Cell-based and stem-cell-based treatments for spinal cord injury: evidence from clinical trials

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Spinal cord injury is a severely disabling neurological condition leading to impaired mobility, pain, and autonomic dysfunction. Most often, a single traumatic event, such as a traffic or recreational accident, leads to primary spinal cord damage through compression and laceration, followed by secondary damage consisting of inflammation and ischaemia, and culminating in substantial tissue loss. Patients need appropriate timely surgical and critical care, followed by neurorehabilitation to facilitate neuronal reorganisation and functional compensation. Although some neurological function might be regained, most patients with initially complete lesions have severe, irreversible neurological impairment. Cell-based and stem-cell-based therapies are recognised as promising candidates to promote functional recovery. However, no trials of these therapies in patients have yet provided reproducible evidence for clinical efficacy, challenged by small effect sizes, low immune suppression, and low sensitivity study designs. Nevertheless, in the past decade, clinical trials have shown the feasibility and long-term safety of cell transplantation into the injured spinal cord. This crucial milestone has paved the way to consider refinements and combined therapies, such as the use of biomaterials to augment the effects of cell transplantation. In the future, emerging cell types, scaffolding, and cell engineering might improve cell survival, integration, and therapeutic efficiency.

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Introduction

Acute traumatic spinal cord injury is a severe neurological condition caused by mechanical trauma and resulting in severe motor loss (ie, paralysis), impaired sensation, and autonomic dysfunction.[1–3] The primary causes are traffic accidents, falls, and sports-related injuries.[4] The resulting lifelong deficits associated with paralysis and sensory loss have a substantial effect on individuals, caregivers, and society, reducing quality of life in some patients (depending on the severity of the injury and comorbidities), and greatly burdening health-care systems worldwide.[5,6]

Although survival rates for traumatic spinal cord injury have steadily increased over the past few decades, mortality rates for individuals with spinal cord injury continue to exceed those for age-matched controls without spinal cord injury.[7] The neurological outcome of spinal cord injury is mainly determined by the initial severity of spinal cord damage, whereas timely medical and surgical care (ie, intensive care unit management, spinal cord decompression, and spine stabilisation) reduce the effects of secondary injury.[8,9] Ideally, acute management would be seamlessly followed by neurorehabilitation, with the aim to maximise neuroplastic reorganisation and residual functions by learning adaptation and compensation strategies.[10] Beyond initial injury severity, only a few reliable predictors of long-term neurological outcome exist, and current management strategies have poor effectiveness. For example, there are no pharmacological or non-pharmacological interventions that enhance the extent of neurological repair from acute spinal cord injury.

Therapies based on transplantation of cells (ie, mature cells) or stem cells (ie, undifferentiated cells or partly differentiated cells that can differentiate and proliferate) are among the most promising strategies for the treatment of spinal cord injury. These therapies have the potential to support repair in several injury phases,[11] and have produced functional improvement in animal models of spinal cord injury.[12] Most studies in animals have entailed transplantation of cells directly into the injured spinal cord, a technique that has previously been considered a major methodological hurdle in human spinal cord injury. However, several clinical trials have provided initial evidence for the feasibility and safety of intraspinal cell transplantation.[13–18] In addition to specific issues related to cell transplantation, several methodological issues exist that impede the success of clinical translation. First, the inherent variability of current standardised outcome measures make small effects difficult to detect in a reproducible way. Second, limitations of conventional designs of studies on rare disorders with high variability of injury conditions hamper the disentanglement of spontaneous recovery and therapeutic effects. Finally, shortcomings in previous study designs, such as underpowered futility analyses, have contributed to preliminary termination of clinical trials.[19]

In this Review, we describe and discuss results from studies of intraspinal cell transplantation, and we highlight obstacles overcome thus far and those that remain. With regard to the challenge of interpreting recovery trajectories, we also summarise the current standards in assessment and prognosis in spinal cord injury, then discuss design considerations and potential avenues for future cell-based trials.

Rationale for use of cell-based treatments

Spontaneous recovery from spinal cord injury is rare, possibly because of several inhibitory modulators, such as extracellular matrix proteins, which attenuate regeneration and plasticity and reduce endogenous repair of the injured cord.[20] Interventions to enhance recovery in spinal cord injury aim to reduce the spread of secondary injury (through neuroprotection, or by modulation of inflammation) and replace lost neural cells and disrupted

neural circuitry (through neural plasticity and regeneration; figure 1). Several transplantable cell types could be useful in the subacute to chronic stages of spinal cord injury, because of their potential to form myelin, promote and guide axonal growth, and bridge the site of injury.[12]

Studies in animals have elucidated crucial windows of opportunity for neuroprotective interventions and interventions promoting plasticity. Neuroprotective treatments must be delivered acutely within hours or a few days after spinal cord injury, to amend ongoing secondary injury, whereas amplification of neural plasticity is possible for weeks after injury.[21] Previously established feasibility and safety profiles of cell-based and stem-cell-based treatments[22] are a major milestone for the field of spinal cord injury and have laid the foundation for forthcoming trials focusing on effectiveness.

Outcome measures for cell-based treatment trials

Many different outcome measures can be used to assess cell-based therapies in clinical trials (figure 2). Neurological impairment after spinal cord injury is classified according to the International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI), published by the American Spinal Injury Association (ASIA) and the International Spinal Cord Society (ISCoS).[23] Although the ISNCSCI has shown high intra-rater and inter-rater reliability, and content and construct validity,[24] limitations due to non-linear measurements reduce its sensitivity for use in clinical trials.[25] In addition to the neurological examination, autonomic function should be assessed in every patient with spinal cord injury.[26] Standardised neurophysiological measures, including motor evoked potentials and sensory evoked potentials, complement the assessment of spinal cord integrity, enable the detection of pre-existing or secondarily developing neural impairment independent of spinal cord injury,[27] and reveal residual spinal cord sparing below the level of clinical detection.[28] Neuroimaging measures (eg, using MRI) can be used to provide information on the type and extent of cord damage and the extent of preserved tissue bridges.[29,30] Functional outcome measures, such as the Spinal Cord Independence Measure, complement neurological assessments and enable clinically meaningful changes to be interpreted.[31,32] These different outcome measures should be included in interventional studies, to provide increased sensitivity towards clinical, functional, and structural changes over time, and maximise the clinical applicability of the findings.

