

# Therapeutic interventions after spinal cord injury

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**Abstract** | Spinal cord injury (SCI) can lead to paraplegia or quadriplegia. Although there are no fully restorative treatments for SCI, various rehabilitative, cellular and molecular therapies have been tested in animal models. Many of these have reached, or are approaching, clinical trials. Here, we review these potential therapies, with an emphasis on the need for reproducible evidence of safety and efficacy. Individual therapies are unlikely to provide a panacea. Rather, we predict that combinations of strategies will lead to improvements in outcome after SCI. Basic scientific research should provide a rational basis for tailoring specific combinations of clinical therapies to different types of SCI.

## Autonomic dysreflexia

A life-threatening condition defined as a sudden and severe increase in blood pressure and a simultaneous decrease in heart rate induced by a noxious stimulus below the level of injury. Individuals with an injury at T6 or above are at risk of developing this condition.

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Worldwide, an estimated 2.5 million people live with spinal cord injury (SCI), with more than 130,000 new injuries reported each year (see [International Campaign for Cures of Spinal Cord Injury Paralysis](#) in Online links box). There are no fully restorative therapies for SCI as yet and so prevention (for example, effective seat belts, weapons restrictions and safety in sports) is the best medicine (see [Foundation for Spinal Cord Injury Prevention, Care and Cure](#) in Online links box). SCI has a significant impact on quality of life, life expectancy and economic burden, with considerable costs associated with primary care and loss of income. In one study, quadriplegics ranked recovery of arm and hand function as a priority, whereas paraplegics rated recovery of sexual function as most important (when measured against recovery of bladder/bowel function, and eradicating autonomic dysreflexia, improving walking movements and trunk stability, regaining normal sensation and eliminating chronic pain)<sup>1</sup>. Therapies addressing these and other important priorities (such as recovery of cardiovascular performance and skeletomuscular properties, and reducing spasticity) have been reviewed elsewhere<sup>2–6</sup>. Here, we focus on the recovery of limb function, which is the focus of most ongoing animal studies and clinical trials for treatments for SCI.

To identify therapies that are unambiguously safe and effective, the scientific and clinical SCI communities increasingly recommend that preclinical studies be reproduced by independent laboratories, and that clinical trials have particular design features. These include an *a priori* unambiguous definition of primary outcome measures and any intended stratification of groups, and the use of methods that are sensitive enough to detect

potentially small increments in function<sup>7–9</sup>. Several pre-clinical studies are now being evaluated independently under contractual arrangements between the National Institute of Neurological Disorders and Stroke (NINDS) and several Facilities of Research Excellence for SCI (FORE-SCI; see [NINDS Facilities of Research Excellence in Spinal Cord Injury](#) in Online links box), including the Miami Project to Cure Paralysis and the Reeve–Irvine Research Centre. For more information on clinical trials, readers are directed to governmental and international consensus statements, and to a description of how US Food and Drug Administration regulatory processes relate to the standing of one SCI drug (see [NINDS workshop on translating promising strategies for spinal cord injury therapy](#) in Online links box)<sup>10–12</sup>.

Here, we stress various cellular and molecular strategies that are supported by more than one peer-reviewed animal experiment and that result in functional improvements after SCI; many of these strategies have reached, or are approaching, clinical trial. Each of the potential therapies described below might produce only small improvements, and a combination of therapies could be needed to improve everyday quality of life. Speciality journals and general audience media need to set reasonable expectations of the safety and efficacy of potential therapies to avoid raising and then dashing the hopes of those living with SCI or those in government, those carrying out research, or the general public.

## Endogenous response to SCI

The normal architecture of the human spinal cord (FIG. 1) can be radically disrupted by injury. SCI is heterogeneous

in cause and outcome<sup>13,14</sup> and can result from contusion, compression, penetration or maceration of the spinal cord. SCI leads to the death of cells, including neurons, oligodendrocytes, astrocytes and precursor cells<sup>15</sup> (FIG. 2), and any resulting cavities and cysts may interrupt descending and ascending axonal tracts, although circumferential white matter is often spared. After the initial insult to the spinal cord, additional structure and function are lost through active secondary processes (for example, ongoing apoptosis of oligodendrocytes and loss of myelin<sup>16</sup>). Demyelinated axons are observed up to a decade after human SCI<sup>17</sup>, and the extent to which these axons survive unmyelinated or become remyelinated by central or peripheral myelin is a subject of ongoing investigation<sup>18</sup>. Resident and invading inflammatory cells (including neutrophils, microglia, macrophages and T cells) can have a range of destructive and reparative roles<sup>19</sup>. SCI culminates in glial scarring, a multifactorial process that involves reactive astrocytes, glial progenitors, microglia and macrophages<sup>20,21</sup>, fibroblasts and Schwann cells<sup>17,22</sup>. The scar is often oriented perpendicular to the neuraxis and appears impenetrable. The scar also contains secreted and transmembrane molecular inhibitors of axon growth<sup>23,24</sup>. Progressive expansion of the injury across more than one segment (syringomyelia) can also occur over months or years, sometimes proving fatal.

In contrast to these destructive events, commonly observed pathological features do indicate some spontaneous repair after SCI<sup>25</sup> (FIG. 2). Whereas there is little or no neurogenesis in the injured spinal cord, proliferation in the ependymal and peri-ependymal canal generates new precursor cells that exclusively differentiate into glial cells<sup>15,26–28</sup>. Limited axon sprouting does occur and lesions might even be spanned by trabeculae containing axon sprouts<sup>25,29</sup>. Sprouting is largely impeded by geometrical and molecular factors<sup>24,30</sup>, and few axons regenerate over long distances back to their original targets. However, various forms of cortical, brainstem and spinal plasticity occur that could contribute to limited compensatory recovery<sup>31,32</sup>. After SCI, new spinal circuits can bypass the lesion, including sprouting of injured corticospinal axons onto spared, long descending propriospinal tracts that increase connectivity with lumbar motor neurons<sup>33,34</sup>. Cortical sensorimotor areas can functionally rearrange<sup>31,33</sup> and, at the subcortical level, the rubrospinal system can reorganize and compensate for much of the function lost after corticospinal injury<sup>31</sup>.

Therefore, although there is some spontaneous repair after CNS injury, it is incomplete. Further recovery of function will require a combination of effective and safe therapeutic interventions (FIG. 3).

**Cellular therapeutic interventions**

Cellular transplantation after SCI has several aims: to bridge any cysts or cavities; to replace dead cells (for example, to provide new neurons or myelinating cells); and to create a favourable environment for axon regeneration.

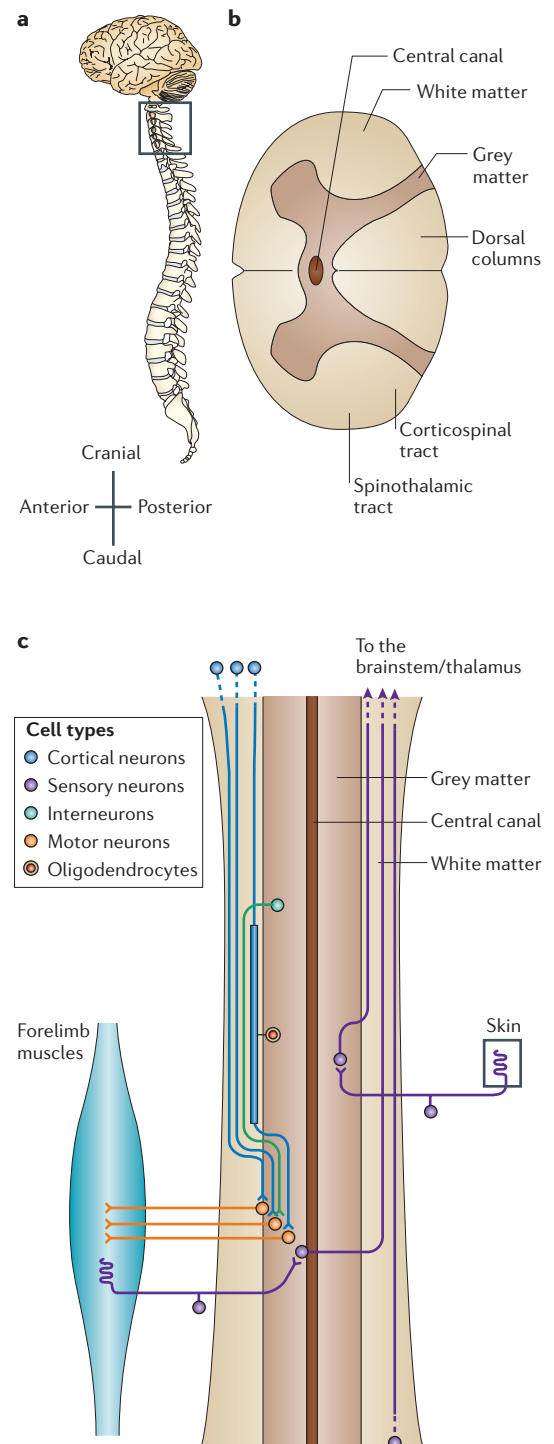


Figure 1 | **Intact spinal cord.** a | Schematic showing a sagittal view through the human CNS. b | Transverse section through human spinal cord showing the relationship between axonal tracts and grey matter. c | Cortical, brainstem and spinal axons project to motor neurons in spinal cord grey matter, which in turn send axons through the PNS to target organs, including muscle. Primary sensory axons send axons through the PNS to second order sensory neurons in the CNS grey matter, which, in turn, send axons through white matter in the dorsal columns to supraspinal regions. Oligodendrocytes myelinate ascending and descending axons.

