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Current Options for Cell Therapy in Spinal Cord Injury

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Spinal cord injury (SCI) is a complex pathology that evolves after primary acute mechanical injury, causing further damage to the spinal cord tissue that exac-erbates clinical outcomes. Based on encouraging results from preclinical experiments, some cell treatments being translated into clinical practice dem-onstrate promising and effective improvement in sensory/motor function. Com-binatorial treatments of cell and drug/biological factors have been demonstrated to be more effective than cell treatments alone. Recent advances have led to the development of biomaterials aiming to promote in situ cell delivery for SCI, together with combinatorial strategies using drugs/biomole-cules to achieve a maximized multitarget approach. This review provides an overview of single and combinatorial regenerative cell treatments as well as potential delivery options to treat SCI.

Clinical and Pathological Features of SCI

SCI is an overwhelming neurological disorder that affects approximately 180 000 new individ-uals each year and a total of 1.3 million persons worldwide [1]. Causes include vehicle accidents, violence, accidental falls, and other traumatic events [1]. SCI leads to devastating neurological deficits and impairment, provoking partial or total loss of sensory/motor capacity resulting in paraplegia or tetraplegia (see Glossary). It can be aggravated by other frequent dysfunctions, such as infections, cardiac problems, respiratory, and bladder and bowel malfunctions, as well as by some pain syndromes (nociceptive and/or neuropathic). All these deleterious conditions have an enormous impact on the lives of SCI patients, with a heavy burden for society in terms of healthcare costs [1]. Most of the [779_TD$DIFF]post-traumatic degeneration of the tissue is caused by multifactorial secondary injury including several interconnected processes: blood–brain barrier dysfunction, local inflammation, neuronal death, demyelination, and disrupted nerve pathways [2]. Current treatment for SCI includes one drug accepted by both European Medicine Agencies and the FDA, methylprednisolone (MP), which is adminis-tered in the acute phase at a high dose for 48 h [3]. MP is a corticosteroid that inhibits lipid peroxidation, acting as a free radical scavenger. It also limits the inflammatory response and preserves the blood–spinal cord barrier, enhancing spinal cord blood flow. However, its efficacy is controversial, and important side effects include increased risk of urinary tract, respiratory, and wound infections [4] which limit its use [3]. Current treatment to ameliorate SCI outcomes can also include surgery to decompress and stabilize the spinal cord [5], as well as treatment of spasticity and rehabilitative care [6]. Different mechanisms have been suggested to facilitate recovery in preclinical studies, and diverse therapeutic approaches are being tested to relieve the secondary damage and maximize regeneration following SCI [2]. Molecular therapies (reviewed in [7]) act mostly on protecting the spinal cord and/or promoting regenerative mechanisms [2,7,8]. Unfortunately, many have shown no relevant efficacy when translated into clinical trials [7]. A possible reason could be that most therapeutic strategies have used treatments directed towards a single pathophysiological mechanism; however, SCI has a

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multifaceted nature, and concomitant and consecutive pathological events occurring during the progression of the secondary injury must be treated to achieve a global therapeutic effect [2]. Other reasons could be associated with the limited pharmacological treatment by conventional administration, mainly because of the low concentration achieved at the injured site and/or potential unacceptable side effects [9]. To overcome these limitations there is an urgent need for potential treatments to counteract secondary injury progression, and consequently a multitarget therapeutic approach might be promising for SCI patients. In this scenario, stem cell
therapy is potentially protective in view of its own broad-spectrum efficacy, and has been extensively investigated preclinically in different SCI models [8]. In particular, the regenerative potential of mesenchymal stem cells (MSCs), embryonic/neuronal stem cells (ESCs), induced pluripotent stem cells (iPSCs), olfactory ensheathing cells (OECs), and Schwann cells in the putative treatment of SCI is discussed in this review (Figure 1 and Figure 2, Key Figure).

Pathophysiology of SCI

Trauma

After a traumatic event due to **contusion**, massive **compression**, or laceration of the spinal cord, mechanical destruction of the tissue leads to acute neurological damage termed the **primary injury**. However, it is now well known that most of the post-traumatic degeneration of the cord is due to secondary injury, which occurs over time, from minutes to years, and leads to further neurological damage [2]. In mammals, this secondary injury includes a wide spectrum of subsequent events: blood–brain barrier dysfunction due to the vascular changes, and thrombosis resulting in edema and ischemia, free radical formation, and increased glutamate release that leads to neuronal death [2]. These in turn trigger an uncontrolled degenerative cascade with concomitant death of oligodendrocytes in **white matter tracts** that continues for several weeks after injury [2].

Generally, SCI involves pathophysiological processes that can be characterized by three consecutive phases developing over time in the injured cord: acute, subacute, and chronic [2]. The acute phase leads to dramatic degeneration of cells and parenchyma in the days following SCI, releasing many molecules such as excitatory neurotransmitters, free radicals, and inflammatory molecules that, together with hypoxic perfusion, produce a cytotoxic environment for any potential therapeutic cell transplantation [2]. However, the subacute phase, defined as the period between the acute and chronic phases (about 2 months in rodent SCI models and 12 months in humans) seems to be more permissive for cell engraftment, with better viability and efficacy [8].

Inflammation

A striking inflammatory response following trauma has been documented, and resident microglia and macrophages have been implicated as key players. Indeed, their selective modulation is deemed crucial for disease outcome [10–12]: the tissue microenvironment can trigger very early activation of inflammatory cells (primarily microglia) that acquire a proinflammatory phenotype in the first stage of injury, promoting early self-propelling local inflammation [10–12]. This proinflammatory environment recruits many peripheral monocytes/macrophages *in situ* [13], with different phenotypes – some could potentially have harmful effects, such as proinflammatory **M1 macrophages** [13] that exacerbate neurodegeneration and tissue loss, and **M2 macrophages**, presumably beneficial, that support neuroprotection and regeneration in different animal models [13]. Unfortunately, a proinflammatory effect orchestrated by M1 macrophages appears predominate in SCI rat and mouse models [14], and this might further impair and limit recovery of the tissue and motor function. This suggests that the timecourse of changes in inflammation and related ensuing responses is decisive in determining a destructive or constructive outcome [11,12].

Neurodegeneration

Weeks after the initial injury the pathological condition may become chronic, with further **white matter demyelination**, neuronal death, **reactive gliosis,** and deposits of extracellular matrix that can lead to scar formation, preventing axon regeneration in SCI animal models [2]. The progression of the pathology may be also exacerbated by molecules with growth-inhibitory effects that are released in the damaged spinal cord and act on multiple receptors expressed on the neuronal membrane. These molecules can limit the regenerative process by inhibiting axonal outgrowth [15–17]. Specifically, disruption of the white matter in the primary injury, and during progression of the secondary injury, can lead to significant oligodendrocyte death, which in turn releases myelin debris that contains several myelin-associated inhibitory molecules [18]. Nogo-A is a well-characterized protein expressed at the plasma membrane of oligodendrocytes that, when exposed in the environment, causes growth inhibition and **growth cone** collapse by interacting with its receptor NgR1 on neurons in mouse and rat SCI models [16,17,19]. Another molecule is myelin-associated glycoprotein (MAG) which, like Nogo A, is produced by oligodendrocytes and is a strong inhibitor of axonal regrowth *in vitro* [15,20]. A further barrier to spontaneous axonal regeneration is a group of molecules belonging to the proteoglycan family (**chondroitin sulfate proteoglycans**, CSPGs) [21]. Proteoglycans are expressed by different cells in the central nervous system (CNS), such as astrocytes, meningeal cells, and oligodendrocytes, which are all involved in glial scar formation in different SCI animal models [2,21]. However, others have shown a beneficial role of the glial scar in limiting and restraining cavity formation. This demonstrates that glial scars play a more complex spatio-temporal role in SCI. On the one hand, at the acute/subacute stages of injury the glial scar isolates the lesion area to preserve healthy tissue, and limits disruption and amplification of the injury [22]. On the other, the glial scar shows a detrimental effect, constituting a physical and chemical barrier to axonal regrowth in different rodent SCI models [22]. Together, these pathological mechanisms suggest that SCI is a multifaceted pathology, and finding treatment strategies poses a major challenge.