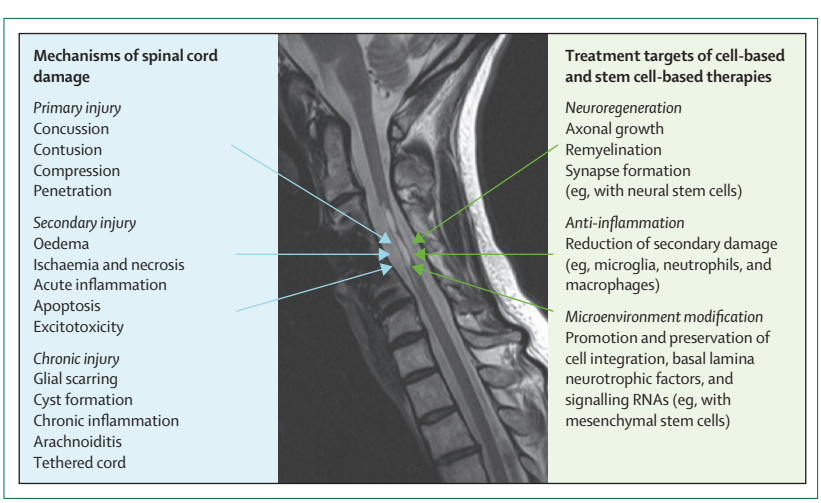

Figure 1: **Mechanisms of spinal cord injury (left) and cell-based and stem cell-based approaches to treatment (right)**
The MRI shows the location and extent of lesion, the development of a post-traumatic cyst, and the presence of preserved dorsal tissue bridges.

Feasibility and safety of cell-based treatments

13 cell transplantation studies were identified by our search strategy, including six that entailed intramedullary injection and seven that involved intrathecal or intravenous routes of administration. Intrathecal and intravenous cell therapies (mostly with bone marrow-derived stem cells) are not discussed in detail, because the focus of this Review is the intramedullar application of cells—an approach that allows a focal and high-volume delivery of cells to the immediate site of spinal cord injury (table).

Five of the six trials defined feasibility and safety of cell-based treatments as their primary or co-primary outcomes,[13–17] comprising protocol compliance, stability of neurological function, study retention, or adverse events. Two of these five trials also assessed preliminary efficacy measures as co-primary outcomes, using the ISNCSCI.[13,16,33] In the sixth study, only efficacy parameters defined according to the ISNCSCI were assessed as the primary outcome (table).[18] Although feasibility and safety of cell-based treatments were shown in the five studies that assessed those outcomes, none of the trials assessing efficacy reached that outcome.

The strengths of these six trials include their well characterised study populations spanning all phases of spinal cord injury, predefined and meaningful outcome measures, and a follow-up period that was of sufficient duration to capture long-term adverse events such as tumorous growth (ie, minimum 1–2 years, with one study >6 years). Additionally, the clinical trial designs provided high levels of evidence (ie, they were multicentre and controlled studies).

Despite these strengths, some limitations of the studies warrant attention. Efficacy outcomes were underpowered and findings could not be replicated independently because the six studies used different types of cells and stem cells. Although clinical assessments used in the trials were standard tests, the rehabilitation procedures used in each trial were not harmonised across participating centres of different studies. Moreover, imaging protocols were not standardised, and included different modalities. Additionally, dosage of cells was adapted to test safety, not efficacy. Finally, cells were implanted as a suspension

