Mesenchymal Stem Cells for Spinal Cord Injury: Current Options, Limitations, and Future of Cell Therapy

Fabio Cofano 1,*,†, Marina Boido 2,*, Matteo Monticelli 1, Francesco Zenga 1, Alessandro Ducati 1, Alessandro Vercelli 2 and Diego Garbossa 1

Abstract: Spinal cord injury (SCI) constitutes an inestimable public health issue. The most crucial phase in the pathophysiological process of SCI concerns the well-known secondary injury, which is the uncontrolled and destructive cascade occurring later with aberrant molecular signaling, inflammation, vascular changes, and secondary cellular dysfunctions. The use of mesenchymal stem cells (MSCs) represents one of the most important and promising tested strategies. Their appeal, among the other sources and types of stem cells, increased because of their ease of isolation/preservation and their properties. Nevertheless, encouraging promise from preclinical studies was followed by weak and conflicting results in clinical trials. In this review, the therapeutic role of MSCs is discussed, together with their properties, application, limitations, and future perspectives.

Keywords: mesenchymal stem cells; spinal cord injury; regenerative medicine; translational medicine

1. Introduction

Spinal cord injury (SCI) constitutes an inestimable public health issue, with an incidence of 40–80 per million people per year [1]. Generally, young adults are involved, where the burden of permanent neurological damage is unbearable for patients, for their caregivers, and for the health system. Prevention plays of course a key role, such as in cases of road accidents, criminal acts, or secondary causes (tumors, degenerative diseases); however, the real challenge involving scientists is about therapy, given the absence of a gold standard or effective treatment. Most of the post-traumatic degeneration of the nervous system is caused by multifactorial secondary damage including different molecular processes such as inflammation, neuronal death, ionic dysregulation, free radicals and lipid peroxidation, disconnection of normal nerve pathways, blood–brain barrier dysfunction, apoptosis, and necrosis, followed by cavitation processes and retrograde degeneration. In traumatic SCI, an early surgical decompression seems to be important in preventing secondary damage, in the range between 8 and 24 h after injury [2–5], together with spinal fixation to allow correct nursing and rehabilitation.

One of the most important and promising tested strategies involves the use of stem cells. Among them, mesenchymal stem cells (MSCs) are particularly appealing and showed hopeful promise in preclinical research, followed by weak and conflicting results in clinical trials. In this review, the
therapeutic role of MSCs is discussed, together with their properties, application, limitations, and future perspectives.

2. Spinal Cord Injury

The spinal cord of mammals is organized in ten laminae of neurons, named dorsoventrally, according to the Rexed description (1952 and 1954) [6,7]. The neurons are mostly multipolar and vary in size. In the dorsal laminae, sensory neurons are found which receive inputs from the dorsal root ganglion cells and project to other spinal levels or to the upper centers of the sensory pathways. In the ventral laminae, cholinergic large motoneurons are devoted to the control of muscle contraction with motor axons. Somewhat in between, interneurons of different morphologies receive the descending projections and recurrent axonal fibers from spinal motoneurons, and influence motoneuron activity (Mai and Paxinos, 2011) [8]. Spinal cord neurons form intraspinal circuits which are controlled by descending pathways. The reflex arc is the most elementary intraspinal circuit.

The acute phase of SCI depends on the mechanism of trauma, which could be caused by contusion, laceration, stretch, compression, or direct massive destruction. The events related to the trauma constitute the primary injury, with disruption of neuronal pathways [9]. During the immediate phase (occurring within the first two hours) (Rowland et al., 2008) [10], neurons and glial cells at the lesion site die either by necrosis or by apoptosis (Zhang et al., 2012) [11]. Therefore, spinal cord repair should aim first to restore intraspinal circuits, and then to obtain regrowth of descending pathways to regain voluntary control of these intraspinal circuits.

It is well known that the most crucial phase in the pathophysiological process of SCI concerns the secondary injury, which is the uncontrolled and destructive cascade occurring later with aberrant molecular signaling, inflammation, vascular changes, and secondary cellular dysfunctions [12–15].

2.1. Secondary Injury

In the injured spinal cord, to a greater or lesser extent depending on the primary injury, a large amount of destructive processes upset the environment. Taking into account the vascular scenario, a global reduction of blood flow is observed, as a result of vasospasm, together with focal microhemorrhages or thrombosis, causing a global disfunction of the blood–spinal cord barrier [15,16]. The cascade of events also affects electrolytic homeostasis around cellular membranes and their ion pumps/transporters. Potassium (K+) increases its extracellular concentration, while sodium (Na+) and calcium (Ca2+) concentrations increase intracellularly [17,18]. This leads to the blockage of neuronal transmission. The influx of water caused by acidosis promotes cytotoxic edema followed by cellular death [18–20]. Many molecules are released, such as free radicals and neurotransmitters. The inflammatory process involves an immune response mediated by cellular invasion after disruption of the blood–spinal cord barrier. T cells, macrophages, microglia, and neutrophils infiltrate the neuronal tissue, acquiring a proinflammatory phenotype. The environment develops with the production of cytokines such as interleukin-1 beta (IL-1β), interleukin-1 alpha (IL-1α), tumor necrosis factor alpha (TNF-α), and interleukin-6 (IL-6), recruiting more cells in loco promoting neurodegeneration [21–24].

2.2. Chronic Phase and Neurodegeneration

The chronic phase is characterized by scar formation after gliosis and deposit of the extracellular matrix. Molecules with growth-inhibitory effects are released and target neuronal receptors. Oligodendrocyte death in the primary injury seems to be a crucial point in the SCI, because myelin debris contains inhibitory molecules preventing axonal growth in animal models, such as Nogo-A protein or myelin associated glycoprotein (MAG) [25–28]. Proteoglycans are also involved in the chronic phase and show a different pattern of functions in the pathophysiological process; while most of them constitute a limitation for axonal regrowth with their inhibitory features, others seem to border and limit the scar, preventing further amplification of tissue damage [29,30].
In this scenario, the removal of cellular debris and the cell environment is a key point for neuroregeneration; the modulation of macrophages, with their different phenotypes (M1 and M2) and effects in supporting neuroprotection or boosting inflammation, is then a multifactorial and crucial step in determining final outcomes [21–24].

Noteworthy, the regeneration of neurons within the injured spinal cord seems a pipe dream in mammals, but it is innate in the axolotl (salamander) where specific molecules may regulate glial reaction after SCI and promote the proliferation and migration of glial cells to replace the missing neural tube and stimulate axonal growth [31]. The identification of the cellular mechanisms which control neural regeneration is fundamental to promoting spinal cord repair after injury. The modulation of intraneuronal signaling networks and of the extracellular milieu is pivotal to enhance axonal regeneration, thus stimulating the regrowth of intraspinal circuits and of the descending and ascending pathways of the spinal cord [32]. The modulation of glial scar formation and of the alterations in the perineuronal nets, and the control of neuroinflammation following SCI are mandatory for spinal cord repair, even though far from being achieved [33]. Finally, axonal sprouting, synapse plasticity, and remodeling, in part cell-autonomous, may be differently regulated by many cells and molecules in the different compartments of the lesioned spinal cord [34].

3. Stem Cell Therapy and Appeal of MSCs

Stem cell division gives birth to an asymmetrical offspring with an additional progenitor cell and a daughter stem cell. A stem cell is able to differentiate into different phenotypes, thereby determining its potency. Totipotency is defined in the case where all terminal cell populations could be achieved, while multipotency describes the possibility to pursue a more restricted pattern of phenotypes.

The promotion of synapse formation or axon elongation by transplanted neuronal progenitors after damage was described in animal models [35]. Direct modulation in the differentiation of stem cells into terminal phenotypes expanded the focus of research, while promising studies showed encouraging recovery of neurological deficits after transplantation of derived cellular populations from embryonic stem cells (ESCs) in rodents after SCI [36–40].

Since then, researchers multiplied their fields of interest, ranging from the modulation of phenotypic pathways and optimization of transplant techniques, to imaging techniques in order to obtain spatial and temporal information on the grafts [41,42], up to clinical studies starting with the Geron clinical trial which promoted the use of human ESC-derived oligodendrocyte progenitor cells (OPCs) in the site of injury [43]. Although mechanisms are far from being elucidated, stem cell functions seem linked mostly to their paracrine effects and trophic support as shown in other neurological degenerative diseases [16,44–46]. Given by a relatively high number of studies focusing on SCI and neuronal repair, both in vivo and in vitro, evidence shows that a combinatory strategy involving not only stem cells, but also gene therapy, biomolecular targets and drugs, and biomaterials as scaffolds could dramatically improve the functional outcomes after SCI [16].

In this charming landscape, mesenchymal stem cells (MSCs) gained attention because of their easy isolation (from different sources) and preservation, raising no ethical concern [47–49], and of the limited risk of developing tumors [49]. In the case of ESCs, indeed many ethical controversies limited their application because of the problems related to the violation of a human embryo [50]. Additionally, MSCs maintain their regenerative potential even after cryopreservation at 80°C [51]. Their proliferation is very rapid, and a high multilineage differentiation can be obtained [47]. Immunoreactivity or a reaction versus hosts is minimal or absent.

Finally, MSCs show properties of “homing”, being able to migrate toward the site of lesion (Figure 1). According to other authors, we previously observed this phenomenon in SCI experimental models both by immunofluorescence reactions [32] and in MRI experiments [42]. Many authors demonstrated that this cellular behavior is mediated by several inflammatory or chemotactic factors [53]; for example, the vascular endothelial growth factor and hepatocyte growth factor, released at the injury level, can actually attract MSCs [53,54]. Additionally, the SDF-1α/CXCR4 (stromal cell-derived factor-1α/C–X–C
chemokine receptor 4) axis plays an important role in these mechanisms [55]; the impairment or the upregulation of this axis can respectively affect or increase the MSC homing ability [56,57]. Other factors able to positively influence MSC migration include substance P [58] and the granulocyte colony-stimulating factor [59]. However, the precise mechanisms justifying the homing MSC ability are still largely unknown [54].

![Figure 1](image)

**Figure 1.** The main factors and mechanisms influencing mesenchymal stem cell (MSC) homing process are illustrated. (A) When injected either intravenously or intraspinally, MSCs show remarkable properties of “homing”. (B) At the injury site, some molecules (such as VEGF, HGF, cytokines, etc.) are secreted; when transplanted into the spinal parenchyma, MSCs are attracted by chemotactic stimuli and migrate toward the lesion site. (C) Moreover, when injected intravenously, MSCs can interact with endothelial cells through the VLA-4–VCAM-1 interaction; then, the extravasation is mediated by the interaction between the C–X–C chemokine receptor 4 and stromal cell-derived factor-1α (SDF-1), a chemotactic cytokine induced by proinflammatory stimuli. Created with BioRender software.

4. Secretome of MSCs

Although some differences were reported depending on the source, MSCs show a remarkable autocrine and paracrine activity [60,61] (Figure 2).

Through their secretome, MSCs can stimulate proliferation and differentiation of different cell types, including themselves. Notably, it was demonstrated that the release of growth factors, cytokines, and interleukins can also influence MSC migration (see also “homing” mechanism above), via an autocrine loop; indeed, when exposed to conditioned medium (i.e., the medium where MSCs are cultured), the MSC expression of Aquaporin 1 and CXCR4 (two membrane proteins involved in cell migration) significantly increased, by activating Akt and Erk intracellular signal pathways, and caused an enhancement of MSC migration [55].

Moreover, the MSC secretome can also exert immunomodulatory, anti-inflammatory, neurotrophic/neuroprotective and angiogenetic effects on the host microenvironment (as necessary in case of SCI).

The immunomodulation is realized thanks to the expression of the major histocompatibility complex-I on the MSC surface, in this way preventing T-cell recognition and induction of a host immune response [62]. Moreover, MSCs are able to inhibit the proliferation, the activation, and differentiation of T cells [63,64].
Concerning their anti-inflammatory potential, MSCs can secrete a variety of soluble molecules; among the anti-inflammatory cytokines, we can include tumor necrosis factor (TNF) \( \beta_1 \), interleukin (IL)-13, IL-18 binding protein, ciliary neurotrophic factor (CNTF), neurotrophin 3 factor (NT-3), IL-10, IL-12p70, IL-17E, IL-27; moreover, MSCs can also modulate cytokine production of the host, for example, by inhibiting the release of pro-inflammatory cytokines (as interferon-\( \gamma \) and tumor necrosis factor \( \alpha \)) or increasing the release of anti-inflammatory IL-10 \([44,65]\).

To exert neuroprotection, MSCs secrete a number of neurotrophic factors, as brain-derived growth factor (BDNF), glial-derived growth factor (GDNF), nerve growth factor (NGF), NT-1, NT-3, CNTF, and basic fibroblast growth factor (bFGF) \([44,65–69]\); through these factors, MSCs can, on one side, prevent nerve degeneration and apoptosis, and, on the other, support neurogenesis, axonal growth, re-myelination, and cell metabolism \([70–76]\).

