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Does the preclinical evidence for functional remyelination following engraftment into the injured spinal cord support progression to clinical trials?

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Abstract

This article reviews all historical literature in which rodent-derived myelinating cells have been engrafted into the contused adult rodent spinal cord. From 2,500 initial PubMed citations identified, human cells grafts, bone mesenchymal stem cells, olfactory ensheathing cells, non-myelinating cell grafts, and rodent grafts into hemisection or transection models were excluded, resulting in the 67 studies encompassed in this review. Forty five of those involved central nervous system (CNS)-derived cells, including neural stem progenitor cells (NSPCs), neural restricted precursor cells (NRPs) or oligodendrocyte precursor cells (OPCs), and 22 studies involved Schwann cells (SC). Of the NSPC/NPC/OPC grafts, there was no consistency with respect to the types of cells grafted and/or the additional growth factors or cells co-grafted. Enhanced functional recovery was reported in 31/45 studies, but only 20 of those had appropriate controls making conclusive interpretation of the remaining studies impossible. Of those 20, 19 were properly powered and utilized appropriate statistical analyses. Ten of those 19 studies reported the presence of graft-derived myelin, 3 reported evidence of endogenous remyelination or myelin sparing, and 2 reported both. For the SC grafts, 16/21 reported functional improvement, with 11 having appropriate cellular controls and 9/11 using proper statistical analyses. Of those 9, increased myelin was reported in 6 studies. The lack of consistency and replication among these preclinical studies are discussed with respect to the progression of myelinating cell transplantation therapies into the clinic.

Keywords

Remyelination; Spinal Cord Injury; Transplantation; Oligodendrocytes; Neural Precursor Cells; Neural Stem Progenitor Cells; Schwann Cells

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INTRODUCTION

There is universal agreement that oligodendrocyte loss after spinal cord injury (SCI) results in acute demyelination, both in experimental animals (Blight, 1983; Cao et al., 2005b; Crowe et al., 1997; Gledhill et al., 1973; Hesp et al., 2015; Powers et al., 2012; Totoiu and Keirstead, 2005) and humans (Guest et al., 2005; Kakulas, 1999; Norenberg et al., 2004). There is, however, conflicting evidence in experimental SCI as to whether chronic demyelination is a component of the long term pathophysiology. Early studies suggested chronic demyelination after SCI is relevant (Blight, 1983; Totoiu and Keirstead, 2005). More recent studies showed little evidence of demyelinated axons, but rather extensive ongoing remyelination (Hesp et al., 2015; Powers et al., 2012). Human pathological studies similarly do not show substantial evidence of chronic demyelination (Norenberg et al., 2004), although some was noted in a few cases 10 years post-SCI (Guest et al., 2005). Collectively, these studies argue that therapeutic strategies to enhance remyelination chronically are unlikely to be effective clinically. However, acceleration of acute remyelination may prove beneficial.

Many excellent reviews of the SCI cell transplantation literature exist (Papastefanaki and Matsas, 2015; Sabapathy et al., 2015; Sahni and Kessler, 2010; Tetzlaff et al., 2011; Volarevic et al., 2013). This review, therefore, confines its focus only to the literature exploring subacute transplantation of rodent stem cells or SCs into rodents with contusion or crush SCI. Because of the wide variability among cell types due to differences in cell source (age, gender and species), variation in *in vitro* growth conditions, and other confounding laboratory to laboratory differences, we follow Tetzlaff et al. (2011) in emphasizing that a cell type is merely an umbrella term for several subtypes of cells.

There are two experimental approaches to enhance remyelination, and these are not inherently mutually exclusive. Facilitation of endogenous remyelination and transplantation of exogenous cells with myelinating potential have both been undertaken by many laboratories, and some of those data have been recently reviewed (Granger et al., 2014; Plemel et al., 2014). While marginal success has been achieved, neither approach has reproducibly proven to be effective at enhancing functional recovery. Here, we take a different approach to examine this question. We have mined these data to determine if there are any common principles with respect to grafted cell type(s) and/or additional factors that facilitate recovery. What these data show are that in no two studies where functional recovery was reported were similar cells and/or additional reagents or drugs utilized. Moreover, only one of these studies has been replicated (Pearce et al., 2004). Preclinical data do not allow conclusive determination of which cell type and modification should optimally be utilized in clinical trials.

