A Review on Stem Cell Treatment for the Spinal Cord Injury
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Abstract
Stem cell therapy is a potential treatment for spinal cord injury (SCI), and a variety of different stem cell types have been evaluated in animal models and humans with SCI. No consensus exists regarding the type of stem cell, if any, that will prove to be effective therapeutically. Most data suggest that no single therapy will be sufficient to overcome all the biological complications caused by SCI. Rationales for therapeutic use of stem cells for SCI include replacement of damaged neurons and glial cells, secretion of trophic factors, regulation of gliosis and scar formation, prevention of cyst formation, and enhancement of axon elongation. Translation of laboratory-based stem cell research into treatments is likely to take many years and must be conducted with strict oversight. This includes ethical safeguards to protect the welfare of the patient and respect for the moral status of the embryo used in embryonic stem cell research. The attendant risks of stem cell therapy for SCI including tumor formation, or abnormal circuit formation leading to dysfunction must be weighed against the potential benefits of this approach. This Review will examine the biological effects of SCI, the opportunities for stem cell treatment, and the types of stem cells that might be used therapeutically. The limited information available on the possible benefits of stem cell therapy to humans will also be discussed.

Key Words. embryonic stem cells, neural stem cells, stem cell therapy, spinal cord, clinical trials

1. Introduction
The spinal cord is a cylindrical structure of nervous tissue that extends from the foramen magnum to the lower border of the first lumbar vertebra. It is the only part of the adult nervous system that preserved the primitive segmental arrangement of the embryonic neural tube. Each of these segments forms a pair of spinal nerves that leaves the sheltering bony vertebral canal and reaches the periphery. The function of the spinal cord is comparable to a cable that conducts impulses back and forth between the brain and the body. Ascending sensory pathways transfer sensory information from skin, muscle, joints and organs to the brain. Descending motor pathways control voluntary movements and reflex functions of limbs and the trunk (1).

Fractures or dislocation of vertebrae, secondary to traffic accidents, falls and violence, can cause compression of the spinal cord. Traumatic spinal cord injury severing descending and ascending fiber tracts leads to loss of motor and sensory function caudal to the level of injury. Disruption of fibers that control the autonomic nervous system leads to impairment of bowel and bladder function as well as to sexual dysfunction. Spinal cord injury (SCI) is a devastating condition associated with significant functional and sensory deficits, emotional, social, and financial burdens, and an increased risk of cardiovascular complications, deep vein thrombosis, osteoporosis, pressure ulcers, autonomic dysreflexia, and neuropathic pain (2).
Currently, the standard clinical treatment includes surgical stabilization of the vertebral column and application of high doses of steroid. Surgical stabilization prevents posttraumatic instability of the vertebral column and further progression of neurological deficits. Early administration of steroids has been suggested to decrease the amount of tissue damage by lowering the amount of free radicals at the injury site. The effects of this treatment regiment are moderate at best and there is a great need for novel treatment strategies that could significantly restore function following spinal cord injury (3).

Worldwide 90 million people suffer from spinal cord injuries of varying severity. The estimated annual global incidence of SCI is 15-40 cases per million. In the USA, approximately 1.275 million individuals are affected, with over 12,000 new cases each year. Spinal cord injury victims are predominantly male (80%) and more than half of them are injured between the ages of 16 and 30. The most common causes of traumatic SCI are road traffic accidents, falls, occupational and sports-related injuries that result in contusion and compression of the spinal cord. Approximately 55% of SCIs occur at the cervical level (C1 to C7-T1) with a mortality of 10% in the first year following injury and an expected lifespan of only 10-15 years post-injury, and thoracic (T1-T11), thoracolumbar (T11-T12 to L1-L2) and lumbosacral (L2-S5) injuries each account for approximately 15% of SCI. Depending on the age of the patient, severity, and levels of SCI, the lifetime cost of health care and other injury-related expenses can reach $25 million (4,5,6).

Here, we will focus on the advantages, opportunities, and challenges presented by stem cell-based therapeutic strategies for spinal cord injuries, and the reader is referred to recent reviews covering other therapeutic strategies. There are two conceptually different ways to employ stem cells for spinal cord repair. First, one may transplant stem cells, or cells derived from stem cells, to the injured spinal cord. Second, endogenous neural stem cells resident in the adult spinal cord could potentially be recruited or modulated to promote recovery. We will discuss these two possible avenues for stem cells in spinal cord repair. A much larger number of studies have addressed the possibility to improve recovery after spinal cord injury by stem cell transplantation, rather than recruitment of endogenous cells. We therefore limit the description of stem cell transplantation strategies to summarize the efficacy and mechanistic insights (7).