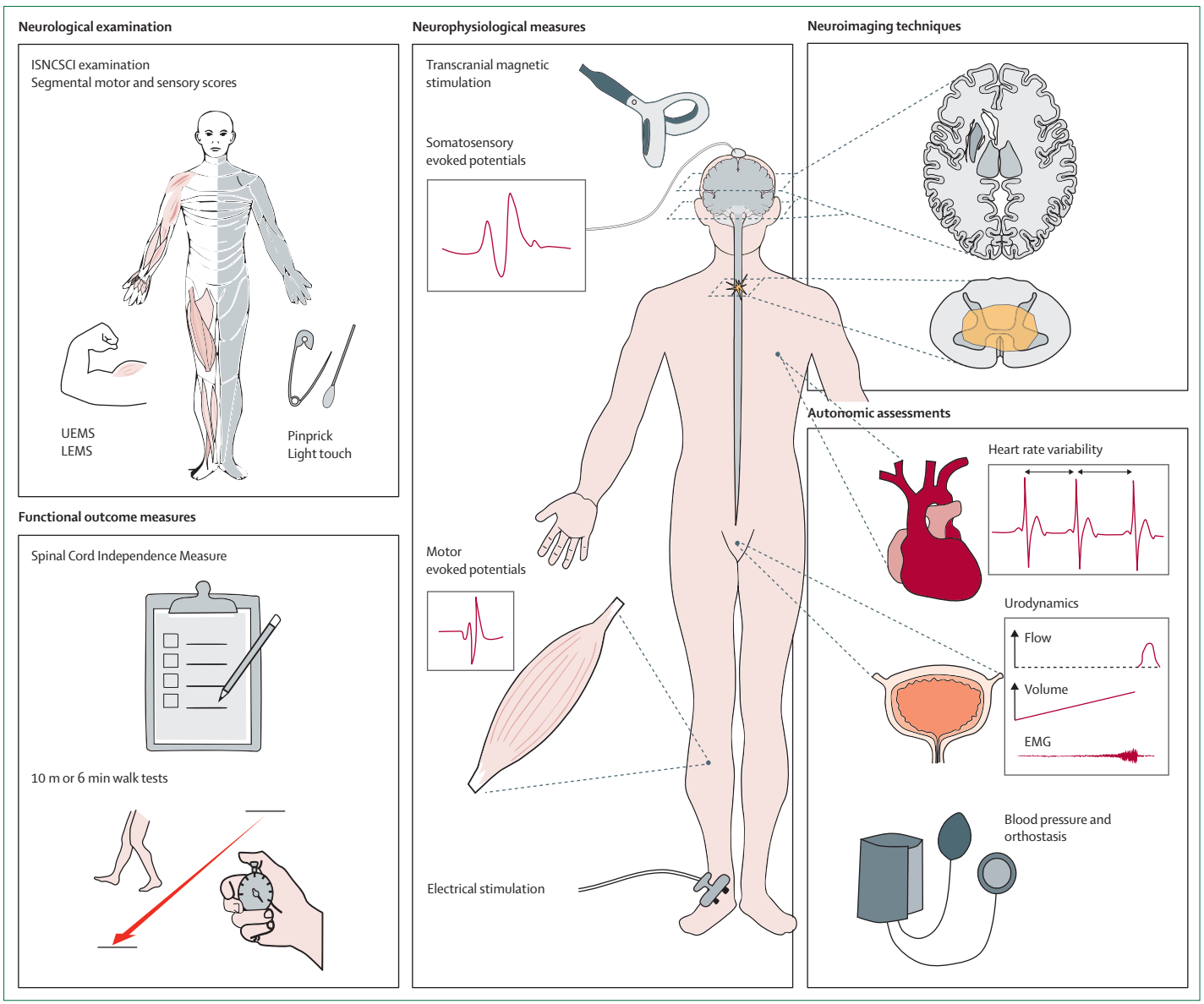

Figure 2: **Diagnostic assessments after spinal cord injury**
Outcome measures can be categorised into neurological examination, neurophysiological measures, autonomic assessments, neuroimaging techniques, and functional outcome measures. The star on the spinal cord indicates the area of spinal cord lesion. Neuroimaging techniques measure the immediate lesion area and secondary remote neural changes. EMG=electromyography. ISNCSCI=International Standards for Neurological Classification of Spinal Cord Injury. LEMS=lower-extremity motor score. UEMS=upper-extremity motor score.

volume, with no attempt made to target cell migration or structural repair of the lesion site.

Neural stem cells

In the 1990s, discovery of adult neural stem cells in the human brain, and their suitability for cell culture, initiated a new direction for cell therapy.[34] Neural stem cells are multipotent, can mature into different cell types, and therefore might be able to promote tissue regeneration and support the formation of new circuits in the injured spinal cord.[35,36] Evidence suggests that transplanted neural stem cells predominantly differentiate into oligodendrocytes and promote remyelination.[37]

In the Swiss-Canadian multicentre, open-label, controlled phase 1/2a trial of neural stem cells by Curt and colleagues,[13] human fetal stem cells derived from the CNS were administered to 12 patients with traumatic, chronic, motor-complete, thoracic spinal cord injury. The mean age of the patients was 33 years, and the time since injury was 5–24 months. Divided into four separate intramedullary microinjections, 20 million cells were injected above and below the lesion level under ultrasound guidance in an open neurosurgical procedure. In

	Location	Participants	Study design and phase	Study concept	Study product and intervention	Immunosuppression	Primary outcome	Main findings
Treatment 4–12 weeks after spinal cord injury								
Anderson et al (2017)[17]	USA	Six individuals with subacute thoracic AIS grade A spinal cord injury	Single centre, open-label, uncontrolled phase 1	Lesion bridging, enhanced axonal growth, and remyelination	Schwann cells, intralesional and acute inpatient rehabilitation	Not administered	Safety	Safety: no safety concerns (assessed by clinical examinations and 12 month follow-up MRI) Efficacy: improvement from AIS grade A to B for one of six patients at 6 months; some positive connectivity on electrophysiology for all patients
Treatment 4–12 months after spinal cord injury								
Curt et al (2020)[13]	Switzerland, Canada	12 individuals with subacute or chronic thoracic AIS grade A or B spinal cord injury	Multicentre, open-label, controlled phase 1/2a	Remyelination and enhanced axonal growth	Neural stem cells, intramedullary	Tacrolimus started pre-transplantation for 9 months, mycophenolate mofetil for 1 month, dexamethasone for 5–10 days	Safety and preliminary efficacy	Safety: surgery-related adverse effects, ie, CSF leak and pseudomeningocele; no further safety concerns over the following 6 years (assessed by follow-up clinical examinations and MRI) Efficacy: segmental sensory improvement in five of 12 patients, with accompanied neurophysiological correlates
Levi et al (2018)[14]	USA, Canada	29 individuals with subacute or chronic cervical or thoracic AIS grade A or B spinal cord injury	Multicentre, single-blind, controlled phase 2	Remyelination	Neural stem cells, intramedullary	Tacrolimus and trimethoprim plus sulfamethoxazole started pre-transplantation for 6 months, mycophenolate mofetil for 1 month, dexamethasone for 7 days	Safety and preliminary efficacy	Safety: surgery-related adverse effects, ie, CSF leak and wound infection; follow-up MRI up to 1 year did not reveal adverse effects Efficacy: upper-extremity motor score (UEMS) and Graded Redefined Assessment of Strength, Sensibility, and Prehension (GRASSP) improved by the time of the interim analysis, but at a magnitude below the defined clinical efficacy threshold
Treatment 1–10 years after spinal cord injury								
Curtis et al (2018)[15]	USA	Four individuals with chronic thoracic AIS grade A spinal cord injury	Single centre, open-label, uncontrolled phase 2	Axonal sprouting	Neural stem cells, intramedullary	Basiliximab pre-transplantation and on day 3 or 4, tacrolimus and mycophenolate mofetil post-transplantation for 3 months	Safety	Safety: no safety concerns (assessed by clinical examinations and 12–24 month follow-up MRI) Efficacy: segmental sensory improvement in two of four patients with accompanied neurophysiological correlates
Oh et al (2016)[18]	South Korea	16 individuals with chronic cervical AIS grade B spinal cord injury	Single centre, open-label, uncontrolled phase 3	Cell proliferation and immunomodulation	Bone marrow-derived mesenchymal stem cells, intramedullary and 1 month standardised rehabilitation	Not administered	Efficacy	Safety: no safety concerns (assessed by clinical examinations and 12 month follow-up MRI) Efficacy: neurological improvement in two of 16 patients
Gant et al (2022)[16]	USA	Eight individuals with chronic cervical or thoracic AIS grade A–C spinal cord injury	Single centre, open-label, uncontrolled phase 1	Myelin repair, lesion bridging, and enhanced axonal growth	Schwann cells, intracavitary and pre-transplant and post-transplant fitness training	Not administered	Safety	Safety: no loss of neurological function and no abnormalities on serial MRI examinations (assessed by clinical examinations and 6–24 month follow-up MRI) Efficacy: motor score improvement in one patient, some reduction in cyst volume on MRI

