. Exp Neurol 2009;216:177–183.

12. Pluchino S, Gritti A, Blezer E, et al. Human neural stem cells ameliorate autoimmune encephalomyelitis in non-human primates. Ann Neurol 2009;66:343–354.

13. Okamura RM, Lebkowski J, Au M, Priest CA, Denham J, Majumdar AS. Immunological properties of human embryonic stem cell-derived oligodendrocyte progenitor cells. J Neuro-immunol 2007;192:134–144.

14. Jaderstad J, Jaderstad LM, Li J, et al. Communication via gap junctions underlies early functional and beneficial interactions between grafted neural stem cells and the host. Proc Natl Acad Sci U S A 2010;107:5184–5189.

15. Bai L, Lennon DP, Eaton V, et al. Human bone marrow-derived mesenchymal stem cells induce Th2-polarized immune response and promote endogenous repair in animal models of multiple sclerosis. Glia 2009;57:1192–1203.

16. Arriola A, Kiel ME, Shi Y, McKinnon RD. Adjunctive MSCs enhance myelin formation by xenogenic oligodendrocyte precursors transplanted in the retina. Cell Res 2010;20:728–731.

17. Zhang J, Wang B, Xiao Z, et al. Olfactory ensheathing cells promote proliferation and inhibit neuronal differentiation of neural progenitor cells through activation of Notch signaling. Neuroscience 2008;153:406–413.

18. Hernandeza J, Torres-Espina A, Navarro X. Adult Stem Cell Trans-plants for Spinal Cord Injury Repair: Current State in Preclinical Research. Curr Stem Cell Res Ther 2011;Sep 1;6(3):273–287.

19. Ruff CA, Wilcox JT, Fehlings MG. Cell-based transplantation strategies to promote plasticity following spinal cord injury. Exp Neurol 2011; Feb 17. PMID:21333647.

20. Ruff CA, Fehlings MG. Neural stem cells in regenerative medicine: bridging the gap. Panminerva Med 2010;52:125–147.

21. Zhang HT, Cheng HY, Cai YQ, et al. Comparison of adult neurospheres derived from different origins for treatment of rat spinal cord injury. Neurosci Lett 2009;458:116–121.

22. Mackay-Sim A, St. John JA. Olfactory ensheathing cells from the nose: Clinical application in human spinal cord injuries. Exp Neurol 2011;229:174–180.

23. Tetzlaff W, Okon EB, Karimi-Abdolrezaee S, et al. A systematic review of cellular transplantation therapies for spinal cord injury. J Neurotrauma 2011;28:1611–1682.

24. 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.

25. Kwon BK, Sekhon LH, Fehlings MG. Emerging repair, regeneration, and translational research advances for spinal cord injury. Spine (Phila Pa) 1976;35(21 suppl):S263-S270.

26. Gensel JC, Donnelly DJ, Popovich PG. Spinal cord injury therapies in humans: an overview of current clinical trials and their potential effects on intrinsic CNS macrophages. Expert Opin Ther Targets 2011.

27. Giovanini MA, Reier PJ, Eskin TA, Wirth E, Anderson DK. Characteristics of human fetal spinal cord grafts in the adult rat spinal cord: influences of lesion and grafting conditions. Exp Neurol 1997;148:523–543.

28. Lu P, Blesch A, Tuszynski MH. Induction of bone marrow stromal cells to neurons: differentiation, transdifferentiation, or artifact? J Neurosci Res 2004;77:174–191.

29. Novikova LN, Brohlin M, Kingham PJ, Novikov LN, Wiberg M. Neuroprotective and growth-promoting effects of bone marrow stromal cells after cervical spinal cord injury in adult rats. Cytotherapy 2011.

30. Lu P, Jones LL, Tuszynski MH. Axon regeneration through scars and into sites of chronic spinal cord injury. Exp Neurol 2007;203:8–21.

31. Urdzikova L, Jendelova P, Glogarova K, Burian M, Hajek M, Sykova E. Transplantation of bone marrow stem cells as well as mobilization by granulocyte-colony stimulating factor promotes recovery after spinal cord injury in rats. J Neurotrauma 2006;23:1379–1391.