2. Pathophysiology of Spinal Cord Injury

Several experimental studies concentrated on pathophysiology of acute SCI lead to the concept of primary and secondary lesion. As primary mechanical injury directly results from traumatism it cannot be avoided, it results on cell body destruction, axonal section, loss of tissues, vascular lesion, etc. On the contrary, secondary injury (ischemia, edema, excitotoxicity, apoptosis, inflammation, etc.) may be considered as a good therapeutical target directed toward neuroprotection. Chronic stages of SCI follow the two previously mentioned stages; indeed, spinal cord lesion does not remain static and evolves towards rearrangement of inhibitory and excitatory circuits that may be at the origin of either a certain regain of functionality (useful spasticity) or, in the contrary, deleterious effects such spasticity, detrusor sphincter dyssynergia, and neuropathic pain. Moreover, tissue modifications and cerebrospinal fluid leaks may lead to cyst formation within the spinal cord or to real cavity named syringomyelia. (8)

2.1 Primary lesion

Acute traumatic SCI may result from a variety of mechanical stress applied to the spinal cord:

1. Injuries to surrounding structures of the spinal cord (traumatisms of spinal column)
2. Injuries to artery and/or vein vascularization
3. Direct isolated trauma to the spinal cord

Thus, acute traumatic SCI results from many mechanisms such as hyperbending, hyperstretching, axial compression, rotation or distraction. Most commonly, acute traumatic SCI consists on both impaction and persistent compression, in particular in cases of burst fractures, dislocation, post-traumatic compressive broken disc hernia and in rare cases, of compressive hematoma. A single impact without persistent compression is seen in hyperstretching or distraction injuries, spontaneous dislocation that had been reduced and blast injuries. Transection and/or loss of tissue may result from blade or firearm weapon injuries. One particular case consists on injuries, more often centrospinal, of narrow cervical canal enhanced by an even mild traumatism. Strength applied on the spinal cord, leads to transitory neurological deficits in its milder form (concussion) and to complete and permanent paralysis in its more serious form.

At best, modifications of axonal conduction lead to transitory dysfunctions whereas at worse, primary axonal lesion (not the one of the neuron) results into axonal transection that ultimately leads to distal segment Wallerian degeneration. Locally, cell bodies may be destroyed and induce a “suspended” syndrome; the lesion then spreads from the epicenter to a secondary extend of injured tissues that constitutes secondary lesion initiation (9).

2.2 Secondary lesion

Secondary lesion starts just after initial tissue destruction and may progress over several days or weeks. In addition to locally restricted secondary injuries, secondary systemic consequences such as hypotension, vasoplegia, hypoxia-associated respiratory dysfunction (with more or less hypercapnia), respiratory or even metabolic acidosis in polytraumatisms and finally ionic, glycolic, hormonal and thermal troubles are superimposed. All these factors may significantly worsen SCI outcome. Primary tissular injury induces electrolyte, free radical, membrane fragments and excitatory amino acid releases leading to a toxic increase of intracellular calcium level. Microhemorrhages and thrombosis occur in microvessels. A variety of electrolytic pumps are thus not capable of regulating ionic fluxes anymore. As a consequence, excitatory amino acid release activates glutamate receptors such N-methyl-D-aspartate (NMDA) and alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) that ultimately lead to an increased mobilization of intracellular calcium and an increase in energy consumption. Concomitant increase of Na⁺, Ca²⁺ and Cl⁻ intracellular influx and K⁺ efflux eventually lead to cell death by excitotoxicity. As a result glutamate and calcium are released, contributing to a vicious circle that contributes to the death of neighboring neuronal and glial cells. Cell death extends preferentially in a vertical direction through the gray matter whereas edema extends transversally through the white matter. Inflammatory processes already starts within the few minutes following the lesion and gradually increase from a few hours to several days after traumatism. Microglia becomes activated and blood monocytes and lymphocytes cross the lesioned blood brain barrier. These cells express several chemoattractive factors inducing leucocytes build-up within the lesion area as well as in the lumen of neighboring microvessels. Mechanical phenomena and thrombin release are the substrate of vascularization problems. In addition, energetic depletion worsens ischemic cell death phenomena. Moreover, inflammatory cells express proteolytic enzymes (proteases) that in turn, degrade extracellular matrix proteins and contribute to blood brain barrier degradation and edema extension. Cell death resulting from all these mechanisms occur through both necrotic or apoptotic phenomena or autophagia. Secondly, but still at early stages of inflammation, macrophages also participate to the clearance of hemorrhagic areas and necrosed tissue. These two stages of inflammatory processes participate in the demyelination of still intact axons. These mechanisms start as early as 24 h after traumatism and increase

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during the first few days. Then Wallerian degeneration begins, with retarded death of oligodendrocytes, and cysts formation within the gray and white matters and the building of a scar (mainly constituted of astrocytes and fibroblasts represent the final stage of histopathologic evolution.

These pathophysiological features plus the dearth of available treatment options for SCI have made the injured spinal cord an attractive target for studying cell-based therapies. Importantly, it has been increasingly appreciated that with effective neuroprotection in place provided by donor cells including neural stem cells (NSCs), other treatments aiming to promote axonal regrowth and neural plasticity, if started simultaneously or immediately thereafter, may synergistically enhance functional restoration (10).