Studies are presented in order of best timing of treatment. AIS=American Spinal Injury Association Impairment Scale.

Table: **Intramedullary cell-based and stem cell-based clinical trials, study characteristics, and main findings**

the first year after transplantation, two severe adverse events were reported (namely, CSF leak and pseudomeningocele), and nine adverse events were related to the transplantation (comprising 9% of total adverse events). Mild to moderate adverse events related to immunosuppression (eg, headache) accounted for 12% of adverse events in the first year of the study. The type and incidence of other adverse events was similar to those reported in a control sample with spinal cord injury (eg, neuropathic pain and urinary tract infection). 12 month, 24 month, and 36 month follow-up MRI scans did not show evidence of additional cord injury or tumorous growth up to 6 years after stem cell transplantation. Segmental sensory improvements were noted in six patients, which were associated with improved electrical and heat perception thresholds, suggesting biological activity from the transplanted neural stem cells.

The North American safety trial of neural stem cells by Levi and colleagues[14] enrolled 29 patients with thoracic (n=12) and cervical (n=17) traumatic, motor-complete spinal cord injury. Age of patients and time since injury were similar to those of the patients in the Swiss-Canadian trial by Curt and colleagues.[13] For transplantation of human fetal stem cells derived from the CNS, spinal cord tissue was exposed and cells were injected above and below the injury site using a hand-held syringe under ultrasound guidance (four to eight injection sites, depending on number of cells administered). Approximately 20 million cells were administered to the 12 patients with thoracic spinal cord injury (the thoracic outcomes were not published). The cervical cohort was divided into two treatment groups. The first group (n=6) was an open-label dose-escalation cohort: a dose of 15 million cells was given to two patients, 30 million cells to two other patients, and 40 million cells to the last two patients. The second group (n=6) was part of a single-blind, randomised controlled trial, in which 40 million cells were transplanted in every patient; an untreated control group (n=4) was included in this study for comparison. In terms of safety, no specific concerns were identified related to the cell transplant or the intramedullary injection technique in both thoracic and cervical cohorts. With respect to clinical outcomes in the cervical cohort, two of six patients showed remarkable improvements in fine motor function when assessed using the Graded Redefined Assessment of Strength, Sensibility, and Prehension (GRASSP), but these improvements were not sustained at 12 months.[33,38] By contrast with some evidence from animal studies, neuropathic pain was not aggravated after cell transplantation.[39]

The Swiss-Canadian trial[13] and the North American trial[14] both included an immunosuppression regimen consisting of tacrolimus, mycophenolate mofetil, and dexamethasone (table). These regimens might also have affected the ongoing immunological dysfunction in patients with chronic spinal cord injury (ie, chronic inflammation and immune suppression).[40] However, no severe adverse events related to immunosuppression were observed, supporting the feasibility of this strategy in the context of cell therapies.

Additional evidence for the safety of intramedullary neural stem cells has been provided by an uncontrolled, small, single-centre case series from the USA.[15] Using a free-floating stereotactic cannula attached to a device for precise 3-axis manipulation, neural stem cells were administered at six perilesional areas to four young adult patients (aged 24–35 years) with chronic, complete, thoracic spinal cord injury. No severe adverse events related to the transplantation were reported. Two patients had a one-level sensory improvement, with sensations that could be perceived one spinal level below the initial sensory level as assessed by ISNCSCI, accompanied by newly detected electromyography activity in the abdominal wall. Although these findings are promising, no control group was included, and the sample size was small.

Schwann cells

For many years, the potential for Schwann cells to contribute to spinal axon regeneration and repair has been of interest.[41] In the 1980s, a study suggested that some spinal axons could regenerate into peripheral nerve grafts, providing enthusiasm to test the efficacy of Schwann cell transplantation for nerve repair.[42] Furthermore, reliable cell culture methods have enabled the preparation of autologous cell grafts, for which immune suppression is not needed.[17]

Schwann cells accompany axons throughout neurodevelopment. During nerve repair, these cells and their secreted basal lamina components create tubular bands through which axons regenerate, when enclosed by cytoplasm of non-myelinating Schwann cells, or contract, when enclosed by extracellular matrix.[43] Known ligands during this nerve repair process are binding of the RGD peptide of laminin to axonal L1-NCAM. Schwann cells undergo a shift from myelinating to non-myelinating phenotype that involves many changes in gene expression.[44] After nerve repair, Schwann cells are able to switch back to a stable myelinating subtype. One of the limitations of transplantation of Schwann cells into the CNS is the sparse integration of the cells with astrocytes and oligodendroglia, which in turn reduces the extent of axonal regeneration beyond the Schwann cell grafts.