MSCs can also induce angiogenesis, an important process by which new vasculature sprouts from pre-existing blood vessels; to this aim, MSCs secrete the tissue inhibitor of metalloproteinase-1, vascular endothelial growth factor, hepatocyte growth factor (HGF), platelet-derived growth factor (PDGF), IL-6, and IL-8. The production of these factors is particularly important for supporting the wound healing processes \([77,78]\).

5. MSCs

MSCs can be obtained from different sources, each of which bears intrinsic characteristics differences, as shown below (Figure 2; Table 1) \([52,79–91]\).
| MSC Type  | Availability [83] | Invasive Procedure of Collection [83] | Proliferation In Vitro [81,85] | Secretome * [79,82,87] | MSC Survival at the Injury Site After Graft [88,89] | Low Immunogenicity in the Host Tissue [84,85] | Anti-Inflammatory Effect in Injured Spinal Cord ** [84] | Glial Scar Reduction [52,80,86,90] | Axonal Regrowth/Sprouting Support [52,80,86,90,91] | Use in Pre-Clinical Studies (This Review) | Use in Clinical Trials (This Review) |
|-----------|------------------|-------------------------------------|-------------------------------|--------------------------|-----------------------------------------------|-----------------------------------------------|---------------------------------------------|-----------------------------------|-----------------------------------|---------------------------------|---------------------------------|
| BM-MSCs   | +++              | +++                                 | ++                            | +++                      | ++                                            | ++                                            | ++                                          | +++                              | +++                              | ++                              | +++                             |
| UC-MSCs   | +                | not invasive                        | +++                           | +++                      | +++                                            | +++                                            | +++                                          | +++                              | +++                              | ++                              | ++                              |
| AD-MSCs   | +++              | ++                                  | +++                           | +++                      | ++                                            | ++                                            | ++                                          | +++                              | +++                              | ++                              | ++                              |
| AF-MSCs   | +                | not invasive                        | +++                           | +++                      | +++                                            | not reported                                  | not reported                               | +++                              | +++                              | +                               | not reported                    |

* Secretion of neurotrophic factors (bFGF, NGF, NT3, NT4, GDNF) is higher for UC-MSCs, whereas the production of pro-angiogenetic factors (VEGF, angiogenin, and PLGF) is higher for BM-MSCs and AD-MSCs. ** Based on the modulation of two inflammatory cytokines of the host tissue (COX-2 and IL-6).
5.1. Bone Marrow Mesenchymal Stem Cells (BM-MSCs)

These cells are found within the adult bone marrow, where they contribute to hematopoiesis and bone regeneration. BM-MSCs can not only be obtained from humans, rodents, or primates, but also from several animal species such as sheep, dogs, cats, and bovines (Figure 3) [88,92–98].

The possibility to differentiate into cells of mesodermal origin and to adhere to plastic distinguishes BM-MSCs from hematopoietic cells. Their range of differentiation is larger than expected, including not only mesenchymal cells such as osteocytes, chondrocytes, and adipocytes, but also a broad range of lineages expressing non-mesenchymal markers [99,100]. Pre-clinical studies collected promising results (Table 2) Wislet-Gendebien et al. addressed the question of differentiation of MSCs in vitro trying to identify neuronal phenotypes. A series of markers were evaluated [100]. Authors found Nestin expression in some groups of cells, a marker for the responsive characteristic of MSCs to extrinsic signals. In Nestin-positive cells, they also registered an overexpression of proteins like sox2, sox10, pax6, fed, erbB2, and erb4. These cells showed a neuron-like conduction, responding to several neurotransmitters (GABA, glycine, glutamate). Compared to neurons, however, trains of action potentials or synaptic activities in co-cultured Nestin-positive MSCs were not observed.

BM-MSCs can not only be transplanted directly into the damaged spinal cord, but also infused with intravenous injections because of the aforementioned homing properties [101–106]. Deng et al. transplanted BM-MSCs two weeks after dorsal SCI in monkeys [104]. A partial functional improvement was noticed in terms of a slight motor recovery (active joints movements in the study group) and in electrophysiological studies with evoked potentials. Monkeys were then studied after three months with characterization of the scar. No neuronal cells were found. The analysis revealed the presence of markers such as neuron-specific enolase (NSE), the neurofilament (NF), and the glial fibrillary acidic protein (GFAP) in approximately 10% of the cells. The true blue, originally injected at the caudal side of injuries, was at the end traceable in the rostral thoracic spinal cord, red nucleus, and sensorymotor cortex. Zurita et al. transplanted BM-MSCs three months after dorsal SCI in pigs [105]. Authors used a motor score (0–10, with 10 considered as animals without deficits). Twelve weeks after transplantation, pigs that underwent stem cell therapy showed a mean score of 6.2 on the motor function scale. Some of the treated animals were even able to get up spontaneously. Recovery of evoked potentials was also noticed.

**Figure 3.** In preclinical experiments, bone marrow (BM)-MSCs can be isolated from femurs of adult mice and expanded in vitro; when cultured, the cells display the typical fibroblast-like shape. Created with BioRender software. Scale bar = 40 μm.
Table 2. Main preclinical studies regarding MSCs.

| Study                          | Type of Stem Cell Transplanted | Type of SCI | Animal | Administration | Scores                                                                 | Adverse Reactions                                                                 | Results                                                                                         | Cells Analysis/Findings in the Scar          |
|--------------------------------|--------------------------------|-------------|--------|----------------|----------------------------------------------------------------------|----------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------|-----------------------------------------------|
| Wislet-Gendebien et al. [100]  | BM-MSC                         | In vitro    | N/A    | N/A            | Anti-gliarial fibrillary acidic protein (GFAP); anti-GLAST; anti-Tuj1; anti-NeuN; anti-SMAD1; anti-MAP2b; anti-synaptophysin; anti-M2; anti-M6; RT-PCR, electrical conductivity | N/A                                                                                      | Neuron-like cells differentiated from Nestin + cells without the mature neuron electrical features; No differentiation in oligodendrocyte-like cells | Nestin + cells, GFAP + cells                  |
| Deng et al. [104]              | BM-MSC                         | Transplantation of BM-MSC 2 weeks after dorsal SCI | Macaco rhesus | Intraleralional | Motor and sensitive improvement (Tarlov behavior assessment), SEP, MEP | None                                                                                   | Motor and sensitive functions improvement (Tarlov 2-3 achieved) in treated monkeys after 3 months follow up; Improvement of SEP and MEP | NSE +, NF +, GFAP + cells                    |
| Zurita et al. [105]            | BM-MSC                         | BM MSC      | Pigs   | Intraleralional | Clinical improvement (from 0 to 10 scale where 0 means paraplegia and 10 constantly useful hike), SEP, MRI | None                                                                                   | 3 months after transplantation improvement of motor functions (mean score of 6.20) and SEP; reduction of the centromedullary cavity | GFAP +, NF +, S100 + cells                   |
| Hofstetter et al. [107]        | BM-MSC                         | Iperacute (immediately after trauma) and acute (transplantation 1 week after dorsal trauma) | Lewis rat | Intraleralional | Fibronectin, vimentin, laminin cells positivity; GFAP, electrical conduction | None                                                                                   | Markers of neuron-like cells, but no depolarization their membrane like mature neurons; No clinical benefit in the iper acute SCI group. In the acute SCI group Ab anti Nestin and GFAP of host astrocytes around and in the scar in the MSC treated population. Immature astrocytes Nestin + GFAP + with the possibility to differentiate into neuron-like cells | Neuron-like cells, host astrocytes closely connected with transplanted MSC cells, astrocyte-like cells |
| Nishio et al. [108]            | HUCB stem cells                | Acute (1 week after dorsal trauma) | Wistar rats | Intraleralional | Basso, Beattie, Bresnahan locomotor scale (BBB), MRI | None                                                                                   | Hindlimb recovery, reduction of cystic cavity, no detection of any double-positive cells for human mitochondria and CD34, of CD4 positive cells, no significant differences between the two groups in the number of OX-42-positive or CD8-positive cells, GAP-43-positive fibers at the epicenter significantly higher than that of the control group | CD45 and human CD44, OX-42, CD4, CD8, GAP-43, 5-HT fibers, T-positive fibers                          |
| Pal et al. [109]               | BM-MSC                         | Acute (1 week after dorsal trauma) | Wistar rats | Intrathecal     | BBB locomotor scale, grid walk, plantar test, inclined plane; cells were tested for CD34, CD44, CD45, CD73, CD90, CD105 and HLA-DR. | None                                                                                   | Improved locomotor and sensory behavioral scores. No graft versus host immune reaction evoked by BM MSC, with the capacity to escape the immune system and be effective in wound healing | Negative astroglial markers, BM MSC            |
| Nemati et al. [110]            | Monkeys NSC                    | Acute (10 days after dorsal trauma) | Macaco rhesus monkeys | Intraleralional | Spontaneous motor activity, Tarlov’s scale, limb pinch test, tail pinch test, sensory test, MRI, evaluation of neural specific markers Tuj1, MAP2, GFAP, Pax6, Sox1 | None                                                                                   | Improvement in the sensory and motor activity, improvement in MRI | Isolated mNSCs express NSC markers such as nestin, Sox1, and Pax6 and could differentiate into mature neurons positive for MAP2 and GFAP |

Int. J. Mol. Sci. 2019, 20, 2698
Table 2. Cont.

| Study                          | Type of Stem Cell Transplanted | Type of SCI | Animal             | Administration | Scores | Adverse Reactions | Results                                                                 | Cells Analysis/Findings in the Scar |
|-------------------------------|-------------------------------|-------------|--------------------|----------------|--------|------------------|-------------------------------------------------------------------------|-------------------------------------|
| Gutierrez et al. [111]         | Human fetal cortex-derived neural progenitor cells (hNPCs) | Iperacute  (immediately after cervical trauma) | Gottingen mini-pig | IntraleSIONal | Tarlov scale, sensory evaluation in the form of a tactile stimulus to the interdigital space | None | Improvement in motor and sensitive functions, no significant decrease in neuronal density between groups; cell engraftment ranged from 12% to 31% | Transplanted MSCs express CD29, Sca-1, and CD45 MHC-I and MHC-II; transplanted MSCs survive and proliferate but do not undergo apoptosis or neural differentiation |
| Hakim et al. [112]            | BM-MSC                        | Acute (24 h after dorsal trauma) | Mice              | IntraleSIONal | Cells were evaluated by flow cytometry, immunohistochemistry, immunocytochemistry, proliferation assay differentiation assay, confocal microscopy and automatic cell quantification | None | MSCs transplanted downregulate genes related to cell-cycle and DNA metabolic/biosynthetic processes and upregulate genes related to immune system response, cytokine production, and phagocytosis/endocytosis; Sca-1 and CD29, MHC-I maintained expression; upregulated expression of CD45 and MHC II; Transplanted MSCs survived and proliferated to a low extent, no expression of Caspase-3, no differentiation into neurons or astrocytes | Transplanted MSCs express CD29, Sca-1, and CD45 MHC-I and MHC-II; transplanted MSCs survive and proliferate but do not undergo apoptosis or neural differentiation |
| Cao et al. [113]              | NSC                            | Acute (10 days after dorsal trauma) | Fischer rats      | IntraleSIONal, intrathecal | Cells were evaluated by immunohistochemistry, confocal microscopy and automatic cell quantification | None | The majority of transplanted cells either differentiated into GFAP + cells or remained nestin +. No Brd-U-positive neurons or oligodendrocytes detected | GFAP+ cells, nestin+ cells, Brd-U+ cells |
| Dasari et al. [114]            | HUCB stem cells               | Acute (1 week after dorsal trauma) | Lewis rat         | IntraleSIONal | BBB locomotor scale, cells were tested for CD44, NF200, CNPase, O1, beta III tubulin, APC, myelin basic protein caspase 3, MAP 2A&2B, confocal/fluorescence microscope, automatic cell quantification, immune blot | None | Improved locomotor and sensory behavioral scores, downregulation HUCB cellsmediated Fas and caspase | NF-200+ cells, CNPase+ cells, CD 44+ cells, co-localization of hUCB with neurons and oligodendrocytes |
| Cho et al. [115]              | HUCB stem cells               | Acute (1 week after lumbar trauma) | Sprague-Dawley rats | IntraleSIONal | BBB locomotor scale, SSEPs, cells were evaluated by immunohistochemistry | None | Improved locomotor and sensory behavioral scores, shortened SSEPs latencies in treated rats | HuNu and GFAP+ cells, MBP+ cells, beta III tubular + cells |
| Khan et al. [92]              | AD-MSCs + BDNF                | Acute (1 week after lumbar trauma) | Beagle dogs       | IntraleSIONal | BBB locomotor scale, cells were tested for Tuj-1, NF, GAP-43, GFA2, Nestin, COX2, TNFa, IL6, STAT3, IL-10, HO-1, BDNF | None | Significant improvement in hindlimb functions, with a higher BBB score | Increase in neuroregeneration, higher expression of Tuj-1, NF-M, and GAP-43, decreased expression of the inflammatory markers interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), and an increased expression of interleukin-10 (IL-10). H&E staining showed more reduced intraparenchymal fibrosis |
Table 2. Cont.

