International Journal of
Molecular Sciences

MDPI

Review

Induced Pluripotent Stem Cell Therapies for Cervical Spinal Cord Injury

Vanessa M. Doulames and Giles W. Plant *

Stanford Partnership for Spinal Cord Injury and Repair, Department of Neurosurgery, Stanford University School of Medicine, 265 Campus Drive Stanford, California, CA 94305, USA; vanessad@stanford.edu
* Correspondence: gplant@stanford.edu; Tel.: +1-650-724-3388; Fax: +1-650-736-1949

Academic Editor: Wenbin Deng
Received: 11 February 2016; Accepted: 28 March 2016; Published: 9 April 2016

Abstract: Cervical-level injuries account for the majority of presented spinal cord injuries (SCIs) to date. Despite the increase in survival rates due to emergency medicine improvements, overall quality of life remains poor, with patients facing variable deficits in respiratory and motor function. Therapies aiming to ameliorate symptoms and restore function, even partially, are urgently needed. Current therapeutic avenues in SCI seek to increase regenerative capacities through trophic and immunomodulatory factors, provide scaffolding to bridge the lesion site and promote regeneration of native axons, and to replace SCI-lost neurons and glia via intraspinal transplantation. Induced pluripotent stem cells (iPSCs) are a clinically viable means to accomplish this; they have no major ethical barriers, sources can be patient-matched and collected using non-invasive methods. In addition, the patient's own cells can be used to establish a starter population capable of producing multiple cell types. To date, there is only a limited pool of research examining iPSC-derived transplants in SCI—even less research that is specific to cervical injury. The purpose of the review herein is to explore both preclinical and clinical recent advances in iPSC therapies with a detailed focus on cervical spinal cord injury.

Keywords: spinal cord injury; cervical; iPSC; induced pluripotent stem cell; embryonic stem cell; intraspinal transplantation

1. Introduction

Spinal Cord Injury (SCI) places a formidable emotional, physical, and financial burden on the United States. Annually, the incidence of survived SCI is estimated at 12,500 cases leading to an estimated total of 276,000 with over half occurring at the cervical level. The causes of general SCI tend to be accident or violence related, however, the weight-bearing and flexible nature of vertebrae at the cervical level make it particularly susceptible to injury [1,2]. Some cervical-specific causes of injury include direct and indirect military-based injuries (via combat or through weaknesses in tactical armor design) [3–7] and lifestyle choices (such as sedentary lifestyles) leading to structural degradation of the cervical spine [8,9]. Ironically, advancements in modern healthcare have also been influential in the increasing incidence of survived cervical SCI; improvements in emergency medicine have led to better survival rates immediately following injury [10] while improvements in preventative care have led to the steady increase of an aging population and therefore age-associated injuries, degeneration, and weaknesses of the cervical spine [11–13].

Less than 1% of patients with SCI leave the hospital with a full neurological recovery; lengthy bouts of hospitalization, outpatient medical care (such as rehabilitation), and the need for full or part-time caretakers are often required. Coupled with injury-related motor deficits, they are often unable to participate in the workforce. In fact, by 10 years post-injury, only around 28% of patients are employed. Medical attention and injury-related lifestyle changes are financially overwhelming; over

the course of a lifetime, they can accrue up to $4.5 million in costs directly associated with SCI. Overall, SCI costs the nation over $20 billion in direct and indirect injury-related expenses [1,2].

Despite survival rates increasing, quality of life still remains drastically poor with cervical SCI patients encountering a gradation of respiratory and upper motor limb dysfunction, full or partial paralysis, neuropathic pain, extensive financial obligations, lack of personal independence, and resulting significant lifestyle adjustments. Therapies to improve quality of life and restore function, even partially, would make a huge impact and help ease the physical, financial, and emotional burden placed on cervical SCI patients and their caretakers (Figure 1).

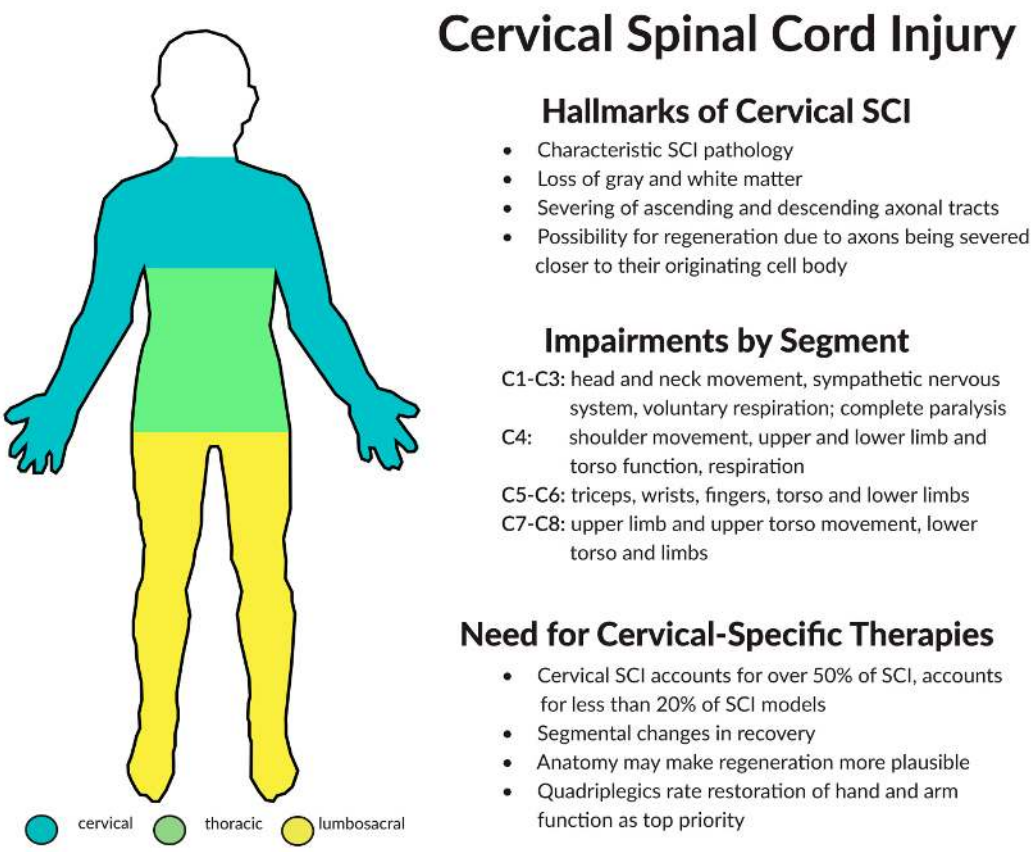

Figure 1. Clinical deficits, segmental differences, and the need for cervical-specific therapies for Spinal Cord Injury (SCI) within the human.

2. Components of Cervical Spinal Cord Injury (SCI)

2.1. Pathophysiology

SCI is characteristically comprised of two phases that, while distinct, still maintain some level of overlap—primary and secondary injury (Figure 2). The primary phase occurs immediately at the time of injury and is directly caused by gross physical trauma to the spinal cord. There are four major categories of primary injury: impact plus persistent compression, impact alone with transient compression, distraction, and laceration or transection. During primary injury, the delicate spinal cord tissue is mechanically compromised due to shearing and compression forces either by direct contact or inadvertently through manipulation of the vertebrae. This initial trauma leads to mechanical injury, disruptions in vasculature, respiratory difficulties, neurogenic shock, inflammation, membrane compromise, alterations in ion and neurotransmitter levels, and ultimately sets the stage for the secondary phase of injury [14–16].

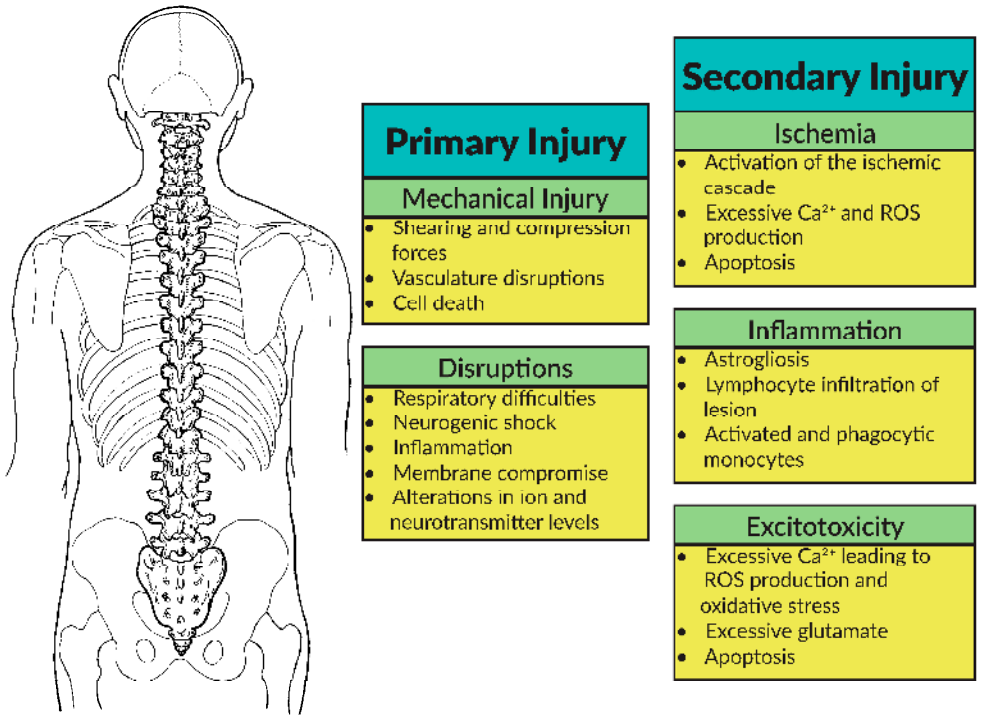

Figure 2. The Spinal Cord Injury (SCI) Cascade is comprised of both a primary and secondary component that ultimately results in ischemia, inflammation, and reactive oxygen species (ROS)-based excitotoxicity.

While primary phase-associated damage leads to an immediate and often serious impairment of neurological function, secondary phase typically dictates the full magnitude of injury. There are approximately 25 well established mechanisms that constitute the secondary phase of injury, yet much remains to be elucidated as to how these pathways converge and play upon each other to determine the full manifestation of injury [17,18]. This biochemical cascade activates the ischemic pathway, which leads to neurotransmitter imbalances that underlie excitotoxicity. Other consequences such as inflammation and immune responses, swelling, and neuronal apoptosis also occur [19–25].

Mammalian SCI triggers large zones of necrosis at the site of injury, leading to cystic cavitation, creating gaps in the circuitry, and preventing communication with rostral centers along the central nervous system (CNS) into the brain. Axons within the spinal cord fail to regenerate after injury and retract towards the soma, with the majority stopping close to the injury lesion border. Overall, this results in a change to normal motor, sensory, and autonomic function depending on injury location. In humans, high cervical injuries are the most severe and often result in full paralysis with respiration, speaking, and bowel function also affected. Lower cervical injuries lead to partial paralysis, and gradations in respiratory, bowel, and arm and hand function. Thoracic injuries are dependent on level with injury to higher levels primarily affecting the full trunk and legs and lower levels affecting the bowels and legs. Injuries at the lumbosacral level lead to impairments in voluntary bowel control and partially affect function in the hips and legs, but patients are often able to retain limited or full walking ability.

2.2. Regeneration and Plasticity

Prior studies have suggested that the adult mammalian CNS does not regenerate, predominantly due to the inability of neurons to regenerate axons through the inhibitory milieu of the glial scar and injured spinal cord lesion [26]. Despite this, some degree of functional recovery is often seen, likely due to reorganization of spared circuitry and heavily dependent on the axonal sprouting of both spared

lesioned and intact fibers [26–28]. Experimental evidence has shown that axonal regeneration and functional recovery can be influenced and promoted via the usage of synergistic therapies such as the addition of neurotrophic growth factors [29–32], the deletion of inhibitory factors typically associated with the lesion [33–35], and rehabilitation regimens and physical activity [36–38]. Despite this, the innate regenerative capabilities of the CNS are often overwhelmed by the extent of injury and functional behavior is drastically affected.

The immense damage following SCI results in pathophysiological heterogeneity and therefore requires complex therapeutic interventions that are engineered to address the individual components of the problem. Preclinical and clinical cell transplantation therapies may provide a solution to this by seeking to ameliorate existing damage and prevent further exacerbation by increasing innate regenerative capacities, providing scaffolding to bridge the lesion site and by replacing SCI-lost neurons and glia via intraspinal transplantation (Figure 3) [39–45].

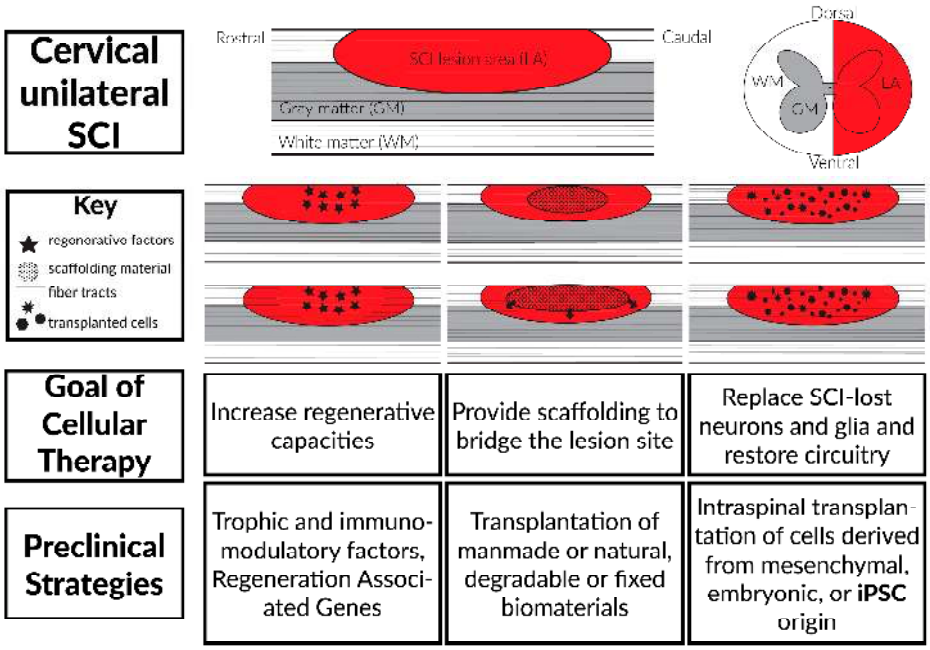

Figure 3. Current SCI therapeutics are calibrated to increase the regenerative capacities of the lesion site, provide a bridge through the lesion site to promote reconnection of rostral and caudal central nervous system (CNS), and to replace SCI-lost neurons and glia via transplantation of mesenchymal, embryonic, and induced pluripotent stem cells (iPSCs).