32. Ohta M, Suzuki Y, Noda T, et al. Bone marrow stromal cells infused into the cerebrospinal fluid promote functional recovery of the injured rat spinal cord with reduced cavity formation. Exp Neurol 2004;187:266–278.

33. Fan L, Du F, Cheng BC, Peng H, Liu SQ. Migration and distribution of bone marrow stromal cells in injured spinal cord with different transplantation techniques. Chin J Traumatol 2008;11:94–97.

34. Zurita M, Vaquero J, Bonilla C, et al. Functional recovery of chronic paraplegic pigs after autologous transplantation of bone marrow stromal cells. Transplantation 2008;86:845–853.

35. Deng YB, Liu XG, Liu ZG, Liu XL, Liu Y, Zhou GQ. Implantation of BM mesenchymal stem cells into injured spinal cord elicits de novo neurogenesis and functional recovery: evidence from a study in rhesus monkeys. Cytotherapy 2006;8:210–214.

36. Kim M. Regeneration of completely transected spinal cord using scaffold of poly(D,L-lactide-co-glycolide)/small intestinal submucosa seeded with rat bone marrow stem cells. Tissue Eng Part A 2011.

37. Neuhuber B, Timothy Himes B, Shumsky JS, Gallo G, Fischer I. Axon growth and recovery of function supported by human bone marrow stromal cells in the injured spinal cord exhibit donor variations. Brain Res 2005;1035:73–85.

38. Vaquero J, Zurita M, Oya S, Santos M. Cell therapy using bone marrow stromal cells in chronic paraplegic rats: systemic or local administration? Neurosci Lett 2006;398:129–134.

39. Zurita M, Vaquero J. Bone marrow stromal cells can achieve cure of chronic paraplegic rats: functional and morphological outcome one year after transplantation. Neurosci Lett 2006;402:51–56.

40. Zurita M, Vaquero J. Functional recovery in chronic paraplegia after bone marrow stromal cells transplantation. Neuroreport 2004;15:1105–1108.

41. de Haro J, Zurita M, Ayllon L, Vaquero J. Detection of 111In-oxine-labeled bone marrow stromal cells after intravenous or intralesional administration in chronic paraplegic rats. Neurosci Lett 2005;377:7–11.

42. Kamada T, Koda M, Dezawa M, et al. Transplantation of bone marrow stromal cell-derived Schwann cells promotes axonal regeneration and functional recovery after complete transection of adult rat spinal cord. J Neuropathol Exp Neurol 2005;64:37–45.

43. Kamada T, Koda M, Dezawa M, et al. Transplantation of human bone marrow stromal cell-derived Schwann cells reduces cystic cavity and promotes functional recovery after contusion injury of adult rat spinal cord. Neuropathology 2011;31:48–58.

44. van der Kooy D, McGinty JF, Koda LY, Gerfen CR, Bloom FE. Visceral cortex: a direct connection from prefrontal cortex to the solitary nucleus in rat. Neurosci Lett 1982;33:123–127.

45. Koda M, Nishio Y, Kamada T, 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.

46. Koda M, Kamada T, Hashimoto M, et al. Adenovirus vector-mediated ex vivo gene transfer of brain-derived neurotrophic factor to bone marrow stromal cells promotes axonal regeneration after transplantation in completely transected adult rat spinal cord. Eur Spine J 2007;16:2206–2214.

47. 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.

48. Chernykh ER, Stupak VV, Muradov GM, 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.

49. Yoon SH, Shim YS, Park YH, 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.

50. Saito F, Nakatani T, Iwase M, et al. Spinal cord injury treatment with intrathecal autologous bone marrow stromal cell transplantation: the first clinical trial case report. J Trauma 2008;64:53–59.

51. Freedman MS, Bar-Or A, Atkins HL, et al. The therapeutic potential of mesenchymal stem cell transplantation as a treatment for multiple sclerosis: consensus report of the International MSCT Study Group. Mult Scler 2010;16:503–510.

52. Atkins H, Freedman M. Immune ablation followed by autologous hematopoietic stem cell transplantation for the treatment of poor prognosis multiple sclerosis. Methods Mol Biol 2009;549:231–246.

53. Amariglio N, Hirshberg A, Scheithauer BW, et al. Donor-derived brain tumor following neural stem cell transplantation in an ataxia telangiectasia patient. PLoS Med 2009;6:e1000029.