**Apoptosis**

Controlled cell death, regulated by an intracellular programme of events.

**Trabeculae**

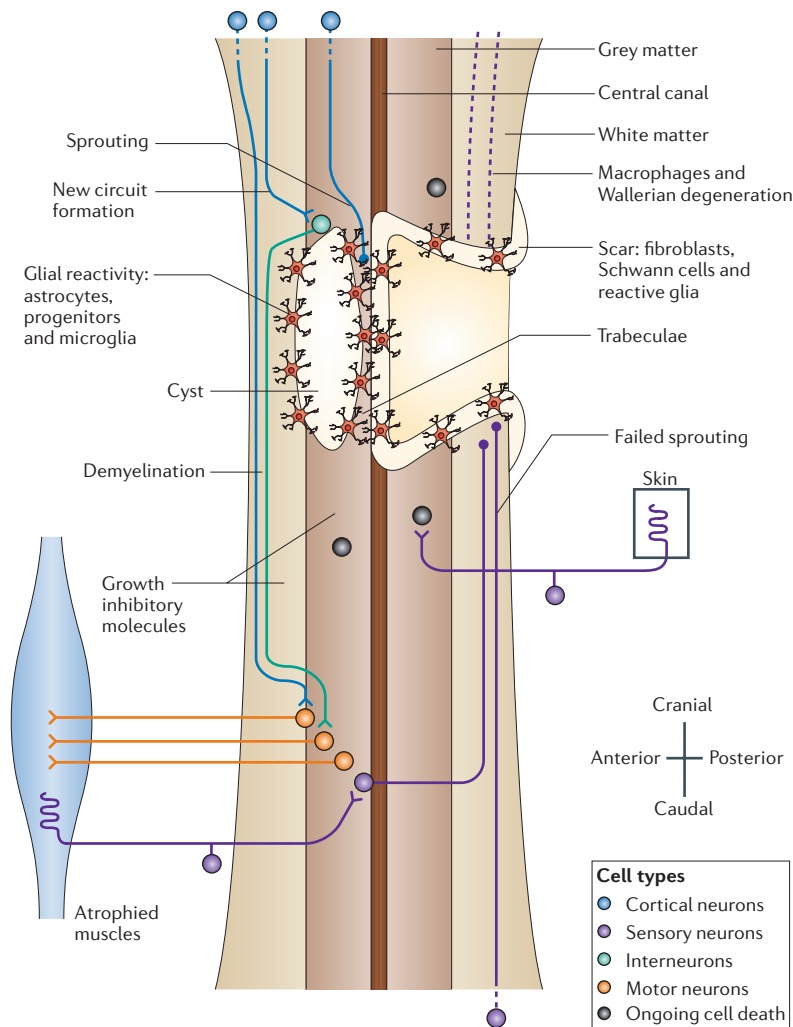
Strands of connective tissue that project into cysts or cavities.

**Plasticity**

Refers to adaptive changes in neurological function. The substrate could be anatomical (for example, collateral sprouting) or physiological (for example, changes in balance between inhibitory and excitatory transmission).

**Autologous transplants**

A transplant is autologous if the recipient also serves as the donor.



**Figure 2 | Spinal cord after injury.** Schematic showing a sagittal view through a region of cervical spinal cord injury (SCI), depicting a combination of features from different types of injury. Many cells die immediately, as well as progressively, after SCI. Cysts usually form after contusion injury. After penetrating injury, cells from the PNS often invade the injury site to form a connective tissue scar that incorporates astrocytes, progenitor cells and microglia. Many ascending and descending axons are interrupted and fail to regenerate over long distances. Some axons form new circuits with motor neurons via interneurons. At the site of cyst formation, axons can sprout into trabeculae that are formed from ependymal cells. Disconnected myelinated axon segments are phagocytosed by macrophages. Some spontaneous remyelination occurs, largely by PNS Schwann cells, whereas denervated (non-spastic) muscles atrophy.

**Olfactory lamina propria**  
A thick vascularized layer of connective tissue beneath the olfactory epithelium that contains nerve fibres wrapped by ensheathing glia.

**Transplantation of peripheral nerve.** After SCI in adult rats, autologous transplants of peripheral nerve were found to support ingrowth of various axonal types but not supraspinal axons<sup>35</sup>. Peripheral nerve grafts with various combinations of therapies (including anti-inflammatory drugs, vertebral wiring, fibrin glue and acidic fibroblast growth factor) promote recovery with regeneration of supraspinal axons into, through and beyond grafts<sup>36–39</sup>.

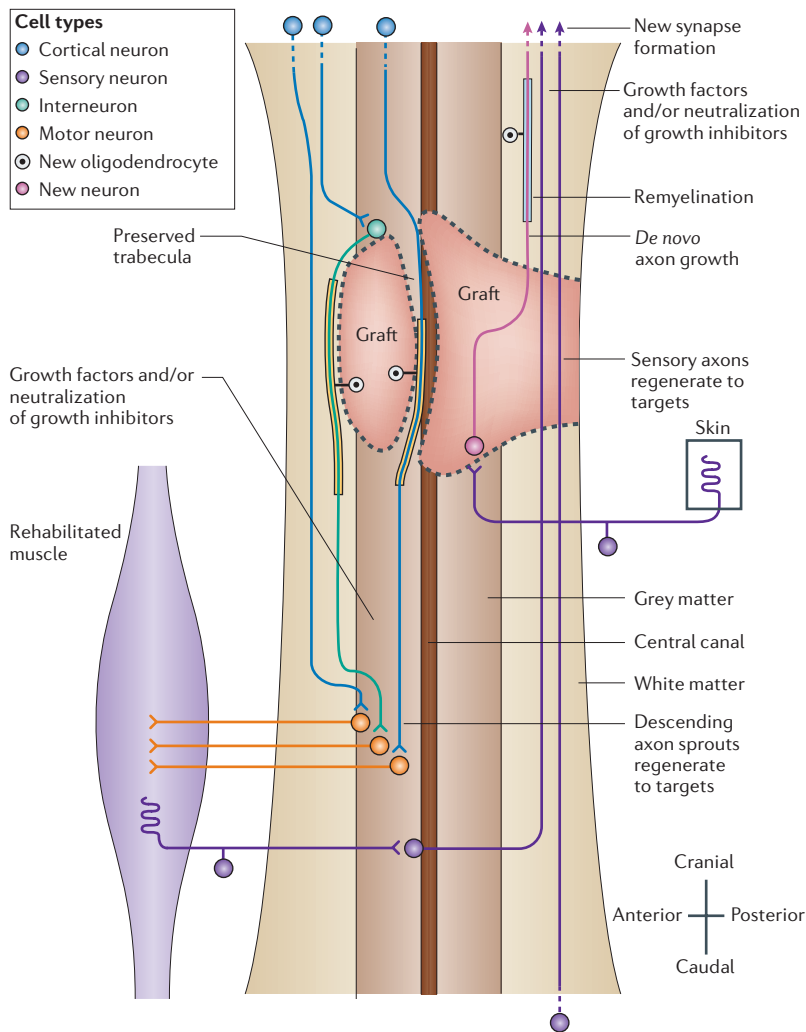
A similar strategy has been tested in non-human primates after lateral spinal hemisection<sup>40</sup>. No functional differences were detected but some spinal axons were found to have regenerated 4 months after injury. This

approach has also been used to treat chronic, incomplete human SCI, with one peer-reviewed report of limited functional recovery in a single patient; however, no control patients were investigated<sup>41</sup>. Anecdotally, this strategy has not proved successful in people with complete SCI<sup>8</sup>. Therefore, much work remains to be done to determine whether therapies that involve peripheral nerve bridge grafting can safely and effectively improve outcome after human SCI.