A phase 1 clinical trial from the USA investigated the safety of intramedullary transplanted autologous Schwann cells in six patients with subacute, complete, thoracic spinal cord injury.[17] Cells were harvested from the sural nerve of each patient, preprocessed in vitro to ensure growth and health of the cells, highly purified (mean 97% purity [SD 2]), and transplanted 30–60 days after injury. The dose was escalated to assess dose-dependent safety, with 5 million cells transplanted in two patients, 10 million cells in two other patients, and 15 million cells in the last two patients. Cells were transplanted into the exposed injury epicentre using a

stereotactic syringe-positioning device under ultrasound guidance. Regarding safety, no adverse events specifically related to nerve harvesting or the transplantation procedure were reported. Documented adverse events were commonly related to spinal cord injury, such as urinary tract infection and decubital ulcers. The clinical improvements noted in the study—ie, from ASIA Impairment Scale (AIS) grade A to AIS grade B—were within the expected range for patients with thoracic spinal cord injury. Neurophysiological examinations showed subclinical improvements of motor cortical connections (motor evoked potentials and electromyography), particularly increased activity below the initial spinal level that injury was detected.[28]

In another phase 1 open-label trial, the safety of Schwann cells was investigated when injected specifically into the lesion cavity.[45,46] Eight patients with chronic, incomplete or complete, cervical or thoracic spinal cord injury were enrolled.[16] An enrolment criterion was that the estimated cavity volume was restricted to less than or equal to 2 cm^3. The injection volume varied between patients because it was tailored to fill the injury cavity with cell suspension. After the cell injections, MRI showed that the lesion volume in six of eight patients decreased on postoperative day 1. At 6 month follow-up, the reduced volume was only maintained in two patients and the survival of the injected cells was unclear.

Mesenchymal stem cells

Originally discovered in the 1970s in bone marrow cultures as adherent cells,[47] interest in bone marrow-derived stem cells as a potential therapeutic agent for numerous conditions has been increasing in the past few years. These cells release cytokines and exosomes that attenuate inflammation, and they have reduced immunogenicity when allografted. The assumed mechanism of action of mesenchymal stem cells in spinal cord injury involves an initial migration of mesenchymal stem cells into the injured cord, followed by a phenotypic change to exhibit a neural cell phenotype that allows the expression of factors promoting repair, instead of replacement of damaged cells.[48]

Autologous bone marrow-derived stem cells are cultured from bone marrow, which can be easily harvested from patients at bedside. Depending on how these stem cells have been administered in various spinal cord injury stages and types (intrathecally, intravenously, intra-arterially, or intramedullary), differences in efficacy were observed, ranging from substantial recovery to a weak effect or no effect.[11] Because the mechanisms of action of bone marrow-derived stem cells are less dependent on intralesional deposition than are those of other cell types, many studies have assessed intrathecal injections, which are simpler to perform compared with intramedullary injections.

In a phase 3 trial, the efficacy of bone marrow-derived stem cells injected above and into the injury cavity, and in the subdural space, was investigated.[18] 16 patients with traumatic, cervical, sensory incomplete spinal cord injury (AIS grade B, except for one patient with AIS grade A) were enrolled at least 12 months after injury. No adverse events associated with transplantation were reported. Efficacy of the cell transplant was low, since motor improvement in the upper extremity arms was only detected in two patients. In the remaining patients, no neurological improvement was noted at the 6 month follow-up visit.

Macrophages

Studies from the 1980s and 1990s have shown that debris removal by macrophages after CNS damage was lower and more delayed than debris removal by macrophages after peripheral nerve damage.[49] These findings led to experimental work in animal models that used peripheral nerve-activated macrophages in spinal cord injury aiming to render macrophages more beneficial for recovery.[50]

The approach of macrophage implantation was tested in 33 patients with acute cervical or thoracic AIS grade A spinal cord injury, who were enrolled in a phase 2, multicentre, clinical trial.[49] Compared with 17 controls, matched for age and sex, who received standard-of-care treatment after similar spinal cord injury, 26 patients who received macrophage therapy did not show any improvement in AIS grade, recovery in motor or sensory level or scores, and self-reported bowel and bladder function. Two severe adverse events were reported that were related to the intervention, which included laminectomy. After intervention, one patient was diagnosed with spinal instability, and the other patient developed pulmonary subsegmental atelectasis.

Olfactory ensheathing cells

Olfactory ensheathing cells are present from the nasal cavity to the olfactory bulb and support the growth of new axons throughout life. This cell type exhibits integration with astrocytes that is superior to that observed for Schwann cells; however, olfactory ensheathing cells are a heterogeneous cell population that expresses a myelinating phenotype in vivo only under specific conditions.[51] These properties of olfactory ensheathing cells led, in the 2000s, to a period of great enthusiasm for their preclinical and clinical testing. However, small amounts of source tissue make culture expansion difficult, and clinical studies have not shown their superiority to Schwann cells.[52] Owing to the scarce availability of source tissue, the use of nasal cavity olfactory mucosa for transplantation was explored. This procedure, however, was linked to serious complications in the past few years, in particular spinal tumour growth, that required surgery.[53,54]

Emerging cell types and strategies to improve cell integration

Although the feasibility and safety of intramedullary cell transplantation for spinal cord injury has been shown for

neural stem cells, Schwann cells, and bone marrow-derived stem cells, no evidence of their efficacy has been reported. This deficit is possibly related to the cells that were transplanted, which might not have survived given the hostile environment after spinal cord injury.[55,56] Patient selection based on imaging biomarkers, such as presence of tissue bridges or cyst volume, and individual recovery profiles (entailing lower-extremity motor score and age) could enhance efficacy in future trials. However, novel cell products or innovative approaches to cell integration might also increase efficacy.