The Therapeutic Potential of Stem Cells in SCI

MSCs: Immunomodulation and Trophic Support for SCI

MSCs are particularly appealing for SCI repair and currently constitute the most promising stem cells in preclinical and clinical research [23] on account of their relative ease of access and efficient *in vitro* expansion [24]. Compared to other stem cells they rouse no ethical concerns, they can be used in **autologous transplants**, and are presumably safe when inserted into the CNS [23]. MSCs can be collected from different sources such as bone marrow, umbilical cord, amniotic liquid, and adipose tissue. MSCs have recently shown desirable properties for therapeutic use in CNS pathologies (Alzheimer's disease [25], stroke [26], Parkinson's disease [25], multiple sclerosis [25], and amyotrophic lateral sclerosis [25]) including as anti-inflammatory, immunomodulatory, trophic, and anti-apoptotic effects in different animal models of CNS disorders [23,25]. These functions might be mediated by transient paracrine bystander mechanisms and/or by migration to injured tissues for cell-to-cell contact, rather than resulting in cell differentiation and replacement as reported in different models of neurological disease [25], although the mechanisms have not been directly demonstrated [25,27]. As discussed below, there are intrinsic differences in MSCs from different sources which may be exploited for SCI therapy.

Bone Marrow (BM)-MSCs

BM-MSCs are distinguished from hematopoietic cells by their ability to adhere to plastic and to differentiate into cells of mesodermal origin. However, specific markers are necessary to unequivocally identify BM-MSCs [28]. MSCs from BM were initially believed to be pluripotent, with the ability to differentiate into neurons and glial cells; however, these findings are now being questioned. Indeed, cell fusion or transdifferentiation rather than cell differentiation might occur

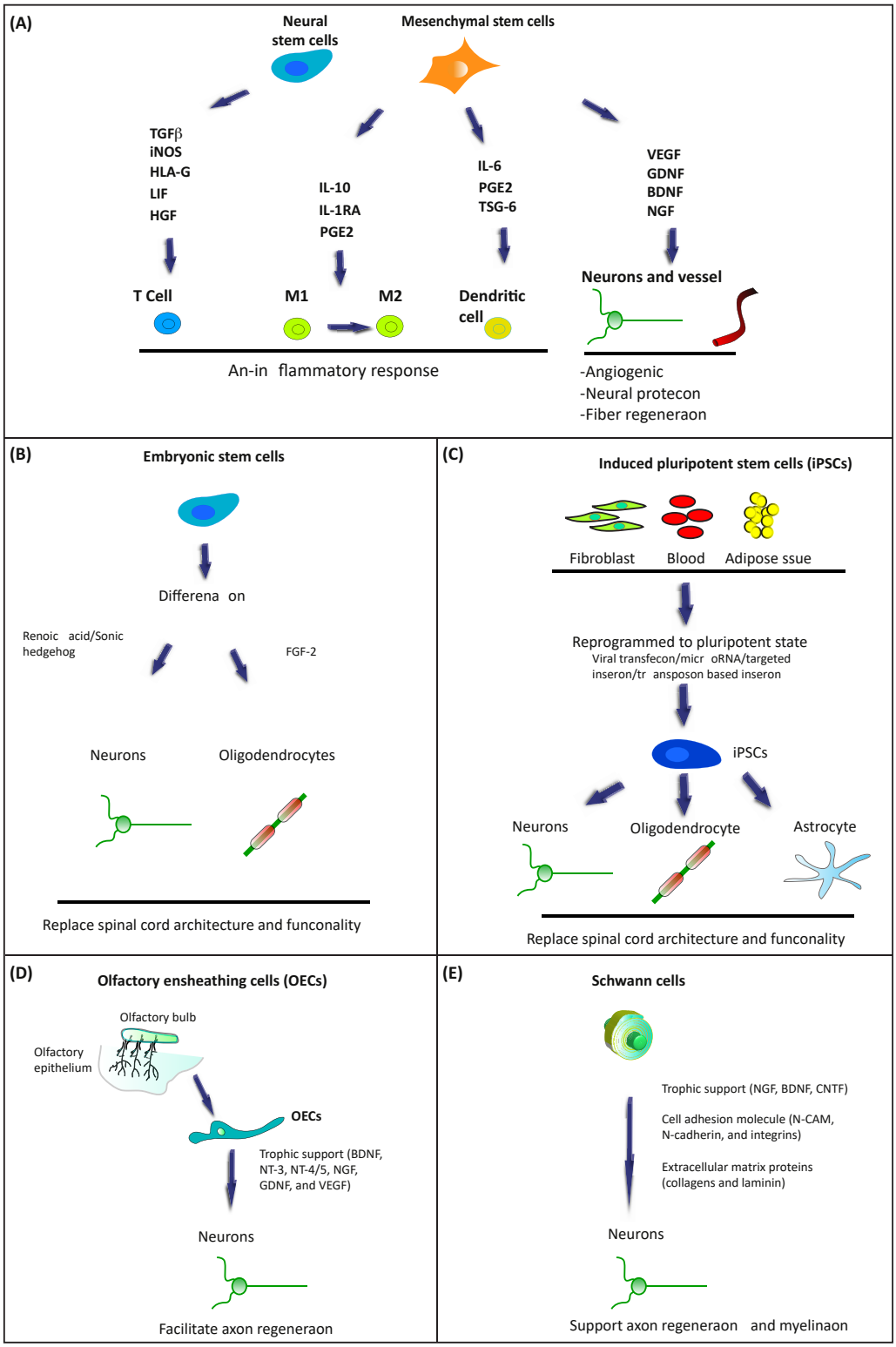

Figure 1. Cell Therapy Approaches to Spinal Cord Injury (SCI) Therapeutics. The cartoon illustrates (A) mesenchymal stem cells (MSCs) and neural stem cells can release several factors able to modulate different immune cells (T cells, macrophages, and dendritic cells) promoting an anti-inflammatory environment. MSCs can