54. Doucette R. PNS-CNS transitional zone of the first cranial nerve. J Comp Neurol 1991;312:451–466.

55. Mackay-Sim A, Feron F, Cochrane J, et al. Autologous olfactory ensheathing cell transplantation in human paraplegia: a 3-year clinical trial. Brain 2008;131(pt 9):2376–2386.

56. Feron F, Perry C, Cochrane J, et al. Autologous olfactory ensheathing cell transplantation in human spinal cord injury. Brain 2005;128(pt 12):2951–2960.

57. Lu J, Feron F, Mackay-Sim A, Waite PM. Olfactory ensheathing cells promote locomotor recovery after delayed transplantation into transected spinal cord. Brain 2002;125(pt 1):14–21.

58. Lu J, Feron F, Ho SM, Mackay-Sim A, Waite PM. Transplantation of nasal olfactory tissue promotes partial recovery in paraplegic adult rats. Brain Res 2001;889:344–357.

59. Zhang X, Klueber KM, Guo Z, et al. Induction of neuronal differentiation of adult human olfactory neuroepithelial-derived progenitors. Brain Res 2006;1073–1074:109–119.

60. 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;29:191–206.

61. Lima C, Escada P, Pratas-Vital J, et al. Olfactory mucosal autografts and rehabilitation for chronic traumatic spinal cord injury. Neurorehabil Neural Repair 2010;24:10–22.

62. Ruitenberg MJ, Plant GW, Hamers FP, et al. Ex vivo adenoviral vector-mediated neurotrophin gene transfer to olfactory ensheathing glia: effects on rubrospinal tract regeneration, lesion size, and functional recovery after implantation in the injured rat spinal cord. J Neurosci 2003;23:7045–7058.

63. Steward O, Sharp K, Selvan G, et al. A re-assessment of the consequences of delayed transplantation of olfactory lamina propria following complete spinal cord transection in rats. Exp Neurol 2006;198:483–499.

64. Deumens R, Koopmans GC, Honig WM, et al. Chronically injured corticospinal axons do not cross large spinal lesion gaps after a multifactorial transplantation strategy using olfactory ensheathing cell/olfactory nerve fibroblast-biomatrix bridges. J Neurosci Res 2006;83:811–820.

65. Deumens R, Koopmans GC, Honig WM, et al. Olfactory ensheathing cells, olfactory nerve fibroblasts and biomatrices to promote long-distance axon regrowth and functional recovery in the dorsally hemisected adult rat spinal cord. Exp Neurol 2006;200:89–103.

66. Lu P, Yang H, Culbertson M, Graham L, Roskams AJ, Tuszynski MH. Olfactory ensheathing cells do not exhibit unique migratory or axonal growth-promoting properties after spinal cord injury. J Neurosci 2006;26:11120–11130.

67. Bretzner F, Liu J, Currie E, Roskams AJ, Tetzlaff W. Undesired effects of a combinatorial treatment for spinal cord injury–transplantation of olfactory ensheathing cells and BDNF infusion to the red nucleus. Eur J Neurosci 2008;28:1795–1807.

68. Richter MW, Fletcher PA, Liu J, Tetzlaff W, Roskams AJ. Lamina propria and olfactory bulb ensheathing cells exhibit differential integration and migration and promote differential axon sprouting in the lesioned spinal cord. J Neurosci 2005;25:10700–10711.

69. Au E, Richter MW, Vincent AJ, et al. SPARC from olfactory ensheathing cells stimulates Schwann cells to promote neurite outgrowth and enhances spinal cord repair. J Neurosci 2007;27:7208–7221.

70. Gorrie CA, Hayward I, Cameron N, et al. Effects of human OEC-derived cell transplants in rodent spinal cord contusion injury. Brain Res 2010;1337:8–20.

71. Deng C, Gorrie C, Hayward I, et al. Survival and migration of human and rat olfactory ensheathing cells in intact and injured spinal cord. J Neurosci Res 2006;83:1201–1212.

72. Pearse DD, Sanchez AR, Pereira FC, et al. Transplantation of Schwann cells and/or olfactory ensheathing glia into the contused spinal cord: survival, migration, axon association, and functional recovery. Glia 2007;55:976–1000.