| Study               | Type of Stem Cell Transplanted | Type of SCI Administration | Animal        | Scores                                | Adverse Reactions | Results                                                                 | Cells Analysis/Findings in the Scar |
|---------------------|--------------------------------|----------------------------|---------------|---------------------------------------|-------------------|--------------------------------------------------------------------------|-------------------------------------|
| Ryu et. al. [88]    | BM-MSC, AD-MSC, UCB MSC, Wharton’s jelly-derived MSC | Acute (1 week after lumbar trauma) | Beagle dogs  | Olby score and Revised Modified Talov scale, BBB locomotor scale, confocal/fluorescence microscope, Immunohistochemistry | None              | Significant differences of neurologic recovery in MSCs groups at 2 weeks following MSC transplantation. Purposeful hind limb motion of all dogs in the MSCs groups. No significant differences observed among the MSCs groups. UCB-derived MSCs (UCSCs) induced more nerve regeneration and anti-inflammation activity. Some MSCs expressed markers for neurons (NF160), neuronal nuclei (NeuN) and astrocytes (GFAP). GFAP-positive reactive astrocytes were observed more often in the control group than in MSCs groups. Lesion sizes were smaller, and fewer microglia and reactive astrocytes were found in the spinal cord epicenter of all MSC groups. |                                      |
| Penha et.al. [93]   | BM-MSC                          | Acute (10 days after dorsal or lumbar trauma) | Dogs         | Clinical evaluation, MRI images       | None              | No changes at the MSC administration site into the spinal cord. Progressive recovery of the panniculus reflex and diminished superficial and deep pain response. Conscious reflex recovery occurred simultaneously with moderate improvement in intestine and urinary bladder functions. | N/A                                 |
| Kim et.al. [94]     | AD-MSCs                         | Acute (1 week after dorsal or lumbar trauma) | Dogs         | Clinical improvement: full recovery (normal neurologic state; grade 0), improved (regained deep pain perception (DPP) and recovery of ambulation, but still had mild ataxia; grade 1–2) and unsuccessful (did not regain DPP or the ability to walk without support; grade 3–5) | None              | Clinical improvement (55.6% of the dogs were in full recovery, 22.2% showed improved outcomes and 22.2% had unsuccessful recovery) | N/A                                 |
| Kim et.al. [95]     | AD-MSCs                         | Iperacute (immediately after lumbar trauma) | Beagle dogs  | Revised Talov scale, gait analysis, cells were evaluated by western blot | None              | Significant enhanced motor function in AD-MSCs group compared with those in the control group at 7 days post treatment. The levels of GFAP, and GaCa were increased in the AD-MSC group. β3-tubulin levels were increased, COX-2, IL-6, and TNFα levels were significantly decreased, 3-NF level was significantly decreased, the level of 4-HNE was significantly decreased; the level of PC was significantly decreased. |                                      |
Many authors investigated BM-MSC transplantation in dogs [88,92–95]. Among them, Ryu et al. recorded improved neurological outcomes in MCSs groups after acute transplantation (one week after trauma), since all dogs had purposeful hind limb motion. He also showed that some MSCs expressed markers for neurons (NF160), neuronal nuclei (NeuN) and astrocytes (GFAP). NF160- and NeuN-positive neurons were found, and GFAP-positive reactive astrocytes were observed more often in the control group than in MSCs groups [88].

Hofstetter et al. in 2002 studied stem cell therapy after both iperacute and acute (one week) transplantation. Populations of neuron-like cells with the presence of neural markers were found, but they were not able to depolarize their membrane-like mature neurons. No clinical benefits were recorded. In the acute SCI group, a population of neuronal progenitors and astrocytes of the host were found in tissues after introduction of BM-MSCs in the lesion site [107].

Other preclinical studies are reported in Table 2 [108–115].

Jeon SR et al. described one of the first applications of these cells in patients with cervical SCI (Table 3). In this case, cells were isolated from iliac bones and then subjected to intramedullary or intradural introduction after expansion in a subacute and chronic state. After six months, most patients showed a slight improvement of motor function in the upper limbs, while magnetic resonance imaging (MRI) showed changes at the level of treatment in terms of the disappearance of the cavity margin and the presence of fiber-like streaks [116]. No evidence of neoplasm growth was observed even at three years follow-up [117]. This study and others showed promising but very limited results (Table 3) [118–126]. Dai et al. [118] tested BM-MSCs in a randomized study with complete and chronic SCI patients. Neurological functions were evaluated with AIS grading, ASIA score, residual urine volume, and neurophysiological examination. In the treatment group (N = 20), 10 had clinical improvement. Mean motor improvement with AIS grading was 0.9 ± 1.07, that with the ASIA score was 11.5 ± 17.07, that with the sensory prick score was 5.2 ± 7.78, and that with the sensory light touch score was 5.4 ± 8.22. Residual urine volume (mL) was decreased with a mean of 61.55 ± 77.43. Patients were followed up for six months after an interval between the injury and stem cell therapy of 51.9 ± 18.3 months. No details about clinical improvements before stem cell therapy or other therapies were mentioned.

In a phase I/II controlled single-blind clinical trial, El-Kehir et al. [119] showed functional improvements over patients in the control group of stem cell therapy and physical therapy using AIS grading and ASIA scores in about half of the cases (46%), especially in patients with thoracic injuries with shorter durations of injury and smaller cord lesion. Motor recovery was recorded and promising but still qualitatively limited. Geffner et al. [120] described a partial efficacy of stem cell therapy with some improvements in ASIA, Barthel (quality of life), Frankel, and Ashworth scoring in eight cases (four acute, four chronic). Karamouzian et al. [121] described the results of a nonrandomized clinical trial of transplantation of BM-MSCs in 11 complete SCI patients against 20 in the control group. Results showed improvements of 45.5% of patients (a two-grade improvement from baseline, i.e., from ASIA A to ASIA C) in the study group vs. 15% in the control group, but were not statistically significant (p = 0.095). The heterogeneity and small number of the patients did not allow a reliable analysis. Mendonca et al. [122], Park et al. [123], and Cheng et al. (NCT01393977) described a slight functional improvement in small groups of patients treated with stem cells. Other papers and reviews questioned the extent of improvement and the correct timing of treatment [63,64]. Park et al. only described six cases with some improvements in motor function and changes in cord enhancement with the MRI [123]. Sykova et al. reported data from 20 patients with complete SCI who received transplants 10 to 467 days post-injury. Patients were then evaluated at three, six, and 12 months after implantation with ASIA protocol, the Frankel score, motor and somatosensory evoked potentials, and MRI evaluation of lesion size. Authors registered improvement in motor and/or sensory functions within three months in five of six patients with intra-arterial application, in five of seven acute patients, and in one of 13 chronic patients. Transplantation of cells appeared safe but there was no evidence that the observed beneficial effects were linked to cell therapy [124].
| Study                  | Type of SCI                          | Administration | n of Transplanted Cells | Transplanted Cells Type | Scores                              | Adverse Reactions | Results                                                                 |
|-----------------------|--------------------------------------|----------------|------------------------|------------------------|-------------------------------------|--------------------|-------------------------------------------------------------------------|
| Jeon et al. [116]     | 10 acute SCI patients                 | Intrathecal    | 8 × 10^6 cells         | Autologous MSCs        | ASIA, Frankel score, EMG, SEP, MRI  | None               | Improvement in ASIA score, EMG, and SEP; improvement in MRI imaging   |
| Dai et al. [118]      | 40 human patients; chronic and complete cervical SCI (AIS A) | Perilesional Suspension with 8 × 10^6 cells/microl | Autologous BM-MSCs expanded in culture | AIS, ASIA, residual urinary volume, EMG, MRI | None | 45% AIS A to B; ASIA total scores were 31.6 prior and 43.1 after treatment (p = 0.01); preoperative urinary volume 235 mL to postop volume 173 mL (p = 0.01), improvement also in EMG and MRI |
| El Kheir et al. [119] | 70 human patients; chronic complete cervical or thoracic SCI | Intrathecal | 2 × 10^6 cells/kg | Autologous BM-MSCs | AIS, ASIA, MRI, SEP | None | AIS conversion from AIS A to AIS B or C and from AIS B to AIS C; Improvement in ASIA score, SEP and in MRI. Higher improvement in the thoracic than in the cervical SCI group |
| Gefner 2008 [120]    | 8 human patients (4 acute SCI, 4 chronic SCI) | Directly into the spinal cord, directly into the spinal canal, and intravenous | / | Autologous BM-MSCs | ASIA, Barthel, Frankel Ashworth score, residual urinary volume, MRI | None | Improvement in all of the parameters |
| Karamouzian et al. [121] | 11 human patients with acute or subacute (2-8 weeks after trauma) SCI | Intrathecal | 7 × 10^5 to 1.2 × 10^6 cells | Autologous BM-MSCs | ASIA (12-33 months follow up) | None | Improvement in the ASIA score but the score was not statistically significant (p = 0.095) |
| Mendonca et al. [122] | 14 human patients with chronic thoraco-lumbar SCI | Intralvesional | 5 × 10^6 cells/cm^3 | BM-derived MSCs expanded in culture | ASIA, SEP, MRI, urodynamic, AIS | None | AIS A to B or C; incomplete injury; urinary function improved in 9 subjects, SEP improved in 1 subject |
| Park et al. [123]     | 6 human patients with cervical SCI treated at 72 h after trauma | Intralvesional | 2 × 10^6 cells | Autologous BM-MSCs | Frankel, AIS, MRI | None | AIS A to B/C; improved MRI |
| Sykova et al. [124]   | 20 human patients with complete SCI transplanted from 10 to 467 days after trauma | Intra arterial vs. intra venous | 89.7 +/- 70.7 × 10^6 cells | Autologous BM-MSCs | Frankel, AIS, ASIA, SEP, MRI | None | Not significant results at 3–6–12 months follow-up; however, there was a positive trend |
| Pal et al. [125]      | 30 human patients with complete cervical or thoracic SCI | Intrathecal | 1 × 10^6 cells | Autologous BM-MSCs expanded in culture | ASIA, Barthel, SSEP, MEP, NCV, MRI | None | No significant results in ASIA score; variable patterns of recovery (especially in bladder functions), no significant variations in SSEP, MEP, NCV. Improved MRI |
| Moviglia et al. [126] | 2 human patients with cervical and thoracic chronic SCI | Intralvesional | 5 × 10^6 to 1 × 10^9 cells | Autologous BM-MSCs | SSEP, MEP, MRI, clinical examination | None | Improvement in all of the parameters |
Therefore, new trials are needed, given the absence of protocols and the poor knowledge about mechanisms and outcomes of BM-MSC transplantation. In the ongoing trials (phase I and II), hundreds of patients should be enrolled, thus trying to improve quality of evidence.

Some studies are trying to explore benefits of different combinatory strategies involving not only BM-MSCs, but also technological tools such as virtual reality or exoskeletal stimulation to face the challenge with more holistic approaches [127]. BM-MSCs showed a very promising anti-inflammatory effect on cell environment. In animal models (rats), they promoted anti-inflammatory phenotypes of macrophages (M2) and suppressed lymphocyte proliferation before sustaining regeneration [128–130]. Furthermore, molecules such as vascular endothelial growth factor (VEGF), the glial cell-derived neurotrophic factor (GDNF), the nerve growth factor (NGF), and the brain-derived neurotrophic factor (BDNF) could be produced by MSCs and are currently related to the ability of MSCs to provide trophic support, studied in vivo with animal models [128,131,132]. This is probably why the genetically modified over-expression of these factors could improve clinical outcomes [133]. Finally, the homing properties of BM-MSCs could sustain targeted delivery of drugs acting like specific vectors [134].

In clinical trials involving SCI patients, BM-MSCs were injected with an intrathecal approach in about half of the cases, while, in the remaining studies, other routes of administration were used (in situ as grafts or with scaffold, intravenous or intramuscular) [16].