3. Clinical Potential of Stem Cells for Spinal Cord Injury

Stem cell therapy involves the introduction of stem cells into damaged tissues in order to treat a disease or injury. For this form of therapy to be successful, scientists must be able to produce large numbers of stem cells, turn them into the desired specialised cell types, transplant the cells and then get these cells to replace the damaged or destroyed tissues. A number of adult stem cell treatments exist already, particularly bone marrow transplants that are used to treat leukemia. Medical researchers hope to be able to use technologies produced from stem cell research to treat a wider variety of illnesses in the future.

Various strategies have shown benefit in experimental animal models, there is still no effective therapy for clinical SCI. This difficult situation, in our opinion, is attributable to the following realities. First, there has been no conclusive evidence favoring one process as the predominant pathophysiological mechanism which can account for all the spinal dysfunction seen following SCI. Most of the pathophysiological processes (e.g secondary molecular events: glutamate toxicity, sodium and calcium influx, free radical insult, cytochrome c release; secondary pathophysiological events: ischemia, anoxia, apoptosis, etc.) apparently exist either simultaneously or sequentially in an interlocked or independent manner throughout the evolution of the injury and represent different facets of this complicated disease entity (11).

Most interventions reported to date target solely one facet of the injury process which, in isolation, is doomed to have limited benefit. To further complicate the situation, a given approach that may be useful when used alone, may become ineffective or even detrimental when used in combination with other interventions, perhaps working at cross purposes. Hence, it is critical to understand the intricate interactions between these options and identify the underlying mechanisms of their actions so that they may be orchestrated in a safe, synergetic, and clinically feasible fashion.

Given these challenges, the use of a “therapeutic anchoring vehicle” such as the NSC has been deemed to be an appealing strategy to address multiple pathological processes simultaneously while effecting functional recovery. In an ideal situation, the “therapeutic anchoring agent” should be
(a) multipotent in terms of assuming the roles of different neural cell lineages and performing different functions, including functions at play early in development such as the regulated release of neurotrophic factors and other homeostasis maintaining agents
(b) capable of modifying the restrictive environment of the post-injury CNS, while not being vulnerable to most of the secondary injury molecules (12).

3.1 Human Embryonic Stem Cells

Embryonic stem cells (ESCs) are pluripotent cells that have the capability to differentiate into nearly all cell types, including neuronal and glial fate cells. Therefore, these cells are a promising source of differentiated oligodendrocytes and motoneurons and could be used to treat neurological disorders and traumas, including SCI. Following SCI, oligodendrocytes were shown to be highly vulnerable to the factors existing in inflamed tissue and may undergo cell death. This loss of myelinating cells will cause abnormal neuronal functionality but hESC-derived oligodendrocyte transplantation can restore the functional outcome in animals initiated by the activation of brain derived neurotrophic factor and IL-6 signaling pathways. However, clinical applications of hESCs...
critically depend on their ability to differentiate toward defined and purified neural cell types in vitro. Due to often lengthy and complex differentiation protocols, current protocols for differentiation of neural progenitors from hESCs include application of undesired cell types and undefined factors (such as neural inducing stroma PA6 and MS5). Some new studies have focused on the improvement of the methods for predifferentiation of hESCs into neural or neuronal precursors before cell transplantation to models of SCI. For instance, vitronectin in combination with retinoic acid, Sonic hedgehog, Noggin, or SB431542 drug (SMAD signaling pathway inhibitor) promote oligodendrocyte differentiation of hESCs, whereas the addition of insulin prevents apoptosis in ESC-derived neural precursor cells (NPCs). Noggin or SB431542 treatment improves the efficiency of neural induction, showing synergistic effects.

The rosette-forming cells (neuroectodermal structures capable of differentiation into neuronal cells) or neuroepithelial stem cell populations isolated from hESCs retain a broad differentiation potential. These cells maintain a neurodifferentiation potentiality even after long-term in vitro growth, retaining their ability to proliferate even after several passages. Further more, the growth of undifferentiated hESCs in chemically defined media without animal-derived components has been well established, and considerable effort is under way to induce targeted neural differentiation of hESCs using animal and serum free conditions. Several reports, including our own, have demonstrated the in vitro capacity of hESCs to generate NPCs, including regionally specific neuronal subtypes. The ability to direct NPCs to regional specific neurons is a distinct property of hESC-derived NPCs, which is a considerable advantage over adult NSCs, in which developmental programming and directed differentiation have not been shown to be effective. The transplantation of hESC-derived NPCs alone may not recover demyelinated axons by a remyelinating activity but the beneficial effect of the transplanted hESC-derived NPCs could be due to a neuroprotective mechanism that is provoked by an immunomodulatory or a suppression effect on T cells. Nevertheless, there are several concerns regarding the safety of transplantation of hESCs in humans, including the controversial formation of teratomas following hESC-derived neural cell engraftment. The possible reason for this conflict could be the usage of different cell lines, various differentiation protocols, and heterogeneous cell populations. Therefore, the application of prolonged differentiation of hESCs, fluorescent activated cell sorting or magnetic activated cell sorting, or inhibition of proliferation signaling pathways by genetic manipulation decreases the incidence of tumor formation and efficiently converts hESCs into neural cells.