73. Takami T, Oudega M, Bates ML, Wood PM, Kleitman N, Bunge MB. Schwann cell but not olfactory ensheathing glia transplants improve hindlimb locomotor performance in the moderately contused adult rat thoracic spinal cord. J Neurosci 2002;22:6670–6681.

74. Barakat DJ, Gaglani SM, Neravetla SR, et al. Survival, integration, and axon growth support of glia transplanted into the chronically contused spinal cord. Cell Transplant 2005;14:225–240.

75. Kalincik T, Choi EA, Feron F, et al. Olfactory ensheathing cells reduce duration of autonomic dysreflexia in rats with high spinal cord injury. Auton Neurosci 2010;154:20–29.

76. Kalincik T, Jozefcikova K, Sutharsan R, Mackay-Sim A, Carrive P, Waite PM. Selected changes in spinal cord morphology after T4 transection and olfactory ensheathing cell transplantation. Auton Neurosci 2010;158:31–38.

77. Draper JS, Moore HD, Ruban LN, Gokhale PJ, Andrews PW. Culture and characterization of human embryonic stem cells. Stem Cells Dev 2004;13:325–336.

78. Draper JS, Smith K, Gokhale P, et al. Recurrent gain of chromosomes 17q and 12 in cultured human embryonic stem cells. Nat Biotechnol 2004;22:53–54.

79. Cloutier F, Siegenthaler MM, Nistor G, Keirstead HS. Transplantation of human embryonic stem cell-derived oligodendrocyte progenitors into rat spinal cord injuries does not cause harm. Regen Med 2006;1:469–479.

80. Keirstead HS, Nistor G, Bernal G, 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.

81. Faulkner J, Keirstead HS. Human embryonic stem cell-derived oligodendrocyte progenitors for the treatment of spinal cord injury. Transpl Immunol 2005;15:131–142.

82. Nistor GI, Totoiu MO, Haque N, Carpenter MK, Keirstead HS. Human embryonic stem cells differentiate into oligodendrocytes in high purity and myelinate after spinal cord transplantation. Glia 2005;49:385–396.

83. 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.

84. Bottai D, Cigognini D, Madaschi L, et al. Embryonic stem cells promote motor recovery and affect inflammatory cell infiltration in spinal cord injured mice. Exp Neurol 2010;223:452–463.

85. Knoller N, Auerbach G, Fulga V, 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.

86. Bretzner F, Gilbert F, Baylis F, Brownstone RM. Target populations for first-in-human embryonic stem cell research in spinal cord injury. Cell Stem Cell 2011;8:468–475.

87. Wirth E 3rd, Lebkowski JS, Lebacqz K. Response to Frederic Bretzner, et al. Target populations for first-in-human embryonic stem cell research in spinal cord injury. Cell Stem Cell 2011;8:476–478.

88. Solbakk JH, Zoloth L. The tragedy of translation: the case of "first use" in human embryonic stem cell research. Cell Stem Cell 2011;8:479–481.

89. Mitsui T, Shumsky JS, Lepore AC, Murray M, Fischer I. Transplantation of neuronal and glial restricted precursors into contused spinal cord improves bladder and motor functions, decreases thermal hypersensitivity, and modifies intraspinal circuitry. J Neurosci 2005;25:9624–9636.

90. Bambakidis NC, Miller RH. Transplantation of oligodendrocyte precursors and sonic hedgehog results in improved function and white matter sparing in the spinal cords of adult rats after contusion. Spine J 2004;4:16–26.

91. Lee KH, Yoon DH, Park YG, Lee BH. Effects of glial transplantation on functional recovery following acute spinal cord injury. J Neurotrauma 2005;22:575–589.

92. Enzmann GU, Benton RL, Woock JP, Howard RM, Tsoulfas P, Whittemore SR. Consequences of noggin expression by neural stem, glial, and neuronal precursor cells engrafted into the injured spinal cord. Exp Neurol 2005;195:293–304.

93. Han SS, Liu Y, Tyler-Polsz C, Rao MS, Fischer I. Transplantation of glial-restricted precursor cells into the adult spinal cord: survival, glial-specific differentiation, and preferential migration in white matter. Glia 2004;45:1–16.

94. Hill CE, Proschel C, Noble M, et al. Acute transplantation of glial-restricted precursor cells into spinal cord contusion injuries: survival, differentiation, and effects on lesion environment and axonal regeneration. Exp Neurol 2004;190:289–310.