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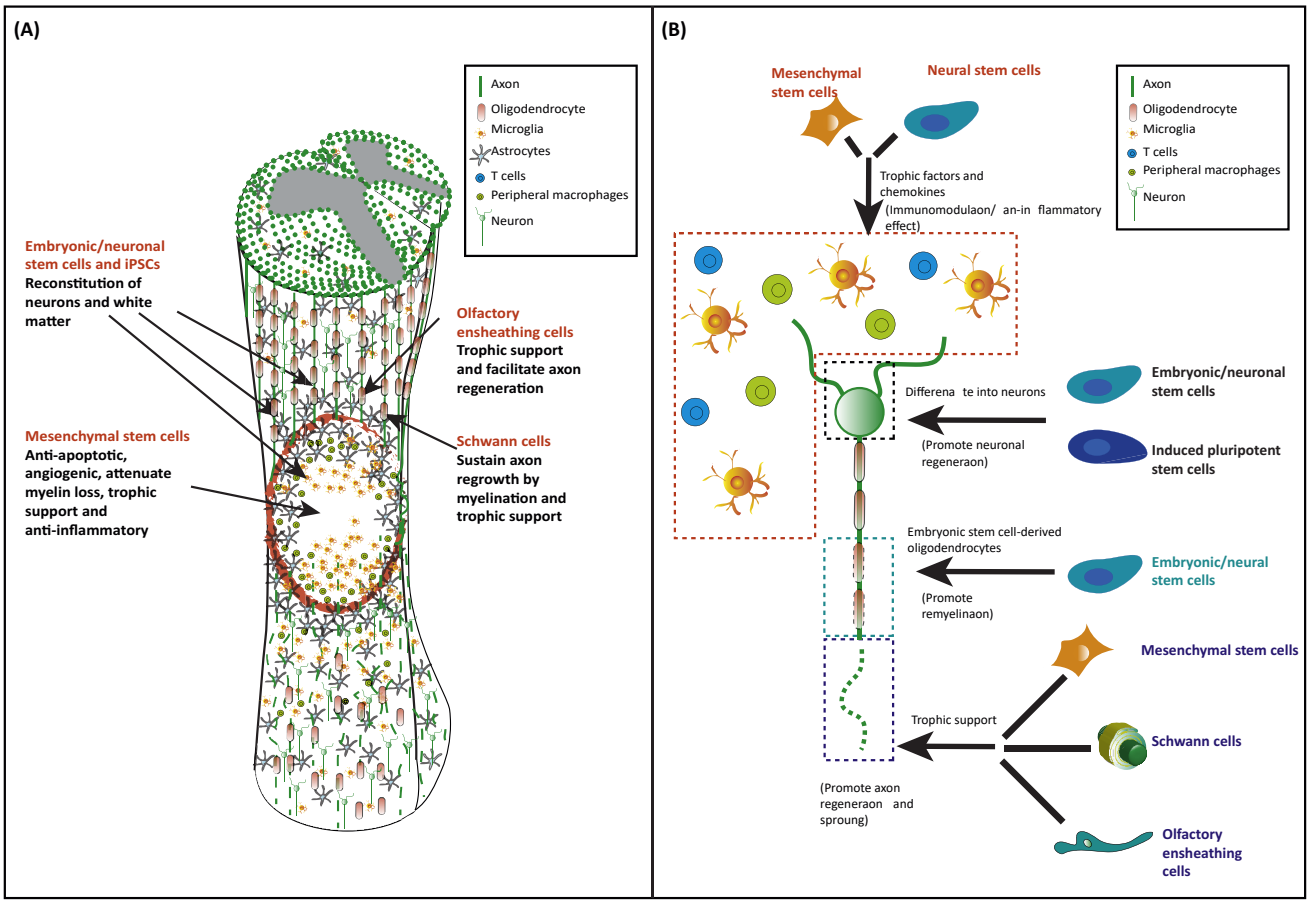
Key Figure

Cellular Targets and Putative Mechanisms of Action in Spinal Cord Injury (SCI) Therapeutics



Trends in Molecular Medicine

Figure 2. The cartoon illustrates (A) the main cellular targets and putative mechanism of action of cell-based approaches in mammalian spinal cord tissue after SCI. (B) A focus on stem cell-based mechanisms driving anti-inflammatory and proregenerative processes on neuronal cytoarchitecture in SCI. Trophic factors and chemokines released by mesenchymal stem cells (MSCs) and neural stem cells (NSCs) can have immunomodulatory effects on microglia, T cells, and peripheral macrophages, promoting a pro-regenerative environment. Embryonic stem cells (ESCs), NSCs, and induced pluripotent stem cells (iPSCs) can differentiate into neurons to support neuronal regeneration. ESCs/NSCs can replace damaged oligodendrocytes to promote remyelination of injured axons. MSCs, Schwann cells, and olfactory ensheathing cells may offer trophic support to regenerate axons.

also induce angiogenesis, neuroprotection and fibers regeneration acting on neurons and vessels. (B) Embryonic stem cells (ESCs) after differentiation into neurons and oligodendrocytes can replace spinal cord architecture and functionality. (C) Induced pluripotent stem cells (iPSCs) derived from different sources can be reprogrammed to differentiate into central nervous system cells and replace damaged spinal cord tissue. (D) Olfactory ensheathing cells (OECs) derived from the olfactory mucosa or epithelium can give trophic support to neurons and facilitate axon regeneration. (E) Schwann cells can support axon regeneration and remyelination after injury. Abbreviations: BDNF, brain-derived neurotrophic factor; CNTF, ciliary neurotrophic factor; FGF, fibroblast growth factor; GDNF, glial cell-derived neurotrophic factor; HGF, hepatocyte growth factor; HLA-G, human leukocyte antigen G; IL, interleukin; iNOS, inducible nitric oxide synthase; LIF, leukemia inhibitory factor; M1 and M2, macrophage phenotypes 1 and 2; NGF, nerve growth factor; NT, neurotrophin; PGE2, prostaglandin E2; TSG-6, TNF-stimulated gene 6; TGF, transforming growth factor; VEGF, vascular endothelial growth factor.

after transplantation [29]. BM-MSCs collected from different sources and transplanted into mouse and rat SCI models exposed to compression, contusion, or **transection** of the spinal cord have shown some improvements in motor activity [8]. The majority of BM-MSCs used in preclinical experiments are of human or rodent origin, although they may also be obtained from primates [30] or pigs [31] (reviewed in [8,23]). Routes of administration include intravenous (IV) infusion [32–35] or, more frequently, cells are grafted directly into damaged spinal cord in preclinical models [8] (Figure 3). The immunosuppressive properties of these cells have been linked to their efficacy in mouse and rat SCI models [25,36]. In addition, BM-MSCs might also play an antiinflammatory protective role [36], suppressing lymphocyte proliferation and differ-entiation [37], thereby prolonging MSC survival *in vivo* in mouse and rat SCI [36], or facilitating