The mechanisms of effect of potential new cell products for treatment of spinal cord injury will clearly vary, because many different cell types exist with distinct mechanisms of action. We anticipate that these mechanisms will either entail permanent integration of cells, as would be the case for functional CNS cells contributing to myelination or neuronal circuitry, or be a transient cell effect, which could result in enhanced repair via supply of trophic factors, modulation of the immune response, or restoration of the integrity of the blood–brain barrier. Bridging of the injury site with a single therapeutic approach has not yet been achieved in clinical trials, and combined approaches (eg, with biomaterials, cells, and neurotrophic factors) might be expected to be more efficient than a single therapeutic approach to bridge the lesion site.[12] Current methods for cell implantation have not yet enabled a cellular organisation that can reconstruct tracts or emulate grey matter–white matter organisation.[57] In some cases, transient engraftment of transplanted cells could be sufficient to improve locomotor or sensory function. In other cases, long-term integration of differentiated cells might be required for repair.

Based on essential mechanisms of repair, many future directions are possible from which to address efficacy. First, many studies have assessed the effects of ex-vivo genetically transduced cells delivered as cell suspensions on axonal responses.[58] Polysialylation has been shown to increase the migration and integration of transplanted Schwann cells.[59] Moreover, this post-translational modification has been shown to amplify the delivery of glial cell line-derived neurotrophic factor, which allows astrocytes to enter Schwann cell grafts and enhances the integration of transplanted cells.[60,61]

Second, transplanted cell populations could be engineered to reduce or prevent allogeneic rejection,[62–66] thereby enhancing both initial and long-term cell survival. Because cessation of immunosuppression in individuals who have received cell transplants has been associated with improvement of neurological levels (ie, reverting back to the original levels) in conditions including both Parkinson's disease and spinal cord injury,[13,67] establishing an optimal immunosuppressive regimen is a pivotal factor that could be crucial for sustaining transplant efficacy. Although still in the early phases of clinical testing, individualised induced pluripotent stem cells might be less subject to immune rejection than might allogeneic transplants.[68]

Third, transplant efficacy also depends on the quality of engraftment, particularly survival and differentiation along specific cell lineages or subtypes within a lineage. For example, the immune molecule C1q is present at high concentrations when the integrity of the blood–brain barrier is compromised due to neurotrauma, including spinal cord injury, and it has been shown to mediate cell signalling through the CD44 receptor.[69] CD44 deletion in transplanted cells, and C1q blockade in the injury area, has been shown to restore the ability of human neural stem cells to both migrate and induce locomotor recovery.[69]

Fourth, biomaterials that can direct cell differentiation or circuit formation are an additional path through which the efficacy of cell transplantation could be enhanced.[70,71] For instance, polymeric bridges,[72] hydrogel scaffolds,[73,74] neurotrophin-3 releasing bioscaffolds,[75] and self-assembling nanoparticles[76] have been shown to increase spinal progenitor survival.

Finally, perhaps the most exciting future path is our expanding understanding of endogenous neural stem cells, which will open new therapeutic avenues, such as the reprogramming of endogenous spinal cord cells to drive repair. For example, findings have shown spinal cord injury-induced neurogenic reprogramming of the NG2 (neuron-glial antigen 2) cell population with SOX2.[77]

Surgical considerations

Cell-based therapies have been administered at regions of maximal injury during different phases after spinal cord injury, by either intralesional injections of cell suspensions or injections into the adjacent spinal cord parenchyma. Surgical planning needs to account for the natural course of spinal cord injury lesion morphology, including variable extension of oedema initially, and cyst formation over time. Cysts are fluid-filled cavities that develop in the lesion area after cell degradation and can have either simple or complex (ie, septated) morphology.

Determining the injury epicentre during dorsal spinal cord surgery can be difficult. Intraoperative ultrasound can greatly help in locating the injury epicentre and in accurate visualisation of the injected cell suspension (figure 3). With spinal instrumentation in place, it is best to limit the number of additional surgical exposures, to avoid interfering with the progress of spinal fusion; however, the removal of rods or crosslinks might be necessary. Often, MRI is used as a guide to identify the epicentre of the spinal cord injury; however, the injury region structure can be altered by dural opening and by loss of cell adhesion. Complex cysts might not be easily identified by standard MRI. Additionally, these septated cysts can be quite stiff, as can be determined when injecting into the injury cavity. Unlike the tissue rim, septated cysts can be complex and not optimally oriented for linear axonal growth from the rostral side to the

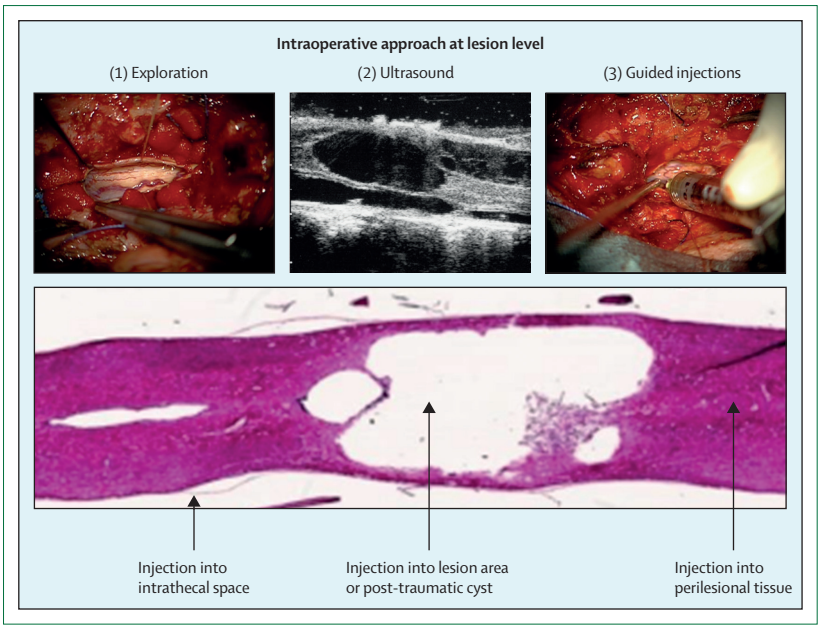

Figure 3: **Intraoperative photos showing ultrasound-guided injections into the injured spinal cord (top), and areas suitable for intraspinal injections (bottom)**