5.2. Umbilical Cord Mesenchymal Stem Cells (UC-MSCs)

These cells are obtained from cord blood or the umbilical cord [49], and grow in colonies with the support of growth factors [16,49]. The risk of graft rejection using these cells is very low as confirmed by studies demonstrating their hypoimmunogenicity [62]. In preclinical studies, using SCI animal models with rats or mouse, UC-MSCs showed a promising profile of neurotrophic, anti-apoptotic, and anti-inflammatory effects [135–138]. Molecular markers and neuron-like characteristics were observed after homogeneous maturation of UC-MSCs [139]. Despite the aforementioned results in pre-clinical studies, only few clinical trials were published describing minor improvements in some SCI patients [130–132]. Kang et al. described a case report of a young female patient with slight improvements after acute transplantation [140]. In the study of Yao et al., 25 patients with traumatic SCI (injury time >6 months) were treated with human umbilical cord blood stem cells via intravenous and intrathecal injection. The follow-up period was 12 months after transplantation. Results reported some autonomic restoration and changes in somatosensory evoked potentials [141]. The trial of Zhu et al. showed more promising, although limited results after transplantation in the chronic phase: 13 out of 20 patients improved their motor and sphincteric functions. Five out of 20 converted from complete to incomplete (two sensory, three motor; p = 0.038) SCI [142]. A phase II ongoing multicenter, randomized, sham-controlled trial (NCT03521336) recently started with patient enrollment, trying to evaluate efficacy of intrathecal transplantation of UC-MSCs. Completion of the study is expected in 2022.

5.3. Adipose-Derived Mesenchymal Stem Cells (AD-MSCs)

AD-MSCs represent an appealing source of transplantable MSCs, given the remarkable population of somatic stem cells and the availability of adipose tissue [143,144]. The ability of AD-MSCs to secrete growth factors, proteases, cytokines, extracellular matrix molecules, and immunomodulatory factors supports their potential of neuroregenerative, anti-apoptotic, angiogenetic, and wound healing actions [145]. Cellular survival pathways and repairing mechanisms in pre-clinical studies involved the upregulation of kinase proteins like ERK1/2 and Akt [40]. AD-MSC transplantation was studied in animal models showing no adverse effects but often unsatisfactory functional results [86,94,146]. Biomolecular and histological analysis revealed promising details. Kolar et al. [146] studied the effect of transplantation in rats with SCI. AD-MSCs were transplanted into the lateral funiculus 1 mm rostral and caudal to the C3–C4 lesion. In animals treated with cyclosporine, BDNF, vascular endothelial
growth factor, and fibroblast growth factor-2 were expressed for about three weeks. An extensive ingrowth of 5HT-positive raphespinal axons was noticed in the trauma zone with some terminal arborizations reaching the caudal spinal cord. Furthermore, sprouting of raphespinal terminals in C2 contralateral ventral horn and C6 ventral horn on both sides was observed. Relative to the lesion scar, astrocytic processes extended into the middle of the trauma zone in association with regenerating axons. Menezes et al. described an abundant deposition of laminin at the lesion site and spinal midline, the appearance of cell clusters composed of neural-like precursors in the areas of laminin deposition, and the appearance of blood vessels [86]. Kim et al. showed a modification of the inflammatory environment after transplantation of AD-MSCs, with decreased astrogliosis-related signal molecules such as phosphorylated signal transducer and activator of transcription. Furthermore, markers like Tuj-1, Nestin, microtubule-associated protein 2, and neurofilament M were expressed as shown in other aforementioned studies [94]. In clinical studies, a slight sensory improvement was recorded in the majority of patients after intrathecal transplantation, but longitudinal clinical trials with concrete motor responses are still lacking [40]. Coadministration of other compounds, such as 17β-estradiol, and overexpression of Bcl-2 or chondroitinase ABC, were able to enhance therapeutic efficacy in dog models [147]. On this premis, a series of ongoing trials of AD-MSC transplantation is currently underway [40] (Table 3).

5.4. Amniotic Fetal Mesenchymal Stem Cells (AF-MSCs)

These cells can be obtained from amniotic membrane or amniotic fluid. Several features are attributed to AF-MSCs such as their multipotency, ease of isolation, and ability of proliferation, together with a low immunogenicity [148]. Despite this, only few pre-clinical studies in animal models were performed. They showed preliminary and limited results in terms of reduced inflammation and apoptosis, promoted angiogenesis, and provided trophic support [149–151]. No effective clinical trials followed pre-clinical investigations.

6. Biomaterials and Scaffolds for Stem Cell Therapy

Due to technological advances, researchers started to investigate biomaterials with the aim of promoting tissue repair, improving stem cell survival, and supporting their functions [152]. This strategy could be pursued using biomaterials as carriers, thereby ensuring stem cell biofactor delivery, or as a scaffold, offering a structural support for tissue regeneration [16].

Among synthetic polymers, biodegradable hydrogels (such as polyactic acid (PLA), polyglycol acid (PGA), and polyethylene glycol (PEG)) were developed to promote cellular survival and carry several advantages. They easily fill the lesion cavity after injection and show high flexibility, gas permeability, no toxicity, and a favorable mechanical profile [16]. Drugs, biomolecules, and biofactors can be loaded and released locally by hydrogels [153]. To improve proper micro-structure and ensure correct support, three-dimensional (3D) printing nano-architecture was developed to recreate a sustainable and attractive stem cell niche [16,154]. Usually, hydrogels are injected at the site of the lesion. The possibility to use them with a minimally invasive injection reduces the risk of a surgical procedure. Furthermore, they have the ability to load hydrophilic drugs and biomolecules with controlled release. Among the disadvantages, it is important to highlight that the kinetics and delivery of drugs could be inadequate because of uncontrolled diffusion or an unfavorable environment. For example, molecules with low steric hindrance cannot be controlled easily and might diffuse without reproducible control. Furthermore, the loading of hydrophobic drugs with a reduced affinity for the aqueous environment constitutes a real limitation. Bonds between drugs molecules could be built to increase or reduce the rate of release, depending on the ease of breakage of links, thereby offering controlled stem cell biofactor delivery [16].

Among natural scaffolds, a variety of materials were evaluated. Because of its biocompatibility, plasticity, and flexibility, fibrin was shown to promote regeneration and delay accumulation of astrocytes at the site of the injury. Enriched with stem cells or growth factors, fibrin improved survival and
migration of transplanted cells, also increasing neural fiber density. Collagen and hyaluronic acid were proposed and used because of their elasticity, time of degradation, and ability to support cell adhesion and migration. Time of degradation plays a key role in letting the matrix produced by transplanted cells progressively replace the scaffold during the repairing process. An example of an innovative composite implant was described by Rochkind et al., cross-linked hyaluronic acid with growth factors and the adhesive molecule laminin (NVR-Gel), showing promising results. Natural scaffolds were able to reduce neuro-inflammation in the acute stage and support synaptic plasticity, as well as axonal outgrowth [106]. That said, despite this promising neuroengineering background, no concrete results were observed in clinical trials [16,155–158]. The field of polymeric scaffolds was developed to support stem cell survival and efficacy after transplantation, but favorable biomechanical properties did not translate encouraging pre-clinical studies into clinical success, highlighting the necessity for new comprehensive experimentations.

7. Limitations of Current Evidence and Future Directions

Stem cell technology is a growing and evolving field with an unquestionable appeal, as testified by many research papers and state-of-the-art reviews published in the English literature [16,40,80,158,159]. In our experience with MSCs or other stem cells [52,160], like in many other aforementioned papers, tests with animal models showed promising results. Despite this, many caveats arise and, thus, elicit caution against inordinate enthusiasm.

First of all, studies involving animal models are usually performed applying standardized protocols of lesions, treatments, and specific timings of transplantation in each group of investigation. These conditions are often inimitable in human patients with SCI, when timing and treatments are dependent on chance and emergency setting, or where lesions at the cord site could differ a lot from tailored laboratory damage. Most in vivo studies are necessarily performed with rodents, and, despite many anatomical or behavioral correspondences, human clinical trials should be the unavoidable aim of stem cell research.

Therefore, completed human trials showed only limited results. On the one hand, the use of MSCs in SCIs seems caused no harm; different trials [118–126,161] described, above all, the safety of stem cell therapy showing no adverse reactions or side effects. On the other hand, results in terms of clinical outcomes were poor compared to expectations. Among the others, few studies seemed particularly to encourage cell therapy.

Dai et al. [118] tested BM-MSCs in a randomized study with complete and chronic SCI patients. Neurological functions were evaluated with AIS grading, ASIA score, residual urine volume, and neurophysiological examination. In the treatment group (N = 20), 10 had clinical improvement. As already mentioned, patients were followed up for six months and details about clinical improvements before stem cell therapy or other therapies were not mentioned. Outcomes seemed limited, even if promising.

In the trial of El-Kehir et al. [119], functional improvements were noted but were confined and particularly involved patients with smaller and thoracic lesions. Geffner et al. [120] described partial efficacy of stem cell therapy with some improvements in ASIA, Barthel (quality of life), Frankel, and Ashworth scoring in eight cases (four acute, four chronic). The other clinical studies confirmed the trend of confined (Mendonca et al. [122], Park et al. [123], and Cheng et al. (NCT01393977)) or no significant improvements (Karamouzian et al. [121]). Many other trials failed to report satisfactory outcomes (NCT01186679, NCT02027246, NCT01769872, NCT01873547, NCT01624779, NCT01328860, NCT02237547, NCT01694927, and NCT01730183). There is a marked lack of large phase III trials of therapeutic efficacy, due to financial, ethical, and logistics reasons. [40] The phase III study of Oh et al. [162] showed weak efficacy in functional recovery, although some limitations could have compromised clinical results. For instance, only a single administration was given because of a restrictive government policy.
Finally, even if immunochemistry, molecular markers, and morphological tracts show that MSCs, once transplanted, present neuron-like characteristics, it is hard to consider them as such [16,129]; indeed, the expression of neuronal antigens can be simply due to the extremely immature nature of MSCs [163]. Moreover, cell fusion (between MSCs and neurons) was sometimes documented [164]; furthermore, when forced to transdifferentiate by chemical means (such as DMSO), MSCs showed evident morphological changes, which finally were simply attributed to cell shrinkage and changes in the cytoskeleton [165]. More sophisticated protocols are continuously being developed in order to differentiate MCSs into neurons [166,167]. However, currently, the efficacy of MSCs still seems in particular related to their paracrine activity, rather than to cellular replacement mechanisms [168].

Ongoing trials (Table 4) will probably help researchers improve knowledge about the clinical impact of stem cell therapy. Encouraging data from preclinical experiments were not concretely translated into clinical practice. This probably reflects the multifactorial and complex physiopathology of SCI, requiring a multimodal therapeutic approach. As a matter of fact, many points need to be further clarified and depicted, as listed below.

1. The optimal therapeutic protocols regarding the preparation, type, and number of stem cells transplanted;
2. The timing of transplantation and route of administration;
3. The paracrine effects and their influence on functional recovery;
4. The importance of biomaterials and scaffold;
5. The importance of microenvironment;
6. The plasticity and ability to recreate connections of neuronal cells.
7. Additionally, logistics, ethical, and financial problems related to this field of research constitute a real challenge to face in order to channel basic science studies into clinical practice.
Table 4. Ongoing trials about MSCs. IANR-SCIRFS = International Association of Neural Restoration Spinal Cord Injury Functional Rating Scale; NSC = neural stem cell; SCIM III = Spinal Cord Independence Measure III; UC = umbilical cord.

| ClinicalTrials.gov Identifier | Title | MSC Type | Enrolled Subjects | Phase(s) | I End Point | II End Point | Date of Completion | Site of Administration | Intervention | Status |
|-------------------------------|-------|----------|-------------------|----------|------------|-------------|-------------------|-----------------------|--------------|--------|
| NCT03521336                  | Intrathecal transplantation of UC-MSC in patients with sub-acute spinal cord injury | UC-MSCs | 130 | II | ASIA score | IANR-SCIRFS score; EMG; residual urine | Dec 2022 | Intrathecal | Allogeneic UC-MSCs | Recruiting |
| NCT03308565                  | Adipose stem cells for traumatic spinal cord injury | AD-MSCs | 10 | I | Acute-adverse event | ASIA; MEPs; SSEPs; MRI; functional changes | Nov 2023 | Intrathecal | Autologous AD-MSCs | Recruiting |
| NCT03225625                  | Stem cell spinal cord injury exoskeleton and virtual reality treatment study | BM-MSCs | 40 | N/A | ASIA score | ANS function; general well-being | Jul 2022 | Paraspinal; intravenous; intranasal | Autologous BM-MSCs | Recruiting |
| NCT02917291                  | Safety and preliminary efficacy of FAB117-HC in patients with acute traumatic spinal cord injury | AD-MSCs | 46 | I/II | Safety | ISNC-SCI; SCIM III; SSEPs; MEPs | Jan 2020 | Intramedullary | Autologous AD-MSCs | Recruiting |
| NCT01676441                  | Safety and efficacy of autologous mesenchymal stem cells in chronic spinal cord injury | BM-MSCs | 32 | II/III | Treatment | / | Dec 2020 | Intramedullary | Autologous BM-MSC | Recruiting |
| NCT03505034                  | Intrathecal transplantation of UC-MSC in patients with late stage of chronic spinal cord injury | UC-MSCs | 43 | II | ASIA score | IANR-SCIRFS score; EMG; residual urine | Dec 2021 | Intrathecal | Umbilical cord mesenchymal stem cells | Recruiting |
| NCT02574572                  | Autologous mesenchymal stem cell transplantation in cervical chronic and complete spinal cord injury | BM-MSCs | 10 | I | N of participants with treatment-related adverse events as assessed by MRI | ASIA score, ASIA impairment scale, improvement in sensorial mapping and neuropathic pain | Jun 2020 | Intralesional | Autologous BM-MSC | Recruiting |
| NCT03521323                  | Intrathecal transplantation of UC-MSC in patients with early stage of chronic spinal cord injury | UC-MSCs | 66 | I/II | ASIA score | IANR-SCIRFS score; EMG; residual urine | Dec 2021 | Intrathecal | Umbilical cord mesenchymal stem cells | Recruiting |
| NCT02574585                  | Autologous mesenchymal stem cell transplantation in thoracolumbar chronic and complete spinal cord injury | BM-MSCs | 40 | II | N of participants with treatment-related adverse events as assessed by MRI | ASIA score, AIS score, improving in sensorial mapping and neuropathic pain | Jan 2022 | Percutaneous | Autologous BM-MSC | Not yet recruiting |
8. Conclusions