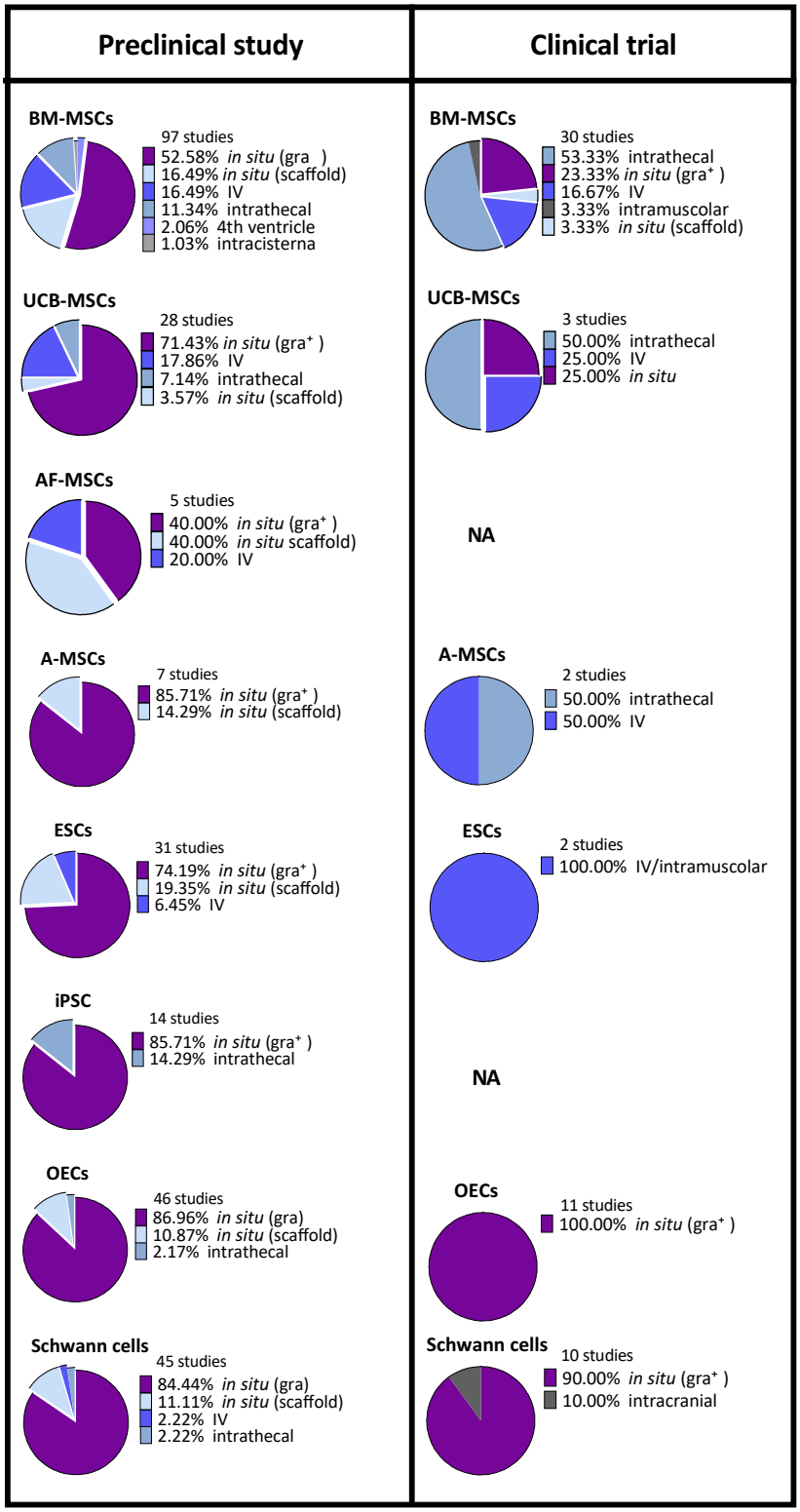
the transition of macrophages from the M1 proinflammatory phenotype to the M2 anti-inflammatory and regenerative phenotype in these animal models [38] (Figures 1 and 2). Furthermore, BM-MSCs may protect the injured spinal cord from further cellular damage via trophic support and neuroprotective activities [39,40]: among the trophic factors, the best-studied are vascular endothelial growth factor (VEGF), nerve growth factor (NGF), glial cellderived neurotrophic factor (GDNF), and brain-derived neurotrophic factor (BDNF) that are known to support neural protection and fiber regeneration in SCI rat models [36]. As such, combinatorial therapeutic approaches using a variety of molecules or factors have been tested experimentally in SCI rodents, further improving motor performance (Table S1 in the supple-mental information online). In addition, BM-MSCs could be used as optimal vectors for therapeutic agent delivery on account of their ability to migrate toward damaged tissues [41]. Specifically, BM-MSCs genetically modified to overexpress neurotrophin 3 were found to sustain axon regeneration after transplantation in SCI rodent models, leading to improved motor activity [41]. Gene-modified BM-MSCs overexpressing BDNF have been implanted into injured sites in a rat SCI model, resulting in improved neurological function, namely the sprouting of injured corticospinal tract and its serotonergic projections, and improving motor function outcome [42]. Given these encouraging results in preclinical experiments, some treatments have been rapidly translated into clinical practice, demonstrating safe BM-MSC transplantation in SCI patients, where partial efficacy has been seen in some cases [43–49]. However, in several clinical trials, unlike preclinical studies, intrathecal rather than *in situ* injection has mostly been adopted for SCI patients (Figure 3).

Umbilical Cord (UC)-MSCs

UC-MSCs are easily obtained by treating umbilical cord or cord blood from the newborn can be stored at cryogenic temperatures until use. They are hypoimmunogenic and cause less graft rejection than other stem cells [50]. They can be expanded with different growth factors with excellent colony-forming ability and can potentially be used for autologous cell therapy [24]. Many preclinical studies have shown their broad therapeutic capacity [51] with multifaceted efficacy in several rat and mouse SCI animal models, including neurotrophic [52], anti-inflam-matory [52,53], anti-apoptotic [54] and angiogenic actions [55] (Figures 1 and 2). The most frequent route of administration in preclinical studies is *in situ* (Figure 3). Despite numerous

promising preclinical studies, few clinical trials have been published on the safety and efficacy of UC-MSCs [56–58] (Table S2). A clinical trial of human UC-MSCs transplanted directly into the damaged spinal cord of a female patient aged 37 years with SCI has been reported; adminis-tration was safe and movement and sensory perception improved within 41 days of treatment [56]. Another study documented the treatment of 25 SCI patients with UC-MSCs by IV or intrathecal injection, which partially restored autonomic nerve functions and somatosensory evoked potentials within 12 months after the treatment [57]. Others have evaluated therapeutic

efficacy of UC-MSCs, directly injected into the spinal cord, in combination with locomotor training in 28 patients with chronic complete SCI, and found no severe adverse reactions after transplantation, with improvement of the motor performance in some SCI patients [58].



Trends in Molecular Medicine

Figure 3. Routes of Cell Administration in Preclinical and Clinical Studies in Spinal Cord Injury (SCI) Therapeutics. The number of studies and the route of administration (percentage of total) are represented for different

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Amniotic Fetal (AF)-MSCs

AF-MSCs can be derived from amniotic fluid or amniotic membrane (a component of the placenta) that are both important to sustain and protect the fetus and organ development during pregnancy [59]. AF-MSCs can be considered as a valid alternative source of MSCs for regenerative medicine in SCI [59]. They offer several advantages such as minimal invasive isolation and no ethical issues. They also show multipotency, efficient proliferative activity, non-tumorigenicity, and low immunogenicity [59]. They can also be used for autologous transplantation of cells from patient tissues. A few studies suggest that these stem cells can improve functional recovery in preclinical models [60,61]. One study found that IV injection of AF-MSCs in a rat SCI contusion model could attenuate myelin loss in the damaged spinal cord, promote angiogenesis, and reduce inflammatory cell migration into the lesion site, enhancing motor recovery [60]. These effects were proposed to be mediated by a specific cytokine, hepatocyte growth factor, that supports angiogenesis and remyelination [60]. Another report documented anti-inflammatory and anti-apoptotic effects of a combined treatment of AF-MSCs with MP after SCI in rats that was more effective in motor functional recovery than AF-MSCs or MP alone [61] (Table S1). In addition, another study showed that amniotic cells transplanted into the transection cavity of the spinal cord of monkeys supported the growth of axons [62], demonstrating an effective treatment, even in primates.

Adipose Tissue MSCs (A-MSCs)

A-MSCs can be easily obtained in a minimally invasive manner from adipose tissue in large amounts using different techniques such as **lipoplasty** or liposuction. Several features have been attributed to A-MSCs such as secretion of trophic growth factors (BDNF and GDNF) [63], modulation of activated immune cells [64], neuroregeneration [65,66], anti-apoptotic action [67], and multilineage differentiation capacity (e.g., adipogenic, chondrogenic, myogenic, smooth muscle, neurogenic, and endothelial cell lineages, as well as Schwann cells) [68], which may confer potential regenerative effects in SCI. The secretome of A-MSCs consists of growth factors, extracellular matrix molecules, proteases, cytokines, and several immunomodulatory molecules that can promote angiogenesis and wound healing; these bioactive molecules have been suggested to aid new tissue growth and lead to immunosuppression and reduction of inflammation by inhibiting the proliferation of activated lymphocytes *in vitro* [68]. Various preclinical and clinical studies have shown a potential regenerative effect of A-MSCs when transplanted directly *in situ* in SCI, their most frequent route of administration (Figure 3). For instance, one study found that transplanted A-MSCs in injured rat cervical spinal cord reduced glial scar formation and stimulated axonal regrowth; however, this treatment did not achieve recovery of forelimb function [65]. A-MSCs have also been directly delivered into the spinal parenchyma of a rat model of spinal cord compression immediately after injury, promoting tissue preservation with abundant deposition of laminin and axonal regeneration, leading to functional recovery, as evidenced by open-field locomotion testing [66]. Early IV injection of A-MSCs after acute SCI in dogs has also been found to be feasible and to improve functional motor recovery, without adverse effects [69].