**Transplantation of Schwann cells.** Schwann cells from peripheral nerves have been transplanted into rat models of SCI, either being injected as suspensions after contusion injury<sup>42</sup> or implanted into channels containing extracellular matrix after lateral hemisection<sup>43</sup> or complete transection<sup>44</sup>. After transection and implantation of Schwann cells, sensory and spinal axons with cell bodies near the grafts extend into these bridge grafts, become myelinated<sup>44</sup> and are electrophysiologically active<sup>45</sup>: regenerating axons do not leave grafts distally to reinnervate the host. After contusion and implantation of Schwann cells, cavitation is reduced and sensory and spinal axons extend into grafts, and many are remyelinated<sup>42</sup>. Recovery of hindlimb function was also reported in some<sup>42</sup>, but not all<sup>46</sup>, studies. Consequently, combination therapies have been evaluated. After thoracic transection, increased regeneration of CNS axons beyond bridges has been reported in response to transplantation of Schwann cells with concomitant delivery of neurotrophins<sup>47,48</sup>, a steroid (methylprednisolone sodium succinate)<sup>49</sup> or olfactory ensheathing glia (OEG)<sup>50</sup>.

Human Schwann cells have also been transplanted into the transected spinal cord of rats with attenuated immune systems. In these rats, brainstem axons regenerated into grafts and spinal axons regenerated distal to grafts. Functional improvements were also reported, although weight-supported stepping was observed in only one rat<sup>51</sup>. Finding the most effective, safe and reproducible combination therapy involving Schwann cells remains crucial. One important step towards human clinical trials would be to test the safety and efficacy of transplanting autologous Schwann cells into non-human primates after contusive SCI<sup>52</sup>. So far, there have been no peer-reviewed reports of clinical trials involving the transplantation of Schwann cells after SCI.

**Transplantation of olfactory nervous system cells.** Cells from the embryonic and adult olfactory bulb or mucosa (FIG. 4) have been transplanted after SCI. Indeed, porcine, primate and human cells are now being tested in rodent and non-human primate models of SCI and demyelination<sup>53–57</sup>. Functional recovery and/or CNS axon regeneration has been reported when olfactory nervous system-derived cells are transplanted immediately or up to 2 months after SCI in adult rats<sup>58–65</sup>. After lateral cervical hemisection in adult rats, injection of cells from the olfactory bulb led to improvements in respiratory function and enhanced performance on a climbing task<sup>58</sup>. These transplants might also prevent loss of



**Figure 3 | Injured spinal cord after combination treatments.** Schematic showing a sagittal view through injured cervical spinal cord after a hypothetical combination of potential therapies. Cysts are filled by vascularized grafts and trabeculae are spared. Grafts provide remyelinating cells, and inhibitory molecules in the scar regions and in intact spinal cord are neutralized using antibodies, peptides or enzymes. Grafted neurons allow the formation of new relay circuits or the regeneration of injured axons back to their original targets. Furthermore, rehabilitation may allow correct synapses to be stabilized and reverses muscle atrophy.

neural tissue<sup>42,66,67</sup> and may enhance myelination after SCI<sup>68</sup>, although whether OEG directly myelinate axons after SCI remains controversial<sup>69</sup>. Transplants of cells from the olfactory nervous system do not, however, promote CNS axon regeneration and functional recovery under all circumstances<sup>42,67,70,71</sup>. FORE-SCI re-assessment of delayed transplantation of olfactory *lamina propria* after transection of adult rat spinal cord failed to find any improvement in hindlimb function, although some serotonergic axons were found in caudal spinal cord tissue<sup>72</sup>.

Transplants of cells derived from fetal olfactory bulbs or the adult mucosa have reportedly already been carried out in more than 400 humans in China, Portugal and Colombia<sup>8,73,74</sup>. Many procedures do not meet international standards for a clinical trial<sup>74</sup>, and, because controls are not included and comprehensive follow-up

studies have not been performed, it is difficult to gauge the safety and efficacy of this intervention, although there are reports of improvements in motor and sensory function<sup>8,73</sup>. One independent case report describes rapid segmental improvement in a single patient classified as having a ‘complete’ injury — according to the American Spinal Injury Association (ASIA) Impairment Scale (BOX 1) — who nonetheless remained neurologically quadriplegic<sup>75</sup>. An additional seven patients have been independently assessed pre- and post-operatively, with only complications (including meningitis), and no clinically useful improvements, being observed. The view was expressed that physicians should not recommend this procedure to patients<sup>74</sup>.

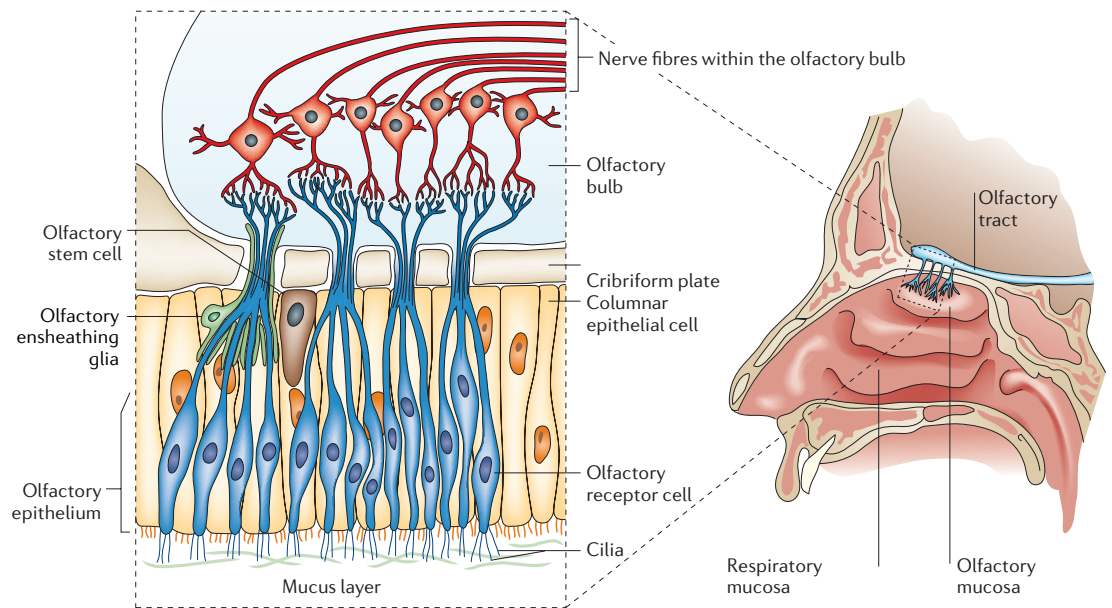
Elsewhere, formal veterinary and human clinical trials using cells derived from the adult olfactory nervous system are now advancing. SCI in dogs often occurs as a result of road traffic accidents or intravertebral disc extrusion, and presents an opportunity to test potential therapeutics in a large, heterogeneous patient population with variable injury severity<sup>76</sup>. Cells from the olfactory bulb have been transplanted autologously into nine dogs after naturally occurring thoracolumbar severe SCI, with no adverse events up to 2 years later<sup>76</sup>. Some hindlimb function was recovered (including weight-supported plantar steps) but, as the authors note, controls and blind testing are required in future trials.

In one Phase I clinical trial in humans, cells were collected from the adult human *lamina propria* and transplanted autologously into the spinal cord of three patients with thoracic injuries that had occurred at least 6 months previously<sup>54,77</sup>; three matched controls were included. No adverse consequences were reported in these patients after 1 year, although no details of the methods used for neurological assessment were reported: a 3-year follow-up study is planned. A large number of injections (240) were made and sensitive testing will be required to rule out the possibility that functional tissue has been damaged in these neurologically complete patients, particularly before applying this therapy to incomplete injuries.

It is necessary to establish whether there are conditions under which transplantation of cells from the olfactory nervous system works reproducibly to promote plasticity, regeneration, remyelination, neuroprotection and/or functional recovery. Important issues to be resolved include the optimal source of cells (*lamina propria* versus olfactory bulb), age of cells (embryonic versus adult) and graft strategy (for example, injection of suspensions or transfer within cellular matrix). It will also be important to determine whether enriching cultures for specific phenotypes of cells improves outcome<sup>58,69,78,79</sup>.