The goal of this latter approach is to repair connectivity along the CNS by overcoming glial scar formation, thus serving as a cellular relay system or via promoting neurite regeneration [46]. In recent decades, the therapeutic promise of replacing neurons and glia by intraspinal transplantation has gained significant interest and some have eventuated to clinical trials. Numerous pre-clinical experiments have been developed including peripheral nerve bridges, Schwann cells, olfactory glia, mesenchymal stem cells and neural stem cells [47–54]. Initially, embryonic neural tissue was grafted into SCI lesion sites leading to some restoration of anatomy and function. The heterogeneity and source of the implanted tissue made direct "bench to bedside" translatability of this approach difficult and unlikely to gain required FDA approval as a clinical treatment [55]. Embryonic neural progenitor cells provide an alternative means to addressing cell loss and support following SCI, yet also add an additional ethical barrier that may endanger its applicability as a viable clinical treatment [56,57]. Therapies derived from human induced pluripotent stem cells (iPSCs) are in their infancy, but overcome many of the barriers of alternative existing cellular approaches. They provide the significant additional benefit of patient-matching, therefore surmounting the need for immunosuppression between host and

donor cells. The review herein explores both preclinical and clinical recent advances in iPSC therapies targeting cervical SCI.

2.3. Considerations for Treating Cervical SCI

Cervical level injuries account for the majority of SCI, however, over 80% of studies have used various mid-thoracic injury models [58]. Although gross similarities in pathophysiology exist between the levels, there are key distinctions that underlie the need for cervical-specific models and therapeutics.

Anatomically, injuries at the cervical level are characterized by severing of the ascending and descending axonal tracts of the white matter and substantial cell loss within the gray matter. At the thoracic level, injuries tend to involve white-matter damage. This translates to distinct segmental differences in motor function at the cervical level that simply do not apply to thoracic injuries. Clinically, restoring function in a mid to lower cervical injury in even a singular segment would translate to greater patient independence. One study reported that restoration of function at the 5th and 6th cervical (C5/C6) level would allow a patient to independently type, eat, drink, wash, shave, dress their upper body, have more control over their wheelchair, and be able to transfer themselves from a chair to a bed or a car [59]. Restoration of a singular segment at the thoracic level would not translate to the same magnitude of functional recovery.

Furthermore, axonal tracts descending from rostral centers within the CNS are damaged closer to their originating soma in cervical *versus* thoracic SCI. There is substantial evidence that long descending axons rarely regenerate in injuries at the mid-thoracic level or lower but can at the cervical level [60–62]. Interestingly in mammalian quadruped models of SCI, animals that receive thoracic injuries are often able to regain some level (if not all) of locomotion, presumably due to the presence of a central pattern generator in the lumbar segments and the restructuring of propriospinal circuitry [63,64]. Supporting'this was a key study in which decerebrate cats received a full spinal transection in the lower thoracic region and were still able to perform basic walking motions when electrophysiologically stimulated, thus suggesting that the supraspinal tracts originating in the motor cortex may not even be imperative to basic function [65–67]. In contrast, in rat models of cervical SCI, unilateral hemisection injury in the lower cervical levels leads to the irreversible loss of fine motor control of the forepaws and substantial motor deficits in the biceps and triceps brachii muscles [68–71]. Moreover, during reach and grab behavioral assessments, the recruitment pattern for proximal and distal pairs of antagonist muscles showed highly disorganized activation patterns [72].

Survivors of cervical SCI are faced with quadriplegia and all the sensorimotor deficits that accompany it. In a survey distributed to the SCI community and composed of 681 responses, the top priority of quadriplegics was restoration of hand and arm function—even above locomotion [73]. Restoration of function at a singular cervical segment could mean the difference between independence and full-time caretakers. Based on anatomical and functional differences between spinal levels, therapies that target regeneration of the descending tracts at the cervical level may be worth pursuing, further indicating that thoracic SCI models are not always fully translatable towards cervical SCI.

3. Stem Cell Transplantation Therapies

3.1. Background

Stem cells are naturally occurring, undifferentiated cells that have the unique ability to both divide to produce more stem cells for self-renewal, and, differentiate into specific cell lineages (potency) under particular physiological conditions. Stem cells act as a repair and turnover system in both the developing embryo and adult, with the additional role of differentiating into all germ lines for organ formation within the embryo. Whereas self-renewal is essentially the same *in vivo* for cells of embryonic or adult somatic origin, potency is variable. Embryonic stem cells (ESCs) are harvested from the inner cell mass of blastocysts within four to five days post fertilization whereas adult stem cells (also termed mesenchymal stem cells; MSCs) are predominantly harvested from the bone marrow,

adipose tissue, and occasionally the umbilical cord tissue and blood, molars, and several other locations. ESCs from the blastocyst are pluripotent—capable of differentiating into all three germ lines whereas MSCs are multipotent and are limited to lineages of the mesodermal layer. The ability to harvest and culture naturally-occuring stem cells and the subsequent ability to differentiate them towards specific phenotypes has instigated a surge in advancements in developmental biology, disease pathogenesis, and regenerative medicine.

It is beyond the scope of this review to detail all the *in vitro* and *in vivo* capabilities and progress using both ESCs and MSCs as this has already been accomplished by several elegant reviews [74–85]. The following sections briefly overview preclinical and clinical uses of stem cells in cervical SCI.

3.2. Mesenchymal Stem Cells (MSCs)

MSCs are commonly classified and identified by their ability to adhere to plastic, their expression of CD73, CD90, and CD105, the lack of expression of CD14/CD11b, CD79, CD19, CD34, CD45, and HLA-DR surface markers, and their multipotent ability to differentiate into mesodermal lineages *in vitro* [85–90]. The distribution of MSCs in a variety of adult somatic sources, their ability to respond to cues produced by tissue damage based on their association with the vasculature, the potential for autologous transplants, their trophic and immunomodulatory secretion capabilities, their ease and rapidity in harvesting and expansion, and minimal risk of tumorigenicity have made them potential candidates for stem cell transplantation following SCI [91–104]. Furthermore, MSCs transplantation has been tested in clinical trials looking at neurological, cardiovascular, and immunological disease and has been deemed safe [105]. MSCs are multipotent, indicating their restriction towards mesodermal lineages. The ability to differentiate beyond this capacity towards neuronal and glial lineages is a hotly debated topic, in part due their weak expression of neuronal marker NeuN (neuronal specific nuclear marker) and neurotrophic/neuroprotective properties [106–109]. Despite this, several groups have demonstrated that MSCs are incapable of a "true" neuronal fate [75,89,108].

MSCs are most commonly derived from the bone marrow of the iliac crest and, as such, represent the bulk of studies examining MSCs transplantation following cervical SCI. Human umbilical cord blood also provides a rich source of adult stem cells and has therefore generated much interest in stroke, traumatic brain injury, and SCI. Generally, MSCs transplantation following SCI leads to amelioration of inflammation, apoptosis, and glial scarring in conjunction with increased axonal regrowth, angiogenesis, and tissue sparing in both cervical [110–117] and thoracic models [106,118–132]. In clinical trials of thoracic SCI, autologous bone marrow transplants delivered either intravenously or intra-arterial in patients were found to be safe. Although motor and sensory function did improve, it is inconclusive whether therapeutic benefit was due to the transplants [118]. In one clinical case study utilizing MSCs derived from umbilical cord blood demonstrated improved sensory perception and movement in the patient's hips and slight regeneration in and caudal to the lesion within 41 days transplantation following SCI [133].

3.3. Embryonic Stem Cells (ESCs)

ESCs are harvested from the blastocyst and half the capacities for both self-renewal and pluripotency. In addition, the characterization of 59 lines of demonstrated similar expression patterns of SSEA3, SSEA4, TRA-1-60, TRA-1-81, GCTM2, GCT343, CD9, Thy1 (also known as CD90), tissue-nonspecific alkaline phosphatase and class 1 HLA, and developmental genes *Nanog*, *Oct4*, *TDGF1*, *DNMT3B*, *GABRB3* and *GDF3* that maintain the potency state and help reduce unwanted differentiation [134]. In the field of SCI, current research studies using ESCs have been promising, in part due to the versatility in potency and decades of cell culture experience.

Embryonic neural tissue grafts became a popular endeavor during the 1970s and 1980s and transplantation within animal models showed positive outcomes in axonal projections between graft and host tissue, the secretion of glial proliferation-inhibiting factors, and demonstrated integration of host astrocytes into the donor graft. Despite this, the tissue did not survive or integrate well in large

lesion areas (such as with a complete transection) or if the age of the donor graft was inappropriate, and required a rich vascular surface [135,136]. The culturing of ESCs began in the early 1980s using murine sources demonstrated *in vitro* survivability without the support of fibroblast feeder layers but required leukemia inhibitory factor (LIF) [137,138]. Overall this led to earlier xeno-free expansion and characterization than human sources, which originated in the late 1990s and were found to rely on fibroblast growth factor (FGF) to retain their pluripotency in culture [139–141].

The pluripotent capacities of ESCs also make them an extremely versatile and attractive option in studying neurodegenerative pathophysiology following injury or illness. Unlike MSCs that are limited to cell fates within a mesodermal lineage, ESCs can be driven towards ectodermal lineages, or more specifically, neural subtypes. *In vivo* transplantation of undifferentiated ESCs often leads to teratoma formation, so there has been a strong push to develop high-caliber differentiation protocols to mitigate the risk of tumor formation and create specialized cell populations that enhance the therapeutic potential within the heterogeneous niche of the spinal cord lesion [142]. ESCs driven towards and neural lineage and grafted provide an innovative approach in solving the loss of connectivity following SCI. Neural stem and progenitor cells have been found to overcome the inhibitory milieu of an SCI lesion site by promoting axon growth across the injury, remyelinating host axons, and facilitating synaptogenesis between host and donor; their use in cellular transplantation strategies therefore have significant potential to improve measurable functionality in SCI models [143–145]. A substantial portion of ESCs differentiation protocols supports the selection of astrocyte and oligodendrocyte populations over neurons in part due to glial proliferation, and has been found to assist in improving myelination, weight support, and gait in a model of thoracic SCI [58,146–157]. Other directed differentiation protocols have led to the derivation of neural progenitor cells and additional neuronal phenotypes such as spinal motor neurons, cholinergic, serotonergic, dopaminergic, noradrenergic, medium spiny striatal neuronal, and deep cortical pyramidal [156–167] for use in thoracic SCI models as well as other neurodegeneration-based pathologies.

In models of cervical SCI, the transplantation of whole fetal spinal cords or fetal neural progenitor cells taken from brain and spinal tissue has been associated with supraspinal growth, axonal projection and growth, differentiation into all three neuronal lineages, long distance cell migration, and improvement in skilled forelimb function [168–172]. Since 2013, human fetal-derived tissue and stem cell transplantation for cervical and thoracic SCI has eventuated into several clinical trials in which safety and efficacy are being assessed, based on promising results seen in thoracic SCI and amyotrophic lateral sclerosis models [173–177].

In contrast to transplantation of derivations of fetal tissue, several groups examined the transplantation of ESCs cultured towards different lineages within cervical SCI. Sharp and colleagues (2010) transplanted human ESC-derived oligodendrocyte progenitor cells seven days following a severe midline contusion in a rat model of cervical SCI [151]. Transplantation led to a decrease in lesion size, white and gray matter sparing, preservation of host motor neuron pools, minimal migration of transplanted cells, differential changes in spinal cord gene expression of neurons, growth factors, apoptosis, and inflammation, and increased forelimb function. Sun and colleagues (2013) also utilized human ESC-derived oligodendrocyte progenitor cells four months post cervical irradiation injury in a rat model and witnessed markedly less demyelination and improved forelimb locomotion. Furthermore, transplanted cells were shown to differentiate into mature oligodendrocyte phenotypes that expressed myelin basic protein [178].

In a change of direction, Rossi and colleagues (2010) drove human ESCs towards a progenitor motor neuron phenotype that *in vitro* were shown to be Olig $1/2^+$, $Tuj1^+$, and $Hb9^+$, had functional glutamate receptors, and could innervate human and rodent muscle [179]. When transplanted following a cervical contusion in a rodent model of SCI, the cells displayed variation in differentiation pathways and phenotypes dependent on location, reduced lesion size, increased survival and growth of host neurons, and correlated with improved performance in motor tasks.

3.4. Potential Drawbacks of Adult and Embryonic Stem Cell Therapies

Current literature clearly demonstrates that stem cell transplantation therapies have shown promise in treating SCI, however, there are certain potential drawbacks that need to be considered and addressed when assessing this line of therapeutics for clinical purposes.

MSCs can easily be harvested from abundant adult somatic sources, are quick to culture *in vitro*, and are genomically stable. They have distinctive immunomodulatory and growth factor secretion capabilities, and clinically allow for the use of autografts thus circumventing the need for immunosuppression. Despite this their multipotency limits their ability to replace SCI-lost cells. They are less plastic than ESCs and there is evidence that MSCs do not persist and integrate following transplantation; overall, functional recovery post treatment remains limited to modest improvements [180–186].

ESCs are pluripotent, thus allowing for the manipulation of specific neuronal lineages and phenotypes following SCI. They rapidly proliferate in culture and have demonstrated significant functional recovery and amelioration of SCI pathology following transplantation. However, there are some concerns that potentially limit their use in clinical applications. ESCs are harvested from the blastocyst or from fetal-derived tissue, and thus are subject to ethical constraints and limited availability [187]. Their pluripotent capacity also introduces the risk of tumor formation following transplantation of undifferentiated cell populations, although this can be curbed by the use of more maturated cell types [188–193]. In culture, prolonged passage also leads to karyotypic abnormalities and genetic amplification that can contribute to oncogenesis [194–196]. Additionally, ESC allograft transplantation results in the need for immunosuppression, which can further confound the complicated and integral role the innate immune response plays following SCI.

Transplanting stem cells and their derivatives to treat SCI is a logical approach as they are capable of replacing cell phenotypes lost during injury, can induce powerful regenerative and immunological changes within the host tissue, and can potentially bridge the inhibitory environment of the lesion so that circuitry can hopefully be restored. The ideal cell type would be able to combine the advantages of both MSCs and ESCs while bypassing their individual pitfalls.

4. Induced Pluripotent Stem Cell-Derived Therapies

4.1. Induced Pluripotent Stem Cells

Induced pluripotent stem cells (iPSCs) offer a promising alternative to the potential drawbacks of embryonic and adult somatic stem cells. Whereas MSCs are limited in their potency, and ESCs are harvested via the manipulation of a pre-implantation embryo, iPSCs are a type of pluripotent stem cell that can be created from adult somatic cells by harvesting these cells and "reprogramming" them towards a stem cell state via the transduction of pluripotency genes.

The discovery that mature cells could be reprogrammed to pluripotency was initially pioneered by the Yamanaka laboratory in 2006. Therein it was demonstrated that iPSCs could be induced from mouse fibroblasts (and later using adult human fibroblasts) via retroviral delivery of Oct3/4, Sox2, c-Myc, and Klf4 without the need for Nanog [197–200]. Since then, iPSCs technology has progressed and multiple processes can be used to induce pluripotency in somatic cells. Viral transduction is easy to use, reproducible, yields iPSCs efficiently, and is controlled. However there is an increased risk of insertional mutagenesis and transgene reactivation, incomplete slicing, and clone-to-clone variation [201–203]. Reprogramming factors can also be fused to cell-penetrating peptides or introduced through plasmids, which requires no genomic modification but is also a very slow and inefficient process [204]. Finally, iPSCs can be induced via mRNA introduction. It is a highly efficient (and faster) method, requires no genomic modification, and is safe due to the transient nature of mRNA; however, repeated transfections are typically required [205,206].