Transplantation of hESC-derived neural progenitor cells with cellular matrix protein-based synthetic three-dimensional biodegradable scaffolds such as laminin, fibronectin, or collagen could be of advantage because these environments provide an adhesive support and may also release some growth factors such as NT-3 and PDGF. This strategy has been confirmed after transplantation of hESC-derived neural progenitors into a rat model of SCI using collagen scaffolds.

With the improvement of growth and differentiation conditions, the first FDA approval (http://www.fda.gov) for the preclinical usage of differentiated hESCs for the treatment of SCI makes hESCs a very attractive source for clinical applications. However, in August 2009, the FDA put a clinical hold on hESC clinical trials because further characterization of differentiated cells and more nonclinical trials/applications of hESC-derived neural cells into animal models have been requested. Nevertheless, compared with the other sources of cell therapy, hESCs are one of the most attractive cell sources for spinal cord therapy and this strategy has been validated recently, as hESC-derived motoneurons can survive and integrate into the spinal cord (13).
3.2 Neural Stem Cells

Recruitment of endogenous NSCs or transplantation of NSCs would be alternative strategies for the treatment of SCI. Neural stem cells are multipotent cells with the potential to differentiate into neurons, oligodendrocytes, and astrocytes and can be efficiently propagated in vitro. These cells can be obtained from the spinal cord and their characteristics are different from NSCs obtained from the forebrain. In the adult CNS, the tissue adjacent to ventricles and the ependymal cells directly lining the lateral ventricles are known to be rich sources of multipotent NSCs. The proliferation in the central ependymal canal of the spinal cord produces many new progenitor cells that are capable of differentiating toward cells with neural and neuronal characteristics. Following SCI, resident ependymal stem cells are activated and proliferate; after contusion, nearly 2 million new cells are produced at the injury site within a month, peaking at 3-7 days after injury. However, this activation is not sufficient itself to promote recovery. The ependymal stem cell progeny that proliferates in response to SCI migrates to the lesion site and contributes to the glial scar. The majority of these cells show immunoreactivity for Sox9 and vimentin, with astrocyte-like morphology (but they are GFAP negative) and a smaller cell population expresses Olig2, an immature oligodendrocyte phenotype. We have recently reported a functional motor recovery after transplantation of ependymal stem progenitor cells that were derived from adult rat spinal cord suffering a traumatic lesion (14).

The cells were propagated and differentiated in vitro to obtain oligodendrocytes precursor cells (OPCs), which engrafted after cell transplantation. In general, it is believed that following SCI, endogenous or transplanted NSCs differentiate mostly into oligodendrocytes and astrocytes, contributing to remyelination of axons and helping in the recovery. Because demyelination is a progressive problem following SCI, OPC transplantation of glial-restricted Ronaghi, Erceg, Moreno-Manzano et al. progenitor cells (GRP) and OPCs differentiated from NSCs could be a promising strategy to treat SCI. Indeed, astrocytes mediate neuroprotection, because the transplantation of lineage-restricted astrocyte precursors, also called GRPs, has been shown to be able to decrease focal motor neuron loss. Mature oligodendrocytes differentiated from OPCs are expected to remyelinate the axons in white matter, and astrocytes differentiated from GRPs secrete many neurotrophic factors that could support axonal regeneration and cell survival. However, the glial scar formation blocks the access of OPCs to demyelinated axons, and the expression of some inhibitory molecules by astrocytes (such as Jagged1) inhibits OPC differentiation and proliferation. These two processes represent very important barriers for endogenous OPC remyelination.

Grown under in vitro conditions, the NSCs maintain their capacity for self-renewal after several passages and are capable of secreting neurotrophic factors. One disadvantage of in vitro-derived NSCs for clinical therapies is their decreased potential of differentiation after several passages. Moreover, differentiation of NSCs into pure neural populations has not been reported yet. Endogenous neural progenitors are inefficient in differentiation toward motoneurons because the ratio of Ngn2 to Olig2, which determines motoneuron versus oligodendrocyte differentiation, is 10-fold lower in neural progenitors than in ESCs. This problem could be solved by overexpressing some genes involved in motoneuron development including HB9, NKX6.1, and NGN2 or by a coculture system with endothelial cells that enhance the motoneuron differentiation from NSCs where NSC-derived motoneuron progenitors promote functional recovery of a SCI model.