To enhance the survival and therapeutic efficacy of A-MSCs *in situ,* coadministration of different compounds has been studied (Table S1), such as 17β-estradiol (to increase the secretion of growth factors from A-MSCs) [70], or overexpression of Bcl-2 (anti-apoptotic effect) [71]. Alternatively, chondroitinase ABC (ChABC, an enzyme from *Proteus vulgaris* bacteria that

cell treatments. BM-MSCs are the prevalent cell population used in both preclinical and clinical studies. The best-evaluated route of administration for cell therapy in preclinical studies is *in situ*, whereas in clinical trials intrathecal administration is highly represented for mesenchymal stem cells (BM-MSCs, UCB-MSCs, and A-MSCs), and *in situ* for OECs and stem cells. Data: Pubmed. Abbreviations: A-MSCs, adipose tissue mesenchymal stem cells (MSCs); AF-MSCs, amniotic fetal MSCs; BM-MSCs, bone marrow MSCs; ESCs, embryonic stem cells; IV, intravenous; NA, not available; OECs, olfactory ensheathing cells; UCB-MSCs, umbilical cord blood MSCs.

enzymatically removes glial scars) has been combined with A-MSCs in a chronic SCI dog model [72]. All the combinatorial approaches listed above have shown increased efficacy and improved motor function recovery compared to single treatments [70,71,72]. On the basis of these preclinical studies, several clinical trials have been proposed to confirm safety following intrathecal injection of these cells [73–75] (Table S2).

Using Stem Cells to Replace Spinal Cord Architecture and Functionality

Embryonic Stem Cells (ESCs)

ESC-based therapeutic strategies have been investigated in many diseases as well as for their potential to repair mechanically damaged nerve tissue in SCI. The pluripotent nature of ESCs may allow them to generate new cells in human or animal CNS tissue, including neurons and glial cells [76]. One of the major strategies for treating the injured spinal cord is to induce ESCs to differentiate into specific phenotypes to replace the desired cell (neurons or glia) or to produce factors that could limit the damage and sustain regeneration of the tissue (Figures 1 and 2). Several ESC-based therapeutic approaches have been proposed and evaluated for treating SCI, and different groups have shown improvements in motor and sensory function after transplantation of neuron or glia pre-differentiated mouse ESCs in rat or mouse SCI animal models. For instance, differentiation of ESCs into motor neurons using a combination of retinoic acid and Sonic hedgehog protein was demonstrated *in vitro*, as well as following transplanta-tion *in vivo* into the spinal cord of a paralyzed adult rat [77] (Figures 1 and 2). One study showed that allogenic neural stem cells, derived from ESCs and grafted into the damaged spinal cord of adult marmosets, promoted functional recovery, as demonstrated from behavioral analyses using an open-field rating scale, as well as from evidence of preserved myelin and axons [78]. Others have demonstrated that stem cells clonally derived from ESCs and transplanted into a mouse thoracic SCI model gave significant preservation of cells and tissue [79]. Moreover, porcine ESCs transplanted into the contused spinal cord of rats differentiated into neuronal cells, and animals showed significant functional recovery of motor function [80]. In addition, a combinatorial therapeutic strategy with growth factors (NT-3 and PDGF) has been tested in a subacute model of SCI, and was found to enhance survival and differentiation into neurons [81](Table S1). ESCs differentiated into oligodendrocytes have been used to treat SCI, achieving some improvements of motor activity after reconstitution of part of the white matter in transection or contusion SCI rat models [82,83]. In clinical settings (Table S2), Geron Corpo-ration proposed in 2010 a trial recruiting SCI patients to assess ESC-derived oligodendrocytes grafted in the damaged spinal cord. Unfortunately, the company stopped the trial 1 year later for lack of funds, now restarted by Asterias Biotherapeutics. However, some ethical issues have been raised regarding the approval process and management of this trial [84].

Neural Stem Cells (NSCs)

NSCs are multipotent, self-renewing progenitor or stem cells isolated from the subventricular zone of the hippocampus of the brain and a region of central canal of the spinal cord [85]. These cells can differentiate into specific neuronal or glial phenotypes to replace lost tissue or produce pro-regenerative factors. Different studies in rodent compression SCI models have shown that transplantation of NSCs into lesioned spinal cord leads to functional recovery, sustained through neuronal cell replacement that was able to reconstitute lost neuronal and glial tissue, with trophic support (BDNF, CNTF, GDNF, NGF, and IGF-1) [86], preserving damaged cells and axons [87]. The potential effect of immunomodulation (T cells and macrophages) has been demonstrated in other neurodegenerative disease such as in inflammatory demyelinating disorders (e.g., multiple sclerosis), showing reduced accumulation of $CD4^+$ T cells in the CNS together with reduced demyelination, shifting microglia from a harmful to a neuropro-tective phenotype [88]. Clinical trials have so far demonstrated that NSC transplantation into the injury site of SCI patients can be done safely, but effects on recovery have not yet been documented [89,90].

Induced Pluripotent Stem Cells (iPSCs)

The recent development of iPSCs provides a valid alternative to ESCs. These cells are generated by reprogramming somatic cells in the presence of the necessary transcription factors (Yamanaka factors), and by different methods including viral transfection, microRNA delivery, targeted insertion, transposon-based insertion, and protein transfection (Figure 1) [91]. Unlike traditional ES cell lines, iPSCs circumvent ethical concerns regarding the use of embryos and allow autologous transplantation of pluripotent cells which should reduce the risk of rejection. Nevertheless, iPSCs and ESCs share some of the same disadvantages, such as the risk of forming teratomas [91]. However, studies have demonstrated iPSC safety and efficacy for cell therapy in SCI after *in situ* injection (Figure 3) [92]. For example, the conversion of iPSCs into oligodendrocytes or neuronal cells has been reported to improve motor activity in contusion models of mouse, rat, or marmoset spinal cord [82,93], as well as conversion into neuronal cells in compression/contusion models in mouse or rat [94–98]. Although these results provide strong encouragement for using iPSCs in cell therapy, further investigation will evidently be necessary to rigorously clarify their potential role as an effective treatment for SCI.