METHODS

From over 2,500 citations identified from an initial PubMed search performed in October of 2015 using the search terms “spinal cord injury and transplantation” in conjunction with “Schwann cells”, “stem cells”, or “myelinating cells”, we further narrowed relevant studies by eliminating those studies that utilized human cells or grafted rodent cells into non-

contusion/compression injuries. Those studies that met this *initial* inclusion criteria were formatted into Table 1 (for CNS-derived cells) or Table 3 (for Schwann cells) describing the experiment's injury model, cell type(s) transplanted, additional genetic or pharmacological manipulation, reported behavior, myelin sparing, and additional reported tissue sparing. To obtain a non-biased set of publications to analyze for their potential support of translational application, we restricted our analysis to those that: 1) claimed functional recovery, and 2) were properly controlled. The *latter* criteria required that a cellular graft control was included in the analysis, as non-neural tissue grafts can enhance host reparative responses (Toft et al., 2013). Culture medium was not allowed as an acceptable control. Those studies that passed those two criteria were then statistically evaluated for appropriate power and proper use of statistical analyses (Burke et al., 2013). Briefly, a study was determined to contain inadequate or inappropriate data analysis when a high number (> 25%) of Student's t-tests were applied without correction, resulting in a high probability of Type I errors occurring (i.e., falsely rejecting the Null hypothesis and stating a significant effect). Taking into account each study's experimental design and the specific outcome measures upon which conclusions were based, sample sizes of 4 or less in the experimental group were considered as likely too low to illustrate sufficient power to confidently replicate the results, although it should be emphasized that given the modest behavioral effects observed, even this sample size criteria is generous. This last group of manuscripts was then evaluated for evidence of increased exogenous or endogenous/spared white matter myelin.

RESULTS

CNS-derived myelinating cells

With respect to the CNS-derived myelinating umbrella of cell types, 45 papers that satisfied our initial inclusion criteria were identified (Table 1). Table 2 presents those studies that met the latter inclusion criteria and demonstrated at least some evidence for both improvements in remyelination/myelin sparing and functional recovery. Figure 1 illustrates the selectivity of our criteria. Functional recovery was reported in 31 studies. Twenty of those reports were properly controlled, and nineteen of those were sufficiently powered and utilized appropriate statistical analyses. That pool of 19 studies was used to analyze the relationship between exogenous and endogenous myelin and functional recovery. Evidence of exogenous or endogenous remyelination was reported in 10 or 3 studies, respectively, with 2 studies providing evidence of both (Fig. 1). Here and throughout this review, we use a less rigorous definition of the term 'remyelination' to indicate any increase in the remyelination of demyelinated axons, myelination of newly sprouted axons, or the sparing of existing, myelinated axons. Short of anterograde tracing spared axons and quantifying myelinating cells (Powers et al., 2012), traditional methods of quantifying myelinating cells cannot exclude these possibilities.

Of the studies that provided at least some kind of evidence for exogenous cell host integration post-transplantation, most studies utilized GFP or AP-expressing cells to track transplant survival, migration, axon ensheathment and/or remyelination. These studies, then, were primarily focused on the potential for cell transplantation to promote exogenous remyelination following injury, and generally less effort was made on investigating

transplantation effects on endogenous remyelination, sparing of endogenous oligodendrocytes, or myelination of newly sprouted axons. Immuno-electron microscopy (EM) remains the best method to verify remyelination of host axons with engrafted cells. Only 20% of these studies provided evidence of exogenous remyelination via immune-EM, although five studies used confocal microscopy to identify close apposition of oligodendrocyte marker-expressing, fluorescent protein expressing-engrafted cells to host neurofilament-expressing axons (Hawryluk et al., 2014; Hu et al., 2013; Karimi-Abdolrezaee et al., 2010; Plemel et al., 2011; Wilcox et al., 2014). The ability of grafted cells to simply differentiate into oligodendrocytes with the potential to remyelinate was investigated using immunohistochemical staining against markers for mature oligodendrocytes (i.e. APC/CCI, MBP, GAL-C, CNPase, O1) in most of the studies (88%), while the remaining studies reported the expression of only immature oligodendrocyte markers (i.e. PDGF α R, RIP, O4) on engrafted cells, leaving open the question of whether the cells could differentiate later into fully mature, remyelination-capable oligodendrocytes.

Of the 15 studies which provide evidence for the ability of transplanted cells to increase white matter following injury, five reports indicated that cell transplantation promoted either endogenous remyelination or sparing of existing oligodendrocytes. Hu et al. (2012) demonstrated that Olig2 overexpression increased MBP expression in transplanted NSPCs, but the increase in spared white matter observed post-injury primarily correlated with addition of MBP-activated T cells, not the NSPC graft, indicating facilitation of endogenous remyelination. Indeed, quantitation of remyelinated axons indicated that MBP-activated T cell treatment promoted endogenous Schwann cell remyelination, whereas NSPC transplantation promoted exogenous oligodendrocyte remyelination. Lu et al. (2010a) demonstrated a small degree of differentiation of transplanted OPCs into early oligodendrocytes, but the vast majority differentiated into astrocytes, and the observed increase in spared white matter and behavioral recovery was exclusively attributed to the immunosuppressive effects of cyclosporine A, not cell engraftment. Mothe et al. (2013) provided evidence that transplantation of adult NSPCs in a hydrogel blend covalently modified with recombinant rat platelet-derived growth factor-A (rPDGF-A) significantly increased the number of host oligodendrocytes rostral to the injury and transplant site. However, the extent to which this effect was due to the modified hydrogel matrix alone, and not the interaction of the transplanted cells and matrix, is difficult to determine without a hydrogel alone control.