**Transplantation of embryonic CNS tissue.** After spinal cord transection in animal models and transplantation of fetal spinal cord into the lesion site, a small number of host axons regenerate into the transplant but terminate near the host transplant border<sup>80,81</sup>. Small but significant functional recovery is observed in rats<sup>82,83</sup> and cats<sup>84</sup>. This recovery is independent of long-distance growth into, through and beyond grafts, and the authors suggest that

**Phase I clinical trial**  
A clinical trial that includes a small number of human participants to determine the safety of a new drug or invasive medical device; allows the determination of drug dosage or toxicity limits.



**Figure 4 | The olfactory nervous system.** Schematic of a sagittal section through the human head, showing the olfactory nervous system (right), with a section of the olfactory nervous system depicted in greater detail (inset). Stem cells at the base of the olfactory epithelium generate new olfactory receptor neurons throughout life, which extend axons *de novo* to the olfactory bulb. These axons are wrapped by olfactory ensheathing glia as they pass through the *lamina propria* from olfactory mucosa and into the CNS via the cribriform plate<sup>69</sup>. Modified, with permission, from REF. 275 © (1996) TM Higher Education Group.

it is instead caused by the transplants acting as relays, affording transmission of signals via transplanted neurons, which are innervated by proximal host neurons and project in turn to distal host neurons. Grafts might also provide growth factors or improve conduction in spared axons<sup>85,86</sup>. When fetal spinal cord transplants are combined with neurotrophin delivery after complete spinal cord transection in adult rats, recovery of function is observed<sup>87</sup>, with some supraspinal and propriospinal axons growing into the caudal spinal cord<sup>86</sup>.

Intraspinal transplantation of fetal spinal cord has been tested in a clinical trial involving patients with syringomyelia<sup>88–90</sup>. No complications were observed and cysts were obliterated in all the patients. These trials have not led to improvements in the standard treatment for SCI or syringomyelia<sup>7</sup>, perhaps because of the difficulties associated with obtaining fetal tissue for transplantation.

**Transplantation of embryonic stem/progenitor cells.** Multipotent progenitor cells can differentiate into many cell types, and stem cells can self-renew indefinitely and differentiate into any cell type. Three of the major challenges for stem cell-mediated repair after SCI are controlling the survival, integration and differentiation of transplanted cells. These cells might promote functional recovery by reconstituting damaged circuits, remyelinating axons, and increasing plasticity and/or axon regeneration. Many groups have studied the fate of stem cells<sup>91,92</sup> or progenitor cells<sup>93–99</sup> that were derived from rodent embryonic CNS or human umbilical cord blood and transplanted into the injured adult rodent spinal cord. Some groups have even reported modest improvements in functional recovery<sup>91,100,101</sup>.

The potential of human fetal stem cells in animal models of SCI is currently being investigated. Neural progenitors derived from human fetuses have been transplanted into immunosuppressed mice<sup>102</sup> and non-human primates<sup>103</sup> after contusion. In both cases, the transplanted cells survived and differentiated into cells with characteristics of oligodendrocytes and neurons, and were associated with locomotor improvements<sup>102,103</sup>.

The most recent successful approach with embryonic CNS-derived stem/progenitor cells is to use progenitor cells that have been pre-differentiated to a desired lineage before transplantation. Transplantation in rats of neuron- and glia-restricted precursors after contusion injury improved bladder and motor function. The cells survived, filled the lesion site, and differentiated into cells with some characteristics of neurons and glia, resulting in sparing/sprouting of descending pathways<sup>104</sup>. Transplantation of human embryonic stem cell (ESC)-derived oligodendrocyte-restricted progenitor cells into the adult rat spinal cord 7 days after injury enhanced remyelination and promoted improvement of motor function. The cells survived, migrated over short distances and differentiated into oligodendrocytes. By contrast, when cells were transplanted 10 months after injury, there was no enhanced remyelination or locomotor recovery<sup>105,106</sup>. This study is being considered for FORE-SCI replication at the [Miami Project to Cure Paralysis](#) (see Online links box).

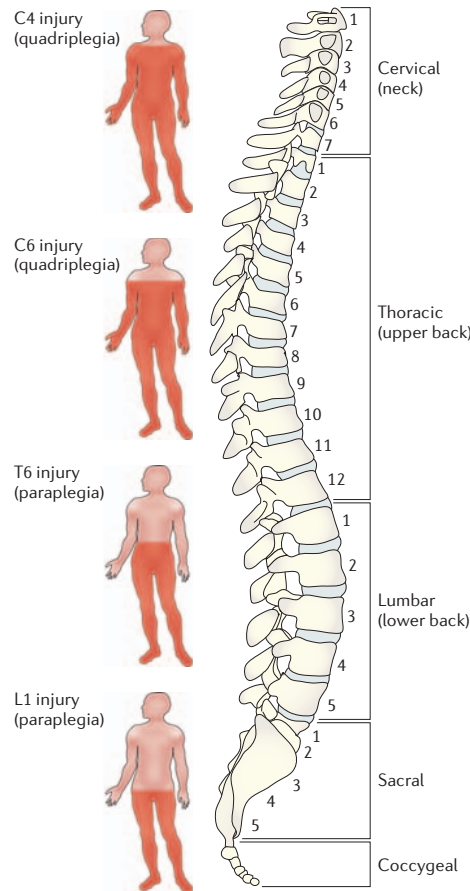
**Transplantation of adult stem/progenitor cells.** Adult stem cells are now being considered for CNS transplantation<sup>107</sup>. In contrast to ESC transplantation, adult stem cell

Box 1 | The ASIA Impairment Scale

Classification of spinal cord injury (SCI) severity using the American Spinal Injury Association (ASIA) Impairment Scale. The main categories of the Impairment Scale are as follows:

- A (complete): No motor or sensory function is preserved in the sacral segments S4–S5.
- B (incomplete): Sensory but not motor function is preserved below the neurological level and includes the sacral segments S4–S5.
- C (incomplete): Motor function is preserved below the neurological level, and more than a half of key muscles below the neurological level have a muscle grade of <3.
- D (incomplete): Motor function is preserved below the neurological level, and at least a half of key muscles below the neurological level have a muscle grade of ≥3.
- E (normal): Motor and sensory functions are normal.

Extent of injury after damage to specific spinal segments is illustrated in the figure (see [American Spinal Injury Association](#) in Online links box for the complete standard neurological classification of SCI).



transplantation should reduce ethical concerns (BOX 2) and autologous transplants should not be rejected. Various adult progenitor cells have been implanted in rodent models of SCI, ranging from cells from the olfactory system (see above) to bone marrow-derived stem cells, cultured spinal cord and brainstem cells and dermis-derived stem cells<sup>107</sup> (FIG. 5).

Adult bone marrow contains several different stem cell populations, including haematopoietic stem cells (HSCs) and bone marrow stromal cells (BMSCs), also known as mesenchymal stem cells (BOX 3). Transplantation of HSCs promotes functional recovery after compression-induced SCI in mice<sup>108,109</sup> and transplantation of BMSCs significantly improves hindlimb function after SCI in mice and rats<sup>109–111</sup>. However, the potential mechanisms by which BMSCs act are currently unclear, although neurotrophic and axonal elongation-facilitating actions have been proposed<sup>110</sup>. Also, the functional outcomes reported must be interpreted with caution because many are primarily based on one evaluation protocol without other behavioural/electrophysiological assessments. A small-scale human trial was conducted in which autologous BMSCs were intravenously delivered to nine patients

with SCI<sup>88</sup>. The improvements observed appeared to fall within an expected range of spontaneous recovery, and one participant advanced from ASIA category B to D. Nevertheless, without controls or some indication of cell viability within those lesions, we can conclude only that a measure of procedural safety was demonstrated. To our knowledge, peer-reviewed results have yet to be presented for this study<sup>8</sup>.

Adult neural progenitor cells (NPCs), isolated from the dentate gyrus, the subventricular zone or spinal cord, have been shown to self-renew, and to be multipotent *in vitro* and after transplantation into the CNS<sup>112,113</sup>. After transplantation of adult NPCs into the intact and injured murine spinal cord, differentiation into only astrocytes or oligodendrocytes is observed<sup>114–116</sup>. Recently, mouse brain-derived adult NPCs were transplanted into the injured spinal cord of adult rats. This treatment was combined with growth factors to selectively increase the number of oligodendrocyte precursors after transplantation. NPCs transplanted 2 weeks post-injury survived, migrated, integrated in the injured spinal cord tissue, generated mature oligodendrocytes that remyelinated the injured axons, and promoted some functional recovery. However, NPCs transplanted 8 weeks post-injury did not survive, and failed to exert similar effects<sup>117</sup>. Therefore, there is a need to find and neutralize the inhibitory obstacles present in chronic SCI that interfere with NPC survival after transplantation.