Initial work suggested that iPSCs and ESCs were transcriptionally different possibly due to differential promoter binding by the reprogramming factors, variances in genetic background, or

studying small set numbers [207–210]. There is a large body of research though that concludes that ESCs and iPSCs are molecularly and functionally equivalent after accounting for differences in genetic background in that they share the same morphology, gene markers and expression, mitochondrial properties, and tumorigenicity [209–217].

The ability to create pluripotent stem cells from adult somatic cells circumvents some of the difficulties of using ESCs and MSCs–ethical barriers are minimized, cells can be driven towards any lineage, and, in the case of potential transplants, cells can be harvested directly from the patient therefore avoiding the need for immunosuppression [218,219]. Furthermore, there has been a strong push in Japan and the USA to create a global library of iPSC lines from donor somatic cells that are homozygous at several gene loci to match a patient's individual HLA type, thus making grafts without immunosuppression possible [218,219]. While iPSCs introduce exciting possibilities and advancements in regenerative medicine, their use still presents potential difficulties. When considering using autografts in clinical cases of SCI, the ideal time for transplantation is within two to four weeks post injury yet the generation and induction of iPSCs towards a neural lineage takes at least four to six months, with another year necessary for quality control [219]. The method of reprogramming can also be of concern due to the use of various viruses, possibility for mutagenesis and transgene reactivation [218,219]. There is also an argument that iPSCs never truly become "blank" stem cells and instead retain an epigenetic "memory" of their tissue source, which can then possibly influence the nuances of differentiation and final phenotype [220,221]. Finally, while exciting, iPSCs are a technology in its infancy that does not have decades of research supporting it as with MSCs or ESCs; many questions remain to be answered.

4.2. Differentiation of Induced Pluripotent Stem Cells (iPSCs)

A chief advantage of iPSCs technology is that iPSCs can be directed into the three main neural types *in vitro*. A major effort has been put forth to develop reliable iPSCs differentiation protocols that consistently generate functional neurons and glial cells so as to study the differences in neurons and neuronal networks in both healthy and impaired states [222–231].

In one protocol, human iPSCs were driven towards a neuroepithelial lineage using exogenous patterning molecules. The presence of mitogens allowed for the generation of astroglial progenitors, which could then be differentiated into functional astrocytes via ciliary neurotrophic factor [232]. In another protocol, mouse and rat fibroblasts were directly reprogrammed into oligodendrocyte precursor cells via forced expression of Sox10, Olig2, and Zfb536. These precursors exhibited expected morphologies and gene expression and gave rise to mature oligodendrocytes that could ensheath dorsal root ganglion cells *in vitro* and form myelin *in vivo* [233]. In another study reporting on the creation of neurons from iPSCs, the overexpression of Neurogenin-2 quickly and efficiently transformed iPSCs into neurons that formed spontaneous excitatory synaptic networks, which exhibited plasticity and synaptically integrated once transplanted into the mouse brain [234]. Another report showed that adult human fibroblasts could directly be reprogrammed into functional neurons that formed synapses via a cocktail of miR-124, BRN2, and MYT1L [235]. In addition to the aforementioned cell types, other groups have shown that iPSCs can be successfully driven towards glutamatergic, GABAergic, motor, and retinal neuron phenotypes, amongst others [236–248]. While not specific to SCI, these results demonstrate that developing differentiation protocols that generate specific neural subtypes can open up new avenues in understanding and creating therapies for neuropathologies.

Currently, preclinical studies have used iPSCs technology and differentiation strategies for disease modeling *in vitro* and *in vivo*. Despite being a young technology, some of these have already eventuated into clinical trials or are in progress to evaluate the safety and efficacy of iPSCs. Various studies are exploring a variety of degenerative states such as macular degeneration, recessive dystrophic epidermolysis bullosa, Parkinson's disease, thrombocytopenia, Multiple Sclerosis, spinal cord injury, corneal endothelial dysfunction, Stevens-Johnson syndrome, heart failure, retinitis pigmentosa, and refractory thrombocytopenia ([219]). Not surprisingly, considering the substantial lack of preclinical

and clinical stem cell transplantation studies following cervical SCI, there are no clinical studies that examine iPSC transplantation in treating cervical SCI.

4.3. Treating Cervical SCI with iPSC Technologies

The cervical spinal cord contains the long tracts connecting the rostral and caudal portions of the CNS, as well as the sensory and motor neurons for upper limb function and diaphragm-mediated respiration [249]. Despite the fact that SCI in the cervical region accounts for more than half of all presented cases, there is a tremendous lack of research to date exploring potential targeted therapies for this region. To date, there are currently four preclinical studies looking at iPSCs transplantation within the cervical cord.

One study using iPSCs in a cervical SCI model examined transplantation within what could be described as an "early chronic" window. The majority of transplantation studies utilize an acute injury model, transplanting by two weeks post SCI as this has been empirically determined to be the ideal clinically relevant time point [219]. However, acute injury models do not assist patients living with chronic SCI. Nutt and colleagues (2013) developed an early chronic injury model that mimics the deficits seen in humans using rats [250]. Four weeks following a cervical contusion injury at C4, rats received intraspinal transplantations of iPSC-derived neural progenitor cells and fibroblast rostral and caudal to the lesion site. By eight weeks post-transplant, NeuN/FOX-3 labeling showed a portion of transplanted cells differentiated into mature neurons. Despite intermingling between transplanted cells and host neurons, transplanted cells did not express glutamate receptors. Additionally, transplanted cells did not express serotonin but were positive for GABA and were shown to localize with host positive choline acetyltransferase. Behaviorally, grasping and weight bearing were only slightly improved by the transplants.

Another study by Li and colleagues (2015) evaluated respiratory function following iPSC-derived astrocyte transplants (following a standard differentiation protocol and also engineered to overexpress GLT1 [251]. In this work, both rats and mice underwent a C4 contusion injury resulting in chronic diaphragm dysfunction and phrenic motor neuron deterioration. Immediately post injury, they received an intraspinal transplant comprised of two separate injections rostral and caudal to the lesion and within the ventral horn. At two day, two week, and four week post injury/transplant time points, grafts survived and differentiated into astrocytes (GFAP positive), did not display any tumorigenicity, and had less than 10% proliferation (Ki67 staining). In addition, following transplant of GLT1-overexpressing astrocytes, lesion area and total lesion volume were reduced within one millimeter rostral and caudal to the lesion epicenter and preserved innervation of the diaphragm neuromuscular junction. By analyzing spontaneous EMG activity, it was also demonstrated that GLT1-overexpressing astrocyte transplants significantly increased EMG amplitude in the dorsal region of the hemi diaphragm, thus indicating preservation of diaphragmatic respiratory function.

Lu and colleagues (2014) examined the effect of sub-acute human iPSC-derived neural stem cells (NSCs) transplantation from an 86-year-old male in a C5 lateral hemisection in a rat model [252]. While not extensively characterized, the NSCs *in vitro* displayed a dramatic reduction in Tra1-81 and SSEA4 (pluripotency markers), and maintained expression of Nestin and Sox2 (NSC-associated markers). These NSCs were embedded in a fibrin matrix alongside a cocktail of growth factors and administered via intraspinal transplantation two weeks post injury. By three months post transplantation, grafts had survived and distributed throughout the lesion. The majority of grafted cells expressed NeuN (neuronal specific nuclear marker), but rarely doublecortin (NSC marker). Mature neuronal markers MAP2 and Tuj1 were expressed along with mature astrocytic marker GFAP, suggesting favored differentiation into neuronal and astrocytic lineages. There was also evidence of a small percentage of grafted cells expressing ChAT (characteristic of spinal motor neurons) and expressing Ki67, which is indicative of proliferation within the graft. No grafted cells expressed the serotonergic marker 5-HT. Most notably in this study was the robust axonal outgrowth of grafted cells throughout the entirety of the host CNS (from the lesion site rostral to the olfactory bulbs and caudally

to lumbar spine sections). In addition, there was evidence of integration of host axons into the grafted lesion site. Despite these interesting results, no behavioral recovery was observed.

Kobayashi and colleagues (2012) chose to examine the safety and efficacy of sub-acute transplantation of iPSC-derived neural stem cell transplants following cervical SCI in a non-human primate model [253]. Human iPSCs were cultured and neuronally induced to form neurospheres. Primary neurospheres were passed into secondary and tertiary neurospheres prior to transplantation. Adult female marmosets were given a moderate contusion at the C5 level and 9 days later received an intraspinal injection of cultured iPSC-neurospheres in the lesion epicenter. By 12 weeks post-transplant, hematoxylin-eosin staining revealed that the grafted cells survived and differentiated into all 3 neural subtypes (NeuN, GFAP, Olig1); the transplanted animals displayed a significant difference in cystic cavity size and no evidence of tumorigenicity was found in any of the animals. Undifferentiated iPSCs stained positive for Oct4, while cells that were positive for HNu did not. While severe demyelination was evident surrounding the lesion site in both transplanted and control groups, quantification revealed significantly higher degrees of demyelination in the control group at 12 weeks post-transplant. In accordance with these findings, conventional MRI and Myelin-mapping revealed more myelin sparing in the transplanted group and an intramedullary high-signal intensity area in the lesion site of the control group. Grafts had a higher number of neurofilaments and descending motor axons at the lesion center than the control group. Furthermore, this was coupled with evidence of increased angiogenesis, as demonstrated by staining of platelet endothelial cell adhesion molecule-1. Calcitonin generated peptide fibers, which are involved in spinal pain mechanisms, did not differ between transplanted and control groups. Behaviorally, contusion at the C5 level led to tetraplegia in the marmosets with a gradual improvement in motor function, as was expected in a severe central cord injury model. By eight weeks following SCI, significant differences in the open field test were found between transplanted and control groups, which stayed consistent throughout the study. Additionally, the bar grip strength and cage climbing tests were also found to be statistically significant between the groups by eight weeks post-transplant.

5. Conclusions and Future Considerations

Cervical-level SCIs account for the majority of presented cases and place a formidable lifetime financial burden on the nation. Despite the increase in patient survival rates due to improvements in modern medicine, there has been little advancement in ameliorating subsequent deficits in respiratory and motor dysfunction and quality of life still remains poor. Therapies that aim to restore function, even partially, are urgently needed and would make a substantial impact in helping patients regain independence. Cell transplantation therapies in SCI seek to accomplish this by increasing the innate host regenerative capacities through donor-secreted trophic and immunomodulatory factors, providing scaffolding to bridge the lesion site to promote regeneration of native axons and restoration of rostral and caudal circuitry, and by replacing SCI-lost neurons and glia.

Stem cell transplantations are a logical step in achieving this as they have the ability to replace specific phenotypes within the lesion and can evoke a strong immunological, regenerative, and healing response from the host. MSC and ESC-derived grafts for treatment of cervical SCI have been found to positively influence the inhibitory environment of the lesion and have occasionally assisted in behavioral recovery. However, these cell types raise concerns that make their use in commercial and clinical applications difficult. MSCs are limited by their potency, transient presence following transplantation, and functional outcomes. ESCs are subject to ethical constraints, carry karyotypic abnormality and tumorigenicity risks, and require immunosuppression. The use of iPSCs circumvents some of the pitfalls of MSCs and ESCs, namely potency in conjunction with source. The use of reprogrammed cells is a developing technology and not without its own concerns though; reprogramming methods can be inefficient and difficult to safely clinically translate, iPSCs are subject to karyotypic instability and may retain an epigenetic memory, and there is simply not an abundance of preclinical and clinical data.

To date, there is only a limited pool of research examining iPSC-derived transplants in SCI—even fewer of those research studies being specific to cervical injury. Current research predominantly uses rat animal modeling, and in one case, non-human primates. In these studies there was evidence of transplanted cell survivability, differentiation into mature neurons (conflicting evidence exists regarding synaptic activity of these mature neurons), intermingling between transplanted cells and host neurons, reduced lesion area and volume, increased angiogenesis, no tumorigenicity, and amelioration of motor deficits.

These results are certainly promising, especially when considered in conjunction with the positive findings associated with alternative stem cell-derived transplants. However, there still exists a significant research gap in this area, and underlies a major need to identify, examine and develop clinically relevant cervical SCI therapeutics. With so few studies existing using iPSC technology at the cervical level, it is difficult to speculate as to what factors would improve the efficacy of transplantation. At this current time, it may be of value to consider the experimental design of studies in this niche to have better clinical translation. The use of appropriate animal models, inherent capabilities of the transplanted cell type, timing of transplantation, and the chosen behavioral assessments to assess functional recovery have importance.

Experimentally, it is important to choose an anatomically appropriate model that mimics human cervical SCI pathology and motor deficits. The authors recognize that no individual animal model will completely embody every human clinical manifestation of cervical SCI, however, it is worthwhile to be able to recognize the strengths and weaknesses of each type within the context of achieved results. In rodent species, rats are superior to mice in that a contusion injury will form cystic cavitation that resembles what is seen in humans while mice develop matrices of connective tissue instead [254–256]. Furthermore, rats are able to perform detailed tasks predominantly using the biceps and triceps brachii and extensor and flexor carpi radialis muscles of the forelimbs and digits—an ability that is eviscerated by damage to the descending tracts following cervical injury, much as in humans. The usage of non-optimal behavioral tasks and assessments (or relying on a singular task) may confound what is defined as "functional recovery". Rats are cost-effective, easily trainable, and mimic some of the anatomical and motor deficits seen in clinical presentations of cervical SCI, however, the corticospinal tract projection is predominantly located in the dorsal column and decussates at the brainstem meaning that axons will have derived from the contralateral motor cortex. In contrast, the location of the primate's corticospinal tracts are predominantly in the dorsolateral column and experience many decussations over the spinal midline indicating that axons will have derived from both the left and right motor cortex [72]. This is significant because it means that following cervical SCI, primates may be more adept at circuitry rewiring and restoration than rats. Therapies that achieve limited or no functional recovery in rodents may have more potent effects on primates.

Additionally, the predominant derivation of transplanted iPSCs populations has been neural progenitors (NPCs). NPCs certainly show survivability, integration and colocalization with host cells, evidence of differentiation via the presence of mature markers, and are associated with improved functional outcomes. However, they are often transplanted as uncharacterized or mixed populations, which limit experimental control and clinical translation. Once transplanted, differentiation and phenotype are beyond the experimenter's control. Restoring circuitry within the injured cord requires that host axons are able to overcome the inhibitory environment of the lesion and reconnect or that transplanted cells within the lesion can form a relay system to connect the rostral and caudal CNS. It may be relevant to transplant cells that are further matured towards a neuronal lineage to accomplish this *versus* NPCs that will predominantly yield supporting glial phenotypes.