MSC therapy represents an intriguing field of research trying to face the burden of SCI. MSCs of different origin, together with scaffolds, can release immunomodulating and neuroprotective factors which may support neuron survival, axonal growth, and control of glial scarring in absence of significant side effects. Despite promising preclinical findings, clinical trials failed to keep their promises and are still far from obtaining functional recovery and restoring neural circuits. Further studies are needed to improve our knowledge on their mechanisms of action and on the cellular mechanisms preventing restoration of neural circuits after SCI, while combinatory strategies involving stem cells, biomaterials, and modifications of cell environment could be the key to translate fascinating premises into clinical practice. A better relationship between preclinical and clinical studies with a back-and-forth approach is mandatory to enhance the efficacy of cell therapy. Nevertheless, stem cell therapy in SCI injury remains an experimental therapy, possibly in association with others, and should be tested and provided at no cost for the patient. Moreover, patients should be aware of the poor clinical results obtained thus far in clinical trials to prevent exaggerated expectations and dramatic psychological consequences in the case of failure to obtain significant results.

Funding: This study was supported by Ministero dell’Istruzione, dell’Università e della Ricerca—MIUR project “Dipartimenti di eccellenza 2018-2022”.

Conflicts of Interest: The authors declare no conflicts of interest.

References
1. International Perspectives on Spinal Cord Injury. Available online: http://apps.who.int/iris/bitstream/10665/94190/1/9789241564663_eng.pdf (accessed on 31 October 2014).
2. Yılmaz, T.; Kaptanoğlu, E. Current and future medical therapeutic strategies for the functional repair of spinal cord injury. World J. Orthop. 2015, 6, 42–55. [CrossRef] [PubMed]
3. Wilson, J.R.; Forgione, N.; Fehlings, M.G. Emerging therapies for acute traumatic spinal cord injury. CMAJ 2013, 185, 485–492. [CrossRef] [PubMed]
4. Wilson, J.R.; Tetreault, L.A.; Kwon, B.K.; Arnold, P.M.; Mroz, T.E.; Shaffrey, C.; Harrop, J.S.; Chapman, J.R.; Casha, S.; Skelly, A.C.; et al. Timing of Decompression in Patients with Acute Spinal Cord Injury: A Systematic Review. Glob. Spine J. 2017, 7, 955–1155. [CrossRef] [PubMed]
5. Fehlings, M.G.; Perrin, R.G. The role and timing of early decompression for cervical spinal cord injury: Update with a review of recent clinical evidence. Injury 2005, 36, S13–S26. [CrossRef] [PubMed]
6. Rexed, B. The cytoarchitectonic organization of the spinal cord in the cat. J. Comp. Neurol. 1952, 96, 414–495. [CrossRef] [PubMed]
7. Rexed, B. A cytoarchitectonic atlas of the spinal cord in the cat. J. Comp. Neurol. 1954, 100, 297–379. [CrossRef] [PubMed]
8. Mai, J.K.; Paxinos, G. The Human Nervous System, 3rd ed.; Academic Press: Cambridge, MA, USA, 2011; ISBN 9780123742360.
9. Ackery, A.; Tator, C.; Krassioukov, A. A global perspective on spinal cord injury epidemiology. J. Neurotrauma 2004, 21, 1355–1370. [CrossRef]
10. Rowland, J.W.; Hawryluk, G.W.; Kwon, B.; Fehlings, M.G. Current status of acute spinal cord injury pathophysiology and emerging therapies: Promise on the horizon. Neurosurg. Focus 2008, 25, E2. [CrossRef]
11. Zhang, N.; Yin, Y.; Xu, S.J.; Wu, Y.P.; Chen, W.S. Inflammation & apoptosis in spinal cord injury. Indian J. Med. Res. 2012, 135, 287–296.
12. Beattie, M.S.; Li, Q.; Bresnahan, J.C. Cell death and plasticity after experimental spinal cord injury. Prog. Brain Res. 2000, 128, 9–21.
13. Blight, A.R. Spinal cord injury models: Neuropathology. J. Neurotrauma 1992, 9, 147–150. [CrossRef] [PubMed]
14. Grossman, S.D.; Rosenberg, L.J.; Wrathall, J.R. Relationship of altered glutamate receptor subunit mRNA expression to acute cell loss after spinal cord contusion. Exp. Neurol. 2001, 168, 283–289. [CrossRef] [PubMed]
15. Silva, N.A.; Sousa, N.; Reis, R.L.; Salgado, A.J. From basics to clinical: A comprehensive review on spinal cord injury. Prog. Neurobiol. 2014, 114, 25–57. [CrossRef] [PubMed]
16. Vismara, I.; Papa, S.; Rossi, F.; Forloni, G.; Vegliano, P. Current Options for Cell Therapy in Spinal Cord Injury. Trends Mol. Med. 2017, 23, 831–849. [CrossRef]
17. Fehlings, M.G.; Nakashima, H.; Nagoshi, N.; Chow, D.S.; Grossman, R.G.; Kopjar, B. Rationale, design and critical end points for the Riluzole in Acute Spinal Cord Injury Study (RISCIS): A randomized, double-blinded, placebo-controlled parallel multi-center trial. Spinal Cord 2016, 54, 8–15. [CrossRef] [PubMed]
18. Gazdic, M.; Volarevic, V.; Harrell, C.R.; Fellbaum, C.; Jovicic, N.; Arsenijevic, N.; Stojkovic, M. Stem Cells Therapy for Spinal Cord Injury. Int. J. Mol. Sci. 2018, 19, 1039. [CrossRef] [PubMed]
19. Garcia, E.; Aguilar-Cevallos, J.; Silva-Garcia, R.; Ibarra, A. Cytokine and Growth Factor Activation In Vivo and In Vitro after Spinal Cord Injury. Mediat. Inflamm. 2016, 2016, 9476020. [CrossRef] [PubMed]
20. Hayta, E.; Elden, H. Acute spinal cord injury: A review of pathophysiology and potential of non-steroidal anti-inflammatory drugs for pharmacological intervention. J. Chem. Neuroanat. 2017, 87, 25–31. [CrossRef] [PubMed]
21. David, S.; Lopez Vales, R.; Wee Yong, V. Harmful and beneficial effects of inflammation after spinal cord injury: Potential therapeutic implications. Handb. Clin. Neurol. 2012, 109, 485–502. [PubMed]
22. Papa, S.; Caron, I.; Erba, E.; Panini, N.; De Paola, M.; Mariani, A.; Colombo, C.; Ferrari, R.; Pozzer, D.; Zanier, E.R.; et al. Early modulation of pro-inflammatory microglia by minocycline loaded nanoparticles confers long lasting protection after spinal cord injury. Biomaterials 2016, 75, 13–24. [CrossRef] [PubMed]
23. Papa, S.; Caron, I.; Rossi, F.; Vegliano, P. Modulators of microglia: A patent review. Expert Opin. Ther. Pat. 2016, 26, 427–437. [CrossRef] [PubMed]
24. Shechter, R.; Schwartz, M. Harnessing monocyte-derived macrophages to control central nervous system pathologies: No longer ‘if’ but ‘how’. J. Pathol. 2013, 229, 332–346. [CrossRef] [PubMed]
25. Fournier, A.E.; GrandPre, T.; Strittmatter, S.M. Identification of a receptor mediating Nogo-66 inhibition of axonal regeneration. Nature 2001, 409, 341–346. [CrossRef] [PubMed]
26. GrandPre, T.; Nakamura, F.; Vartanian, T.; Strittmatter, S.M. Identification of the Nogo inhibitor of axon regeneration as a reticulon protein. Nature 2000, 403, 439–444. [CrossRef] [PubMed]
27. Schwab, M.E.; Strittmatter, S.M. Nogo limits neural plasticity and recovery from injury. Curr. Opin. Neurobiol. 2014, 27, 53–60. [CrossRef] [PubMed]
28. Schwab, M.E. Nogo and axon regeneration. Curr. Opin. Neurobiol. 2004, 14, 118–124. [CrossRef] [PubMed]
29. Beller, J.A.; Snow, D.M. Proteoglycans: Road signs for neurite outgrowth. Neural Regen. Res. 2014, 9, 343–355.
30. Yuan, Y.-M.; He, C. The glial scar in spinal cord injury and repair. Neurosci. Bull. 2013, 29, 421–435. [CrossRef] [PubMed]
31. Sabin, K.Z.; Jiang, P.; Gearhart, M.D.; Stewart, R.; Echeverri, K. AP-1 (cFos/JunB) and miR-200a regulate the pro-regenerative glial cell response during axolotl spinal cord regeneration. Commun. Biol. 2019, 2, 91. [CrossRef]
32. Van Niekerk, E.A.; Tuszynski, M.H.; Lu, P.; Dulin, J.N. Molecular and Cellular Mechanisms of Axonal Regeneration After Spinal Cord Injury. Mol. Cell Proteom. 2016, 15, 394–408. [CrossRef]
33. Tran, A.P.; Warren, P.M.; Silver, J. The Biology of Regeneration Failure and Success After Spinal Cord Injury. Physiol. Rev. 2018, 98, 881–917. [CrossRef] [PubMed]
34. O'Shea, T.M.; Burda, J.E.; Sofroniew, M.V. Cell biology of spinal cord injury and repair. J. Clin. Investig 2017, 127, 3259–3270. [CrossRef] [PubMed]
35. Lu, P.; Wang, Y.; Graham, L.; McHale, K.; Gao, M.; Wu, D.; Brock, J.; Blesch, A.; Rosenzweig, E.S.; Havton, L.A.; et al. Long-distance growth and connectivity of neural stem cells after severe spinal cord injury. Cell 2012, 150, 1264–1273. [CrossRef] [PubMed]
36. Keirstead, H.S.; Nistor, G.; Bernal, G.; Totoiu, M.; Cloutier, F.; Sharp, K.; Steward, O. Human embryonic stem cell-derived oligodendrocyte progenitor cell transplants remyelinate and restore locomotion after spinal cord injury. J. Neurosci. 2005, 25, 4694–4705. [CrossRef] [PubMed]
37. Deshpande, D.M.; Kim, Y.S.; Martinez, T.; Carmen, J.; Dike, S.; Shats, I.; Rubin, L.L.; Drummond, J.; Krishnan, C.; Hoke, A.; et al. Recovery from paralysis in adult rats using embryonic stem cells. Ann. Neurol. 2006, 60, 32–44. [CrossRef] [PubMed]
38. Pera, M.F.; Andrade, J.; Houssami, S.; Reubinoff, B.; Trounson, A.; Stanley, E.G.; Oostwaard, D.W.-v.; Mummery, C. Regulation of human embryonic stem cell differentiation by BMP-2 and its antagonist noggin. J. Cell Sci. 2004, 117, 1269–1280. [CrossRef] [PubMed]
39. McDonald, J.W.; Liu, X.Z.; Qu, Y.; Liu, S.; Mickey, S.K.; Turetsky, D.; Gottlieb, D.I.; Choi, D.W. Transplanted embryonic stem cells survive, differentiate and promote recovery in injured rat spinal cord. Nat. Med. 1999, 5, 1410–1412. [CrossRef]

40. Jin, M.C.; Medress, Z.A.; Azad, T.D.; Doulames, V.M.; Veeravagu, A. Stem cell therapies for acute spinal cord injury in humans: A review. Neurosurg. Focus 2019, 46, E10. [CrossRef]

41. Filippi, M.; Boido, M.; Terreno, E. Imaging of MSC transplantation in neuroscience. Oncotarget 2017, 8, 10781–10782. [CrossRef]

42. Filippi, M.; Boido, M.; Pasquino, C.; Garello, F.; Fagioli, F.; Temi, S.; Mazzini, L.; Ferrero, I.; Mareschi, K.; Vizzini, A.; Temi, S.; Mazzini, L.; Ferrero, I.; Boido, M.; Rupa, R.; Garbossa, D.; Ducati, A.; Vercelli, A. Embryonic and adult stem cells promote raphespinal axon outgrowth and improve functional outcome following spinal hemisection in mice. Exp. Neurol. 2016, 282, 66–77. [CrossRef]