There are very few reports that describe the mechanism of integration of NSCs and how they promote functional recovery after SCI. Recently, Hooshmand et al. have shown that there is no change in the host microenvironment following analysis of NSC engrafted models of SCI. Lesion size, tissue sparing, glial scar, and expression of proteins such as fibronectin, NG2, versican, GFAP, and PECAM1 showed no change following cell implantation. However, the source of transplanted NSCs and methods of isolation and preparation

of cells prior to implantation seem to be very critical in cell survival and integration after implantation. For instance, monolayer cell cultures enhance the proliferation of multipotent NSCs, whereas neurosphere cultures result in more restricted NSC populations. Interestingly, monolayer and neurosphere cultures do not behave in the same manner following transplantation. Additionally, the type of animal model, immunosuppression used, time of transplantation, and type of injury affect the mechanism(s) of recovery. For instance, contusion injuries result in neural and neuronal apoptosis in rostral and caudal parts of lesion sites and are accompanied by secondary inflammatory responses, whereas transection injuries result in a small zone of tissue damage but have active necrotic mechanisms. A part of the scientific community believes that NSCs are more preferable than hESCs for clinical applications because they are considered safer for cell therapy, as NSCs have less potential to form tumors compared with ESCs. However, many critical challenges remain using NSCs for clinical applications, including the need for pure populations of differentiated cells, inefficient tracking systems, and moderate cell survival after transplantation. Additional obstacles of axonal regeneration and extension by cell replacement by either endogenous or exogenous NSCs remain: the formation of glial scars, the lack of neurotrophic factors, inhibitory myelin-associated molecules, and decreased levels of Camp. (3.3 Mesenchymal Stem cells)

Mesenchymal stem cells (MSCs), also known as multipotent mesenchymal stromal cells, are self-renewing cells that can be found in almost all postnatal organs and tissues. MSCs were first recognised by Alexander Friedenstein and associates in the bone marrow by their ability to form fibroblastic colonies and differentiate into osteoblasts, chondrocytes and adipocytes. MSCs can be easily harvested from a variety of tissues including the umbilical cord blood and adult adipose, bone marrow and skin tissues. They have also recently been isolated from olfactory tissue. It has been reported that when bone marrow-derived MSCs are cultured under specific conditions such as in the presence of EGF or BDNF, they develop neuronal morphologies and begin to express neural markers such as Nestin, GFAP and NeuN. Other investigators have used 2% dimethylsulfoxide (DMSO) to induce MSCs to express neuronal markers such as NeuN, NSE and tau. These findings have been interpreted as the ability of MSCs to differentiate into neurons, hence suggesting a potential for their use in cell replacement following CNS injury and disease. (16)

There are many studies involving autologous therapies and some allogenic therapies, based on the recovery of mobilized bone marrow cells, including mesenchymal stem cells (MSCs) and adipose derived stem cells that also include the stromal or adherent cell type that has an MSC phenotype. Human umbilical cord blood cells have been used in a large number of trials for paraplegia, ataxia, multiple sclerosis, amyotrophic lateral sclerosis, cerebrovascular disease, multiple system atrophy, motor neuron disease, among other indications, without severe immunological response. (17)

Placenta derived stem cells are being considered for similar uses and are in Phase III clinical trial for critical limb ischemia by Israel’s Pluristem Therapeutics. A significant proportion of clinical studies that are underway involve bone marrow and cord blood stem cells for blood and immune disorders and cancers. Several of those are now considered applicable for patient treatments beyond the need for regulated clinical trials. We have chosen to concentrate on the emerging therapeutics that broadly involves a wide range of cell types in clinical trials registered on the National Institutes of Health’s clinical trials web site. (18)

Clinical trials involving use of MSCs for the treatment of neurological disorders is also relatively common, despite little evidence for their conversion to neural cells in vivo. Autologous MSCs isolated from bone marrow and injected intrathecally into spinal cord cerebrospinal fluid, allowing access to the brain and spinal column, can be accomplished safely in patients with multiple sclerosis and amyotrophic lateral sclerosis (ALS). Karussis et al. provided some evidence of immunomodulatory effects of MSCs within 24 hours of

intrathecal injection but claims of ferumoxides labeled MSCs persisting after three to six months was less persuasive. (19)


Different sources and types of cells have been and/or are being tested in clinical trials for SCI, including embryonic stem cells (ESCs), neural progenitor cells (NPCs), bone marrow mesenchymal cells (BMSCs) and non-stem cells such as olfactory ensheathing cells and Schwann cells. Other cell types are being developed for the clinic, including other sources of mesenchymal cells (fetal blood, adipose tissue, umbilical cord adult and immortalized neural progenitors, skin-derived progenitors, induced pluripotent stem cells and endogenous spinal cord progenitors. The advantages and disadvantages of each cell source and type being considered or already in clinical trials for SCI have been extensively described and compared elsewhere, and reflect their potential in the clinic Table 1. There are currently more than a dozen cell therapy clinical trials for SCI listed on clinicaltrials.gov. Most are Phase I or I/II clinical safety and feasibility studies, indicating that cellular treatments for SCI developed in the laboratory are still in the very early stages of clinical translation.