The use of iPSCs represents an exciting interface between disease modeling, developmental biology, and regenerative medicine. Treating cervical SCI with iPSCs is currently limited by a significant lack of published studies despite its overwhelming clinical relevance. Furthermore, discrepancies within experimental design may contribute to confound results. As iPSC technology increases and knowledge of differentiation protocols progress, it is our belief that iPSC-derived transplants (possibly

in conjunction with combinatorial treatments) will provide an encouraging avenue to positively address the unique and multifaceted requirements triggered by cervical SCI.

Acknowledgments: The authors would like to thank the following funders: Wings for Life, International Spinal Research Trust, California Institute for Regenerative Medicine, The Dennis Chan Research Fund, the Coulter Foundation, The Klein Family Research Fund, Saunders Family Neuroscience Fund, Eileen Bond Neuroscience Fund, James Doty Neurosurgery Fund, and Stanford Neuroscience Institute.

Author Contributions: Vanessa M. Doulames: manuscript writing and final approval of manuscript; Giles W. Plant: design, conception, manuscript writing, fundraising, final approval of manuscript.

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

References

1. National Spinal Cord Injury Statistical Center. *Facts and Figures at a Glance*; University of Alabama: Birmingham, AL, USA, 2015.
2. Berkowitz, M. Spinal cord injury: An analysis of medical and social costs. *Am. J. Phys. Med. Rehabil.* **1999**, *78*, 568–569.
3. Schoenfeld, A.J.; Sielski, B.; Rivera, K.P.; Bader, J.O.; Harris, M.B. Epidemiology of cervical spine fractures in the US military. *Spine J.* **2012**, *12*, 777–783. [CrossRef] [PubMed]
4. Yoganandan, N.; Stemper, B.D.; Pintar, F.A.; Maiman, D.J.; McEntire, B.J.; Chancey, V.C. Cervical spine injury biomechanics: Applications for under body blast loadings in military environments. *Clin. Biomech.* **2013**, *28*, 602–609. [CrossRef] [PubMed]
5. Inoue, T.; Lin, A.; Ma, X.; McKenna, S.L.; Creasey, G.H.; Manley, G.T.; Ferguson, A.R.; Bresnahan, J.C.; Beattie, M.S. Combined SCI and TBI: Recovery of forelimb function after unilateral cervical spinal cord injury (SCI) is retarded by contralateral traumatic brain injury (TBI), and ipsilateral TBI balances the effects of SCI on paw placement. *Exp. Neurol.* **2013**, *248*, 136–147. [CrossRef] [PubMed]
6. Breeze, J.; Fryer, R.; Hare, J.; Delaney, R.; Hunt, N.C.; Lewis, E.A.; Clasper, J.C. Clinical and post mortem analysis of combat neck injury used to inform a novel coverage of armour tool. *Injury* **2015**, *46*, 629–633. [CrossRef] [PubMed]
7. Wagner, L.S.C.; Lehman, L.R.A. Cervical spine and neck injuries. In *Musculoskeletal Injuries in the Military*; Springer: New York, NY, USA, 2016; pp. 229–245.
8. Satyanand, V.; Gopalakrishnaiah, T.; Panneerselvam, E.; Mahaboobvali, S.; Basha, S.A.; Sarala, V. Effects of yogasanas on cervical spondylosis. *Int. Arch. Integr. Med.* **2015**, *2*, 6–10.
9. Smith, L.; Louw, Q.; Crous, L.; Grimmer-Somers, K. Prevalence of neck pain and headaches: Impact of computer use and other associative factors. *Cephalalgia* **2009**, *29*, 250–257. [CrossRef] [PubMed]
10. Ropper, A.E.; Matthew, T.N.; Nicholas, T. Acute management of traumatic cervical spinal cord injury. *Pract. Neurol.* **2015**, *15*, 266–272. [CrossRef] [PubMed]
11. Tetreault, L.A.; Karpova, A.; Fehlings, M.G. Predictors of outcome in patients with degenerative cervical spondylotic myelopathy undergoing surgical treatment: Results of a systematic review. *Eur. Spine J.* **2015**, *24*, 236–251. [CrossRef] [PubMed]
12. Laing, A.C.; Brenneman, E.C.; Yung, A.; Liu, J.; Kozlowski, P.; Oxland, T. The effects of age on the morphometry of the cervical spinal cord and spinal column in adult rats: An MRI-based study. *Anat. Rec.* **2014**, *297*, 1885–1895. [CrossRef] [PubMed]
13. Wang, C.; Tian, F.; Zhou, Y.; He, W.; Cai, Z. The incidence of cervical spondylosis decreases with aging in the elderly, and increases with aging in the young and adult population: A hospital-based clinical analysis. *Clin. Interv. Aging* **2016**, *11*, 47–53. [PubMed]
14. Sabapathy, V.; George, T.; Sanjay, K. Cell therapy augments functional recovery subsequent to spinal cord injury under experimental conditions. *Stem Cells Int.* **2014**, *2015*, 640–645. [CrossRef] [PubMed]
15. Peitzman, A.B., Fabian, T.C., Rhodes, M., Yealy, D.M., Schwab, C.W., Eds.; *The Trauma Manual: Trauma and Acute Care Surgery*; Lippincott Williams & Wilkins: Pasadena, CA, USA, 2012.
16. Newman, M.F.; Lee, A.F.; Mitchell, P.F. *Perioperative Medicine: Managing for Outcome*; Elsevier Health Sciences: New York, NY, USA, 2008.
17. Tator, C.H. Biology of neurological recovery and functional restoration after spinal cord injury. *Neurosurgery* **1998**, *42*, 696–707. [CrossRef] [PubMed]

18. Ramer, L.M.; Ramer, M.S.; Steeves, J.D. Setting the stage for functional repair of spinal cord injuries: A cast of thousands. *Spinal Cord* **2005**, *43*, 134–161. [CrossRef] [PubMed]
19. Park, E.; Alexander, A.V.; Michael, G.F. The role of excitotoxicity in secondary mechanisms of spinal cord injury: A review with an emphasis on the implications for white matter degeneration. *J. Neurotrauma* **2004**, *21*, 754–774. [CrossRef] [PubMed]
20. Dumont, R.J.; Okonkwo, D.O.; Verma, S.; Hurlbert, R.J.; Boulos, P.T.; Ellegala, D.B.; Dumont, A.S. Acute spinal cord injury, part I: Pathophysiologic mechanisms. *Clin. Neuropharmacol.* **2001**, *24*, 254–264. [CrossRef] [PubMed]
21. Evelyne, E.; Aldana, P.; Bunge, M.B.; Puckett, W.; Srinivasan, A.; Keane, R.W.; Bethea, J.; Levi, A.D.O. Apoptosis after traumatic human spinal cord injury. *J. Neurosurg.* **1998**, *89*, 911–920.
22. Hausmann, O.N. Post-traumatic inflammation following spinal cord injury. *Spinal Cord* **2003**, *41*, 369–378. [CrossRef] [PubMed]
23. Beattie, M.S.; Akhlaq, A.F.; Jacqueline, C.B. Review of current evidence for apoptosis after spinal cord injury. *J. Neurotrauma* **2000**, *17*, 915–925. [CrossRef] [PubMed]
24. Lu, J.; Ken, W.S.A.; Phil, W. Advances in secondary spinal cord injury: Role of apoptosis. *Spine* **2000**, *25*, 1859–1866. [CrossRef] [PubMed]
25. Mautes, A.E.; Weinzierl, M.R.; Donovan, F.; Noble, L.J. Vascular events after spinal cord injury: Contribution to secondary pathogenesis. *Phys. Ther.* **2000**, *80*, 673–687. [PubMed]
26. Horner, P.J.; Fred, H.G. Regenerating the damaged central nervous system. *Nature* **2000**, *407*, 963–970. [PubMed]
27. Ramer, M.S.; John, V.P.; Stephen, B.M. Functional regeneration of sensory axons into the adult spinal cord. *Nature* **2000**, *403*, 312–316. [CrossRef] [PubMed]
28. Bernstein, J.J.; Mary, E.B. Axonal regeneration and formation of synapses proximal to the site of lesion following hemisection of the rat spinal cord. *Exp. Neurol.* **1971**, *30*, 336–351. [CrossRef]
29. Ye, J.-H.; Houle, J.D. Treatment of the chronically injured spinal cord with neurotrophic factors can promote axonal regeneration from supraspinal neurons. *Exp. Neurol.* **1997**, *143*, 70–81. [CrossRef] [PubMed]
30. Namiki, J.; Kojima, A.; Tator, C.H. Effect of brain-derived neurotrophic factor, nerve growth factor, and neurotrophin-3 on functional recovery and regeneration after spinal cord injury in adult rats. *J. Neurotrauma* **2000**, *17*, 1219–1231. [CrossRef] [PubMed]
31. McTigue, D.M.; Horner, P.J.; Stokes, B.T.; Gage, F.H. Neurotrophin-3 and brain-derived neurotrophic factor induce oligodendrocyte proliferation and myelination of regenerating axons in the contused adult rat spinal cord. *J. Neurosci.* **1998**, *18*, 5354–5365. [PubMed]
32. Grill, R.; Murai, K.; Blesch, A.; Gage, F.H.; Tuszynski, M.H. Cellular delivery of neurotrophin-3 promotes corticospinal axonal growth and partial functional recovery after spinal cord injury. *J. Neurosci.* **1997**, *17*, 5560–5572. [PubMed]
33. Simonen, M.; Pedersen, V.; Weinmann, O.; Schnell, L.; Buss, A.; Ledermann, B.; Christ, F.; Sansig, G.; van der Putten, H.; Schwab, M.E. Systemic deletion of the myelin-associated outgrowth inhibitor Nogo-A improves regenerative and plastic responses after spinal cord injury. *Neuron* **2003**, *38*, 201–211. [CrossRef]
34. Kim, G.-M.; Xu, J.; Xu, J.; Song, S.K.; Yan, P.; Ku, G.; Xu, X.M.; Hsu, C.Y. Tumor necrosis factor receptor deletion reduces nuclear factor-κB activation, cellular inhibitor of apoptosis protein 2 expression, and functional recovery after traumatic spinal cord injury. *J. Neurosci.* **2001**, *21*, 6617–6625. [PubMed]
35. Nishio, Y.; Koda, M.; Hashimoto, M.; Kamada, T.; Koshizuka, S.; Yoshinaga, K.; Onodera, S.; Nishihira, J.; Okawa, A.; Yamazaki, M. Deletion of macrophage migration inhibitory factor attenuates neuronal death and promotes functional recovery after compression-induced spinal cord injury in mice. *Acta Neuropathol.* **2009**, *117*, 321–328. [CrossRef] [PubMed]
36. Hamid, S.; Ray, H. Role of electrical stimulation for rehabilitation and regeneration after spinal cord injury: An overview. *Eur. Spine J.* **2008**, *17*, 1256–1269. [CrossRef] [PubMed]
37. Smith, R.R.; Shum-Siu, A.; Baltzley, R.; Bunger, M.; Baldini, A.; Burke, D.A.; Magnuson, D.S. Effects of swimming on functional recovery after incomplete spinal cord injury in rats. *J. Neurotrauma* **2006**, *23*, 908–919. [CrossRef] [PubMed]
38. Engesser-Cesar, C.; Anderson, A.J.; Basso, D.M.; Edgerton, V.R.; Cotman, C.W. Voluntary wheel running improves recovery from a moderate spinal cord injury. *J. Neurotrauma* **2005**, *22*, 157–171. [CrossRef] [PubMed]
39. Donnelly, D.J.; Phillip, G.P. Inflammation and its role in neuroprotection, axonal regeneration and functional recovery after spinal cord injury. *Exp. Neurol.* **2008**, *209*, 378–388. [CrossRef] [PubMed]

40. Popovich, P.G.; Guan, Z.; Wei, P.; Huitinga, I.; van Rooijen, N.; Stokes, B.T. Depletion of hematogenous macrophages promotes partial hindlimb recovery and neuroanatomical repair after experimental spinal cord injury. *Exp. Neurol.* **1999**, *158*, 351–365. [CrossRef] [PubMed]
41. Ikegami, T.; Nakamura, M.; Yamane, J.; Katoh, H.; Okada, S.; Iwanami, A.; Watanabe, K.; Ishii, K.; Kato, F.; Fujita, H.; *et al.* Chondroitinase ABC combined with neural stem/progenitor cell transplantation enhances graft cell migration and outgrowth of growth-associated protein-43-positive fibers after rat spinal cord injury. *Eur. J. Neurosci.* **2005**, *22*, 3036–3046. [CrossRef] [PubMed]
42. Bregman, B.S.; McAtee, M.; Dai, H.N.; Kuhn, P.L. Neurotrophic factors increase axonal growth after spinal cord injury and transplantation in the adult rat. *Exp. Neurol.* **1997**, *148*, 475–494. [CrossRef] [PubMed]
43. Chopp, M.; Zhang, X.H.; Li, Y.; Wang, L.; Chen, J.; Lu, D.; Lu, M.; Rosenblum, M. Spinal cord injury in rat: Treatment with bone marrow stromal cell transplantation. *Neuroreport* **2000**, *11*, 3001–3005. [CrossRef] [PubMed]
44. Jones, L.L.; Oudega, M.; Bunge, M.B.; Tuszynski, M.H. Neurotrophic factors, cellular bridges and gene therapy for spinal cord injury. *J. Physiol.* **2001**, *533*, 83–89. [CrossRef] [PubMed]
45. Raisman, G. Olfactory ensheathing cells—Another miracle cure for spinal cord injury? *Nat. Rev. Neurosci.* **2001**, *2*, 369–375. [CrossRef] [PubMed]
46. Thuret, S.; Lawrence, D.F.M.; Fred, H.G. Therapeutic interventions after spinal cord injury. *Nat. Rev. Neurosci.* **2006**, *7*, 628–643. [CrossRef] [PubMed]
47. Plant, G.W.; Margaret, L.B.; Mary, B.B. Inhibitory proteoglycan immunoreactivity is higher at the caudal than the rostral Schwann cell graft-transected spinal cord interface. *Mol. Cell. Neurosci.* **2001**, *17*, 471–487. [CrossRef] [PubMed]
48. Ruitenberg, M.J.; Plant, G.W.; Christensen, C.L.; Blits, B.; Niclou, S.P.; Harvey, A.R.; Boer, G.J.; Verhaagen, J. Viral vector-mediated gene expression in olfactory ensheathing glia implants in the lesioned rat spinal cord. *Gene Ther.* **2002**, *9*, 135–146. [CrossRef] [PubMed]
49. Ramer, L.M.; Au, E.; Richter, M.W.; Liu, J.; Tetzlaff, W.; Roskams, A.J. Peripheral olfactory ensheathing cells reduce scar and cavity formation and promote regeneration after spinal cord injury. *J. Comp. Neurol.* **2004**, *473*, 1–15. [CrossRef] [PubMed]
50. Kwon, B.K.; Tetzlaff, W.; Grauer, J.N.; Beiner, J.; Vaccaro, A.R. Pathophysiology and pharmacologic treatment of acute spinal cord injury. *Spine J.* **2004**, *4*, 451–464. [CrossRef] [PubMed]
51. Snyder, E.Y.; Yang, D.T. Stem cells and spinal cord repair. *N. Engl. J. Med.* **2012**, *366*, 1940–1942. [CrossRef] [PubMed]
52. Barry, F.P.; Murphy, J.M. Mesenchymal stem cells: Clinical applications and biological characterization. *Int. J. Biochem. Cell Biol.* **2004**, *36*, 568–584. [CrossRef] [PubMed]
53. Parr, A.M.; Kulbatski, I.; Zahir, T.; Wang, X.; Yue, C.; Keating, A.; Tator, C.H. Transplanted adult spinal cord–Derived neural stem/progenitor cells promote early functional recovery after rat spinal cord injury. *Neuroscience* **2008**, *155*, 760–770. [CrossRef] [PubMed]
54. Cummings, B.J.; Uchida, N.; Tamaki, S.J.; Salazar, D.L.; Hooshmand, M.; Summers, R.; Gage, F.H.; Anderson, A.J. Human neural stem cells differentiate and promote locomotor recovery in spinal cord-injured mice. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 14069–14074. [CrossRef] [PubMed]
55. Reier, P.J.; Bregman, B.S.; Wujek, J.R. Intraspinal transplantation of embyronic spinal cord tissue in neonatal and adult rats. *J. Comp. Neurol.* **1986**, *247*, 275–296. [CrossRef] [PubMed]
56. Reubinoff, B.E.; Itsykson, P.; Turetsky, T.; Pera, M.F.; Reinhartz, E.; Itzik, A.; Ben-Hur, T. Neural progenitors from human embryonic stem cells. *Nat. Biotechnol.* **2001**, *19*, 1134–1140. [CrossRef] [PubMed]
57. Carpenter, M.K.; Rosler, E.; Rao, M.S. Characterization and differentiation of human embryonic stem cells. *Cloning Stem Cells* **2003**, *5*, 79–88. [CrossRef] [PubMed]
58. 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] [PubMed]
59. Hedel, H.J.A.V.; Armin, C. Fighting for each segment: Estimating the clinical value of cervical and thoracic segments in SCI. *J. Neurotrauma* **2006**, *23*, 1621–1631. [CrossRef] [PubMed]
60. Hill, C.E.; Beattie, M.S.; Bresnahan, J.C. Degeneration and sprouting of identified descending supraspinal axons after contusive spinal cord injury in the rat. *Exp. Neurol.* **2001**, *171*, 153–169. [CrossRef] [PubMed]