43. Frantz, S. Embryonic stem cell pioneer Geron exits field, cuts losses. Nat. Biotechnol. 2012, 30, 12–13. [CrossRef]

44. Boido, M.; Piras, A.; Valsecchi, V.; Spigolon, G.; Mareschi, K.; Ferrero, I.; Vizzini, A.; Temi, S.; Mazzini, L.; Fagioli, F.; et al. Human mesenchymal stromal cell transplantation modulates neuroinflammatory milieu in a mouse model of amyotrophic lateral sclerosis. Cytotherapy 2014, 16, 1059–1072. [CrossRef]

45. Mazzini, L.; Vercelli, A.; Ferrero, I.; Boido, M.; Cantello, R.; Fagioli, F. Transplantation of mesenchymal stem cells in ALS. Prog. Brain Res. 2012, 201, 333–359. [PubMed]

46. Gunetti, M.; Tomasi, S.; Giammò, A.; Boido, M.; Rustichelli, D.; Mareschi, K.; Errichiello, E.; Parola, M.; Ferrero, I.; Fagioli, F.; et al. Myogenic potential of whole bone marrow mesenchymal stem cells in vitro and in vivo for usage in urinary incontinence. PLoS ONE 2012, 7, e45538. [CrossRef]

47. Lee, M.W.; Yang, M.S.; Park, J.S.; Kim, H.C.; Kim, Y.J.; Choi, J. Isolation of mesenchymal stem cells from cryopreserved human umbilical cord blood. Int. J. Hematol. 2005, 81, 126–130. [CrossRef]

48. Dasari, V.R.; Veeravalli, K.K.; Dinh, D.H. Mesenchymal stem cell transplantation in the treatment of spinal cord injuries. World J. Stem Cells 2014, 6, 120–133. [CrossRef]

49. Lu, L.L.; Liu, Y.J.; Yang, S.G.; Zhao, Q.J.; Wang, X.; Han, Z.B.; Xu, Z.S.; Lu, Y.X.; Liu, D.; et al. Isolation and characterization of human umbilical cord mesenchymal stem cells with hematopoiesis-supportive function and other potentials. Haematologica 2006, 91, 1017–1026.

50. Lo, B.; Parham, L. Ethical Issues in Stem Cell Research. Endocr. Rev. 2009, 30, 204–213. [CrossRef]

51. Kotobuki, N.; Hirose, M.; Takakura, Y.; Ohgushi, H. Cultured autologous human cells for hard tissue regeneration: Prepa-ration and characterization of mesenchymal stem cells from bone marrow. Artif. Organs 2004, 28, 33–39. [CrossRef]

52. Boido, M.; Rupa, R.; Garbossa, D.; Fontanella, M.; Ducati, A.; Vercelli, A. Embryonic and adult stem cells promote raphespinal axon outgrowth and improve functional outcome following spinal hemisection in mice. Eur. J. Neurosci. 2009, 30, 833–846. [CrossRef]

53. Zachar, L.; Bačenková, D.; Rosocha, J. Activation, homing, and role of the mesenchymal stem cells in the inflammatory environment. J. Inflamm. Res. 2016, 9, 231–240. [CrossRef]

54. Qu, J.; Zhang, H. Roles of Mesenchymal Stem Cells in Spinal Cord Injury. Stem Cells Int. 2017, 2017, 5251313. [CrossRef]

55. Pelagalli, A.; Nardelli, A.; Lucarelli, E.; Zannetti, A.; Brunetti, A. Autocrine signals increase ovine mesenchymal stem cells migration through Aquaporin-1 and CXCR4 overexpression. J. Cell. Physiol. 2018, 233, 6241–6249. [CrossRef]

56. Xu, Q.; Wang, J.; He, J.; Zhou, M.; Adi, J.; Webster, K.A.; Yu, H. Impaired CXCR4 expression and cell engraftment of bone marrow-derived cells from aged atherogenic mice. Atherosclerosis 2011, 219, 92–99. [CrossRef]

57. Marquez-Curtis, L.A.; Gul-Uludag, H.; Xu, P.; Chen, J.; Janowska-Wieczorek, A. CXCR4 transfection of cord blood mesenchymal stromal cells with the use of cationic liposome enhances their migration toward stromal cell-derived factor-1. Stem Cells Transl. Med. 2013, 15, 840–849. [CrossRef]

58. Hong, H.S.; Lee, J.; Lee, E.; Kwon, Y.S.; Lee, E.; Ahn, W.; Jiang, M.H.; Kim, J.C.; Son, Y. A new role of substance P as an injury-inducible messenger for mobilization of CD29(+)/stromal-like cells. Nat. Med. 2009, 15, 425–435. [CrossRef]

59. Petit, I.; Szyper-Kравitz, M.; Nagler, A.; Lahav, M.; Peled, A.; Habler, L.; Ponomaryov, T.; Taichman, R.S.; Arenzana-Seisdedos, F.; Fuji, N.; et al. G-CSF induces stem cell mobilization by decreasing bone marrow SDF-1 and up-regulating CXCR4. Nat. Immunol. 2002, 3, 687. [CrossRef]
60. Sobacchi, C.; Palagano, E.; Villa, A.; Menale, C. Soluble Factors on Stage to Direct Mesenchymal Stem Cells Fate. Front. Bioeng. Biotechnol. 2017, 5, 32. [CrossRef]

61. Baez-Jurado, E.; Hidalgo-Lanussa, O.; Barrera-Bailón, B.; Sahebkar, A.; Ashraf, G.M.; Echeverria, V.; Barreto, G.E. Secretome of Mesenchymal Stem Cells and Its Potential Protective Effects on Brain Pathologies. Mol. Neurobiol. 2019, 1–26. [CrossRef]

62. Ryan, J.M.; Barry, F.P.; Murphy, J.M.; Mahon, B.P. Mesenchymal stem cells avoid allogeneic rejection. J. Inflamm. 2005, 2, 8. [CrossRef]

63. Di Nicola, M.; Carlo-Stella, C.; Magni, M.; Milanesi, M.; Longoni, P.D.; Matteucci, P.; Grisanti, S.; Gianni, A.M. Human bone marrow stromal cells suppress T-lymphocyte proliferation induced by cellular or nonspecific mitogenic stimuli. Blood 2002, 99, 3838–3843. [CrossRef]

64. Liang, X.; Ding, Y.; Zhang, Y.; Tse, H.F.; Lian, Q. Paracrine mechanisms of mesenchymal stem cell-based therapy: Current status and perspectives. Cell Transplant. 2014, 23, 1045–1059. [CrossRef]

65. Vizzoso, F.J.; Eiro, N.; Cid, S.; Schneider, J.; Perez-Fernandez, R. Mesenchymal Stem Cell Secretome: Toward Cell-Free Therapeutic Strategies in Regenerative Medicine. Int. J. Mol. Sci. 2017, 25, 1852. [CrossRef]

66. Mead, B.; Logan, A.; Berry, M.; Leadbeater, W.; Scheven, B.A. Paracrine-mediated neuroprotection and neuritogenesis of axotomised retinal ganglion cells by human dental pulp stem cells: Comparison with human bone marrow and adipose-derived mesenchymal stem cells. PLoS ONE 2014, 9, e109305. [CrossRef]

67. Kolar, M.K.; Itte, V.N.; Kingham, P.J.; Novikov, L.N.; Wiberg, M.; Kelk, P. The neurotrophic effects of different human dental mesenchymal stem cells. Sci. Rep. 2017, 7, 12605. [CrossRef]

68. Teixeira, F.G.; Carvalho, M.M.; Neves-Carvalho, A.; Panchalingam, K.M.; Behie, L.A.; Pinto, L.; Sousa, N.; Salgado, A.J. Secretome of mesenchymal progenitors from the umbilical cord acts as modulator of neural/glial proliferation and differentiation. Stem Cell Rev. 2015, 11, 288–297. [CrossRef]

69. Potapova, I.A.; Gaudette, G.R.; Brink, P.R.; Robinson, R.B.; Rosen, M.R.; Cohen, I.S.; Doronin, S.V. Mesenchymal stem cells support migration, extracellular matrix invasion, proliferation, and survival of endothelial cells in vitro. Stem Cells 2007, 25, 1761–1768. [CrossRef]

70. Hofer, H.R.; Tuan, R.S. Secreted trophic factors of mesenchymal stem cells support neurovascular and musculoskeletal therapies. Stem Cell Res. Ther. 2016, 7, 131. [CrossRef]

71. Razavi, S.; Ghasemi, N.; Mardani, M.; Salehi, H. Remyelination improvement after neurotrophic factors secreting cells transplantation in rat spinal cord injury. Iran. J. Basic Med. Sci. 2017, 20, 392–398.

72. Lu, P.; Jones, L.L.; Tuszyński, M.H. BDNF-expressing marrow stromal cells support extensive axonal growth at sites of spinal cord injury. Exp. Neurol. 2005, 191, 344–360. [CrossRef]

73. Neuhuber, B.; Timothy Hilmes, B.; Shumsky, J.S.; Gallo, G.; Fischer, I. Axon growth and recovery of function reduces glial cyst and improves functional outcome after spinal cord compression. World Neurosurg. 2014, 81, 183–190. [CrossRef]
81. Choudhery, M.S.; Badowski, M.; Muise, A.; Harris, D.T. Comparison of human mesenchymal stem cells derived from adipose and cord tissue. *Cytotheraphy* **2013**, *15*, 330–343. [CrossRef]

82. Giampà, C.; Alvino, A.; Magatti, M.; Silini, A.R.; Cardinale, A.; Paldino, E.; Fusco, F.R.; Parolini, O. Conditioned medium from amniotic cells protects striatal degeneration and ameliorates motor deficits in the R6/2 mouse model of Huntington’s disease. *J. Cell. Mol. Med.* **2019**, *23*, 1581–1592. [CrossRef]

83. Hass, R.; Kasper, C.; Böhms, S.; Jacobs, R. Different populations and sources of human mesenchymal stem cells (MSC): A comparison of adult and neonatal tissue-derived MSC. *Cell Commun. Signal.* **2011**, *9*, 12. [CrossRef]

84. Leto Barone, A.A.; Khalifian, S.; Lee, W.P.; Brandacher, G. Immunomodulatory effects of adipose-derived stem cells: Fact or fiction? *BioMed Res. Int.* **2013**, *2013*, 383685. [CrossRef]

85. Ma, J.; Wu, J.; Han, L.; Jiang, X.; Yan, L.; Hao, J.; Wang, H. Comparative analysis of mesenchymal stem cells derived from amniotic membrane, umbilical cord, and chorionic plate under serum-free condition. *Stem Cell Res. Ther.* **2019**, *10*, 19. [CrossRef]

86. Menezes, K.; Nascimento, M.A.; Gonçalves, J.P.; Cruz, A.S.; Lopes, D.V.; Curzio, B.; Bonaminio, M.; de Menezes, J.R.; Borovevic, R.; Rossi, M.I.; et al. Human mesenchymal cells from adipose tissue deposit laminin and promote regeneration of injured spinal cord in rats. *PLoS ONE* **2014**, *9*, e96020. [CrossRef]

87. Pisciutti, F.; Brunelli, L.; Romele, P.; Silini, A.; Sammali, E.; Paracchini, L.; Marchini, S.; Talamini, L.; Bigini, P.; Boncoraglio, G.B.; et al. Protection of brain injury by amniotic mesenchymal stromal cell-secreted metabolites. *Crit. Care Med.* **2016**, *44*, e1118–e1131. [CrossRef]

88. Ryu, H.H.; Kang, B.J.; Park, S.S.; Kim, Y.; Sung, G.J.; Woo, H.M.; Kim, W.H.; Kweon, O.K. Comparison of mesenchymal stem cells derived from fat, bone marrow, Wharton’s jelly, and umbilical cord blood for treating spinal cord injuries in dogs. *J. Vet. Med. Sci.* **2012**, *74*, 1617–1630. [CrossRef]

89. Takahashi, A.; Nakajima, H.; Uchida, K.; Takeura, N.; Honjoh, K.; Watanabe, S.; Kitade, M.; Kokubo, Y.; Johnson, W.E.B.; Matsumine, A. Comparison of Mesenchymal Stromal Cells Isolated from Murine Adipose Tissue and Bone Marrow in the Treatment of Spinal Cord Injury. *Cell Transplant.* **2018**, *27*, 1126–1139. [CrossRef]

90. Yang, C.; Wang, G.; Ma, F.; Yu, B.; Chen, F.; Yang, J.; Feng, J.; Wang, Q. Repeated injections of human umbilical cord blood-derived mesenchymal stem cells significantly promotes functional recovery in rabbits with spinal cord injury of two noncontinuous segments. *Stem Cell Res. Ther.* **2018**, *9*, 136. [CrossRef]