Table 1. Neural stem cell (NSC) clinical trials underway

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<th>StemCells Inc., CA</th>
<th>HuCNS-SC® (fetal derived human NSCs)</th>
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<td>Phase I – completed</td>
<td>Batten’s Disease (NCL)</td>
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<td>Phase Ib</td>
<td>Discontinued for lack of enrollment</td>
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<td>Phase I</td>
<td>Pelizaeus-Merzbacher Disease (PMD)</td>
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<td>Phase I/II</td>
<td>Chronic Spinal Cord Injury</td>
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<td>Phase I – completed</td>
<td>Advanced Parkinson’s Disease</td>
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<td>Phase II – clinical hold</td>
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<td>ALS (Lou Gehrig’s Disease)</td>
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<th>ReNeuron, UK</th>
<th>ReN001 Immortalized huNSCs</th>
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<td>Phase I</td>
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<th>Targeted Delivery of Therapeutics</th>
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Studies utilizing NSCs from StemCells Inc. for chronic thoracic spinal cord injury are beginning clinical trial in Switzerland in 2011. The NSCs are injected into the spinal cord and migrate to the area of injury to form neurons and oligodendrocytes, critical for remyelinating damaged neuronal axons for recovery of nerve function. Fetal NSC preparations are in clinical trial by the company Neuralstem for the treatment of ALS (Lou Gehrig’s disease). The NSCs are injected into multiple (five to ten) grey matter sites of the lumbar region of the spinal cord. The first six non-ambulatory patients showed no adverse effects of NSC engraftment. The aim is to protect healthy neural cells and repair those that have ceased communication with the patient’s muscles and return ambulatory function.

Autologous NSCs obtained from patient brain biopsies have been used to treat Parkinson’s disease by the company NeuroGeneration Inc., which has trial sites in California (University of California Los Angeles and Cedars-Sinai Medical Center), Italy (University of Milan) and Estonia (University of Tallin). Biopsied brain tissue is cultured in vitro for several months and the expanded neural stem cells differentiated into neurons, astrocytes and oligodendrocytes. These include GABAnergic (60%) and dopaminergic (15%) neurons and the mixed neurons and glia are implanted at multiple sites in the post commissural putamen. Patients showed some motor recovery (not always sustained) and increased dopamine uptake in the transplanted putamen and clinical benefits that persist (20).

4.1 Phase 1 Clinical Trial Data

Data were presented on four patients with neurologically complete American Spinal Injury Association (ASIA) Impairment Scale grade A thoracic spinal cord injuries, who received GRNOPC1 at a dose of two million cells delivered by injection into the lesion site using a syringe positioning device designed by Geron. GRNOPC1 was administered between 7 and 14 days after injury. Low-dose tacrolimus was given for temporary immune-suppression from the time of injection for 46 days, at which point the dose was tapered and withdrawn completely at 60 days.

Endpoints of the trial are safety and evaluation of neurological function, using standardized testing at specified time points to monitor sensory and lower extremity motor function. The trial protocol also includes multiple MRI scans. Initial follow-up of patients is one year. One patient in the trial has completed the Day 365 follow-up visit. The most recent patient to be enrolled in the clinical trial has completed the Day 30 follow-up. After one year the patients enter a period of long-term follow-up that includes annual in-person visits for the first five years and subsequent yearly check-ups via telephone for an additional nine years.

Safety data to date from the trial has shown:

- No surgical complications during or after the procedures.
- No adverse events related to the injection procedures or to GRNOPC1.
- A few mild adverse events related to tacrolimus.
- No evidence of cavitation in the spinal cord at the injury sites on MRI.
- No unexpected neurological changes.
- No evidence of immune responses to GRNOPC1.

GRNOPC1 was delivered to four spinal cord injured patients at a dose of two million cells without complications from either the cells or the surgical procedure itself, and without any negative effects on the spinal cord or neurological function of the patients to date. The only side-effects observed were due to the immunosuppressive drug tacrolimus, which is administered for the first two months after injection of

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GRNOPC1. Furthermore, there is no evidence to date of immune rejection of GRNOPC1, an allogeneic cell therapy, including after withdrawal of immunosuppressive drug.

4.2 About GRNOPC1

GRNOPC1 contains hESC-derived oligodendrocyte progenitor cells that have demonstrated remyelinating, nerve growth stimulating and angiogenic properties leading to restoration of function in rodent models of acute spinal cord injury. Preclinical studies have shown that administration of GRNOPC1 significantly improved locomotor activity and kinematic scores of rodents with spinal cord injuries when injected seven days after the injury. Histological examination of the injured spinal cords treated with GRNOPC1 showed improved axon survival and extensive remyelination surrounding the rodent axons (21).

4.3 StemCells, Inc. Announces Positive Spinal Cord Injury Trial Results

The first phase of the clinical trial appears to be "well-tolerated." according to a press release May 22, 2012. The Phase I/II clinical trial of StemCells, Inc.’s HuCNS-SC® purified human adult neural stem cells was designed to assess both safety and preliminary efficacy. Twelve patients with thoracic (chest-level) neurological injuries at the T2-T11 level are planned for enrollment. The Company had dosed the first three patients all of whom have injuries classified as AIS A, in which there was no neurological function below the injury level. The second and third cohorts will be patients classified as AIS B and AIS C, those with less severe injury, in which there was some preservation of sensory or motor function. The injuries were classified according to the American Spinal Injury Association Impairment Scale (AIS). In addition to assessing safety, the trial will assess preliminary efficacy based on defined clinical endpoints, such as changes in sensation, motor and bowel/bladder function. All patients will receive HuCNS-SC cells through direct transplantation into the spinal cord and will be temporarily immunosuppressed. Patients will be evaluated regularly in the post-transplant period in order to monitor and assess the safety of the HuCNS-SC cells, the surgery and the immunosuppression, as well as to measure any recovery of neurological function below the injury site.