Adult neural stem cells also reside in the spinal cord<sup>118</sup>, and the ability to regulate their numbers and fate after injury might provide an alternative to transplantation. To regulate their numbers and fate to promote recovery, it will be necessary to determine which molecules are involved in governing neural stem cell proliferation, migration and differentiation. Therapies using endogenous stem cells would require no exogenous stem cell sources and would therefore circumvent the obstacle of immune rejection, as well as the ethical and moral considerations associated with their use.

**Transplantation of engineered stem/progenitor cells.** The injured adult spinal cord is a poor micro-environment for cell survival, neuronal differentiation and maturation. Therefore, to enhance the capacity of stem cells in CNS repair, researchers have recently begun to engineer stem cells to have better survival, and desired differentiation and maturation properties<sup>110</sup>. In an attempt to increase the survival of transplanted rat ESCs, ESCs were genetically modified to overexpress BCL2, an anti-apoptotic protein. This led to tumour-like growth of cells, accompanied by increased morbidity and mortality<sup>119</sup>. More promisingly, when transplanted in the compressed mouse spinal cord, engineered mouse ESCs expressing the cell adhesion molecule L1 survived longer and migrated rostrally and caudally from the lesion. Corticospinal axons showed interdigitation with L1-transfected ESCs and extended into and, in some cases, beyond the lesion site<sup>120</sup>.

It is apparent that transplantation alone of stem/progenitor cells after SCI will not lead to optimal recovery; combination strategies will be necessary for optimum return of function. Advances in molecular biology (for

example, viral vectors) have facilitated the manipulation of these cells to express molecules of interest<sup>121,122</sup>. These types of combination strategy are promising but need further development and careful animal testing, individually and jointly, before any clinical trial can be started.

**Transplantation of activated macrophages.** It has been suggested that the failure of the spinal cord to repair can be attributed to the nature of the macrophage response<sup>123</sup>, which differs from that observed in the regenerative PNS. In rats, recovery of hindlimb function has been reported after transection and transplantation of activated macrophages that had been incubated with PNS or skin tissue. Fibres were shown to extend through the lesion, and re-transection of the spinal cord abolished the previously recovered functions<sup>124,125</sup>. However, the degree of recovery was comparable to that obtained using transplants of other cell types and occurred only in a subgroup of rats<sup>126</sup>.

By contrast, activation of intrinsic macrophages at the spinal contusion site with micro-injections of a pro-inflammatory agent has negative effects on hindlimb functional recovery and tissue survival<sup>127</sup>. Depletion of macrophages has also led to significantly better hindlimb usage during overground locomotion, more extensive white matter sparing and decreased tissue cavitation<sup>128</sup>.

There is, therefore, evidence that macrophages have deleterious effects on functional recovery, as well as beneficial properties, but it would be advantageous to replicate these studies in independent laboratories. Moreover, no peer-reviewed studies on the transplantation of activated macrophages in non-human primates have been described.

Proneuron sponsored Phase I clinical trials on the transplantation of activated macrophages in humans from 2000 to 2003 in Israel and Belgium (see [Proneuron](#) in Online links box). Blood-derived monocytes activated using biopsied skin were transplanted into eight

ASIA category A participants between 9 and 14 days after injury. No irresolvable adverse effects have been reported. Three participants improved to ASIA category C, which was claimed to be well above the expected rate of natural recovery<sup>129</sup>. A multicentre, randomized controlled, Phase II clinical trial for ASIA category A participants is underway at hospitals in the USA and Israel, but recruitment for this clinical trial has currently been suspended for financial reasons (D. Snyder, Proneuron, personal communication).

### Molecular therapeutic interventions

Molecular therapies after SCI have several aims: to protect neurons from secondary cell death; to promote axonal growth; and to enhance conduction.

**Neuroprotective therapies.** Substantial effort has been devoted to limiting the evolution of secondary damage through the development of neuroprotective measures for acute SCI (and, potentially, for accompanying surgical interventions for chronic SCI)<sup>130</sup>. Delivery of antibodies against a cell adhesion molecule present on neutrophils and monocytes/macrophages reduces secondary damage after SCI in rats and improves motor function while reducing both autonomic dysreflexia and mechanical allodynia<sup>131</sup>. Erythropoietin has been reported to improve outcome<sup>130</sup>, although this finding might not be reproducible (see [Miami Project to Cure Paralysis](#) in Online links box). Several studies have also recently reported that intravenous minocycline reduces cell death and improves hindlimb function in mouse and rat models of SCI<sup>132–134</sup>, and this commonly used antibiotic could progress to clinical trials for SCI<sup>9</sup>.

Intravenous steroids (for example, methylprednisolone sodium succinate; MP) have been registered for clinical use in acute SCI in many countries<sup>135</sup>. There is considerable debate as to whether MP has been proved to be safe and efficacious for acute SCI<sup>135–140</sup>. Treatment is claimed, controversially, to be beneficial if an appropriate regimen is given that is dependent on the type of injury and whether more than 3 or 8 hours have elapsed since incurring the injury; however, prolonged or delayed treatment, incorrect dosing or treatment of penetrating SCI has been shown to be detrimental<sup>135,138</sup>. A recent review of randomized trials examined whether modest improvements have been shown using MP, GM-1 ganglioside, thyrotropin-releasing hormone (TRH), nimopidine and the NMDA (*N*-methyl-D-aspartate) antagonist gacyclidine<sup>8</sup>. The authors concluded that, in most trials, primary outcome measures were not significant and placebo controls were lacking. When small effects or trends were observed, these were often based on *post hoc* stratification, and severe side effects were also reported in some groups. Nevertheless, trials of neuroprotective agents have shown that large multi-centre, double-blind studies for SCI are feasible. Randomized and placebo-controlled Phase III clinical trials, with primary outcomes clearly recorded *a priori*, should enable the development of highly effective and safe neuroprotective therapies for human SCI<sup>10,11</sup>.

#### Phase II clinical trial

A clinical trial that includes a larger number of human participants than a Phase I trial and is intended to evaluate the efficacy of a treatment; side effects are also monitored.

#### Allodynia

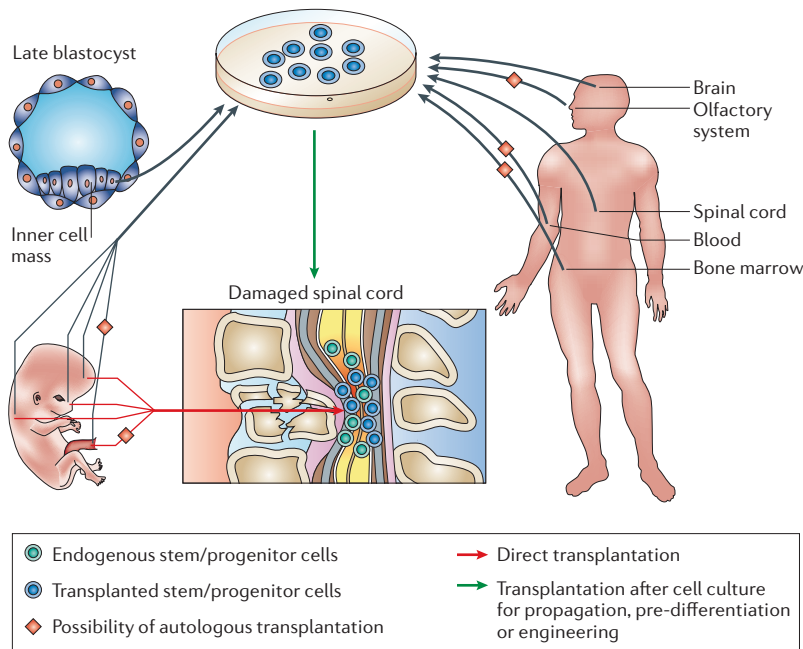
A type of neuropathic pain that manifests as increased sensitivity to normally innocuous stimuli.

#### Phase III clinical trial

A clinical trial that includes high numbers of human participants to test a treatment or drug that has been shown to be efficacious with tolerable side effects in Phase I and Phase II clinical trials.