95. Cao Q, Xu XM, Devries WH, et al. Functional recovery in traumatic spinal cord injury after transplantation of multineurotrophin-expressing glial-restricted precursor cells. J Neurosci 2005;25:6947–6957.

96. Iwanami A, Yamane J, Katoh H, et al. Establishment of graded spinal cord injury model in a nonhuman primate: the common marmoset. J Neurosci Res 2005;80:172–181.

97. Iwanami A, Kaneko S, Nakamura M, et al. Transplantation of human neural stem cells for spinal cord injury in primates. J Neurosci Res 2005;80:182–190.

98. Yamane J, Nakamura M, Iwanami A, et al. Transplantation of galectin-1-expressing human neural stem cells into the injured spinal cord of adult common marmosets. J Neurosci Res 2010;88:1394–1405.

99. 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.

100. 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.

101. Cummings BJ, Uchida N, Tamaki SJ, Anderson AJ. Human neural stem cell differentiation following transplantation into spinal cord injured mice: association with recovery of locomotor function. Neurol Res 2006;28:474–481.

102. Cummings BJ, Uchida N, Tamaki SJ, et al. Human neural stem cells differentiate and promote locomotor recovery in spinal cord-injured mice. Proc Natl Acad Sci U S A 2005;102:14069–14074.

103. 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.

104. Duncan ID, Milward EA. Glial cell transplants: experimental therapies of myelin diseases. Brain Pathol 1995;5:301–310.

105. Biernaskie J, Sparling JS, Liu J, et al. Skin-derived precursors generate myelinating Schwann cells that promote remyelination and functional recovery after contusion spinal cord injury. J Neurosci 2007;27:9545–9559.

106. Hill CE, Moon LD, Wood PM, Bunge MB. Labeled Schwann cell transplantation: cell loss, host Schwann cell replacement, and strategies to enhance survival. Glia 2006;53:338–343.

107. Bruce JH, Norenberg MD, Kraydieh S, Puckett W, Marcillo A, Dietrich D. Schwannosis: role of gliosis and proteoglycan in human spinal cord injury. J Neurotrauma 2000;17:781–788.

108. Agudo M, Woodhoo A, Webber D, Mirsky R, Jessen KR, McMahon SB. Schwann cell precursors transplanted into the injured spinal cord multiply, integrate and are permissive for axon growth. Glia 2008;56:1263–1270.

109. Firouzi M, Moshayedi P, Saberi H, et al. Transplantation of Schwann cells to subarachnoid space induces repair in contused rat spinal cord. Neurosci Lett 2006;402:66–70.

110. Golden KL, Pearse DD, Blits B, et al. Transduced Schwann cells promote axon growth and myelination after spinal cord injury. Exp Neurol 2007;207:203–217.

111. Meijs MF, Timmers L, Pearse DD, et al. Basic fibroblast growth factor promotes neuronal survival but not behavioral recovery in the transected and Schwann cell implanted rat thoracic spinal cord. J Neurotrauma 2004;21:1415–1430.

112. Pearse DD, Marcillo AE, Oudega M, Lynch MP, Wood PM, Bunge MB. Transplantation of Schwann cells and olfactory ensheathing glia after spinal cord injury: does pretreatment with methylprednisolone and interleukin-10 enhance recovery? J Neurotrauma 2004;21:1223–1239.

113. Pearse DD, Pereira FC, Marcillo AE, et al. cAMP and Schwann cells promote axonal growth and functional recovery after spinal cord injury. Nat Med 2004;10:610–616.

114. Fouad K, Schnell L, Bunge MB, Schwab ME, Liebscher T, Pearse DD. Combining Schwann cell bridges and olfactory-ensheathing glia grafts with chondroitinase promotes locomotor recovery after complete transection of the spinal cord. J Neurosci 2005;25:1169–1178.

115. Saberi H, Moshayedi P, Aghayan HR, et al. Treatment of chronic thoracic spinal cord injury patients with autologous Schwann cell transplantation: an interim report on safety considerations and possible outcomes. Neurosci Lett 2008;443:46–50.