The Company intends to follow the effects of this therapy long-term, and a separate four-year observational study will be initiated at the conclusion of this trial. The trial was being conducted at Balgrist University Hospital, University of Zurich, a world leading medical center for spinal cord injury and rehabilitation, and was open for enrollment to patients in Europe, Canada and the United States.

Further Phase II studies are presently on hold while manufacturing methods are established. NSCs are also entering clinical trials for targeting the destruction of inoperable glioblastoma. NSCs home to tumors and scientists at the City of Hope, California are genetically modifying the NSCs so they produce a pro-drug activating enzyme (cytosine deaminase) that converts a non-toxic prodrug (5-Fluorocytosine, 5-FC) to a cytotoxic anticancer drug (5-Fluorouracil, 5-FU). The high local cytotoxicity will destroy glioblastomas. This is a very aggressive disease and patients are under treatment in the initial Phase I/II study (22).

Ongoing clinical studies and those carried out to date have enrolled small patient numbers and have used autologous marrow-derived cells rather than purified stromal cells. recently published dose-escalation trial examined autologous BMSCs in patients with chronic SCI. Although BMSCs were safe, they were not found to be beneficial in this cohort of patients. Having clearly established the safety and feasibility of the clinical use of BM-derived cells specifically for SCI in these trials, the continued testing of BMSCs in the context of SCI appears justified although the use of this intervention in complete thoracic cases may not be optimal. Based on the mechanism of action of BMSCs, which appear to provide trophic support to the penumbra zone of the...
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Acutely and subacutely injured cord, trials in patients with subacute severe, but incomplete spinal cord lesions are a consideration (23).

4.4 Websites

A tremendous number of information is now available on the Internet. We have listed on Table 2 some of the major websites that are reliable and informative for healthcare professional and patients. This is a non-exhaustive list that summarizes non profit-organization institutions involved in basis research and or support SCI patients in France and worldwide. Regarding basic research, almost all research directions are illustrated (epidemiology, pathophysiology, neuroanatomy, neurophysiology, neurobiology, neuroprotection, axonal regeneration, remyelination, synaptic formation, neurotrophic factors, embryonic and/or adult cell grafting, biologic material, stem cell, gene therapy, electric stimulations, etc.).

<table>
<thead>
<tr>
<th>Name</th>
<th>Internet address</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>International campaign for cures of spinal cord injury paralysis (ICCP)</td>
<td><a href="http://www.campaignforcure.org">http://www.campaignforcure.org</a></td>
<td>International</td>
</tr>
<tr>
<td>Association des paralyses de France (APF) and in particular the site for para- and tetraplegic</td>
<td><a href="http://www.apf.asso.fr">http://www.apf.asso.fr</a></td>
<td>France</td>
</tr>
<tr>
<td>Institut pour la recherche sur la moelle epiniere and l’Encephale (IRME)</td>
<td><a href="http://www.paratetra.apf.asso.fr">http://www.paratetra.apf.asso.fr</a></td>
<td>France</td>
</tr>
<tr>
<td>Association libre d’aide a la recherche sur la moelle epiniere (Alarme)</td>
<td><a href="http://www.ime.org/fr/index.phphttp://alarme.asso.fr">http://www.ime.org/fr/index.phphttp://alarme.asso.fr</a></td>
<td>France</td>
</tr>
<tr>
<td>Association « Demain Debout »</td>
<td><a href="http://www.demaindebout.com">http://www.demaindebout.com</a></td>
<td>France</td>
</tr>
<tr>
<td>Association Combattre la paralysie</td>
<td><a href="http://www.combattrelaparalysie.com">http://www.combattrelaparalysie.com</a></td>
<td>France</td>
</tr>
<tr>
<td>Fondation pour la recherche sur la moelle epiniere</td>
<td><a href="http://www.moelle-epiniere.com">http://www.moelle-epiniere.com</a></td>
<td>Canada</td>
</tr>
<tr>
<td>Rich Hansen Foundation</td>
<td><a href="http://www.rickhansen.com">http://www.rickhansen.com</a></td>
<td>Canada</td>
</tr>
<tr>
<td>Fondation internationale pour la recherche en paraplegie (IRP)</td>
<td><a href="http://www.irp.com">http://www.irp.com</a></td>
<td>Switzerland</td>
</tr>
<tr>
<td>Spinal Research</td>
<td><a href="http://www.spinal-research.org">http://www.spinal-research.org</a></td>
<td>England</td>
</tr>
<tr>
<td>Wings for life</td>
<td><a href="http://www.wingsforlife.com">http://www.wingsforlife.com</a></td>
<td>Austria</td>
</tr>
<tr>
<td>The Miami Project to cure paralysis</td>
<td><a href="http://www.themiamiproject.org">http://www.themiamiproject.org</a></td>
<td>USA</td>
</tr>
<tr>
<td>Paralyzed Veterans of America</td>
<td><a href="http://www.pva.org/site/PageServer">http://www.pva.org/site/PageServer</a></td>
<td>USA</td>
</tr>
<tr>
<td>Christopher and Dana Reeve Foundation</td>
<td><a href="http://www.christopherreeve.org">http://www.christopherreeve.org</a></td>
<td>USA</td>
</tr>
<tr>
<td>Spinal Cure Australia</td>
<td><a href="http://www.spinalcure.org.au">http://www.spinalcure.org.au</a></td>
<td>Australia</td>
</tr>
</tbody>
</table>