### Box 2 | Ethics of spinal cord injury research and clinical trials

Research with animals and humans can involve ethical challenges. For example, animals are typically used to evaluate whether a therapy is safe and efficacious. In the UK, to minimize suffering, researchers are instructed, whenever possible, to replace (with other methods of investigation), refine (techniques) and reduce (numbers). Some potential therapies are in themselves ethically controversial. Transplantation of stem cells derived from human embryos is a particularly divisive issue, largely because many object on social, moral and/or religious grounds to the destruction of the donor to generate the graft material. Choosing controls for therapies involving surgical intervention is also ethically problematic: without incision or injection, control patients will probably perceive that they are 'control', and so any placebo effect might no longer be identifiable. However, anaesthesia, incision and injection pose special risks for people with acute spinal cord injuries. 'Sham' surgeries therefore require careful justification. Different cultures can also have different attitudes towards experiments using humans: although most Western scientists and clinicians would argue that controls are necessary to identify unambiguously whether a therapy is safe and effective, some clinicians have claimed that withholding a potential therapy from a patient with spinal cord injury is in itself unethical. Finally, high ethical standards are required by researchers, clinicians and journalists to ensure that results are communicated to the general public in a manner that reflects honestly the safety and efficacy of a potential therapy.



**Figure 5 | Potential sources of stem/progenitor cells for transplantation into the injured spinal cord.** Stem/progenitor cells can be collected at three different stages of development: from the inner cell mass layer of the mature blastocyst; from the brain, spinal cord, olfactory system or umbilical cord of the fetus; and from the brain, spinal cord, olfactory system, bone marrow or blood of the adult. Each of these cell populations can be propagated in cell culture and engineered to produce a molecule of interest, or be restricted to a particular cell fate before transplantation in the injured spinal cord. Some of these cells (those of fetal CNS origin and umbilical cord blood cells) could eventually be transplanted directly into the injured cord. Some of these cells have the potential to be used for autologous transplantation, including cells from the olfactory system, and umbilical cord blood cells (which can be frozen at birth for use in later life), haematopoietic stem cells and bone marrow stromal cells. Also, endogenous stem/progenitor cells are present at the injury site and are actively dividing. Controlling their proliferation and fate might provide an alternative to transplantation. This diagram is based on published data<sup>82–87,91–105,108–111,113–122</sup> from stem/progenitor cell transplantation after SCI in animals.

**Enhancing conduction.** Electrophysiological studies of humans with chronic SCI indicate that some axons can survive demyelination and that only a proportion become remyelinated (although denuded axons might die)<sup>17</sup>. Therapies that enhance remyelination (by host or transplanted glia) or enhance conduction could yet prove useful. A potassium channel blocker (4-aminopyridine) that can improve axonal conduction has been tested in several double-blind, placebo-controlled trials in humans with chronic SCI<sup>17</sup>. However, Acorda Therapeutics' Phase III clinical trials of an oral, sustained-release preparation of 4-aminopyridine showed a trend for improvements only in spasticity (see [Acorda Therapeutics](#) in Online links box).

**Delivery of growth factors.** Growth factors modulate neuronal survival, neurite outgrowth, synaptic plasticity and neurotransmission. Exogenous administration of growth factors has been proposed as one potential therapeutic treatment for SCI. The effectiveness of this approach has been tested using, for example, brain-derived neurotrophic factor (BDNF)<sup>141–147</sup>, basic

fibroblast growth factor<sup>148</sup>, glial cell-derived neurotrophic factor (GDNF)<sup>147,149</sup>, nerve growth factor (NGF)<sup>144,150</sup>, and neurotrophin 3 (NT3)<sup>141,145,146,151,152</sup>, NT4 and NT5 (REF. 153). Growth factors have been applied to the lesioned spinal cord by transient injection<sup>154</sup>, continuous infusion<sup>143,144</sup> or insertion of an artificial carrier saturated with neurotrophic factors<sup>142</sup>. *Ex vivo* gene therapy involves grafting cells, usually fibroblasts, that have been transduced with genes that encode growth factors<sup>145,146,151–153</sup>. *In vivo* delivery of growth factors has also been achieved using various viral vectors, including adenovirus<sup>155,156</sup>, adeno-associated virus (AAV) and lentivirus<sup>157</sup> (see below).

After SCI, the exogenous delivery of NGF in rats can induce growth of coeruleospinal axons<sup>158,159</sup>, whereas NT3 elicits growth of corticospinal axons<sup>160</sup>. BDNF induces recovery of forelimb function after cervical lateral hemisection and induces axonal growth of rubrospinal, reticulospinal, vestibulospinal, raphespinal, and local sensory and motor axons<sup>161–164</sup>. A combination of NT3 and BDNF improves bladder and hindlimb function after a mid-thoracic contusion<sup>152</sup>, and GDNF induces growth of motor and dorsal column sensory axons after partial and complete spinal cord transections and induces remyelination<sup>149</sup>. Because exogenous delivery of growth factors alone leads to only partial recovery, researchers are now combining the delivery of promising growth factors with other therapeutic approaches: OEG transplants and NT3 (REF. 67); marrow stromal cell transplants and BDNF<sup>165</sup>; and exercise with serotonergic agonists and NT3 (REF. 166). In addition, delayed delivery of growth factors might be less effective than acute delivery because axons of chronically injured neurons can lack appropriate growth factor receptors<sup>167</sup>.

Unfortunately, clinical trials using systemic delivery of growth factors for various disorders have failed either as a result of lack of efficacy or unacceptable side effects, or both<sup>168</sup>. Obviously, to avoid adverse effects, growth factors must be delivered in sufficient quantities to have an effect but their distribution must be restricted to the site at which they are needed. A recent Phase I trial of *ex vivo* NGF gene delivery in patients with Alzheimer's disease by implanting autologous fibroblasts genetically modified to express human NGF in the forebrain showed promising results, with no side effects attributable to the delivery of NGF itself and a slowing of the rate of cognitive decline<sup>169</sup>. However, to move forward with the clinical application of growth factor delivery after SCI, further work is required to show whether this promotes CNS axon regeneration and leads to functional recovery in injured non-human primates.

**Delivery of cAMP or small GTPases.** Cyclic AMP (cAMP) can induce axonal sprouting of cultured neurons *in vitro*<sup>170–172</sup> and of injured adult rat spinal sensory neurons *in vivo* when prophylactically applied<sup>171,173,174</sup>. Obviously, to be viable clinically, a therapy needs to be effective when applied after SCI. In zebrafish, post-injury application of cAMP led to regeneration of severed CNS axons and restored function<sup>175</sup>. After injury, the CNS environment is more permissive for growth in fish than in mammals.

**GTPases**  
Small enzymes that interact with GTP, a molecule that is used as a source of cellular energy.

Box 3 | **Bone marrow cells**

Bone marrow cells have been the subject of extensive interest because of their stem cell-like characteristics and pluripotency<sup>259,260</sup>. These cells were attractive for CNS repair because they could apparently give rise to neurons and glia<sup>261–264</sup>. However, those findings have not been confirmed<sup>265</sup> and have been questioned on cell fusion and transdifferentiation issues<sup>266–268</sup>. Furthermore, cells that express neuronal markers had no distinct neuronal morphological features and properties<sup>269–271</sup>. Unexpectedly, recent observations<sup>272,273</sup> that suggest that human haematopoietic cells can transdifferentiate into neurons, astrocytes and microglia in a long-term setting without fusing have revived the issue of transdifferentiation potential. Whether this can apply to bone marrow stromal cells (BMSCs) is not currently clear and requires further investigation. Like olfactory ensheathing glia and Schwann cells, BMSCs remain attractive for autologous transplantation and spinal cord repair because they can be easily procured, expanded in culture and delivered<sup>274</sup>.

Therefore, elevating cAMP levels after SCI has been tried in combination with other treatments. In rodents, some improvements in locomotion were observed<sup>176,177</sup> after delivery of Rolipram (which prevents the hydrolysis of cAMP) combined with fetal tissue transplants<sup>176</sup>, and after administration of the combination of Schwann cells, a cAMP analogue and Rolipram<sup>177</sup>. However, before any human clinical tests can begin, therapeutic windows of delivery of cAMP analogues must be defined, and doses and methods of delivery established, ideally in contusion injury models in rodents or primates.

Other strategies targeting molecules that are intrinsic to neurons could be viable, with modulation of GTPases being one potential approach. Many factors that limit axon regeneration (see below) signal to the neuronal cytoskeleton through GTPases, including Rho and Rac<sup>178–180</sup>. Inhibition of Rho by a bacterial toxin, C3-ADP-ribosyltransferase, promotes CNS axon regeneration and a degree of functional recovery after dorsal hemisection injury in adult rats<sup>181</sup>, although these results were not observed in another study<sup>182</sup>. Side effects have also been reported<sup>182,183</sup> and, although potential explanations have been offered<sup>184</sup>, the safety and the efficacy of small GTPase modulation need to be further evaluated before their use for human SCI<sup>182,183,185</sup>. BioAxone Therapeutic has developed a cell-permeable variant of a Rho inhibitor known as Cethrin (BA-210). Cethrin will enter an open-label, multi-centre Phase I/IIa trial that will include ASIA category A patients who are scheduled to receive spinal decompression within 7 days of thoracic SCI; Cethrin will be applied using fibrin<sup>184</sup> (see **BioAxone Therapeutic** in Online links box).