116. Schaal SM, Kitay BM, Cho KS, et al. Schwann cell transplantation improves reticulospinal axon growth and forelimb strength after severe cervical spinal cord contusion. Cell Transplant 2007;16:207–228.

117. Guest JD, Hesse D, Schnell L, Schwab ME, Bunge MB, Bunge RP. Influence of IN-1 antibody and acidic FGF-fibrin glue on the response of injured corticospinal tract axons to human Schwann cell grafts. J Neurosci Res 1997;50:888–905.

118. Guest JD, Rao A, Olson L, Bunge MB, Bunge RP. The ability of human Schwann cell grafts to promote regeneration in the transected nude rat spinal cord. Exp Neurol 1997;148:502–522.

119. Kohama I, Lankford KL, Preiningerova J, White FA, Vollmer TL, Kocsis JD. Transplantation of cryopreserved adult human Schwann cells enhances axonal conduction in demyelinated spinal cord. J Neurosci 2001;21:944–950.

120. Brierley CM, Crang AJ, Iwashita Y, et al. Remyelination of demyelinated CNS axons by transplanted human schwann cells: the deleterious effect of contaminating fibroblasts. Cell Transplant 2001;10:305–315.

121. Halfpenny C, Benn T, Scolding N. Cell transplantation, myelin repair, and multiple sclerosis. Lancet Neurol 2002;1:31–40.

122. Ikegami T, Nakamura M, Yamane J, et al. Chondroitinase ABC combined with neural stem/progenitor cell transplantation enhances graft cell migration and outgrowth of growth-associated protein-43-positive fibers after rat spinal cord injury. Eur J Neurosci 2005;22:3036–3046.

123. Karimi-Abdolrezaee S, Eftekharpour E, Wang J, Schut D, Fehlings MG. Synergistic effects of transplanted adult neural stem/progenitor cells, chondroitinase, and growth factors promote functional repair and plasticity of the chronically injured spinal cord. J Neurosci 2010;30:1657–1676.

124. Bottai D, Madaschi L, Di Giulio AM, Gorio A. Viability-dependent promoting action of adult neural precursors in spinal cord injury. Mol Med 2008;14:634–644.

125. Hofstetter CP, Holmstrom NA, Lilja JA, et al. Allodynia limits the usefulness of intraspinal neural stem cell grafts; directed differentiation improves outcome. Nat Neurosci 2005;8:346–353.

126. Okada S, Ishii K, Yamane J, et al. In vivo imaging of engrafted neural stem cells: its application in evaluating the optimal timing of transplantation for spinal cord injury. Faseb J 2005;19:1839–1841.

127. Mitchell KE, Weiss ML, Mitchell BM, et al. Matrix cells from Wharton's jelly form neurons and glia. Stem Cells 2003;21:50–60.

128. Wang HS, Hung SC, Peng ST, et al. Mesenchymal stem cells in the Wharton's jelly of the human umbilical cord. Stem Cells 2004;22:1330–1337.

129. Weiss ML, Medicetty S, Bledsoe AR, et al. Human umbilical cord matrix stem cells: preliminary characterization and effect of transplantation in a rodent model of Parkinson's disease. Stem Cells 2006;24:781–792.

130. La Rocca G, Anzalone R, Corrao S, et al. Isolation and characterization of Oct-4+/HLA-G + mesenchymal stem cells from human umbilical cord matrix: differentiation potential and detection of new markers. Histochem Cell Biol 2009;131:267–282.

131. Vawda R, Kennea NK, Mehmet H. Stem cells in neurodegeneration and injury. In: Potten CS, Clarke RB, Wilson J, Renehan AG, eds. Tissue stem cells: London: Taylor & Francis Pubs, 2006:271–293.

132. Vawda R, Woodbury J, Covey M, Levison SW, Mehmet H. Stem cell therapies for perinatal brain injuries. Semin Fetal Neonatal Med 2007;12:259–272.

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

134. Weiss ML, Anderson C, Medicetty S, et al. Immune properties of human umbilical cord Wharton's jelly-derived cells. Stem Cells 2008;26:2865–2874.

135. Weiss ML, Mitchell KE, Hix JE, et al. Transplantation of porcine umbilical cord matrix cells into the rat brain. Exp Neurol 2003;182:288–299.