Olfactory Ensheathing Cells (OECs)

OECs are glial cell types which play an important role in neural regeneration of olfactory neurons by supporting and guiding their constant replacement and axon growth from the peripheral nervous system into the CNS. OECs can be obtained through nasal biopsies from the olfactory mucosa (OM) located in the nasal cavity, or alternatively from the olfactory bulb (OB) [99]. OECs hold great promise for SCI regenerative treatment because after implantation in the damaged spinal cord they can create a permissive environment for axonal regeneration that can cross the injured site in several rodent SCI models [99]. Several potential mechanisms have been proposed to contribute to the efficacy of OECs in supporting axon regeneration, such as providing a large amount of neurotrophic molecules (BDNF, NT-3, NT-4/5, NGF, GDNF, and VEGF) (Figures 1 and 2). For example, they have been reported to regulate glial scar formation and remyelination, and to counteract diffusion of inhibitory factors released by axons of dead neurons phagocytizing their debris *in vitro* [99]. Many laboratories have also reported that OECs transplanted into the spinal cord can facilitate axon regeneration and ameliorate motor function, mostly in rat SCI models [100,101]. One study indicated that OECs transplanted into trans-ected spinal cord of paraplegic rats promoted tissue regeneration after 3 months, and this persisted for up to 7 months after treatment [102]. In addition, OECs derived from the olfactory mucosa have been documented to promote regeneration when implanted 4 weeks after transection of the rat spinal cord [103]. Another example has been the application of OECs at the upper cervical level of an injured rat spinal cord as a treatment to restore supraspinal control of breathing and climbing after SCI [104]. Others have reported that OEC transplanta-tion in the damaged site of the spinal cord can improve hindlimb motor performance in paraplegic rats and, when combined with task-specific training, the effect on motor activity was enhanced [105,106].

Combinatorial therapy has been proposed to improve pro-regenerative efficacy of OECs in rat SCI models, including co-treatment with stem cells [107], Schwann cells [108], or ChABC [109], or when seeded into a biodegradable poly-lactic acid/polylactic-co-glycolic acid 3D scaffold [110] (Table S1). Nonetheless, the potential of OECs to promote tissue regeneration remains controversial despite findings from preclinical and clinical studies [111]. This contro-versy is presumably based on the observation that OECs can comprise several subpopulations that are not well described phenotypically or functionally, and that, furthermore, different protocols and purification methods have been used which might not result in pure homogenous populations [112] (Tables S1,S2). A key aspect in defining their potential for transplantation therapies is to characterize the cell phenotypes thoroughly and draw up reproducible protocols to validate their therapeutic potential in SCI.

Schwann Cells

Schwann cells in peripheral nerves support axonal regeneration after damage, and this has suggested their potential application in spinal cord injury [113]. Schwann cells could contribute to regeneration after injury by sustaining axonal regrowth and myelination; this is necessary for appropriate axonal functioning [113]. Schwann cells offer several properties that could enhance recovery after SCI, such as the production of a variety of growth factors (including NGF, BDNF, and CNTF), cell adhesion molecules (N-CAM, N-cadherin, and integrins), and extracellular matrix proteins (collagens and laminin) [113] (Figures 1 and 2). Their potential efficacy has been demonstrated in a variety of rat SCI models, showing increased numbers of myelinated axons in damaged sites, reduced **cystic cavities**, white matter sparing, and axonal regeneration [114]. Indeed, several proposals have been made to increase the therapeutic effect of Schwann cells after transplantation *in situ*. Combination treatments directed against different targets using trophic factors, stem cells, anti-inflammatory drugs, drugs to improve axon regrowth, and enzymes (e.g., chABC) have been evaluated, demonstrating a more effective outcome than transplantation of Schwann cells alone, resulting in increased numbers of myelinated axons and improved motor function recovery [115] (Table S1). Schwann cells genetically modified to produce high levels of factors (glial cell line-derived neurotrophic factor or cell adhesion molecule L1, a protein promoting neurite outgrowth) have also been evaluated in rat SCI models, improving spinal cord repair and motor function [116,117]. Clinical trials using Schwann cells have suggested their safety and potential efficacy in humans as a single transplantation *in situ* [118,119] or in combination with BM-MSCs [120] or OECs [121]; there have been no noteworthy relevant side effects in SCI patients, and functional improvement has been observed in some cases [119] (Table S2).

Adult Endogenous Stem Cells (AESCs)

AESCs are a population of stem cells that are present in adult neural tissue. In the spinal cord, ependymal cells, located in proximity to the central canal, have stem cell properties [122]. After an acute injury, they proliferate and constitute mostly new glial cells in the injured spinal cord [122]. The regenerative response of these cells after insult has been shown in different mouse or rat SCI models [122]. One study reported functional motor recovery after transplantation of spinal cord-derived precursor cells in paraplegic rats, and these were able to differentiate preferentially into glial cells when transplanted *in situ* [123]. Others found that the ependymal cell population has stem cell properties, and these cells differentiate mostly into new astrocytes, forming glial scars, while oligodendrocyte progenitors could reconstitute myelin and astrocytes in rat SCI models [122]. These findings have raised hope for future non-invasive therapy for SCI. However, understanding the molecular regulation of these processes is fundamental in identi-fying potential therapeutic targets and for developing realistic regenerative strategies to promote recovery after SCI. Indeed, controversy still surrounds many of these approaches, and they evidently have advantages, disadvantages, and true limitations (Boxes 1 –3). Thus, despite their potential use in SCI regeneration, extensive research on the potential use of stem cell populations remains a priority.

Future Considerations for Stem Cell Therapy in SCI

The multipotency of pluripotent stem cells may offer a valuable solution for SCI by providing replacement neurons and glial cells to restore motor function. However, many problems remain regarding their clinical application. Ethical debate surrounds ESC research on account of their source, which limits clinical applicability [124]. Transplantation of ESCs might also result in teratomas because of uncontrollable cell proliferation [125], further mitigating enthusiasm for their potential application in repair strategies for CNS disorders.

New research has indicated great potential for iPSCs that have been proposed as a valid alternative to ESCs, and moreover overcome ethical obstacles because iPSCs can be obtained

Box 1. Potential Targets for SCI Cell-Based Therapy

The efficacy of cell transplantation is being intensively studied for its potential in treating SCI. However, less is known about the mechanisms through which transplanted cells promote functional improvements. Depending on the candidate cell types, different mechanisms have been proposed.

Tissue Protection

Preservation of the tissue after injury is one of the best-documented mechanisms underlying functional improvement following cell transplantation. Many cell types are able to promote tissue sparing, including MSCs, OECs, Schwann cells, and oligodendrocyte progenitor cells. It is broadly proposed that bioactive molecules such as trophic factors and cytokines secreted by transplanted cells support neuroprotection and preservation of the cytoarchitecture. Specifically, these biomolecules might enhance cell survival, modulate gliosis, and improve blood vessel repair (Figures 1 and 2) [134].

Immunomodulation

Among the mechanisms involved in secondary injury, there is a proinflammatory response that might exacerbate the SCI outcome [13]. Cell transplantation can offer benefits through immunomodulation by stimulating beneficial or reducing detrimental responses. MSCs or NSCs can modify immune responses after injury by releasing anti-inflammatory cytokines or factors. Specifically, MSCs could modulate the M1/M2 balance of macrophages and microglia in the injured site, promoting an M2 anti-inflammatory and regenerative phenotype, whereas NSCs might act by modulating T cell-mediated responses to ameliorate pathology (Figures 1 and 2) [88].

Axon Regeneration

Enhancing axon growth is considered a significant challenge in SCI therapeutic strategies. NSCs, Schwann cells, OECs, and MSCs can promote axon regeneration and sprouting. NSCs can differentiate into neurons that may be used to reconnect portions of the tissue through the formation of structural and functional circuits. OECs and MSCs can give trophic support to neurons sustaining axon regeneration. Scaffolds based on biocompatible material might be used to promote cell viability after transplantation [9] and support axon outgrowth for networking neuronal cells (Figures 1 and 2) [139].