Rho kinase (ROCK) acts as a downstream effector of Rho<sup>186</sup>. Inhibition of ROCK by a peptide-based inhibitor and two small-molecule inhibitors stimulated or accelerated functional recovery, and had a neuroprotective effect in different mouse and rat SCI models when given locally or systemically immediately after injury either as a single dose or over several weeks<sup>181–183,187,188</sup>. However, it should be kept in mind that ROCK inhibitors have teratogenic potential<sup>189</sup> and that the diversity of cellular functions of small GTPases might reduce the therapeutic specificity of the compounds that modulate small GTPase activity.

**Modulation of interactions with myelin inhibitors.** Intact and injured CNS myelin contains several growth inhibitory molecules (including Nogo-A, myelin-associated glycoprotein, oligodendrocyte myelin glycoprotein, chondroitin sulphate and ephrin B3)<sup>190–193</sup>. Various therapies have been developed to target and overcome these inhibitors of axon growth. After delivery of anti-Nogo therapeutics, independent laboratories report CNS axon growth and recovery of limb function in many<sup>194–199</sup>, although not all<sup>200,201</sup>, rodent models of SCI, and report no nociceptive effects<sup>202</sup>. Promisingly, antibodies against Nogo-A have recently been shown to promote growth of corticospinal tract axons after unilateral dorsal thoracic hemisection in four out of five marmoset monkeys tested<sup>192</sup>. Future experiments might show whether anti-Nogo therapies safely improve outcome in contusion or compression models of SCI. Phase I clinical trials using humanized antibodies against Nogo-A are in progress for ASIA category A patients with thoracic SCI in association with Novartis (M. Schwab, personal communication).

Therapies targeting molecules in receptor complexes for Nogo-A<sup>203</sup> are also being tested. In some studies, genetic deletion of Nogo-A, Nogo receptor or NGF receptor leads to CNS axon growth and functional recovery<sup>204–208</sup>, confounding factors in the negative studies need to be elucidated because these could be important future targets for therapies. Intrathecal delivery of NgR(310)ecto-Fc enhances corticospinal and raphespinal axon growth after dorsal thoracic overhemisection in adult rats and enhances electrophysiological and behavioural recovery<sup>209,210</sup>. Delayed, subcutaneous treatment with NEP1–40 promotes growth of corticospinal axons and serotonergic fibres and a degree of locomotor recovery after thoracic dorsal hemisection<sup>211,212</sup>; independent testing of NEP1–40 by one FORE-SCI centre is underway (see **Reeve–Irvine Research Centre** in Online links box).

**Extracellular matrix modifiers.** Transient suppression of collagen synthesis promotes CNS axon growth after brain injury<sup>213</sup>, and, when combined with an analogue of cAMP, it has been reported to promote CNS axon regeneration and functional recovery after acute SCI<sup>214</sup> (but see REF. 215 for a contrasting result). Neuraxo has reported its intention to investigate the efficacy of this combination therapy, which they have designated Cordaneurin, in human SCI (see **Neuraxo Biopharmaceuticals** in Online links box). However, it would be valuable to reproduce these results independently, and to carry out studies in non-human primates.

In adult rats, degradation of growth-inhibitory chondroitin sulphate by delivery of the bacterial enzyme chondroitinase ABC (ChABC) promotes regeneration of injured CNS axons and recovery of function after dorsal column crush<sup>216,217</sup>. Moreover, after spinal cord hemisection in adult rats, delivery of ChABC promotes regrowth of axons from spinal cord neurons into peripheral nerve grafts<sup>218</sup> and regrowth of CNS axons into the spinal cord beyond hemichannel bridges containing Schwann cells<sup>219</sup>. After complete transection and implantation of

**Chondroitin sulphate**

A glycosaminoglycan that can limit the growth of axons.

**NgR(310)ecto-Fc**

Soluble function-blocking protein made by fusing part of the ectodomain of NgR to rat IgG1 Fc. It could act by providing a decoy, non-signalling receptor that competes for binding of myelin-associated inhibitors of axon growth.

**NEP1–40**

Nogo-A has two inhibitory regions; amino-Nogo and Nogo-66. NEP1–40 is a peptide derived from Nogo-66 that antagonizes stimulation of NgR1 *in vitro*.

**Chondroitinase ABC**

A bacterial enzyme that degrades chondroitin sulphate.

**Functional electrical stimulation**

(FES). FES involves electrophysiological stimulation of spinal cord or peripheral nerves or muscle.

channels containing Schwann cells, delivery of ChABC and OEG promotes regeneration of serotonergic axons beyond grafts<sup>220</sup>. Intrathecal delivery of ChABC also promotes recovery of bladder and hindlimb function following severe (although not moderate) thoracic contusion injury in adult rats<sup>221</sup>. Tests for efficacy and safety of ChABC in non-human primate models of SCI remain to be reported. Seikagaku is testing ChABC in Phase II clinical trials for herniated lumbar discs (see [Seikagaku Corporation](#) in Online links box), which could aid translation to treatment of SCI.

### Rehabilitative training

Improved locomotor function is often seen in mammals with incomplete and even complete SCI following exercise or rehabilitation<sup>222</sup>. Locomotor training even enhances the ability of many spinally transected mammals to walk on a treadmill when body-weight support is provided<sup>31,223,224</sup>. This improvement occurs because, after SCI, the spinal circuitry below the lesion site does not become silent but maintains active and functional neuronal properties, and can respond to peripheral input from below the level of the injury. It can generate oscillating coordinated motor patterns and is capable of considerable plasticity<sup>225–227</sup>. Increasing numbers of animal experiments combine rehabilitation/physical therapy with other strategies for promoting CNS axon regeneration and recovery of limb function<sup>228–231</sup>.

Many SCI clinical trials that are currently recruiting participants or are already in progress address aspects of rehabilitation, including upper-extremity exercise, body-weight-supported treadmill training, robotic or manually assisted training, and/or functional electrical stimulation (FES) (see [Clinical Trials.gov](#) in Online links box)<sup>2</sup>. Such trials are vital to establish empirically which types of locomotor training and rehabilitation are optimal for recovery of function<sup>232,233</sup>. Studies show that locomotor training enhances the ability of humans with neurologically complete SCI to walk on a treadmill, especially when body-weight support is provided<sup>31,223,224</sup>, although rehabilitation does not yet enable patients with neurologically complete SCI to walk unassisted overground<sup>233</sup>. FES of the dorsal surface of the spinal cord can induce step-like movements accompanied by corresponding electromyographic activity in the leg muscles in patients with complete SCI<sup>226</sup>. A single-blind, randomized, multi-centre trial has shown that many patients with recent, incomplete SCI achieve independent walking when trained to step/stand, either using conventional devices or using body-weight-supported treadmill training<sup>234,235</sup>. People with chronic, incomplete SCI also benefit from treadmill or overground locomotor training: for example, improvements are seen in overground walking speed (although outside the testing environment, participants did not walk independently of their wheelchairs)<sup>233</sup>. Another study with an ASIA category C patient reported that a combination of treadmill training and spinal cord epidural FES improved the quality and quantity of stepping during the training session and resulted in an immediate improvement in the quality of overground walking superior to that obtained with only treadmill training<sup>236</sup>. Therefore,

the combination of centrally (epidural stimulation) and peripherally (locomotor training) induced stepping appears to be an effective method for restoring locomotor activity in the absence of normal supraspinal input and should be explored further. Improvements in health have also been seen after rehabilitation, including improved cardiovascular performance and reductions in spasticity, bone loss and bladder/bowel complications<sup>2,235,237</sup>.

The mechanisms by which physical therapy or rehabilitation improve function after SCI need to be better understood because they could allow for rational improvement in therapy. Experimentation is also vital to identify safe and effective rehabilitative therapies: exercise can pose special risks to people with SCI, including autonomic dysreflexia, fracture or muscular injury and hyperthermia<sup>2</sup>. People with SCI have atypical physiological responses to exercise (for example, abnormal heart rates), which can limit their ability to sustain intense exercise<sup>2</sup>. Inappropriate exercise could also be detrimental after SCI<sup>238,239</sup>. Furthermore, rehabilitation is a potential confounding factor in clinical trials because it is difficult to control, although we should not restrict patient activity without strong justification.