caudal side of the injury. Furthermore, the cysts might not communicate with each other, complicating intralesional injections, because multiple injection sites would be required to administer enough cell volume into the lesion sites. Injection into the injury epicentre is much easier before cyst maturation.

See Online for appendix

Additionally, the choice of surgical procedure, and the resulting clinical outcome, might be affected depending on the distribution of cysts and the location of tissue bridges,[29] if present, which, in MRI scans, are preserved tissue located dorsally or ventrally to post-traumatic cysts (figure 4).[30] Despite these challenges, a controlled intraparenchymal injection is needed if the treating clinician has concerns about aberrant biodistribution of cells. The principles of spinal cord injection of cell suspensions include controlled delivery rate, small volumes, prevention of haemorrhage, and minimal movement of the injection needles.

Design considerations for future cell-based trials

Prediction of outcome

Measures of spinal cord injury prognosis are very important when designing clinical trials. The transition from acute to chronic traumatic spinal cord injury is highly variable—some individuals gain an extensive amount of sensory and motor function in the initial weeks to months after injury, whereas others show very little improvement.[78] Major neurological recovery, as measured by ISNCSCI, is typically observed within the first 6 months after injury, then it plateaus, leaving individuals with permanent neurological deficits. Because of these variable recovery profiles, the prediction of neurological recovery is challenging.

Only a few reliable predictors of recovery exist, such as the initial severity of injury (eg, assessed by AIS) and the neurological (ie, spinal) level of injury. However, even individuals with seemingly identical initial injury severities can recover very differently. Typically, more severe (complete) injuries and thoracic injuries have poorer prognosis for recovery than incomplete injuries and cervical injuries.[10] Other factors that could predict recovery include age at injury,[79] acute care management (eg, early surgical decompression and blood pressure regulation),[9,80] comorbidities and adverse events after spinal cord injury,[81] and medications administered to treat secondary complications (eg, gabapentinoids for neuropathic pain).[82] In addition to these factors, emerging MRI-derived biomarkers might allow for better prediction of clinical outcomes.[30] Lesion morphology can vary from patient to patient, and it dynamically evolves during the natural course of spinal cord injury (figure 4).

Trends in study design

Clinical trials of novel cell-based and stem-cell-based therapies for spinal cord injury are affected by variability in spontaneous neurological recovery, which can obscure treatment effects. The field has substantially progressed in understanding recovery profiles after acute spinal cord injury, due to the initiation of large, ongoing, prospective observational studies in international clinical networks, including the European Multicenter Study about Spinal Cord Injury (EMSCI), the Rick Hansen Spinal Cord Injury Registry (RHSCIR), the US Model Systems, and the North American Clinical Trials Network (NACTN) (appendix). Future cell-based and stem-cell-based therapies will need to account for heterogeneity in spontaneous neurological recovery among patients, despite the variability of recovery profiles being consistent across studies in different regions worldwide.[83] To this end, techniques to identify homogeneous subgroups, such as recursive partitioning and cluster analyses,[84,85] will be useful in the design of future trials (eg, to define inclusion and exclusion criteria or injury-specific tailored outcome definitions), and in predicting people who might respond to cell-based treatments.

Another important design consideration is the determination of a minimally clinically important difference (MCID) in spinal cord injury.[86] Even if studies show statistical significance, such findings might not necessarily translate into meaningful functional gains. Moreover, the MCID might differ between individuals with spinal cord injury—eg, an improvement of 5 motor score points on the ISNCSCI could be clinically meaningful for a high cervical injury, whereas the same number of motor points recovered in a thoracic injury might not correlate with meaningful functional recovery. Because the field has no landmark clinical trials on which to benchmark benefits from novel interventions, the MCID for cell-based trials will rely on comparisons with historical progression studies, patient input, and clinical