Myelin Regeneration

Demyelination in white matter has been observed in experimental and human SCI [135]. Preserved myelin seems to be related to the ability to improve motor function [136], and some transplanted cells can potentially improve myelination. However, it is difficult to experimentally differentiate new myelinated axons from spared myelinated neurites [136]. Thus, debates remain concerning the potential of remyelination after cell transplantation [136]. It has been proposed that oligodendrocyte progenitor cells or NSCs might be differentiated and integrated as oligodendrocytes to enhance myelin regeneration after SCI; Schwann cells might contribute to regeneration by sustaining axon regrowth by trophic factors, and biomaterials might be used to improve the survival of these cells and create a permissive environment for axon regeneration and myelination (Figures 1 and 2) [9].

by reprogramming differentiated adult cells, but some concerns remain about their efficacy and safety [126]. Somatic stem cells (i.e., MSCs, OECs, and Schwann cells) are free from the ethical controversy because they are collected from adults. MSCs may provide several therapeutic options for SCI, preventing inflammatory cell activation, supporting axonal regrowth and the reestablishment of blood supply to damaged tracts [27]. MSCs, potentially collected from bone marrow, adipose tissue, or amniotic fluid of SCI patients, or alternatively from cord blood for possible future use, make autologous cell transplantation possible, in principle eliminating immune rejection.

Hematopoietic stem cells are currently used to treat many diseases (e.g., several cancers) and have been used in transplantation for over 20 years; they may therefore be acceptable for SCI clinical application. Among MSCs, BM-MSCs are the most frequently studied in preclinical paradigms and several clinical trials have been initiated [127] (Figure 3). Studies including BM-MSC transplantation into rodent models of SCI have shown improved functional recovery [8]. Several trials recruiting patients with cervical SCI have demonstrated promising efficacy of BM-MSC transplantation, with motor and sensory improvement demonstrated by a clinical score

Box 2. Limits of Stem Cell Treatment in SCI

Although the application of stem cells is appealing, optimal therapeutic protocols in terms of the preparation, type, and number of cells, as well as the timing and route of administration, will require future preclinical study. Concerning delivery methods, different methods have been tested to release therapeutic cells into the injured spinal cord. Cells can be injected directly into the damaged site, intrathecally, or systemically (Figure 3). The direct injection of cells into the injury site is the most widely used approach and is an attractive alternative to systemic administration. IV injection of cells could have contraindications for treatment of the spinal cord, such as an unequal distribution of cells in the target tissue and greater risk of potential side effects. Concerns remain about cells intrathecally injected that often leave the zone of injection and about *in situ* administration where a hostile environment could limit their efficacy. Other issues regard the non-uniform origin of the therapeutic cells and the different protocols tested, giving rise to variability in the experiments that might compromise the apparent efficacy in SCI. For a clinical-grade formulation cells must be prepared in accordance with current good manufacturing practice to ensure the safety and quality of the products, and this is also needed in preclinical experiments. Additional effort will also be necessary to reveal the fundamental, detailed biological mechanisms of the efficacy of cell therapy. Mounting evidence suggests that therapeutic potential is related to the paracrine action of these cells, suggesting that this is essential to achieve a response. Some groups suggest using only the secretome produced by therapeutic cells to approach SCI therapy [137,138]. Relying on the secretome may provide various advantages, such as elimination of the variability in cell survival *in situ* and the potential for sustainable release of factors that might be modified according to diverse therapeutic needs.

developed by the American Spinal Injury Association, electromyography, and magnetic resonance imaging [43,44,127–129]. No significant adverse reactions have been observed after several months of follow-up [43,44,127–129]. Thus, BM-MSC transplantation could be a promising treatment to improve neurological outcome in SCI. However, it is advisable to thoroughly evaluate the clinical benefits of other MSC types that have been less well investigated to select which may be the most effective.

The time of intervention is also an important issue for different types of therapeutic cells. As previously mentioned, the success of cell engraftment may depend on the acute, subacute, and chronic phases of SCI injury. Specifically, several clinical trials have demonstrated the efficacy of cells injected in the chronic phase (after 1 year) in SCI patients, suggesting that SCI may be treatable with a cell therapeutic approach during this time-window [43,44]. However, randomized trials in larger cohorts will still be necessary to confirm and validate these results.

Despite promising results so far, the reproducibility of cell treatments remains a challenge that can be overcome only by standardizing cell sources [130], maintenance protocols, and the number of cells transplanted (several million cells have seemed to be sufficient for therapeutic effect [8,127], even if the numbers of surviving stem cells at the injured site have remained relatively low, varying considerably from animal to animal (reviewed in [8]). The number of cells might be drastically reduced by using a biopolymer scaffold to ensure a more controlled delivery procedure, limiting the dispersion of cells in spinal fluid and preserving them from the hostile environment [53].

Another consideration regarding the preclinical paradigms used to demonstrate cell efficacy is that rat SCI models are still considered to be better predictive models for translational approaches than mouse models, given that the pathological outcomes are more similar to human SCI [131]. Functional recovery in these models is described mostly as statistically significant hindlimb motor function improvement compared to untreated groups [132]. Cervical spine trauma, instead of lumbar transection/contusion, is the most frequent injury in human SCI patients [1], but this has been poorly investigated in preclinical models, and efforts to improve this injury paradigm might facilitate predicting treatment efficacy for translational approaches.

To maximize the cell therapeutic effect, combinatorial strategies have also been proposed, and a multitherapeutic approach might thus be more effective than individual therapies. The use of biomaterials (e.g., hydrogel; reviewed in [9]) (Box 3) could be considered as a promising strategy to complement multitherapeutic clinical needs. Simultaneous stem cell transplantation

Box 3. Engineered Biomaterials for Stem Cell Delivery in SCI

Recent advances in materials science have led to biomaterials that aim to promote functional tissue repair following SCI [9]. This approach could ameliorate repair in two ways: biomaterials can act as carriers that can maintain and release their payload (e.g., stem cells and their own biofactors) and, from a structural point of view, can act as supporting materials for tissue regeneration (scaffolds) [139]. Thus, biomaterials should guarantee high stem cell viability and then guide axon regrowth across their structure, thereby bridging to the opposite side of the cavity. To obtain these results several characteristics are fundamental: (i) biocompatibility, (ii) biodegradability, (iii) cytocompatibility, and (iv) adaptive mechanical properties (reviewed in [140]).

Hydrogels represent a promising strategy to support cell survival *in situ*: they are 'soft matter' that can be injected and easily fills the irregular conformation of the lesion cavity [141]. They present high flexibility, gas permeability, no toxicity, and good mechanical properties. Their intimate structure can be easily oriented (aligned fibers or pores) and can be prepared following proper nano-architecture through 3D printing [142]. Furthermore, their injectability is very important because in SCI repair the necessity to avoid risks due to surgery is mandatory, and minimally invasive placement is a fundamental prerequisite for therapy. Other advantages are their ability to load hydrophilic drugs and biomolecules that could be released with carefully controlled kinetics [141]. Indeed, loaded hydrogels are usually injected intrathecally and remain localized at the site of injection, potentially carrying cells and delivering the loaded drugs to the spinal cord [141].

Limitations of hydrogels include inadequate properties related to the control of delivery (e.g., low steric hindrance drugs that might diffuse uncontrollably), as well as unfavorable loading of hydrophobic drugs with low affinity in an aqueous environment. For control of delivery, polymer chains of hydrogel can be functionalized with several post-polymerization strategies to link, with a cleavable bond, drug molecules to reactive points on the polymer network. Following this strategy, the main mechanism related to drug release is the breakage of the link which can be chosen depending on the medical need: the weaker the bond, the higher the release rate [143]. Regarding hydrophobic drug molecules, these can be loaded into polymeric nanoparticles, which may be in turn loaded into the hydrogel [144].