136. Lund RD, Wang S, Lu B, et al. Cells isolated from umbilical cord tissue rescue photoreceptors and visual functions in a rodent model of retinal disease. Stem Cells 2007;25:602–611.

137. Fong CY, Gauthaman K, Bongso A. Teratomas from pluripotent stem cells: A clinical hurdle. J Cell Biochem 2010;111:769–781.

138. Ganta C, Chiyo D, Ayuzawa R, et al. Rat umbilical cord stem cells completely abolish rat mammary carcinomas with no evidence of metastasis or recurrence 100 days post-tumor cell inoculation. Cancer Res 2009;69:1815–1820.

139. Ayuzawa R, Doi C, Rachakatla RS, et al. Naive human umbilical cord matrix derived stem cells significantly attenuate growth of human breast cancer cells in vitro and in vivo. Cancer Lett 2009;280:31–37.

140. Yang CC, Shih YH, Ko MH, Hsu SY, Cheng H, Fu YS. Transplantation of human umbilical mesenchymal stem cells from Wharton's jelly after complete transection of the rat spinal cord. PLoS ONE 2008;3:e3336.

141. Zhang L, Zhang HT, Hong SQ, Ma X, Jiang XD, Xu RX. Cografted Wharton's jelly cells-derived neurospheres and BDNF promote functional recovery after rat spinal cord transection. Neurochem Res 2009.

142. Campagnoli C, Roberts IA, Kumar S, Bennett PR, Bellantuono I, Fisk NM. Identification of mesenchymal stem/progenitor cells in human first-trimester fetal blood, liver, and bone marrow. Blood 2001;98:2396–2402.

143. Le Blanc K, Gotherstrom C, Ringden O, et al. Fetal mesenchymal stem-cell engraftment in bone after in utero transplantation in a patient with severe osteogenesis imperfecta. Transplantation 2005;79:1607–1614.

144. Toma JG, Akhavan M, Fernandes KJ, et al. Isolation of multipotent adult stem cells from the dermis of mammalian skin. Nat Cell Biol 2001;3:778–784.

145. Toma JG, McKenzie IA, Bagli D, Miller FD. Isolation and characterization of multipotent skin-derived precursors from human skin. Stem Cells 2005;23:727–737.

146. Fernandes KJ, McKenzie IA, Mill P, et al. A dermal niche for multipotent adult skin-derived precursor cells. Nat Cell Biol 2004;6:1082–1093.

147. Biernaskie JA, McKenzie IA, Toma JG, Miller FD. Isolation of skin-derived precursors (SKPs) and differentiation and enrichment of their Schwann cell progeny. Nat Protoc 2006;1:2803–2812.

148. Fernandes KJ, Kobayashi NR, Gallagher CJ, et al. Analysis of the neurogenic potential of multipotent skin-derived precursors. Exp Neurol 2006;201:32–48.

149. Fernandes KJ, Miller FD. Isolation, expansion, and differentiation of mouse skin-derived precursors. Methods Mol Biol 2009;482:159–170.

150. Fernandes KJ, Toma JG, Miller FD. Multipotent skin-derived precursors: adult neural crest-related precursors with therapeutic potential. Philos Trans R Soc Lond B Biol Sci 2008;363:185–198.

151. Biernaskie J, Miller FD. White matter repair: skin-derived precursors as a source of myelinating cells. Can J Neurol Sci 2010;37(suppl 2):S34-S41.

152. McKenzie IA, Biernaskie J, Toma JG, Midha R, Miller FD. Skin-derived precursors generate myelinating Schwann cells for the injured and dysmyelinated nervous system. J Neurosci 2006;26:6651–6660.

153. Zhang F, Citra F, Wang DA. Prospects of induced pluripotent stem cell technology in regenerative medicine. Tissue Eng Part B Rev 2011;17:115–124.

154. Salewski RP, Eftekharpour E, Fehlings MG. Are induced pluripotent stem cells the future of cell-based regenerative therapies for spinal cord injury? J Cell Physiol 2010;222:515–521.

155. Parsons XH, Teng YD, Snyder EY. Important precautions when deriving patient-specific neural elements from pluripotent cells. Cytotherapy 2009;25:1–11.

156. Tsuji O, Miura K, Okada Y, et al. Therapeutic potential of appropriately evaluated safe-induced pluripotent stem cells for spinal cord injury. Proc Natl Acad Sci U S A 2010;107:12704–12709.