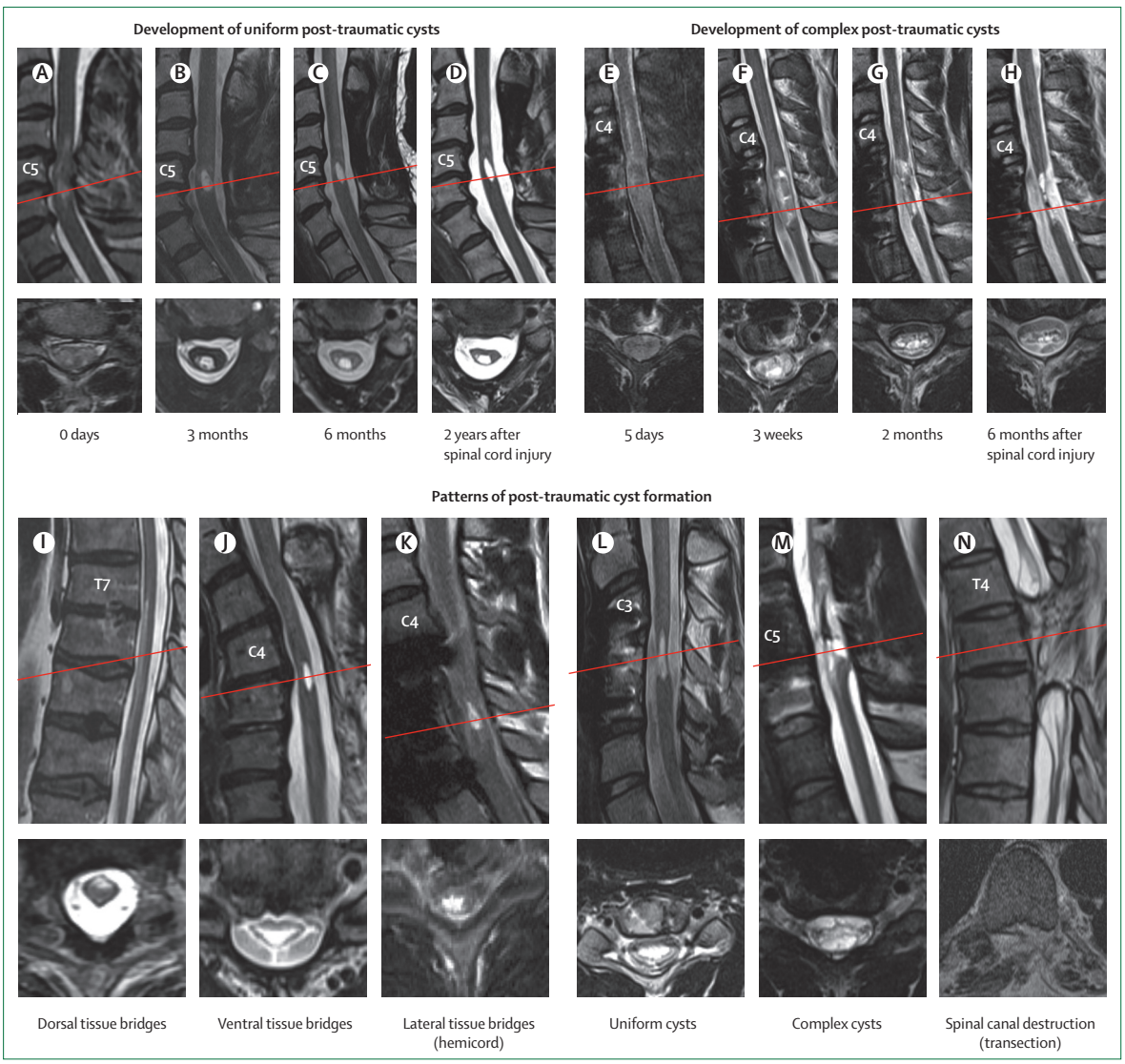

Figure 4: **Chronic cyst evolution after acute lesion (top), and patterns of cyst formation in spinal cord injury (bottom)**
Cervical MRI of two patients with spinal cord injury (A–D and E–H) shown over a period of several weeks or months, and patterns of chronic cervical cyst formation (J, K, L, and M) and thoracic cyst formation (I and N) observed in MRI scans of different patients. MRIs show correlates of secondary damage (eg, oedema) in the acute stages of spinal cord injury (A and E), and post-traumatic cysts in the chronic stages (B–D and F–H), developing within months or years. The presence of tissue bridges might contribute to the prediction of clinical outcomes (I–K). Cysts can be uniform (L) or complex (M), and complete cord transection is possible (N).

experience, with the caveat that this perception could change depending on the time since injury. The MCID will also need to account for the risks and costs of the cell-based treatments, because a small improvement might be acceptable for a low-risk cell-based therapy, whereas a high-risk therapy might require larger gains in neurological function. Thus, the MCID could be tailored on the basis of specific injury (or other prognostic) characteristics. Finally, multimodal assessment of outcome measures will be important in the design of future clinical trials. For example, in addition to ISNCSCI examinations, neurophysiological techniques, such as motor evoked potentials and sensory evoked potentials, can also be used to track progression.

Conclusions and future directions

For decades, the field of spinal cord injury has stalled because of difficulties with translation of experimental work to patients and inconclusive results from early-phase clinical trials. Many years ago, both the feasibility and safety of cell transplantation into the spinal cord were considered a major barrier for cell-based therapies for spinal cord injury. However, the safety and feasibility of cell and stem cell administration into the injured spinal cord have now been shown in multiple international studies. Although this evidence is a key milestone in the field of spinal cord injury, efficacy has not yet been proven. To this end, clinical efficacy could be achieved through use of more effective cell types and

Search strategy and selection criteria

We searched PubMed, Web of Science, and Scopus for original research articles published in English between Jan 1, 2016, and Oct 31, 2021, using the search string "spinal cord injury" AND "stem cells" OR "stem cell therapy" OR "cell therapy" OR "cell transplantation" NOT review [Publication Type]. From this search, we only included clinical trials. Additionally, we included seminal clinical studies published before Jan 1, 2016, if deemed relevant to the scope of this review, and if the associated methods and results sections were described in sufficient detail.

transplantation strategies, to achieve lesion remodelling and bridging, to improve methods that reduce immune rejection, and to create and stabilise useful circuits. Based on current safety data, emerging cell and bioengineering technologies can also be more rigorously translated to human spinal cord injury. Progress in the understanding of recovery profiles and lesion morphology could facilitate the interpretation of efficacy measures. Eventually, cell therapies will need to be tailored to the individual patient depending on the condition of spinal cord injury, stage, and expectations of the injured individual. The current gold standard in management of spinal cord injury, including timely surgery, acute medical care, neurorehabilitation, and lifelong care specific for spinal cord injury, must be pursued in every patient undergoing experimental cell therapies.

Contributors

CMZ and AC conceptualised the Review. All authors contributed to the literature search. AC supervised the interpretation of study findings. CMZ, JJC, CRJ, and AC contributed to the design of the table and figures. CMZ and AC wrote the original draft of the manuscript. All authors contributed to the writing and editing of the Review.

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