Responding to the crucial issue of limited viability and presence of stem cells at the damaged site, several studies have suggested biopolymer support for cell delivery [145], providing localized targeted therapy to maximize the efficacy of these treatments (Table S3). Loaded hydrogels can remain temporally localized in the spinal cord after implantation, delivering factors that are secreted by stem cells [53] or structurally sustaining them to fill the gap at the site of damage. In addition, the hydrogel can preserve cells from the detrimental environment generated by the damaged spinal cord [53]. For instance, to maximize BM-MSCs efficacy after transplantation *in vivo*, smart 3D support has been proposed to mimic the stem cell niche, creating a more sustainable and permissive environment for cell viability and axonal regeneration [146–151]. Several studies have aimed to increase the survival and efficacy of UC-MSCs by supporting the graft with polymeric scaffolds that mimic a physiological niche *in situ* and that can preserve them from hostile environment while concomitantly permitting paracrine release of factors *in situ* [53,152]. To facilitate connection of the damaged spinal cord segment, several biodegradable scaffolds have been transplanted with A-MSCs to address the regenerative processes promoted by these stem cells in rat and dog SCI models [153,154].

To sustain ESC viability in the damaged spinal cord, polymeric scaffolds have been developed for therapeutic intervention that offer several advantages in supporting stem cell survival after transplantation [81,155]. Natural and synthetic polymer scaffolds have also been developed to support stem cell survival and augment the efficacy of the treatment [156–159].

and cell/drug delivery through a scaffold might prove a useful way to boost treatment efficacy for SCI. More than one dose of cells may be needed to counteract the degenerative dynamic evolution in SCI. For instance, cell therapy with an anti-inflammatory trigger could be used given the possibility of eliciting a proinflammatory response during progression of secondary injury [11,133]. Furthermore, the 3D scaffold may prove to be useful to achieve sustained delivery of these putative therapeutics. It is clear that rigorous testing will be necessary to further test these possibilities.

Concluding Remarks

SCI is a complex pathological condition that evolves over time, causing further damage to the spinal cord tissue after a primary injury, exacerbating clinical outcome. Some encouraging results have been seen in preclinical experiments with stem cell transplantation, but they are not

sufficiently successful for translation into clinical practice. This may reflect a limited understanding of SCI neuropathology, especially regarding the therapeutic strategies tested so far, and particularly those used individually against one specific target, whereas many concomitant

processes and pathways are clearly involved in SCI [2]. Consequently, multiple therapeutic pathways may need to be targeted.

In this scenario, the multitherapeutic ability of stem cells, that are able to release many potential beneficial factors at the damaged tissue site, is being evaluated as one of the most promising strategies to treat SCI. Success with stem cell therapy holds promise because many stem cells act on various pathological outcomes, combining trophic support, anti-inflammatory, immunodulatory, and anti-apoptotic effects, as well as neutralizing inhibitory factors and reducing scar formation [134]. There are several other important issues that might be solved with OEC, neuronal stem cell-derived iPSC, or Schwann cell transplantation, where a favorable axon regeneration environment and myelin cell replacement might promote and bridge any cysts, thereby rewiring the nervous system [99,113,126]. Moreover, several preclinical experiments report the efficacy of cell therapy in SCI – as described in this review – and these have heavily promoted different clinical trials with promising cell types. However, a stronger impact needs to be achieved in clinical trials for the application of these cell therapies in SCI. A realistic therapeutic challenge for SCI should be to rescue sufficient nervous tissue and connections that, together with an appropriate rehabilitative therapy, might further improve clinical outcome. In conclusion, although many questions and challenges remain (see Outstanding Questions and Box 3), the success achieved with several novel cell transplantation protocols to treat SCI offers a promising foothold for future treatments in humans, hopefully optimized to achieve positive clinical outcomes.

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Glossary

Autologous transplants: a generic term for the administration of tissues or cells isolated from the same patients, thereby avoiding any risk of genetic incompatibility or tissue rejection.
Chondroitin sulfate proteoglycans (CSPGs): proteoglycans consisting of a protein core and a chondroitin sulfate side chain; these are widely expressed in the normal central nervous system (CNS) and serve as guidance cues during development and modulate synaptic connections in the adult. In pathological conditions CSPGs can accumulate in damaged tissue and limit neuronal regeneration.
Compression: the act of exerting a strong pressure on the spinal cord. This develops when the spinal cord is compressed by mechanical trauma associated with vertebral fracture or with tumor or abscess that involves the spinal cord.
Contusion: damage caused by crushing the spinal cord, with part of its tissue being spared, and ventral nerve fibers connecting the spinal cord rostral and caudal to the injury remain physically intact.
Cystic cavities: fluid-filled cysts caused by an excessive proinflammatory response around the injured spinal cord. These are composed of astrocytes, fibroblasts, ependymal cells, and collagen fibers that persist after acute degenerative trauma.
Growth cone: a large actin-supported motile structure at the distal tip of an elongating neuronal axon or regenerating neurite seeking its synaptic target.
Lipoplasty: a type of surgery characterized by removal of adipose tissue.
M1 macrophages: is one of the major phenotypes of 'classically activated' macrophages that encourage inflammation through the secretion of inflammatory cytokines (TNF-α, IFN-γ, IL-6, IL-1) and the production of nitric oxide.
M2 macrophages: immune cells that are often described as anti-inflammatory cells because they express high levels of IL-10 and TGF-β.
Paraplegia: partial or complete paralysis of the lower half of the body (most often as a result of trauma).
Primary injury: neurological damage of the spinal cord due to laceration or maceration by a sharp penetrating force, contusion, or compression by a blunt force, or due to infarction associated with a vascular insult.
Reactive gliosis: a nonspecific reactive response of glial cells to damage to the CNS. In most cases gliosis involves the proliferation or hypertrophy of several different types of glial cells (astrocytes, microglia, and oligodendrocytes). In its most extreme form the proliferation associated with gliosis leads to the formation of a glial scar.
Secondary injury: a condition that occurs from minutes to weeks after the primary injury, and that leads to further neurological damage via a continuation of some events from the acute phase – electrolyte shifts, edema, and necrotic cell death – as well as through novel processes including the formation of free radicals, delayed calcium influx, immune system response/inflammation, and apoptotic cell death.
Tetraplegia: also known as quadriplegia, partial or complete paralysis of both the arms and legs that is usually due to injury or disease of the spinal cord in the region of the neck.
Transection: an injury in which axons which run inside the vertebral column are cut, usually as the result of a significant traumatic injury.
White matter demyelination: damage to and loss of myelin surrounding neuronal axons, leading to impaired neurotransmission.
White matter tracts: also known as white matter fibers, areas of the CNS that are mainly made up of myelinated axons.

Outstanding Questions

What is the optimal SCI therapeutic protocol for specific stem cell popula-tions, in terms of the preparation and number of cells?

What are the molecular mechanisms by which specific transplanted stem cells ameliorate SCI outcome?

Which delivery option is the most promising for SCI cell-based therapy, and what is the best timing of intervention?

Is a single SCI treatment a good choice, or will more than one dose be necessary to counteract the dynamic evolution of degeneration in SCI?

Is stem cell therapy a challenge or opportunity in patients with chronic SCI? Many interventions have so far been proposed in the acute–subacute phases of SCI.

Could a multitherapeutic approach be more effective than individual cell ther-apy for SCI, and which one is the most promising?

Could biomaterials indeed be a good strategy to support and enhance stem cell therapy in SCI; which one is most promising, and for what cells?

Could the secretome of stem cells constitute a good alternative to cell-based approaches for SCI therapy?Can it reliably eliminate the variability in cell survival *in situ*, and can it provide a sustainable release of factors that could be adjusted based on specific therapeutic needs?