157. Zhao T, Zhang ZN, Rong Z, Xu Y. Immunogenicity of induced pluripotent stem cells. Nature 2011;474:212–215.

158. Meletis K, Barnabe-Heider F, Carlen M, et al. Spinal cord injury reveals multilineage differentiation of ependymal cells. PLoS Biol 2008;6:e182.

159. Hawryluk GW, Fehlings MG. The center of the spinal cord may be central to its repair. Cell Stem Cell 2008;3:230–232.

160. Yamamoto S, Yamamoto N, Kitamura T, Nakamura K, Nakafuku M. Proliferation of parenchymal neural progenitors in response to injury in the adult rat spinal cord. Exp Neurol 2001;172:115–127.

161. Martens DJ, Seaberg RM, van der Kooy D. *In vivo* infusions of exogenous growth factors into the fourth ventricle of the adult mouse brain increase the proliferation of neural progenitors around the fourth ventricle and the central canal of the spinal cord. Eur J Neurosci 2002;16:1045–1057.

162. Dietrich WD. Confirming an experimental therapy prior to transfer to humans: what is the ideal? J Rehabil Res Dev 2003;40(4 suppl 1):63–69.

163. Fawcett JW, Curt A, Steeves JD, 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.

164. Lammertse D, Tuszynski MH, Steeves JD, 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.

165. Steeves JD, Lammertse D, Curt A, 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.

166. Tuszynski MH, Steeves JD, Fawcett JW, 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.

167. Roy NS, Cleren C, Singh SK, Yang L, Beal MF, Goldman SA. Functional engraftment of human ES cell-derived dopaminergic neurons enriched by coculture with telomerase-immortalized midbrain astrocytes. Nat Med 2006;12:1259–1268.

168. Aboody K, Capela A, Niazi N, Stern JH, Temple S. Translating stem cell studies to the clinic for CNS repair: current state of the art and the need for a Rosetta stone. Neuron 2011;70:597–613.

169. Hyun I, Lindvall O, Ahrlund-Richter L, et al. New ISSCR guidelines underscore major principles for responsible translational stem cell research. Cell Stem Cell 2008;3:607–609.

170. Clemente CD, Windle WF. Regeneration of severed nerve fibers in the spinal cord of the adult cat. J Comp Neurol 1954;101:691–731.

171. Windle WF. Regeneration in the spinal cord of the cat in relation to observations on a human subject. Trans Am Neurol Assoc 1954;13:233–5.

172. Guth L, Brewer CR, Collins WF, Goldberger ME, Perl ER. Criteria for evaluating spinal cord regeneration experiments. Surg Neurol 1980;14:392.

173. Cheng H, Cao Y, Olson L. Spinal cord repair in adult paraplegic rats: partial restoration of hind limb function. Science 1996;273:510–513.

174. Navarro R, Usvald D, Motlik J, et al. Chronic spinal compression model in mini pig: a systematic behavioral and quantitative neuropathological study. J Crit Care 2010;25:e21-e.

175. Raore B, Federici T, Taub J, et al. Cervical multilevel intraspinal stem cell therapy: assessment of surgical risks in Gottingen minipigs. Spine (Phila Pa 1976) 2011;36:E164-E171.

176. Lim JH, Piedrahita JA, Jackson L, Ghashghaei T, Olby NJ. Development of a model of sacrocaudal spinal cord injury in cloned Yucatan minipigs for cellular transplantation research. Cell Reprogram 2010;12:689–697.

177. Guizar-Sahagun G, Grijalva I, Hernandez-Godinez B, et al. New approach for graded compression spinal cord injuries in Rhesus macaque: method feasibility and preliminary observations. J Med Primatol 2011.

178. Hamers FP, Koopmans GC, Joosten EA. Cat Walk-assisted gait analysis in the assessment of spinal cord injury. J Neurotrauma 2006;23:537–548.

179. Mackay-Sim A, St. John JA. Olfactory ensheathing cells from the nose: Clinical application in human spinal cord injuries. Exp Neurol 2010.

180. Jones LA, et al. A phase 2 autologous cellular therapy trial in patients with acute, complete spinal cord injury: pragmatics, recruitment, and demographics. Spinal Cord 2010;48:798–807.