61. Fernandes, K.J.; Fan, D.P.; Tsui, B.J.; Cassar, S.L.; Tetzlaff, W. Influence of the axotomy to cell body distance in rat rubrospinal and spinal motoneurons: Differential regulation of GAP-43, tubulins, and neurofilament-M. *J. Comp. Neurol.* **1999**, *414*, 495–510. [CrossRef]
62. Richardson, P.M.; Lu, X. Inflammation and axonal regeneration. *J. Neurol.* **1994**, *242*, S57–S60. [CrossRef] [PubMed]
63. Dimitrijevic, M.R.; Gerasimenko, Y.; Pinter, M.M. Evidence for a spinal central pattern generator in humansa. *Ann. N. Y. Acad. Sci.* **1998**, *860*, 360–376. [CrossRef] [PubMed]
64. Bareyre, F.M.; Kerschensteiner, M.O.; Mettenleiter, T.C.; Weinmann, O.; Schwab, M.E. The injured spinal cord spontaneously forms a new intraspinal circuit in adult rats. *Nat. Neurosci.* **2004**, *7*, 269–277. [CrossRef] [PubMed]
65. Grillner, S.; Zangger, P. On the central generation of locomotion in the low spinal cat. *Exp. Brain Res.* **1979**, *34*, 241–261. [CrossRef] [PubMed]
66. Grillner, S.; Peter, Z. The effect of dorsal root transection on the efferent motor pattern in the cat's hindlimb during locomotion. *Acta Physiol. Scand.* **1984**, *120*, 393–405. [CrossRef] [PubMed]
67. Whelan, P.J. Control of locomotion in the decerebrate cat. *Prog. Neurobiol.* **1996**, *49*, 481–515. [CrossRef]
68. Anderson, K.D.; Marim, A.; Oswald, S. Quantitative assessment of deficits and recovery of forelimb motor function after cervical spinal cord injury in mice. *Exp. Neurol.* **2004**, *190*, 184–191. [CrossRef] [PubMed]
69. Webb, A.A.; Gillian, D.M. Sensorimotor behaviour following incomplete cervical spinal cord injury in the rat. *Behav. Brain Res.* **2005**, *165*, 147–159. [CrossRef] [PubMed]
70. Anderson, K.D.; Ardi, G.; Oswald, S. Spinal pathways involved in the control of forelimb motor function in rats. *Exp. Neurol.* **2007**, *206*, 318–331. [CrossRef] [PubMed]
71. Zörner, B.; Filli, L.; Starkey, M.L.; Gonzenbach, R.; Kasper, H.; Röthlisberger, M.; Bolliger, M.; Schwab, M.E. Profiling locomotor recovery: Comprehensive quantification of impairments after CNS damage in rodents. *Nat. Methods* **2010**, *7*, 701–708. [CrossRef] [PubMed]
72. Friedli, L.; Rosenzweig, E.S.; Barraud, Q.; Schubert, M.; Dominici, N.; Awai, L.; Nielson, J.L.; Musienko, P.; Nout-Lomas, Y.; Zhong, H.; *et al.* Pronounced species divergence in corticospinal tract reorganization and functional recovery after lateralized spinal cord injury favors primates. *Sci. Transl. Med.* **2015**, *7*. [CrossRef] [PubMed]
73. Anderson, K.D. Targeting recovery: Priorities of the spinal cord-injured population. *J. Neurotrauma* **2004**, *21*, 1371–1383. [CrossRef] [PubMed]
74. Antonic, A.; Wenger, N.; Gorod, A. Stem cell transplantation in traumatic spinal cord injury: A systematic review and meta-analysis of animal studies. *PLoS Biol.* **2013**, *11*, e1001738. [CrossRef] [PubMed]
75. Vawda, R.; Michael, G.F. Mesenchymal cells in the treatment of spinal cord injury: Current & future perspectives. *Curr. Stem Cell Res. Ther.* **2013**, *8*, 25–38. [PubMed]
76. Caplan, A.I. Adult mesenchymal stem cells for tissue engineering *versus* regenerative medicine. *J. Cell. Physiol.* **2007**, *213*, 341–347. [CrossRef] [PubMed]
77. Chen, Y.; Shao, J.Z.; Xiang, L.X.; Dong, X.J.; Zhang, G.R. Mesenchymal stem cells: A promising candidate in regenerative medicine. *Int. J. Biochem. Cell Biol.* **2008**, *40*, 815–820. [CrossRef] [PubMed]
78. Deans, R.J.; Moseley, A.B. Mesenchymal stem cells: biology and potential clinical uses. *Exp. Hematol.* **2000**, *28*, 875–884. [CrossRef]
79. Richardson, S.M.; Hoyland, J.A.; Mobasheri, R.; Csaki, C.; Shakibaei, M.; Mobasheri, A. Mesenchymal stem cells in regenerative medicine: Opportunities and challenges for articular cartilage and intervertebral disc tissue engineering. *J. Cell. Physiol.* **2010**, *222*, 23–32. [CrossRef] [PubMed]
80. Tabar, V.; Lorenz, S. Pluripotent stem cells in regenerative medicine: Challenges and recent progress. *Nat. Rev. Genet.* **2014**, *15*, 82–92. [CrossRef] [PubMed]
81. Martello, G.; Austin, S. The nature of embryonic stem cells. *Annu. Rev. Cell Dev. Biol.* **2014**, *30*, 647–675. [CrossRef] [PubMed]
82. Mothe, A.J.; Charles, H.T. Review of transplantation of neural stem/progenitor cells for spinal cord injury. *Int. J. Dev. Neurosci.* **2013**, *31*, 701–713. [CrossRef] [PubMed]
83. Vibhu, S.; Kessler, J.A. Stem cell therapies for spinal cord injury. *Nat. Rev. Neurol.* **2010**, *6*, 363–372.
84. Paul, L.; Ahmad, R.; Tuszynski, M.H. Neural Stem Cells for Spinal Cord Injury. *Transl. Neurosci.* **2016**, *5*, 297–315.
85. Youmna, K.; Scadden, D.T. Mesenchymal cell contributions to the stem cell niche. *Cell Stem Cell* **2015**, *16*, 239–253.
86. Morayma, R.; Verfaillie, C.M. Characterization of multipotent adult progenitor cells, a subpopulation of mesenchymal stem cells. *Ann. N. Y. Acad. Sci.* **2001**, *938*, 231–235.

87. Amit, M.; Carpenter, M.K.; Inokuma, M.S.; Chiu, C.P.; Harris, C.P.; Waknitz-Eldor, J.; Thomson, J.A. Clonally derived human embryonic stem cell lines maintain pluripotency and proliferative potential for prolonged periods of culture. *Dev. Biol.* **2000**, *227*, 271–278. [CrossRef] [PubMed]
88. Biswas, A.; Robert, H. Embryonic stem cells. *Stem Cells Dev.* **2007**, *16*, 213–222. [CrossRef] [PubMed]
89. Hodgetts, S.I.; Paul, J.S.; Giles, W.P. Human mesenchymal precursor cells (Stro-1^{+}) from spinal cord injury patients improve functional recovery and tissue sparing in an acute spinal cord injury rat model. *Cell Transplant.* **2013**, *22*, 393–412. [CrossRef] [PubMed]
90. Dominici, M.; Le, B.K.; Mueller, I.; Slaper-Cortenbach, I.; Marini, F.; Krause, D.; Deans, R.; Keating, A.; Prockop, D.J.; Horwitz, E. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy* **2006**, *8*, 315–317. [CrossRef] [PubMed]
91. Wuchter, P.; Bieback, K.; Schrezenmeier, H.; Bornhäuser, M.; Müller, L.P.; Bönig, H.; Wagner, W.; Meisel, R.; Pavel, P.; Tonn, T.; *et al.* Standardization of Good Manufacturing Practice-compliant production of bone marrow-derived human mesenchymal stromal cells for immunotherapeutic applications. *Cytotherapy* **2015**, *17*, 128–139. [CrossRef] [PubMed]
92. Torres-Espín, A.; Corona-Quintanilla, D.L.; Forés, J.; Allodi, I.; González, F.; Udina, E.; Navarro, X. Neuroprotection and axonal regeneration after lumbar ventral root avulsion by re-implantation and mesenchymal stem cells transplant combined therapy. *Neurotherapeutics* **2013**, *10*, 354–368. [CrossRef] [PubMed]
93. Lalu, M.M.; McIntyre, L.; Pugliese, C.; Fergusson, D.; Winston, B.W.; Marshall, J.C.; Granton, J.; Stewart, D.J. Safety of cell therapy with mesenchymal stromal cells (SafeCell): A systematic review and meta-analysis of clinical trials. *PLoS ONE* **2012**, *7*, e47559. [CrossRef] [PubMed]
94. Lalu, M.M.; Lauralyn, L.M.; Duncan, J.S. Mesenchymal stromal cells: Cautious optimism for their potential role in the treatment of acute lung injury. *Crit. Care Med.* **2012**, *40*, 1373–1375. [CrossRef] [PubMed]
95. Ra, J.C.; Shin, I.S.; Kim, S.H.; Kang, S.K.; Kang, B.C.; Lee, H.Y.; Kim, Y.J.; Jo, J.Y.; Yoon, E.J.; Choi, H.J.; *et al.* Safety of intravenous infusion of human adipose tissue-derived mesenchymal stem cells in animals and humans. *Stem Cells Dev.* **2011**, *20*, 1297–1308. [CrossRef] [PubMed]
96. Uccelli, A.; Federica, B.; Alice, L.; Debora, G. Neuroprotective features of mesenchymal stem cells. *Best Pract. Res. Clin. Haematol.* **2011**, *24*, 59–64. [CrossRef] [PubMed]
97. Tollervey, J.R.; Victoria, V.L. Adult stem cells: Simply a tool for regenerative medicine or an additional piece in the puzzle of human aging? *Cell Cycle* **2011**, *10*, 4173–4176. [CrossRef] [PubMed]
98. Tidball, J.G.; Villalta, S.A. Regulatory interactions between muscle and the immune system during muscle regeneration. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **2010**, *298*, R1173–R1187. [CrossRef] [PubMed]
99. Bai, L.; Lennon, D.P.; Eaton, V.; Maier, K.; Caplan, A.I.; Miller, S.D.; Miller, R.H. Human bone marrow-derived mesenchymal stem cells induce Th2-polarized immune response and promote endogenous repair in animal models of multiple sclerosis. *Glia* **2009**, *57*, 1192–1203. [CrossRef] [PubMed]
100. Kong, D.; Li, Y.; Wang, Z.; Banerjee, S.; Ahmad, A.; Kim, H.R.C.; Sarkar, F.H. miR-200 Regulates PDGF-D-mediated epithelial-mesenchymal transition, adhesion, and invasion of prostate cancer cells. *Stem Cells* **2009**, *27*, 1712–1721. [CrossRef] [PubMed]
101. Uccelli, A.; Lorenzo, M.; Vito, P. Mesenchymal stem cells in health and disease. *Nat. Rev. Immunol.* **2008**, *8*, 726–736. [CrossRef] [PubMed]
102. Aggarwal, S.; Mark, F.P. Human mesenchymal stem cells modulate allogeneic immune cell responses. *Blood* **2005**, *105*, 1815–1822. [CrossRef] [PubMed]
103. 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] [PubMed]
104. Sekiya, I.; Larson, B.L.; Smith, J.R.; Pochampally, R.; Cui, J.G.; Prockop, D.J. Expansion of human adult stem cells from bone marrow stroma: Conditions that maximize the yields of early progenitors and evaluate their quality. *Stem Cells* **2002**, *20*, 530–541. [CrossRef] [PubMed]
105. Parekkadan, B.; Jack, M.M. Mesenchymal stem cells as therapeutics. *Annu. Rev. Biomed. Eng.* **2010**, *12*, 87–117. [CrossRef] [PubMed]
106. Koshizuka, S.; Okada, S.; Okawa, A.; Koda, M.; Murasawa, M.; Hashimoto, M.; Kamada, T.; Yoshinaga, K.; Murakami, M.; Moriya, H.; *et al.* Transplanted hematopoietic stem cells from bone marrow differentiate into neural lineage cells and promote functional recovery after spinal cord injury in mice. *J. Neuropathol. Exp. Neurol.* **2004**, *63*, 64–72. [CrossRef] [PubMed]