Despite the documented advantages of exercise and rehabilitation, a US survey of quadriplegics and paraplegics showed that ~19% reported having no access to exercise, and a further ~45% reported having to exercise on their own without supervision by a physical therapist<sup>1</sup>. Therefore, much remains to be done politically to ensure that therapies that are already known to be effective are made available to individuals with SCI.

### Technical aspects

*Translating cellular therapies to the clinic.* Because autologous transplants of cells or tissues might not require immunosuppression to escape immune rejection, they represent an attractive therapeutic option. Saphenous nerves could be a good source for autologous grafts of peripheral nerves or Schwann cells because only a minor deficit in lower leg sensation results from biopsy. The olfactory mucosa is more accessible than the olfactory bulb for autologous transplant in humans<sup>54,240</sup>, although autologous transplants using tissue from the olfactory bulb have been carried out in dogs<sup>76</sup>. Expansion of cell biopsies using neurotrophins<sup>54</sup> or other mitogens<sup>241</sup> might be possible when the amount of tissue is limiting, but the occurrence of deleterious proliferation after transplantation needs to be prevented<sup>242</sup>.

Cellular suspensions can be transplanted into the acute, post-injury milieu or into irregularly shaped cysts or cavities that develop later in the injured spinal cord. Tissue grafts (for example, peripheral nerve grafts) are perhaps more appropriate for regularly shaped (for example, anatomically complete) injuries or for external routing (for example, direct to target nuclei). Less invasive routes of administration might include delivery of cells into the cerebrospinal fluid by lumbar puncture: stem cells could migrate towards the injury and exert a beneficial effect by reducing injury size<sup>243,244</sup>. Lumbar puncture is a promising strategy because of its minimal invasiveness, simplicity and low cost.

Cells could also be genetically modified to deliver therapeutic molecules<sup>145,146,151–153,169</sup>. Cells used in therapeutic delivery include fibroblasts, ESCs, neural stem/progenitor cells, OEG and Schwann cells. However, in many cases, large numbers of transplanted cells die after transplantation and are replaced by host cells<sup>245–247</sup>. Although the procedure of transplantation might still confer benefits, ensuring survival of the cells and controlling regulation of expression will be necessary to ensure optimal transgenic delivery. Identifying transplanted cells requires the use of a marker that neither induces transplant rejection nor transfers to host cells<sup>246</sup>.

The protective or reparative potential of transplants of a given cell type can be established only by using controls transplanted with alternative cell types (rather than merely injections of fluid). With regard to complete injuries, only slight functional recovery occurs regardless of the cell type transplanted<sup>126</sup>; a goal for the future (currently elusive) will be to enable consistent, hindlimb stepping that supports body weight<sup>226</sup>. Finally, it might be short-sighted to select a cell type for a clinical trial without evaluating alternative cell types within a single experiment. If the race to clinical trial results in one cell type becoming a 'gold standard' without it having been evaluated against other cell types, then other (potentially better) cells might not be easily tested. For example, it would be difficult to deny a clinical trial participant a therapy that has already been shown to be partially effective; this has already been the case when evaluating potential drug alternatives to MP<sup>138</sup>.

**Translating molecular therapies to the clinic.** Techniques to deliver molecular therapies into animal models of SCI include intracerebroventricular, intrathecal and intraspinal injection, continuous infusion or insertion of a carrier saturated with the molecule of interest. Viral vector-mediated transfer of molecules to the injured spinal cord is emerging as a new and effective strategy<sup>157</sup>. *In vivo* gene therapy has been tested in models of SCI using viruses, including herpes simplex virus, adenovirus, AAV, lentivirus and Moloney leukaemia virus<sup>248</sup>. Particularly interesting is the finding that AAV, when injected intramuscularly, can be retrogradely transported efficiently to motor neurons of the spinal cord<sup>249</sup>. It is an efficient tool for delivery of insulin-like growth factor 1 and it extends life expectancy in a murine model of motor neuron disease<sup>249</sup>. AAV-mediated intramuscular delivery of paraplegin also rescued peripheral axonopathy in a model of hereditary spastic paraplegia<sup>250</sup>. Therefore, simple intramuscular injections could be a method for delivering a therapeutic molecule after SCI. However, implementation in clinics will require more research to determine the best AAV serotypes to target motor neurons efficiently, and retrograde transport of AAV has yet to be tested in the context of SCI.

An opportunity exists for tailoring therapies to different types of injury. For example, if regeneration of a specific axon tract is desired, knowledge of the receptors expressed on the cell body and axon will inform whether this neuron will respond to a particular neurotrophin, and where this factor might best be applied. Similarly, there might be little value in delivering a therapy that neutralizes a given inhibitory receptor if this molecule is

not expressed by the axons that are injured. Basic science can provide a rational basis for intervening with a given therapy by meticulously investigating the mechanisms underlying a particular effect.

**Preclinical testing.** Many preclinical therapies have not been shown to be safe and efficacious by more than a single laboratory. Independent replication is extremely desirable to determine the general applicability of a therapy. Before moving to clinical trials, potential therapies should be tested in models that closely approximate the human injury subtype to be treated: naturally occurring injuries in dogs, as well as surgically induced injuries in non-human primates, can be used advantageously<sup>76,126</sup>. The primate response to SCI, although studied surprisingly little, has been examined after contusion injury<sup>16,52,150,251–253</sup>. Given the differences between rodent, cat, dog and primate nervous systems<sup>126</sup>, many recommend that therapies are tested in non-human primates for safety and efficacy<sup>10,11</sup>. Despite the paucity of safety and efficacy studies using non-human primates, many clinical studies and trials in humans are currently in progress<sup>8</sup>. This trend is of particular concern given that several potential therapies, including transplants of stem cells or cells from the olfactory nervous system, can induce pain-related behaviours and/or promote growth of sensory and sympathetic axons when tested in rodent models of SCI<sup>72,110,254,255</sup>. Rigorous testing for changes in nociception, autonomic dysreflexia and spasticity should therefore take place in animal models of SCI before moving to human patients to ensure that therapies neither induce adverse consequences nor interfere with the natural, spontaneous recovery of function that can occur. For example, when transplanting cells, care should be taken not to ablate any advantageous axons in trabeculae or axons spared in circumferential white matter<sup>29,256</sup>.

There are also relatively few studies that report outcomes after intervening more than 1 month post-SCI<sup>64,65,72,145,167,197,257,258</sup>, and, of these, many fail to detect improvements in axon growth or functional recovery. This insufficiency requires redressing if repair is to be achieved in individuals with long-standing injuries. Additionally, relatively few studies that report improvements in functional recovery go on to determine whether these changes remain stable beyond 2 or 3 months<sup>228</sup>.

**Clinical trials networks.** Various databases of patients with SCI have been established to follow the longitudinal progression of SCI and to enlist and document patients that might be suitable for particular clinical trials. European and North American clinical trial networks have been established to be ready to implement interventions across multiple centres, giving special attention to standardized evaluation using clinical outcome measures, imaging and neurophysiological stimulation and recording<sup>225</sup>. These researchers and others, including the FORE-SCI groups, are developing additional tests of sensory and motor function that should allow more sensitive assessment of recovery of function after SCI<sup>7</sup>.

## Conclusions

SCI is a devastating condition for which there is as yet no cure. Cellular, molecular and rehabilitative training therapies are being developed and some are now in, or moving towards, clinical trials. Nevertheless, work remains to be done to ascertain whether any of these therapies can safely improve outcome after human SCI. To distinguish

therapies that are unequivocally safe and effective, the scientific and clinical SCI communities recommend that preclinical studies should be reproduced by independent laboratories. Individual therapies are unlikely to emerge as a cure for SCI. Rather, we predict that tailored combinations of strategies will lead to cumulative improvements in outcome after different types of SCI.

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**Competing interests statement**

The authors declare **competing financial interests**: see web version for details.

**FURTHER INFORMATION**

**Acorda Therapeutics**: [http://www.acorda.com/pipeline\\_fampridine\\_sci1.asp](http://www.acorda.com/pipeline_fampridine_sci1.asp)  
**American Spinal Injury Association**: <http://www.asia-spinalinjury.org/publications/index.html>  
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**Clinical Trials.gov**: <http://www.clinicaltrials.gov/ct/search>  
**Foundation for Spinal Cord Injury Prevention, Care and Cure**: <http://www.fscip.org>  
**International Campaign for Cures of Spinal Cord Injury Paralysis**: <http://www.campaignforcure.org>  
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