91. Zhou, H.L.; Zhang, X.J.; Zhang, M.Y.; Yan, Z.J.; Xu, Z.M.; Xu, R.X. Transplantation of Human Amniotic Mesenchymal Stem Cells Promotes Functional Recovery in a Rat Model of Traumatic Spinal Cord Injury. *Neurochem. Res.* **2016**, *41*, 2708–2718. [CrossRef]

92. Khan, I.U.; Yoon, Y.; Kim, A.; Jo, K.R.; Choi, K.U.; Jung, T.; Kim, N.; Son, Y.; Kim, W.H.; Kweon, O.K. Improved Healing after the Co-Transplantation of HO-1 and BDNF Overexpressed Mesenchymal Stem Cells in the Subacute Spinal Cord Injury of Dogs. *Cell Transplant.* **2018**, *27*, 1140–1153. [CrossRef]

93. Penha, E.M.; Meira, C.S.; Guimaraes, E.T.; Mendonca, M.V.P.; Gravely, F.A.; Pinheiro, M.B.; Pinheiro, T.M.B.; Barrousin-Melo, S.M.; Ribeiro dos Santos, R.; Pereira Soares, M.B. Use of Autologous Mesenchymal Stem Cells Derived from Bone Marrow for the Treatment of Naturally Injured Spinal Cord in Dogs. *Stem Cells Int.* **2014**, *2014*, 437521. [CrossRef]

94. Kim, Y.; Lee, S.H.; Kim, W.H.; Kweon, O.K. Transplantation of adipose derived mesenchymal stem cells for acute thoracolumbar disc disease with no deep pain perception in dogs. *J. Vet. Sci.* **2016**, *17*, 123–126. [CrossRef]

95. Kim, Y.; Jo, S.H.; Kim, W.H.; Kweon, O.K. Antioxidant and anti-inflammatory effects of intravenously injected adipose derived mesenchymal stem cells in dogs with acute spinal cord injury. *Stem Cell Res. Ther.* **2015**, *6*, 229. [CrossRef]

96. Zhu, X.; Liu, Z.; Deng, W.; Zhang, Z.; Liu, Y.; Wei, L.; Zhang, Y.; Zhou, L.; Wang, Y. Derivation and characterization of sheep bone marrow derived mesenchymal stem cells induced with telomerase reverse transcriptase. *Saud J. Biol. Sci.* **2017**, *24*, 519–525. [CrossRef]

97. Martin, D.R.; Cox, N.R.; Hatchcock, T.L.; Niemeyer, G.P.; Baker, H.J. Isolation and characterization of multipotent mesenchymal stem cells from feline bone marrow. *Exp. Hematol.* **2002**, *30*, 879–886. [CrossRef]

98. Bosnakovski, D.; Mizuno, M.; Kim, G.; Takagi, S.; Okumura, M.; Fujinaga, T. Isolation and multilineage differentiation of bovine bone marrow mesenchymal stem cells. *Cell Tissue Res.* **2005**, *319*, 243–253. [CrossRef]

99. Exp. Hematol.* **2002**, *30*, 879–886. [CrossRef]

100. Crit. Care Med.* **2018**, *46*, 23–32. [CrossRef]
99. Carrade, D.D.; Aflotter, V.K.; Outerbridge, C.A.; Watson, J.L.; Ga-luppo, L.D.; Buerchner, S.; Kumar, V.; Walker, N.J.; Borjesson, D.L. Intradermal injections of equine allogeneic umbilical cord-derived mesenchymal stem cells are well tolerated and do not elicit immediate or delayed hypersensitivity reactions. *Cytotherapy* 2011, 13, 1180–1192. [CrossRef]

100. Wislet-Gendebien, S.; Hans, G.; Leprince, P.; Rigo, J.M.; Moonen, G.; Rogister, B. Plasticity of cultured mesenchymal stem cells: Switch from nestin-positive to excitable neuron-like phenotype. *Stem Cells* 2005, 23, 392–402. [CrossRef]

101. Mishra, P.J.; Banerjee, D. Activation and Differentiation of Mesenchymal Stem Cells. *Methods Mol. Biol.* 2017, 1554, 201–209.

102. Mortada, I.; Mortada, R. Epigenetic changes in mesenchymal stem cells differentiation. *Eur. J. Med. Genet.* 2018, 61, 114–118. [CrossRef]

103. Kozorovitskiy, Y.; Gould, E. Stem cell fusion in the brain. *Nat. Cell Biol.* 2003, 5, 952–954. [CrossRef]

104. Deng, Y.B.; Liu, X.G.; Liu, Z.G.; Liu, X.L.; Liu, Y.; Zhou, G.Q. Implantation of BM mesenchymal stem cells into injured spinal cord elicits de novo neurogenesis and functional recovery. *Cytotherapy* 2006, 8, 210–214. [CrossRef]

105. Zurita, M.; Vaquero, J.; Bonilla, C.; Santos, M.; De Haro, J.; Oya, S.; Aguayo, C. Functional recovery of chronic paraplegic pigs after autologous transplantation of bone marrow stromal cells. *Transplantation* 2008, 86, 845–853. [CrossRef]

106. Tetzlaff, W.; Okon, E.B.; Karimi-Abdolrezaee, S.; Hill, C.E.; Sparling, J.S.; Plemel, J.R.; Plunet, W.T.; Tsai, E.C.; Baptiste, D.; Smithson, L.J.; et al. A systematic review of cellular transplantation therapies for spinal cord injury. *J. Neurotrauma* 2011, 28, 1611–1682. [CrossRef]

107. Hofstetter, C.P.; Schwarz, E.J.; Hess, D.; Widenfalk, J.; El Manira, A.; Prockop, D.J.; Olson, L. Marrow stromal cells form guiding strands in the injured spinal cord and promote recovery. *Proc. Natl. Acad. Sci. USA* 2002, 99, 2199–2204. [CrossRef]

108. Nishio, Y.; Koda, M.; Kamada, T.; Someya, Y.; Yoshinaga, K.; Okada, S.; Harada, H.; Okawa, A.; Moriya, H.; Yamazaki, M. The use of hemopoietic stem cells derived from human umbilical cord blood to promote restoration of spinal cord tissue and recovery of hindlimb function in adult rats. *J. Neurosurg. Spine* 2006, 5, 424–433. [CrossRef]

109. Pal, R.; Chaitanya, G.; Rao, N.M.; Banerjee, P.; Krishnamororth, V.; Venkataramana, N.K. Functional recovery after transplantation of bone marrow-derived human mesenchymal stromal cells in a rat model of spinal cord injury. *Cytotherapy* 2010, 12, 792–806. [CrossRef]

110. Nemati, S.N.; Jabbari, R.; Hajinasrollah, M.; Mehrjerdi, N.Z.; Azizi, H.; Hemmesi, K.; Moghiminasr, R.; Azhdari, Z.; Talebi, A.; Mohitmafi, S.; et al. Transplantation of Adult Monkey Neural Stem Cells into A Contusion Spinal Cord Injury Model in Rhesus Macaque Monkeys. *Cell J. Yakhteh* 2014, 16, 117–130.

111. Gutierrez, J.; Lamanna, J.J.; Grin, N.; Hurtig, C.V.; Miller, J.H.; Riley, J.; Urquia, L.; Avalos, P.; Svendsen, C.N.; Federici, T.; Boulos, N.M. Preclinical Validation of Multilevel Intraparenchymal Stem Cell Therapy in the Porcine Spinal Cord. *Neurosurgery* 2015, 77, 604–612. [CrossRef]

112. Hakim, R.; Covacu, R.; Zachariadis, V.; Frostell, A.; Sankavaram, S.R.; Brundin, L.; Svensson, M. Mesenchymal stem cells transplanted into spinal cord injury adopt immune cell-like characteristics. *Stem Cell Res. Ther.* 2019, 10, 115. [CrossRef]

113. Cao, Q.; Zhang, P.; Howard, R.M.; Walters, W.M.; Tsoufas, P.; Whittermore, S.R. Pluripotent stem cells engrafted into the normal or lesioned adult rat spinal cord are restricted to glial lineage. *Exp. Neurol.* 2001, 167, 48–58. [CrossRef]

114. Dasari, V.R.; Spomar, D.G.; Gondi, C.S.; Sloffer, C.A.; Saving, K.L.; Gujrati, M.; Rao, J.S.; Dinh, D.H. Axonal remyelination by cord blood stem cells after spinal cord injury. *J. Neurotrauma* 2007, 24, 391–410. [CrossRef]

115. Cho, S.R.; Yang, M.S.; Yim, S.H.; Park, J.H.; Lee, J.E.; Eom, Y.W.; Jang, I.K.; Kim, H.E.; Park, J.S.; Kim, H.O.; et al. Neurally induced umbilical cord blood cells modestly repair injured spinal cords. *Neuroreport* 2008, 19, 1259–1263. [CrossRef]

116. Jeon, S.R.; Park, J.H.; Lee, J.H.; Kim, D.Y.; Kim, H.S.; Sung, I.Y.; Choi, G.H.; Geon, M.H.; Kim, G.G. Treatment of spinal cord injury with bone marrow-derived, cultured autologous mesenchymal stem cells. *Tissue Eng. Regen. Med.* 2010, 7, 316–322.
117. Park, J.H.; Kim, D.Y.; Sung, I.Y.; Choi, G.H.; Jeon, M.H.; Kim, K.K.; Jean, S.R. Long-term results of spinal cord injury therapy using mesenchymal stem cells derived from bone marrow in humans. Neurosurgery 2012, 70, 1238–1247. [CrossRef]  
118. Dai, G.; Liu, X.; Zhang, Z.; Yang, Z.; Dai, Y.; Xu, R. Transplantation of autologous bone marrow mesenchymal stem cells in the treatment of complete and chronic cervical spinal cord injury. Brain Res. 2013, 1533, 73–79. [CrossRef]  
119. El-Kheir, W.A.; Gabr, H.; Awad, M.R.; Ghannam, O.; Barakat, Y.; Farghali, H.A.; El Maadawi, Z.M.; El Hawary, I.E.; El Shazly, A.; El Ramly, M.; et al. Transplantation of autologous bone marrow mesenchymal stem cells to prevent the secondary neuronal death after spinal cord injury. Transplant. Proc. 2015, 47, 1039–1042. [CrossRef]  
120. Ge et al. Human mesenchymal stem cells modulate immune responses in multiple sclerosis. Stem Cells Transl. Med. 2018, 7, 210–224. [CrossRef]  
121. Karamouzian, S.; Nematollahi-Mahani, S.N.; Nahkhaee, N.; Eskandary, H. Clinical safety and primary efficacy of bone marrow mesenchymal stem cell transplantation in subacute spinal cord injured patients. Clin. Neurol. Neurosurg. 2012, 114, 935–939. [CrossRef]  
122. Mendonça, M.V.; Larocca, T.F.; de Freitas Souza, B.S.; Villarreal, C.F.; Silva, L.F.; Matos, A.C.; Novaes, M.A.; Bahia, C.M.; de Oliveira Melo Martinez, A.C.; Kaneto, C.M.; et al. Safety and neurological assessments after autologous transplantation of bone marrow mesenchymal stem cells in subjects with chronic spinal cord injury. Stem Cell Res. Ther. 2014, 5, 126. [CrossRef]  
123. Park, H.C.; Shim, Y.S.; Ha, Y.; Yoon, S.H.; Park, S.R.; Choi, B.H.; Park, H.S. Treatment of complete spinal cord injury patients by autologous bone marrow mesenchymal stem cell transplantation and administration of granulocyte-macrophage colony stimulating factor. Tissue Eng. 2005, 11, 913–922. [CrossRef]  
124. Syková, E.; Homola, A.; Mazanec, R.; Lachmann, H.; Konrádová, S.L.; Kobylka, P.; Páděr, R.; Neuwirth, J.; Komrška, V.; Vávra, V.; et al. Autologous bone marrow transplantation in patients with subacute and chronic spinal cord injury. Cell Transplant. 2006, 15, 675–687. [CrossRef]  
125. Pal, R.; Venkataramana, N.K.; Bansal, A.; Balaraju, S.; Jan, M.; Chandra, R.; Dixit, A.; Rauthan, A.; Murgod, U.; Totey, S. Ex vivo-expanded autologous bone marrow-derived mesenchymal stromal cells in human spinal cord injury/paraplegia: A pilot clinical study. Cytotheraphy 2009, 11, 897–911. [CrossRef]  
126. Moviglia, G.A.; Fernández Viña, R.; Brizuela, J.A.; Saslavsky, J.; Vrsalovic, F.; Varela, G.; Bastos, F.; Farina, P.; Etchegaray, G.; Barbieri, M.; et al. Combined protocol of cell therapy for chronic spinal cord injury. Report on the electrical and functional recovery of two patients. Cytotherapy 2006, 8, 202–209. [CrossRef]  
127. Stem Cell Spinal Cord Injury Exoskeleton and Virtual Reality Treatment Study. Identification No. NCT03225625. Available online: https://clinicaltrials.gov/ct2/show=NCT03225625?term=NCT03225625andrank=1 (accessed on 21 July 2014).  
128. Neirinckx, V.; Cantinieaux, D.; Coste, C.; Rogister, B.; Franzen, R.; Wislet-Gendebien, S. Concise review. Spinal cord injuries: How could adult mesenchymal and neural crest stem cells take up the challenge? Stem Cells 2014, 32, 829–843. [CrossRef]  
129. Corcione, A.; Benvenuto, F.; Ferretti, E.; Giunti, D.; Cappiello, V.; Cazzanti, F.; Risso, M.; Gualandi, F.; Mancardi, G.L.; Pistocchi, V.; et al. Human mesenchymal stem cells modulate B-cell functions. Blood 2006, 107, 367–372. [CrossRef]  
130. Nakajima, H.; Uchida, K.; Guerrero, A.R.; Watanabe, S.; Sugita, D.; Takeura, N.; Yoshihara, A.; Long, G.; Wright, K.T.; Johnson, W.E.; et al. Transplantation of mesenchymal stem cells promotes an alternative pathway of macrophage activation and functional recovery after spinal cord injury. J. Neurotrauma 2012, 29, 1614–1625. [CrossRef]  
131. Kanekiyo, K.; Nakano, N.; Homma, T.; Yamada, Y.; Tamachi, M.; Suzuki, Y.; Fukushima, M.; Saito, F.; Ide, C. Effects of multiple injection of bone marrow mononuclear cells on spinal cord injury of rats. J. Neuro-Trauma 2017, 34, 3003–3011. [CrossRef]  
132. Vaquero, J.; Zurita, M. Functional recovery after severe CNS trauma: Current perspectives for cell therapy with bone marrow stromal cells. Prog. Neurobiol. 2011, 93, 341–349. [CrossRef]  
133. Sasaki, M.; Radtke, C.; Tan, A.M.; Zhao, P.; Hamada, H.; Houkin, K.; Honmou, O.; Kocsis, J.D. BDNF-hypersecreting human mesenchymal stem cells promote functional recovery, axonal sprout-ing, and protection of corticospinal neurons after spinal cord injury. J. Neurosci. 2009, 29, 14932–14941.
134. Wang, L.J.; Zhang, R.P.; Li, J.D. Transplantation of neurotrophin-3-expressing bone mesenchymal stem cells improves recovery in a rat model of spinal cord injury. Acta Neurochir. 2014, 156, 1409–1418. [CrossRef]