107. Martinez, A.M.; Goulart, C.O.; Ramalho, B.S.; Oliveira, J.T.; Almeida, F.M. Neurotrauma and mesenchymal stem cells treatment: From experimental studies to clinical trials. *World J. Stem Cell.* **2014**, *6*, 179–194. [CrossRef] [PubMed]
108. 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] [PubMed]
109. Kopen, G.C.; Darwin, J.P.; Donald, G.P. Marrow stromal cells migrate throughout forebrain and cerebellum, and they differentiate into astrocytes after injection into neonatal mouse brains. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 10711–10716. [CrossRef] [PubMed]
110. Lu, P.; Hong, Y.; Jones, L.L.; Filbin, M.T.; Tuszynski, M.H. Combinatorial therapy with neurotrophins and cAMP promotes axonal regeneration beyond sites of spinal cord injury. *J. Neurosci.* **2004**, *24*, 6402–6409. [CrossRef] [PubMed]
111. Lu, P.; Jones, L.L.; Tuszynski, M.H. BDNF-expressing marrow stromal cells support extensive axonal growth at sites of spinal cord injury. *Exp. Neurol.* **2005**, *191*, 344–360. [CrossRef] [PubMed]
112. Lu, P.; Jones, L.L.; Tuszynski, M.H. Axon regeneration through scars and into sites of chronic spinal cord injury. *Exp. Neurol.* **2007**, *203*, 8–21. [CrossRef] [PubMed]
113. Novikova, L.N.; Brohlin, M.; Kingham, P.J.; Novikov, L.N.; Wiberg, M. Neuroprotective and growth-promoting effects of bone marrow stromal cells after cervical spinal cord injury in adult rats. *Cytotherapy* **2011**, *13*, 873–887. [CrossRef] [PubMed]
114. White, S.V.; Czisch, C.E.; Han, M.H.; Plant, C.D.; Harvey, A.R.; Plant, G.W. Intravenous transplantation of mesenchymal progenitors distribute solely to the lungs and improve outcomes in cervical spinal cord injury. *Stem Cell* **2016**. [CrossRef] [PubMed]
115. Sandner, B.; Rivera, F.J.; Caioni, M.; Nicholson, L.; Eckstein, V.; Bogdahn, U.; Aigner, L.; Blesch, A.; Weidner, N. Bone morphogenetic proteins prevent bone marrow stromal cell-mediated oligodendroglial differentiation of transplanted adult neural progenitor cells in the injured spinal cord. *Stem Cell Res.* **2013**, *11*, 758–771. [CrossRef] [PubMed]
116. Neuhuber, B.; Himes, B.T.; Shumsky, J.S.; Gallo, G.; Fischer, I. Axon growth and recovery of function supported by human bone marrow stromal cells in the injured spinal cord exhibit donor variations. *Brain Res.* **2005**, *1035*, 73–85. [CrossRef] [PubMed]
117. Goldschlager, T.; Jenkin, G.; Ghosh, P.; Zannettino, A.; Victor Rosenfeld, J. Potential applications for using stem cells in spine surgery. *Curr. Stem Cell Res. Ther.* **2010**, *5*, 345–355. [CrossRef] [PubMed]
118. Syková, E.; Homola, A.; Mazanec, R.; Lachmann, H.; Langkramer Konrádová, Š.; Kobylka, P.; Pádr, R.; Neuwirth, J.; Komrska, V.; Vávra, V.; *et al.* Autologous bone marrow transplantation in patients with subacute and chronic spinal cord injury. *Cell Transpl.* **2006**, *15*, 675–687. [CrossRef]
119. Saporta, S.; Kim, J.J.; Willing, A.E.; Fu, E.S.; Davis, C.D.; Sanberg, P.R. Human umbilical cord blood stem cells infusion in spinal cord injury: Engraftment and beneficial influence on behavior. *J. Hematother. Stem Cell Res.* **2003**, *12*, 271–278. [CrossRef] [PubMed]
120. Sanchez-Ramos, J.R.; Song, S.; Kamath, S.G.; Zigova, T.; Willing, A.; Cardozo-Pelaez, F.; Stedeford, T.; Chopp, M.; Sanberg, P.R. Expression of neural markers in human umbilical cord blood. *Exp. Neurol.* **2001**, *171*, 109–115. [CrossRef] [PubMed]
121. 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] [PubMed]
122. Quertainmont, R.; Cantinieaux, D.; Botman, O.; Sid, S.; Schoenen, J.; Franzen, R. Mesenchymal stem cell graft improves recovery after spinal cord injury in adult rats through neurotrophic and pro-angiogenic actions. *PLoS ONE* **2012**, *7*, e39500. [CrossRef] [PubMed]
123. Cantinieaux, D.; Quertainmont, R.; Blacher, S.; Rossi, L.; Wanet, T.; Noël, A.; Brook, G.; Schoenen, J.; Franzen, R. Conditioned medium from bone marrow-derived mesenchymal stem cells improves recovery after spinal cord injury in rats: An original strategy to avoid cell transplantation. *PLoS ONE* **2013**, *8*, e69515. [CrossRef] [PubMed]

124. Boido, M.; Garbossa, D.; Fontanella, M.; Ducati, A.; Vercelli, A. Mesenchymal stem cell transplantation reduces glial cyst and improves functional outcome after spinal cord compression. *World Neurosurg.* **2014**, *81*, 183–190. [CrossRef] [PubMed]
125. Karaoz, E.; Kabatas, S.; Duruksu, G.; Okcu, A.; Subasi, C.; Ay, B.; Musluman, M.; Civelek, E. Reduction of lesion in injured rat spinal cord and partial functional recovery of motility after bone marrow derived mesenchymal stem cell transplantation. *Turk. Neurosurg.* **2012**, *22*, 207–217. [CrossRef] [PubMed]
126. Nakajima, H.; Uchida, K.; Guerrero, A.R.; Watanabe, S.; Sugita, D.; Takeura, N.; Yoshida, 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] [PubMed]
127. 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] [PubMed]
128. Osaka, M.; Honmou, O.; Murakami, T.; Nonaka, T.; Houkin, K.; Hamada, H.; Kocsis, J.D. Intravenous administration of mesenchymal stem cells derived from bone marrow after contusive spinal cord injury improves functional outcome. *Brain Res.* **2010**, *1343*, 226–235. [CrossRef] [PubMed]
129. Ide, C.; Nakai, Y.; Nakano, N.; Seo, T.B.; Yamada, Y.; Endo, K.; Noda, T.; Saito, F.; Suzuki, Y.; Fukushima, M.; *et al.* Bone marrow stromal cell transplantation for treatment of sub-acute spinal cord injury in the rat. *Brain Res.* **2010**, *1332*, 32–47. [CrossRef] [PubMed]
130. Hodgetts, S.I.; Simmons, P.J.; Plant, G.W. A comparison of the behavioral and anatomical outcomes in sub-acute and chronic spinal cord injury models following treatment with human mesenchymal precursor cell transplantation and recombinant decorin. *Exp. Neurol.* **2013**, *248*, 343–359. [CrossRef] [PubMed]
131. Kumagai, G.; Tsoulfas, P.; Toh, S.; McNiece, I.; Bramlett, H.M.; Dietrich, W.D. Genetically modified mesenchymal stem cells (MSCs) promote axonal regeneration and prevent hypersensitivity after spinal cord injury. *Exp. Neurol.* **2013**, *248*, 369–380. [CrossRef] [PubMed]
132. Penha, E.M.; Meira, C.S.; Guimarães, E.T.; Mendonça, M.V.P.; Gravely, F.A.; Pinheiro, C.M.B.; Pinheiro, T.M.B.; Barrouin-Melo, S.M.; Ribeiro-dos-Santos, R.; Soares, M.B.P. Use of autologous mesenchymal stem cells derived from bone marrow for the treatment of naturally injured spinal cord in dogs. *Stem Cell Int.* **2014**, *2014*. [CrossRef] [PubMed]
133. Kang, K.; Kim, S.W.; Oh, Y.H.; Yu, J.W.; Kim, K.Y.; Park, H.K.; 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: A case study. *Cytotherapy* **2005**, *7*, 368–373. [CrossRef] [PubMed]
134. Adewumi, O.; Aflatoonian, B.; Ahrlund-Richter, L.; Amit, M.; Andrews, P.W.; Beighton, G.; Bello, P.A.; Benvenisty, N.; Berry, L.S.; Bevan, S.; *et al.* Characterization of human embryonic stem cell lines by the International Stem Cell Initiative. *Nat. Biotechnol.* **2007**, *25*, 803–816. [CrossRef] [PubMed]
135. Nógrádi, A. *Transplantation of Neural Tissue into the Spinal Cord*; Landes Bioscience: Austin, TX, USA, 2006.
136. Reier, P.J.; Anderson, D.K.; Thompson, F.J.; Stokes, B.T. Neural tissue transplantation and CNS trauma: Anatomical and functional repair of the injured spinal cord. *J. Neurotrauma* **1992**, *9*, S223–S248. [PubMed]
137. Evans, M.J.; Kaufman, M.H. Establishment in culture of pluripotential cells from mouse embryos. *Nature* **1981**, *292*, 154–156. [CrossRef] [PubMed]
138. Martin, G.R. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proc. Natl. Acad. Sci. USA* **1981**, *78*, 7634–7638. [CrossRef] [PubMed]
139. Pera, M.F.; Trounson, A.O. Human embryonic stem cells: Prospects for development. *Development* **2004**, *131*, 5515–5525. [CrossRef] [PubMed]
140. Thomson, J.A.; Itskovitz-Eldor, J.; Shapiro, S.S.; Waknitz, M.A.; Swiergiel, J.J.; Marshall, V.S.; Jones, J.M. Embryonic stem cell lines derived from human blastocysts. *Science* **1998**, *282*, 1145–1147. [CrossRef] [PubMed]
141. Willerth, S.M. Neural tissue engineering using embryonic and induced pluripotent stem cells. *Stem Cell Res. Ther.* **2011**, *2*. [CrossRef] [PubMed]
142. Johnson, P.J.; Tatara, A.; Shiu, A.; Sakiyama-Elbert, S.E. Controlled release of neurotrophin-3 and platelet derived growth factor from fibrin scaffolds containing neural progenitor cells enhances survival and differentiation into neurons in a subacute model of SCI. *Cell Transplant.* **2010**, *19*, 89–101. [CrossRef] [PubMed]

143. Lu, P.; Jonesa, L.L.; Snyderb, E.Y.; Tuszynskia, M.H. Neural stem cells constitutively secrete neurotrophic factors and promote extensive host axonal growth after spinal cord injury. *Exp. Neurol.* **2003**, *181*, 115–129. [CrossRef]
144. Ogawa, Y.; Sawamoto, K.; Miyata, T.; Miyao, S.; Watanabe, M.; Nakamura, M.; Bregman, B.S.; Koike, M.; Uchiyama, Y.; Toyama, Y.; *et al.* Transplantation of *in vitro*—expanded fetal neural progenitor cells results in neurogenesis and functional recovery after spinal cord contusion injury in adult rats. *J. Neurosci. Res.* **2002**, *69*, 925–933. [CrossRef] [PubMed]
145. Iwanami, A.; Kaneko, S.; Nakamura, M.; Kanemura, Y.; Mori, H.; Kobayashi, S.; Yamasaki, M.; Momoshima, S.; Ishii, H.; Ando, K.; *et al.* Transplantation of human neural stem cells for spinal cord injury in primates. *J. Neurosci. Res.* **2005**, *80*, 182–190. [CrossRef] [PubMed]
146. 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]
147. Cloutier, F.; Siegenthaler, M.M.; Nistor, G.; Keirstead, H.S. Transplantation of human embryonic stem cell-derived oligodendrocyte progenitors into rat spinal cord injuries does not cause harm. *Rengen. Med.* **2006**, *1*, 469–479. [CrossRef] [PubMed]
148. Bain, G.; Kitchens, D.; Yao, M.; Huettner, J.E.; Gottlieb, D.I. Embryonic stem cells express neuronal properties *in vitro*. *Dev. Biol.* **1995**, *168*, 342–357. [CrossRef] [PubMed]
149. 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] [PubMed]
150. Kayama, M.; Kurokawa, M.S.; Ueda, Y.; Ueno, H.; Kumagai, Y.; Chiba, S.; Takada, E.; Ueno, S.; Tadokoro, M.; Suzuki, N. Transfection with pax6 gene of mouse embryonic stem cells and subsequent cell cloning induced retinal neuron progenitors, including retinal ganglion cell-like cells, *in vitro*. *Ophthalmic Res.* **2009**, *43*, 79–91. [CrossRef] [PubMed]
151. Sharp, J.; Frame, J.; Siegenthaler, M.; Nistor, G.; Keirstead, H.S. Human embryonic stem cell-derived oligodendrocyte progenitor cell transplants improve recovery after cervical spinal cord injury. *Stem Cells* **2010**, *28*, 152–163. [CrossRef] [PubMed]
152. Karimi-Abdolrezaee, S.; Eftekharpour, E.; Wang, J.; Morshead, C.M.; Fehlings, M.G. Delayed transplantation of adult neural precursor cells promotes remyelination and functional neurological recovery after spinal cord injury. *J. Neurosci.* **2006**, *26*, 3377–3389. [CrossRef] [PubMed]
153. Liu, S.; Qu, Y.; Stewart, T.J.; Howard, M.J.; Chakrabortty, S.; Holekamp, T.F.; McDonald, J.W. Embryonic stem cells differentiate into oligodendrocytes and myelinate in culture and after spinal cord transplantation. *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 6126–6131. [CrossRef] [PubMed]
154. Faulkner, J.; Keirstead, H.S. Human embryonic stem cell-derived oligodendrocyte progenitors for the treatment of spinal cord injury. *Transp. Immunol.* **2005**, *15*, 131–142. [CrossRef] [PubMed]
155. Hasegawa, K.; Chang, Y.W.; Li, H.; Berlin, Y.; Ikeda, O.; Kane-Goldsmith, N.; Grumet, M. Embryonic radial glia bridge spinal cord lesions and promote functional recovery following spinal cord injury. *Exp. Neurol.* **2005**, *193*, 394–410. [CrossRef] [PubMed]
156. Nistor, G.; Siegenthaler, M.M.; Poirier, S.N.; Rossi, S.; Poole, A.J.; Charlton, M.E.; McNeish, J.D.; Airriess, C.N.; Keirstead, H.S. Derivation of high purity neuronal progenitors from human embryonic stem cells. *PLoS ONE* **2011**, *6*, e20692. [CrossRef] [PubMed]
157. Peljto, M.; Hynek, W. Programming embryonic stem cells to neuronal subtypes. *Curr. Opin. Neurobiol.* **2011**, *21*, 43–51. [CrossRef] [PubMed]
158. Gabut, M.; Samavarchi-Tehrani, P.; Wang, X.; Slobodeniuc, V.; O'Hanlon, D.; Sung, H.K.; Alvarez, M.; Talukder, S.; Pan, Q.; Mazzoni, E.O.; *et al.* An alternative splicing switch regulates embryonic stem cell pluripotency and reprogramming. *Cell* **2011**, *147*, 132–146. [CrossRef] [PubMed]
159. Son, E.Y.; Ichida, J.K.; Wainger, B.J.; Toma, J.S.; Rafuse, V.F.; Woolf, C.J.; Eggan, K. Conversion of mouse and human fibroblasts into functional spinal motor neurons. *Cell Stem Cell* **2011**, *9*, 205–218. [CrossRef] [PubMed]
160. Chen, J.A.; Huang, Y.P.; Mazzoni, E.O.; Tan, G.C.; Zavadil, J.; Wichterle, H. miR-17-3p controls spinal neural progenitor patterning by regulating Olig2/Irx3 cross-repressive loop. *Neuron* **2011**, *69*, 721–735. [CrossRef] [PubMed]