Some of the websites present past and actual clinical trials on human. Moreover, one can find contact information of a great number of research teams working on spinal cord repair after injury. Nevertheless, one must keep a critical eye on information presented because media coverage very often overestimates the results, due to the pressure for funds raising, the media coverage of one particular research team and sometimes for pure mercenary reasons (24).

One also must keep in mind that results obtained once in an animal study are not always reproducible and very often not directly transposable to humans. Whatever the pathology, almost one out of five patients (probably more for SCI patients) search for medical and health information on the Internet. In this context, the Haute Autorite de sante (HAS) in France will edit a list of health-labeled websites. Criteria that the websites must meet as well as documents for evaluation of reliability of these Internet sources is already available (25).

5. Ethical and Social Concerns

One of the issues that surround the use of ESCs is the time point at which an embryo is considered to be a person. According to the Roman Catholic Church and other religious institutions, an embryo “must be treated from conception as a living person”. This implies that a blastocyst cannot be used to harvest cells. Others consider an embryo to be a person only after the 20th week of gestation implying that ESCs can be harvested from blastocysts. Also, in that case, ESCs could be harvested from embryos that were generated but not selected for in vitro fertilization. These would otherwise be discarded (26).

Discussions on what constitutes “life” and when does “life” start are often intense because they are driven by moral concerns fueled by religious and political ideas. These issues need to be addressed with respect to all opponents. Rules regarding the harvest and use of stem cells can only be set after full agreement by all groups within a society.

Ethical issues that surround the use of adult stem cells mostly involve their possible misuse. For instance, oocytes can be derived from stem cells of male origin, which allows the production of a child from one or two male biological parents. The potential biological problems and psychological effects on the child are unknown. It would also be possible that the offspring develops defects because of acquisition of pairs of (recessive) genes. Therapeutic cloning and genetic manipulation are other issues that surround the use of stem cells. Cloning of cells, genetically matched for the host, could in theory be beneficial for organ transplantation because it may solve issues such as organ shortage and rejection. Genetic manipulation could convert ESCs into gametes, which would allow germ line gene therapy (GLGT) (27).

6. Future Prospective: Induced Pluripotent Stem Cells

A potential alternative to avoid immunological rejection after nonautologous transplantation of stem cells is the use of reprogrammed adult cell (iPSC) technology, which means derivation of patient specific and pluripotent cells derived from adult somatic cells. These cells have been generated from mouse and human somatic cells by overexpression of several defined factors (28,35).

Recently, generation of iPSCs from human NSCs with a single transcription factor, OCT4, or using direct delivery of recombinant proteins has been described. iPSCs have identical patterns in gene expression, chromatin methylation, and embryoid body and viable chimera formation as ESCs. They are capable of differentiation toward all cell types, including neurons, glia, NPCs, and motoneurons. (36, 40)

Furthermore, the generation of iPSCs using nonviral methods (41) or by chemicals and small molecules (42) including protein iPSCs (43) makes this cell replacement strategy very attractive. Nevertheless, these cell types share similar disadvantages as other cell sources: teratoma formation, aberrant reprogramming, and the

presence of transgenes in iPSC populations are the most concerning obstacles, which should be addressed before their clinical application (44).

7. Conclusion

Regeneration and replacement of neurons and glia that undergo cell death soon after injury are the main goals of all stem cell-based therapies for SCI. Although stem cell transplantation strategies have not yet been clinically approved, they are currently the most effective and efficient way to improve motor function in animal models of SCI. Successful development of stem cell-based therapies for SCI requires more intensive work to obtain a better understanding of stem cell differentiation pathways and stem cell survival upon transplantation. All stem cell replacement strategies should address these two important problems and this may be accomplished through improved differentiation protocols of hESCs/iPSCs, transplantation of OPCs and NPCs, and/or by activation of endogenous sources of neural progenitors. However, the ideal source of stem cells for efficient and safe cell replacement has remained a challenging issue that requires more investigation.

8. References


A Review on Stem Cell Treatment for the Spinal Cord Injury