135. Chua, S.J.; Bielecki, R.; Yamanaka, N.; Fehlings, M.G.; Rogers, I.M.; Casper, R.F. The effect of umbilical cord blood cells on outcomes after experimental traumatic spinal cord injury. Spine 2010, 35, 1520–1526. [CrossRef]

136. Caron, I.; Rossi, F.; Papa, S.; Aloe, R.; Sculco, M.; Mauri, E.; Sacchetti, A.; Erba, E.; Panini, N.; Parazzi, V.; et al. A new three dimensional biomimetic hydrogel to deliver factors secreted by human mesenchymal stem cells in spinal cord injury. Biomaterials 2016, 75, 135–147. [CrossRef]

137. Dasari, V.R.; Veeravalli, K.K.; Tsung, A.J.; Gondi, C.S.; Gujrati, M.; Dinh, D.H.; Rao, J.S. Neuronal apoptosis is inhibited by cord blood stem cells after spinal cord injury. J. Neurotrauma 2009, 26, 2057–2069. [CrossRef]

138. Kao, C.H.; Chen, S.H.; Chio, C.C.; Lin, M.T. Human umbilical cord blood-derived CD34 cells may attenuate spinal cord injury by stimulating vascular endothelial and neurotrophic factors. Shock 2008, 29, 49–55.

139. Kang, K.S.; Kim, S.W.; Oh, Y.H.; Yu, J.W.; Kim, K.Y.; Park, H.K.; Song, C.H.; Han, H. A 37-year-old spinal cord-injured female patient, transplanted of multipotent stem cells from human UC blood, with improved sensory perception and mobility, both functionally and morphologically. Cytotherapy 2005, 7, 368–373.

140. Yao, L.; He, C.; Zhao, Y.; Wang, J.; Tang, M.; Li, J.; Wu, Y.; Ao, L.; Hu, X. Human umbilical cord blood stem cell transplantation for the treatment of chronic spinal cord injury. Neuro Regen. Res. 2013, 8, 397–403.

141. Zhu, H.; Poon, W.; Liu, Y.; Leung, G.K.; Wong, Y.; Feng, Y.; Stephanie, C.P.N.g.; Tsang, K.S.; Sun, D.T.F.; Yeung, D.K.; et al. Phase I-II clinical trial assessing safety and efficacy of umbilical cord blood mononuclear cell transplantation therapy of chronic complete spinal cord injury. Cell Transpl. 2016, 25, 1925–1943. [CrossRef]

142. De Ugarte, D.A.; Morizono, K.; Elbarbary, A.; Alfonso, Z.; Zuk, P.A.; Zhu, M.; Dragoon, J.L.; Ashjian, P.; Thomas, B.; Benhaim, P.; et al. Comparison of multi-lineage cells from human adipose tissue and bone marrow. Cells Tissues Organs 2003, 174, 101–109. [CrossRef]

143. Zuk, P.A.; Zhu, M.; Mizuno, H.; Huang, J.; Futrell, J.W.; Katz, A.J.; Benhaim, P.; Lorenz, H.P.; Hedrick, M.H. Multilineage cells from human adipose tissue: Implications for cell-based therapies. Tissue Eng. 2001, 7, 211–228. [CrossRef]

144. Ohta, Y.; Hamaguchi, A.; Ootaki, M.; Watanabe, M.; Takeba, Y.; Iiiri, T.; Matsumoto, N.; Takenaga, M. Intravenous infusion of adipose-derived stem/stromal cells improves functional recovery of rats with spinal cord injury. Cytotherapy 2017, 19, 839–848. [CrossRef]

145. Kolar, M.K.; Kingham, P.J.; Novikova, L.N.; Wiberg, M.; Novikov, L.N. The therapeutic effects of human adipose-derived stem cells in a rat cervical spinal cord injury model. Stem Cells Dev. 2014, 23, 1659–1674. [CrossRef]

146. Lee, S.H.; Kim, Y.; Rhew, D.; Kuk, M.; Kim, M.; Kim, W.H.; Kweon, O.K. Effect of the combination of mesenchymal stromal cells and chondroitinase ABC on chronic spinal cord injury. Cytotherapy 2015, 17, 1374–1383. [CrossRef]

147. Kim, E.Y.; Lee, K.B.; Kim, M.K. The potential of mesenchymal stem cells derived from amniotic membrane and amniotic fluid for neuronal regeneration therapy. BMB Rep. 2014, 47, 135–140. [CrossRef]

148. Bottai, D.; Scesa, G.; Cigognini, D.; Adami, R.; Nicora, E.; Abrignani, S.; Di Giulio, A.M.; Gorio, A. Third trimester NG2-positive amniotic fluid cells are effective in improving repair in spinal cord injury. Exp. Neurol. 2014, 254, 121–133. [CrossRef]

149. Gao, S.; Ding, J.; Xiao, H.J.; Li, Z.Q.; Chen, Y.; Zhou, X.S.; Wang, J.E.; Wu, J.; Shi, W.Z. Anti-inflammatory and anti-apoptotic effect of combined treatment with methylprednisolone and amniotic membrane mesenchymal stem cells after spinal cord injury in rats. Neurochem. Res. 2014, 39, 1544–1552. [CrossRef]

150. Sankar, V.; Muthusamy, R. Role of human amniotic epithelial cell transplantation in spinal cord injury repair research. Neuroscience 2003, 118, 11–17. [CrossRef]

151. Rossi, F.; Perale, G.; Papa, S.; Forloni, G.; Vegliano, P. Current options for drug delivery to the spinal cord. Expert Opin. Drug Deliv. 2013, 10, 385–396. [CrossRef]

152. Perale, G.; Rossi, F.; Santoro, M.; Peviani, M.; Papa, S.; Llupi, D.; Torriani, P.; Micotti, E.; Previdi, S.; Cervo, L.; et al. Multiple drug delivery hydrogel system for spinal cord injury repair strategies. J. Control. Release 2012, 159, 271–280. [CrossRef]
154. Lee, J.M.; Yeong, W.Y. Design and printing strategies in 3D bioprinting of cell-hydrogels. *Adv. Healthc. Mater.* 2016, 5, 2856–2865. [CrossRef]

155. Chen, B.K.; Knight, A.M.; de Ruiter, G.C.; Spinner, R.J.; Yaszemski, M.J.; Currier, B.L.; Windebank, A.J. Axon regeneration through scaffold into distal spinal cord after transection. *J. Neurotrauma* 2009, 26, 1759–1771. [CrossRef]

156. Lin, X.Y.; Lai, B.Q.; Zeng, X.; Che, M.T.; Ling, E.A.; Wu, W.; Zeng, S.Y. Cell transplantation and neuroengineering approach for spinal cord injury treatment: A summary of current laboratory findings and review of literature. *Cell Transplant.* 2016, 25, 1425–1438. [CrossRef]

157. Führmann, T.; Tam, R.Y.; Ballarin, B.; Coles, B.; Elliott Donaghue, I.; van der Kooy, D.; Nagy, A.; Tator, C.H.; Morshead, C.M.; Shoichet, M.S. Injectable hydrogel promotes early survival of induced pluripotent stem cell-derived oligodendrocytes and attenuates longterm teratoma formation in a spinal cord injury model. *Biomaterials* 2016, 83, 23–36. [CrossRef]

158. Garbossa, D.; Boido, M.; Fontanella, M.; Fronda, C.; Ducati, A.; Vercelli, A. Recent therapeutic strategies for spinal cord injury treatment: Possible role of stem cells. *Neurosurg. Rev.* 2012, 35, 293–311. [CrossRef]

159. Garbossa, D.; Fontanella, M.; Fronda, C.; Benevello, C.; Muraca, G.; Ducati, A.; Vercelli, A. New strategies for repairing the injured spinal cord: The role of stem cells. *Neurol. Res.* 2006, 28, 500–504. [CrossRef]

160. Boido, M.; Garbossa, D.; Vercelli, A. Early graft of neural precursors in spinal cord compression reduces glial cyst and improves function. *J. Neurosurg. Spine* 2011, 15, 97–106. [CrossRef]

161. Satti, H.S.; Waheed, A.; Ahmed, P.; Ahmed, K.; Akram, Z.; Aziz, T.; Satti, T.M.; Shahbaz, N.; Khan, M.A.; Malik, S.A. Autologous mesenchymal stromal cell transplantation for spinal cord injury: A Phase I pilot study. *Cytotherapy* 2016, 18, 518–522. [CrossRef]

162. Oh, S.K.; Choi, K.H.; Yoo, J.Y.; Kim, D.Y.; Kim, S.J.; Jeon, S.R. A phase III clinical trial showing limited efficacy of autologous mesenchymal stem cell therapy for spinal cord injury. *Neurosurgery* 2016, 78, 436–447. [CrossRef]

163. Deng, J.; Petersen, B.E.; Steinzlder, D.A.; Jorgensen, M.L.; Laywell, E.D. Mesenchymal stem cells spontaneously express neural proteins in culture and are neurogenic after transplantation. *Stem Cells* 2006, 4, 1054–1064. [CrossRef]

164. Wurmser, A.E. Gage FH Stem cells: Cell fusion causes confusion. *Nature* 2002, 416, 485–487. [CrossRef]

165. Lu, P.; Blesch, A.; Tuszynski, M.H. Induction of bone marrow stromal cells to neurons: Differentiation, transdifferentiation, or artifact? *J. Neurosci. Res.* 2004, 77, 174–191. [CrossRef]

166. Takeda, Y.S.; Xu, Q. Neuronal Differentiation of Human Mesenchymal Stem Cells Using Exosomes Derived from Differentiating Neuronal Cells. *PLoS ONE* 2015, 10, e0135111. [CrossRef]

167. Cortés-Medina, L.V.; Pasantes-Morales, H.; Aguilera-Castrejon, A.; Picones, A.; Lara-Figueroa, C.O.; Luis, E.; Montesinos, J.J.; Cortés-Morales, V.A.; De la Rosa Ruiz, M.P.; Hernández-Estévez, E.; et al. Neuronal Transdifferentiation Potential of Human Mesenchymal Stem Cells from Neonatal and Adult Sources by a Small Molecule Cocktail. *Stem Cells Int.* 2019, 2019, 7627148. [CrossRef]

168. Park, W.S.; Ahn, S.Y.; Sung, S.I.; Ahn, J.Y.; Chang, Y.S. Strategies to enhance paracrine potency of transplanted mesenchymal stem cells in intractable neonatal disorders. *Pediatr. Res.* 2018, 83, 214–222. [CrossRef]

© 2019 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).