161. Wyatt, T.J.; Rossi, S.L.; Siegenthaler, M.M.; Frame, J.; Robles, R.; Nistor, G.; Keirstead, H.S. Human motor neuron progenitor transplantation leads to endogenous neuronal sparing in 3 models of motor neuron loss. *Stem Cells Int.* **2011**, *2011*. [CrossRef] [PubMed]
162. McCreedy, D.A.; Rieger, C.R.; Gottlieb, D.I.; Sakiyama-Elbert, S.E. Transgenic enrichment of mouse embryonic stem cell-derived progenitor motor neurons. *Stem Cell Res.* **2012**, *8*, 368–378. [CrossRef] [PubMed]
163. Brown, C.R.; Butts, J.C.; McCreedy, D.A.; Sakiyama-Elbert, S.E. Generation of V2a interneurons from mouse embryonic stem cells. *Stem Cells Dev.* **2014**, *23*, 1765–1776. [CrossRef] [PubMed]
164. McCreedy, D.A.; Brown, C.R.; Butts, J.C.; Xu, H.; Huettner, J.E.; Sakiyama-Elbert, S.E. A new method for generating high purity motoneurons from mouse embryonic stem cells. *Biotechnol. Bioeng.* **2014**, *111*, 2041–2055. [CrossRef] [PubMed]
165. McCreedy, D.A.; Wilems, T.S.; Xu, H.; Butts, J.C.; Brown, C.R.; Smith, A.W.; Sakiyama-Elbert, S.E. Survival, differentiation, and migration of high-purity mouse embryonic stem cell-derived progenitor motor neurons in fibrin scaffolds after sub-acute spinal cord injury. *Biomater. Sci.* **2014**, *2*, 1672–1682. [CrossRef] [PubMed]
166. Ideguchi, M.; Palmer, T.D.; Recht, L.D.; Weimann, J.M. Murine embryonic stem cell-derived pyramidal neurons integrate into the cerebral cortex and appropriately project axons to subcortical targets. *J. Neurosci.* **2010**, *30*, 894–904. [CrossRef] [PubMed]
167. Yamamori, T.; Fukada, K.; Aebersold, R.; Korsching, S.; Fann, M.J. The cholinergic neuronal differentiation factor from heart cells is identical to leukemia inhibitory factor. *Science* **1989**, *246*, 1412–1416. [CrossRef] [PubMed]
168. Diener, P.S.; Bregman, B.S. Fetal spinal cord transplants support growth of supraspinal and segmental projections after cervical spinal cord hemisection in the neonatal rat. *J. Neurosci.* **1998**, *18*, 779–793. [PubMed]
169. Lepore, A.C.; Bakshi, A.; Swanger, S.A.; Rao, M.S.; Fischer, I. Neural precursor cells can be delivered into the injured cervical spinal cord by intrathecal injection at the lumbar cord. *Brain Res.* **2005**, *1045*, 206–216. [CrossRef] [PubMed]
170. Mitsui, T.; Shumsky, J.S.; Lepore, A.C.; Murray, M.; Fischer, I. Transplantation of neuronal and glial restricted precursors into contused spinal cord improves bladder and motor functions, decreases thermal hypersensitivity, and modifies intraspinal circuitry. *J. Neurosci.* **2005**, *25*, 9624–9636. [CrossRef] [PubMed]
171. Lepore, A.C.; Fischer, I. Lineage-restricted neural precursors survive, migrate, and differentiate following transplantation into the injured adult spinal cord. *Exp. Neurol.* **2005**, *194*, 230–242. [CrossRef] [PubMed]
172. Ruff, C.A.; Wilcox, J.T.; Fehlings, M.G. Cell-based transplantation strategies to promote plasticity following spinal cord injury. *Exp. Neurol.* **2012**, *235*, 78–90. [CrossRef] [PubMed]
173. Church, E.W.; Halpern, C.H.; Faught, R.W.; Balmuri, U.; Attiah, M.A.; Hayden, S.; Kerr, M.; Maloney-Wilensky, E.; Bynum, J.; Dante, S.J.; *et al.* Cervical laminoforaminotomy for radiculopathy: Symptomatic and functional outcomes in a large cohort with long-term follow-up. *Surg. Neurol. Int.* **2014**, *5*, S536–S543. [CrossRef] [PubMed]
174. Giusto, E.; Donegà, M.; Cossetti, C.; Pluchino, S. Neuro-immune interactions of neural stem cell transplants: From animal disease models to human trials. *Exp. Neurol.* **2014**, *260*, 19–32. [CrossRef] [PubMed]
175. Trounson, A.; Thakar, R.G.; Lomax, G.; Gibbons, D. Clinical trials for stem cell therapies. *BMC Med.* **2011**, *9*. [CrossRef] [PubMed]
176. Guzman, R.; Uchida, N.; Bliss, T.M.; He, D.; Christopherson, K.K.; Stellwagen, D.; Capela, A.; Greve, J.; Malenka, R.C.; Moseley, M.E.; *et al.* Long-term monitoring of transplanted human neural stem cells in developmental and pathological contexts with MRI. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 10211–10216. [CrossRef] [PubMed]
177. Mothe, A.J.; Tator, C.H. Advances in stem cell therapy for spinal cord injury. *J. Clin. Investig.* **2012**, *122*, 3824–3834. [CrossRef] [PubMed]
178. Sun, Y.; Xu, C.C.; Li, J.; Guan, X.Y.; Gao, L.; Ma, L.X.; Li, R.X.; Peng, Y.W.; Zhu, G.P. Transplantation of oligodendrocyte precursor cells improves locomotion deficits in rats with spinal cord irradiation injury. *PLoS ONE* **2013**, *8*, e57534. [CrossRef] [PubMed]
179. Rossi, S.L.; Nistor, G.; Wyatt, T.; Yin, H.Z.; Poole, A.J.; Weiss, J.H.; Gardener, M.J.; Dijkstra, S.; Fischer, D.F.; Keirstead, H.S. Histological and functional benefit following transplantation of motor neuron progenitors to the injured rat spinal cord. *PLoS ONE* **2010**, *5*, e11852. [CrossRef] [PubMed]

180. Sandner, B.; Ciatipis, M.; Motsch, M.; Soljanik, I.; Weidner, N.; Blesch, A. Limited functional effects of subacute syngeneic bone marrow stromal cell transplantation after rat spinal cord contusion injury. *Cell Transplant.* **2016**, *25*, 125–139. [CrossRef] [PubMed]
181. Prockop, D.J. Further proof of the plasticity of adult stem cells and their role in tissue repair. *J. Cell Biol.* **2003**, *160*, 807–809. [CrossRef] [PubMed]
182. Gallo, M.P.; Ramella, R.; Alloatti, G.; Penna, C.; Pagliaro, P.; Marcantoni, A.; Bonafé, F.; Losano, G.; Levi, R. Limited plasticity of mesenchymal stem cells cocultured with adult cardiomyocytes. *J. Cell. Biochem.* **2007**, *100*, 86–99. [CrossRef] [PubMed]
183. Zouani, O.F.; Yifeng, L.; Marie-Christine, D. Pericytes, stem-cell-like cells, but not mesenchymal stem cells are recruited to support microvascular tube stabilization. *Small* **2013**, *9*, 3070–3075. [CrossRef] [PubMed]
184. Prockop, D.J. Repair of tissues by adult stem/progenitor cells (MSCs): Controversies, myths, and changing paradigms. *Mol. Ther.* **2009**, *17*, 939–946. [CrossRef] [PubMed]
185. Prockop, D.J.; Brenner, M.; Fibbe, W.E.; Horwitz, E.; Le Blanc, K.; Phinney, D.G.; Simmons, P.J.; Sensebe, L.; Keating, A. Defining the risks of mesenchymal stromal cell therapy. *Cytotherapy* **2010**, *12*, 576–578. [CrossRef] [PubMed]
186. Ankrum, J.A.; Joon, F.O.; Karp, J.M. Mesenchymal stem cells: Immune evasive, not immune privileged. *Nat. Biotechnol.* **2014**, *32*, 252–260. [CrossRef] [PubMed]
187. Nisbet, M.C.; Dominique, B.; Adrianne, K. Framing science the stem cell controversy in an age of press/politics. *Int. J. Press* **2003**, *8*, 36–70. [CrossRef]
188. Cao, Q.L.; Howard, R.M.; Dennison, J.B.; Whittemore, S.R. Differentiation of engrafted neuronal-restricted precursor cells is inhibited in the traumatically injured spinal cord. *Exp. Neurol.* **2002**, *177*, 349–359. [CrossRef] [PubMed]
189. Cao, Q.L.; Zhang, Y.P.; Howard, R.M.; Walters, W.M.; Tsoulfas, P.; Whittemore, S.R. Pluripotent stem cells engrafted into the normal or lesioned adult rat spinal cord are restricted to a glial lineage. *Exp. Neurol.* **2001**, *167*, 48–58. [CrossRef] [PubMed]
190. Dressel, R. Effects of histocompatibility and host immune responses on the tumorigenicity of pluripotent stem cells. *Semin. Immunopathol.* **2011**, *33*, 573. [CrossRef] [PubMed]
191. Ghosh, Z.; Huang, M.; Hu, S.; Wilson, K.D.; Dey, D.; Wu, J.C. Dissecting the oncogenic and tumorigenic potential of differentiated human induced pluripotent stem cells and human embryonic stem cells. *Cancer Res.* **2011**, *71*, 5030–5039. [CrossRef] [PubMed]
192. Iwai, H.; Shimada, H.; Nishimura, S.; Kobayashi, Y.; Itakura, G.; Hori, K.; Hikishima, K.; Ebise, H.; Negishi, N.; Shibata, S.; *et al.* Allogeneic neural stem/progenitor cells derived from embryonic stem cells promote functional recovery after transplantation into injured spinal cord of nonhuman primates. *Stem Cells Transl. Med.* **2015**. [CrossRef] [PubMed]
193. Blum, B.; Nissim, B. The tumorigenicity of human embryonic stem cells. *Adv. Cancer Res.* **2008**, *100*, 133–158. [PubMed]
194. Baker, D.E.; Harrison, N.J.; Maltby, E.; Smith, K.; Moore, H.D.; Shaw, P.J.; Heath, P.R.; Holden, H.; Andrews, P.W. Adaptation to culture of human embryonic stem cells and oncogenesis *in vivo*. *Nat. Biotechnol.* **2007**, *25*, 207–215. [CrossRef] [PubMed]
195. Lefort, N.; Feyeux, M.; Bas, C.; Féraud, O.; Bennaceur-Griscelli, A.; Tachdjian, G.; Peschanski, M.; Perrier, A.L. Human embryonic stem cells reveal recurrent genomic instability at 20q11.21. *Nat. Biotechnol.* **2008**, *26*, 1364–1366. [CrossRef] [PubMed]
196. Mayshar, Y.; Ofra, Y.; Nissim, B. Teratogen screening using transcriptome profiling of differentiating human embryonic stem cells. *J. Cell. Mol. Med.* **2011**, *15*, 1393–1401. [CrossRef] [PubMed]
197. Klimanskaya, I.; Chung, Y.; Becker, S.; Lu, S.J.; Lanza, R. Human embryonic stem cell lines derived from single blastomeres. *Nature* **2006**, *444*, 481–485. [CrossRef] [PubMed]
198. Takahashi, K.; Shinya, Y. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **2006**, *126*, 663–676. [CrossRef] [PubMed]
199. Miura, K.; Okada, Y.; Aoi, T.; Okada, A.; Takahashi, K.; Okita, K.; Nakagawa, M.; Koyanagi, M.; Tanabe, K.; Ohnuki, M.; *et al.* Variation in the safety of induced pluripotent stem cell lines. *Nat. Biotechnol.* **2009**, *27*, 743–745. [CrossRef] [PubMed]

200. Takahashi, K.; Tanabe, K.; Ohnuki, M.; Narita, M.; Ichisaka, T.; Tomoda, K.; Yamanaka, S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* **2007**, *131*, 861–872. [CrossRef] [PubMed]
201. Yamanaka, S. Patient-specific pluripotent stem cells become even more accessible. *Cell Stem Cell* **2010**, *7*, 1–2. [CrossRef] [PubMed]
202. Fusaki, N.; Ban, H.; Nishiyama, A.; Saeki, K.; Hasegawa, M. Efficient induction of transgene-free human pluripotent stem cells using a vector based on Sendai virus, an RNA virus that does not integrate into the host genome. *Proc. Jpn. Acad. Ser. B* **2009**, *85*, 348–362. [CrossRef]
203. Nori, S.; Okada, Y.; Nishimura, S.; Sasaki, T.; Itakura, G.; Kobayashi, Y.; Renault-Mihara, F.; Shimizu, A.; Koya, I.; Yoshida, R. Long-term safety issues of iPSC-based cell therapy in a spinal cord injury model: Oncogenic transformation with epithelial-mesenchymal transition. *Stem Cell Rep.* **2015**, *4*, 360–373. [CrossRef] [PubMed]
204. Zhou, H.; Wu, S.; Joo, J.Y.; Zhu, S.; Han, D.W.; Lin, T.; Trauger, S.; Bien, G.; Yao, S.; Zhu, Y.; *et al.* Generation of induced pluripotent stem cells using recombinant proteins. *Cell Stem Cell* **2009**, *4*, 381–384. [CrossRef] [PubMed]
205. Warren, L.; Manos, P.D.; Ahfeldt, T.; Loh, Y.H.; Li, H.; Lau, F.; Ebina, W.; Mandal, P.K.; Smith, Z.D.; Meissner, A.; *et al.* Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. *Cell Stem Cell* **2010**, *7*, 618–630. [CrossRef] [PubMed]
206. Rao, M.S.; Nasir, M. Assessing iPSC reprogramming methods for their suitability in translational medicine. *J. Cell. Biochem.* **2012**, *113*, 3061–3068. [CrossRef] [PubMed]
207. Yamanaka, S. Induced pluripotent stem cells: Past, present, and future. *Cell Stem Cell* **2012**, *10*, 678–684. [CrossRef] [PubMed]
208. Chin, M.H.; Mason, M.J.; Xie, W.; Volinia, S.; Singer, M.; Peterson, C.; Ambartsumyan, G.; Aimiuwu, O.; Richter, L.; Zhang, J.; *et al.* Induced pluripotent stem cells and embryonic stem cells are distinguished by gene expression signatures. *Cell Stem Cell* **2009**, *5*, 111–123. [CrossRef] [PubMed]
209. Choi, J.; Lee, S.; Mallard, W.; Clement, K.; Tagliazucchi, G.M.; Lim, H.; Choi, I.Y.; Ferrari, F.; Tsankov, A.M.; Pop, R.; *et al.* A comparison of genetically matched cell lines reveals the equivalence of human iPSCs and ESCs. *Nat. Biotechnol.* **2015**, *33*, 1173–1181. [CrossRef] [PubMed]
210. Phanstiel, D.H.; Brumbaugh, J.; Wenger, C.D.; Tian, S.; Probasco, M.D.; Bailey, D.J.; Swaney, D.L.; Tervo, M.A.; Bolin, J.M.; Ruotti, V.; *et al.* Proteomic and phosphoproteomic comparison of human ES and iPS cells. *Nat. Methods* **2011**, *8*, 821–827. [CrossRef] [PubMed]
211. Guenther, M.G.; Frampton, G.M.; Soldner, F.; Hockemeyer, D.; Mitalipova, M.; Jaenisch, R.; Young, R.A. Chromatin structure and gene expression programs of human embryonic and induced pluripotent stem cells. *Cell Stem Cell* **2010**, *7*, 249–257. [CrossRef] [PubMed]
212. Munoz, J.; Low, T.Y.; Kok, Y.J.; Chin, A.; Frese, C.K.; Ding, V.; Choo, A.; Heck, A.J. The quantitative proteomes of human-induced pluripotent stem cells and embryonic stem cells. *Mol. Syst. Biol.* **2011**, *7*. [CrossRef] [PubMed]
213. Prigione, A.; Fauler, B.; Lurz, R.; Lehrach, H.; Adjaye, J. The senescence-related mitochondrial/oxidative stress pathway is repressed in human induced pluripotent stem cells. *Stem Cells* **2010**, *28*, 721–733. [CrossRef] [PubMed]
214. Puri, M.C.; Andras, N. Concise review: Embryonic stem cells *versus* induced pluripotent stem cells: The game is on. *Stem Cells* **2012**, *30*, 10–14. [CrossRef] [PubMed]
215. Hirasaki, M.; Hiraki-Kamon, K.; Kamon, M.; Suzuki, A.; Katano, M.; Nishimoto, M.; Okuda, A. Striking similarity in the gene expression levels of individual Myc module members among ESCs, EpiSCs, and partial iPSCs. *PLoS ONE* **2013**, *8*, e83769. [CrossRef] [PubMed]
216. Chen, K.G.; Mallon, B.S.; McKay, R.D.; Robey, P.G. Human pluripotent stem cell culture: Considerations for maintenance, expansion, and therapeutics. *Cell Stem Cell* **2014**, *14*, 13–26. [CrossRef] [PubMed]
217. Mallon, B.S.; Hamilton, R.S.; Kozhich, O.A.; Johnson, K.R.; Fann, Y.C.; Rao, M.S.; Robey, P.G. Comparison of the molecular profiles of human embryonic and induced pluripotent stem cells of isogenic origin. *Stem Cell Res.* **2014**, *12*, 376–386. [CrossRef] [PubMed]
218. Taylor, C.J.; Peacock, S.; Chaudhry, A.N.; Bradley, J.A.; Bolton, E.M. Generating an iPSC bank for HLA-matched tissue transplantation based on known donor and recipient HLA types. *Cell Stem Cell* **2012**, *11*, 147–152. [CrossRef] [PubMed]

219. Okano, H.; Shinya, Y. iPS cell technologies: Significance and applications to CNS regeneration and disease. *Mol. Brain* **2014**, *7*. [CrossRef] [PubMed]
220. Polo, J.M.; Liu, S.; Figueroa, M.E.; Kulalert, W.; Eminli, S.; Tan, K.Y.; Apostolou, E.; Stadtfeld, M.; Li, Y.; Shioda, T.; *et al.* Cell type of origin influences the molecular and functional properties of mouse induced pluripotent stem cells. *Nat. Biotechnol.* **2010**, *28*, 848–855. [CrossRef] [PubMed]
221. Kim, K.; Doi, A.; Wen, B.; Ng, K.; Zhao, R.; Cahan, P.; Kim, J.; Aryee, M.J.; Ji, H.; Ehrlich, L.I.R.; *et al.* Epigenetic memory in induced pluripotent stem cells. *Nature* **2010**, *467*, 285–290. [CrossRef] [PubMed]
222. Han, S.W.; Williams, L.A.; Eggan, K.C. Constructing and deconstructing stem cell models of neurological disease. *Neuron* **2011**, *70*, 626–644. [CrossRef] [PubMed]
223. Marchetto, M.C.; Gage, F.H. Modeling brain disease in a dish: Really? *Cell Stem Cell* **2012**, *10*, 642–645. [CrossRef] [PubMed]
224. Brennand, K.J.; Simone, A.; Tran, N.; Gage, F.H. Modeling psychiatric disorders at the cellular and network levels. *Mol. Psychiatr.* **2012**, *17*, 1239–1253. [CrossRef] [PubMed]
225. Barker, R.A. Developing stem cell therapies for Parkinson's disease: Waiting until the time is right. *Cell Stem Cell* **2014**, *15*, 539–542. [CrossRef] [PubMed]
226. Beevers, J.E.; Caffrey, T.M.; Wade-Martins, R. Induced pluripotent stem cell (iPSC)-derived dopaminergic models of Parkinson's disease. *Biochem. Soc. Trans.* **2013**, *41*, 1503–1508. [CrossRef] [PubMed]
227. Perrier, A.; Marc, P. How can human pluripotent stem cells help decipher and cure Huntington's disease? *Cell Stem Cell* **2012**, *11*, 153–161. [CrossRef] [PubMed]
228. Israel, M.A.; Yuan, S.H.; Bardy, C.; Reyna, S.M.; Mu, Y.; Herrera, C.; Hefferan, M.P.; van Gorp, S.; Nazor, K.L.; Boscolo, F.S.; *et al.* Probing sporadic and familial Alzheimer's disease using induced pluripotent stem cells. *Nature* **2012**, *482*, 216–220. [CrossRef] [PubMed]
229. Liu, G.-H.; Zhichao, D.; Belmonte, J.C.I. iPSC technology to study human aging and aging-related disorders. *Curr. Opin. Cell Biol.* **2012**, *24*, 765–774. [CrossRef] [PubMed]
230. Trounson, A.; Kelly, A.S.; DeWitt, N.D. Human disease modeling with induced pluripotent stem cells. *Curr. Opin. Genet. Dev.* **2012**, *22*, 509–516. [CrossRef] [PubMed]
231. Guo, Z.; Zhang, L.; Wu, Z.; Chen, Y.; Wang, F.; Chen, G. *In vivo* direct reprogramming of reactive glial cells into functional neurons after brain injury and in an Alzheimer's disease model. *Cell Stem Cell* **2014**, *14*, 188–202. [CrossRef] [PubMed]
232. Krencik, R.; Su-Chun, Z. Directed differentiation of functional astroglial subtypes from human pluripotent stem cells. *Nat. Protoc.* **2011**, *6*, 1710–1717. [CrossRef] [PubMed]
233. Yang, N.; Zuchero, J.B.; Ahlenius, H.; Marro, S.; Ng, Y.H.; Vierbuchen, T.; Hawkins, J.S.; Geissler, R.; Barres, B.A.; Wernig, M. Generation of oligodendroglial cells by direct lineage conversion. *Nat. Biotechnol.* **2013**, *31*, 434–439. [CrossRef] [PubMed]
234. Zhang, Y.; Pak, C.; Han, Y.; Ahlenius, H.; Zhang, Z.; Chanda, S.; Marro, S.; Patzke, C.; Acuna, C.; Covy, J.; *et al.* Rapid single-step induction of functional neurons from human pluripotent stem cells. *Neuron* **2013**, *78*, 785–798. [CrossRef] [PubMed]
235. Ambasudhan, R.; Talantova, M.; Coleman, R.; Yuan, X.; Zhu, S.; Lipton, S.A.; Ding, S. Direct reprogramming of adult human fibroblasts to functional neurons under defined conditions. *Cell Stem Cell* **2011**, *9*, 113–118. [CrossRef] [PubMed]
236. Hodgetts, S.I.; Michael, E.; Harvey, A.R. The state of play with iPSCs and spinal cord injury models. *J. Clin. Med.* **2015**, *4*, 193–203. [CrossRef] [PubMed]
237. Marchetto, M.C.; Carromeu, C.; Acab, A.; Yu, D.; Yeo, G.W.; Mu, Y.; Chen, G.; Gage, F.H.; Muotri, A.R. A model for neural development and treatment of Rett syndrome using human induced pluripotent stem cells. *Cell* **2010**, *143*, 527–539. [CrossRef] [PubMed]
238. Kim, J.; Efea, J.A.; Zhua, S.; Talantovac, M.; Yuana, X.; Wang, S.; Liptonc, S.A.; Zhang, K.; Ding, S. Direct reprogramming of mouse fibroblasts to neural progenitors. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 7838–7843. [CrossRef] [PubMed]
239. Swistowski, A.; Peng, J.; Liu, Q.; Mali, P.; Rao, M.S.; Cheng, L.; Zeng, X. Efficient generation of functional dopaminergic neurons from human induced pluripotent stem cells under defined conditions. *Stem Cells* **2010**, *28*, 1893–1904. [CrossRef] [PubMed]

240. Takebe, T.; Sekine, K.; Enomura, M.; Koike, H.; Kimura, M.; Ogaeri, T.; Zhang, R.R.; Ueno, Y.; Zheng, Y.W.; Koike, N.; *et al.* Vascularized and functional human liver from an iPSC-derived organ bud transplant. *Nature* **2013**, *499*, 481–484. [CrossRef] [PubMed]
241. Zeng, H.; Guo, M.; Martins-Taylor, K.; Wang, X.; Zhang, Z.; Park, J.W.; Zhan, S.; Kronenberg, M.S.; Lichtler, A.; Liu, H.X.; *et al.* Specification of region-specific neurons including forebrain glutamatergic neurons from human induced pluripotent stem cells. *PLoS ONE* **2010**, *5*, e11853. [CrossRef] [PubMed]
242. Brennand, K.J.; Simone, A.; Jou, J.; Gelboin-Burkhart, C.; Tran, N.; Sangar, S.; Li, Y.; Mu, Y.; Chen, G.; Yu, D.; *et al.* Modelling schizophrenia using human induced pluripotent stem cells. *Nature* **2011**, *473*, 221–225. [CrossRef] [PubMed]
243. Hester, M.E.; Murtha, M.J.; Song, S.; Rao, M.; Miranda, C.J.; Meyer, K.; Tian, J.; Boulting, G.; Schaffer, D.V.; Zhu, M.X.; *et al.* Rapid and efficient generation of functional motor neurons from human pluripotent stem cells using gene delivered transcription factor codes. *Mol. Ther.* **2011**, *19*, 1905–1912. [CrossRef] [PubMed]
244. Boulting, G.L.; Kiskinis, E.; Croft, G.F.; Amoroso, M.W.; Oakley, D.H.; Wainger, B.J.; Williams, D.J.; Kahler, D.J.; Yamaki, M.; Davidow, L.; *et al.* A functionally characterized test set of human induced pluripotent stem cells. *Nat. Biotechnol.* **2011**, *29*, 279–286. [CrossRef] [PubMed]
245. Faravelli, I.; Frattini, E.; Ramirez, A.; Stuppia, G.; Nizzardo, M.; Corti, S. iPSC-based models to unravel key pathogenetic processes underlying motor neuron disease development. *J. Clin. Med.* **2014**, *3*, 1124–1145. [CrossRef] [PubMed]
246. Hu, B.Y.; Weick, J.P.; Yu, J.; Ma, L.X.; Zhang, X.Q.; Thomson, J.A.; Zhang, S.C. Neural differentiation of human induced pluripotent stem cells follows developmental principles but with variable potency. *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 4335–4340. [CrossRef] [PubMed]
247. Tucker, B.A.; Mullins, R.F.; Streb, L.M.; Anfinson, K.; Eyestone, M.E.; Kaalberg, E.; Riker, M.J.; Drack, A.V.; Braun, T.A.; Stone, E.M. Patient-specific iPSC-derived photoreceptor precursor cells as a means to investigate retinitis pigmentosa. *Elife* **2013**, *2*, e00824. [CrossRef] [PubMed]
248. Buchholz, D.E.; Hikita, S.T.; Rowland, T.J.; Friedrich, A.M.; Hinman, C.R.; Johnson, L.V.; Clegg, D.O. Derivation of functional retinal pigmented epithelium from induced pluripotent stem cells. *Stem Cells* **2009**, *27*, 2427–2434. [CrossRef] [PubMed]
249. Gensel, J.C.; Tovar, C.A.; Hamers, F.P.; Deibert, R.J.; Beattie, M.S.; Bresnahan, J.C. Behavioral and histological characterization of unilateral cervical spinal cord contusion injury in rats. *J. Neurotrauma* **2006**, *23*, 36–54. [CrossRef] [PubMed]
250. Nutt, S.E.; Chang, E.A.; Suhr, S.T.; Schlosser, L.O.; Mondello, S.E.; Moritz, C.T.; Cibelli, J.B.; Horner, P.J. Caudalized human iPSC-derived neural progenitor cells produce neurons and glia but fail to restore function in an early chronic spinal cord injury model. *Exp. Neurol.* **2013**, *248*, 491–503. [CrossRef] [PubMed]
251. Li, K.; Javed, E.; Scura, D.; Hala, T.J.; Seetharam, S.; Falnikar, A.; Richard, J.P.; Chorath, A.; Maragakis, N.J.; Wright, M.C.; *et al.* Human iPS cell-derived astrocyte transplants preserve respiratory function after spinal cord injury. *Exp. Neurol.* **2015**, *271*, 479–492. [CrossRef] [PubMed]
252. Lu, P.; Woodruff, G.; Wang, Y.; Graham, L.; Hunt, M.; Wu, D.; Boehle, E.; Ahmad, R.; Poplawski, G.; Brock, J.; Goldstein, L.S. Long-distance axonal growth from human induced pluripotent stem cells after spinal cord injury. *Neuron* **2014**, *83*, 789–796. [CrossRef] [PubMed]
253. Kobayashi, Y.; Okada, Y.; Itakura, G.; Iwai, H.; Nishimura, S.; Yasuda, A.; Nori, S.; Hikishima, K.; Konomi, T.; Fujiyoshi, K.; *et al.* Pre-evaluated safe human iPSC-derived neural stem cells promote functional recovery after spinal cord injury in common marmoset without tumorigenicity. *PLoS ONE* **2012**, *7*, e52787. [CrossRef] [PubMed]
254. Sroga, J.M.; Jones, T.; Kigerl, K.A.; McGaughy, V.M.; Popovich, P.G. Rats and mice exhibit distinct inflammatory reactions after spinal cord injury. *J. Comp. Neurol.* **2003**, *462*, 223–240. [CrossRef] [PubMed]
255. Norenberg, M.D.; Jon, S.; Alex, M. The pathology of human spinal cord injury: Defining the problems. *J. Neurotrauma* **2004**, *21*, 429–440. [CrossRef] [PubMed]
256. Zhang, Y.; Lenart, B.A.; Lee, J.K.; Chen, D.; Shi, P.; Ren, J.; Muehleman, C.; Chen, D.; An, H.S. Histological features of endplates of the mammalian spine: From mice to men. *Spine* **2014**, *39*, E312–E317. [CrossRef] [PubMed]