Two studies provided some evidence of both exogenous and endogenous remyelination after transplantation. Yasuda et al. (2011) convincingly demonstrated significant exogenous remyelination via transplantation of embryonic WT NSPCs. However, the increased myelinated area observed after transplantation of shiverer-derived NSPC substantiated the capacity for transplanted NSPCs to either trophically facilitate endogenous remyelination and/or improve sparing of existing oligodendrocytes after injury. Hawryluk et al. (2014) demonstrated maturation of transplanted adult WT NPCs, ensheathment of host axons, and improved functional recovery after SCI. These results are all indicative of exogenous remyelination, yet transplanted NPCs failed to significantly increase white matter volume or oligodendrocyte count within the injury epicenter, supporting more of a role in providing exogenous trophic support. By contrast, transplanted shiverer NPCs successfully ensheathed

host axons and reduced functional recovery following injury relative to controls, indicating that endogenous remyelination was prevented. Differences in experimental design, including host species and cell type transplanted, make interpretation of the differences in behavioral recovery following shiverer-derived cell transplantation difficult.

Cell types and genetic/pharmacological treatments

Table 2 summarizes 15 studies that met all inclusion criteria. What is obvious is the variability of the cell type transplanted and the genetic/pharmacological manipulations utilized. Three studies transplanted OPCs into the rat 5–8 days after thoracic contusion. Bambakidis and Miller (2004) indicated that transplantation modestly improved functional recovery and spared white matter, but addition of recombinant sonic hedgehog (Shh) during cell transplantation failed to improve either outcome over OPC transplant alone. Knockdown of the TNF receptor TROY increased exogenous remyelination of grafted OPCs as well as hindlimb functional recovery post-injury (Sun et al., 2014). Cao et al. (2010) confirmed the ensheathment and remyelination of grafted OPC-derived oligodendrocytes by immuno-EM and were able to correlate improvements in hindlimb function with improvements in oligodendrocyte remyelination by CNTF-expressing OPCs.

One report studied the effects of NRP transplantation following SCI. Setoguchi et al. (2004) provided evidence that noggin overexpression promotes some differentiation of transplanted NRPs into GST π -expressing oligodendrocytes. This correlated with a modest improvement in hindlimb functional recovery 3 weeks post-injury.

Four studies investigated the effects of adult NSPC transplantation following rat contusion or compression, anywhere from 7 days to 7 weeks post-injury. Hawryluk et al. (2014) reported that the immunosuppressants minocycline and cyclosporine improved both total oligodendrocyte count and functional recovery following injury and transplantation. Hofstetter et al. (2005) found that overexpression of neurogenin 2 (NGN2) increased exogenous differentiation of NSPCs into OPCs. This improved white matter sparing and hindlimb functional recovery as assessed by the Basso, Beattie, Bresnahan locomotor rating scale (BBB) and gridwalking tests. Karimi-Abdolrezaee et al. (2010) established the capacity of chondroitinase ABC (ChABC) infusion (along with EGF, bFGF, and PDGF-AA) to increase the percentage of transplanted NSPC to differentiate into mature, APC+ oligodendrocytes. This correlated with modest improvements in BBB score and gridwalk performance. Mothe et al. (2013) provided some evidence for NSPC/PDGF-A-induced improvements in host oligodendrocyte number, and this correlated with modest improvements in gridwalking performance post-injury.

Embryo-derived NSPCs were used to investigate transplantation effects following injury in 3 separate studies. Hu et al. (2012) found that olig2 overexpression in transplanted NSPCs promoted exogenous oligodendrocyte remyelination. However, the observed improvements in hindlimb functional recovery primarily correlated with co-treatment of myelin basic protein-activated T cells. Lu et al. (2010) demonstrated that Cyclosporin A (CsA) treatment increased myelin sparing and functional recovery following injury, but this effect was likely due to improvements in endogenous oligodendrocyte sparing, as CsA did not significantly affect NSPC differentiation *in vivo*. The improvements in hindlimb motor function observed

by Yasuda et al. (2011) following NSPC transplantation correlated with the degree to which each cell type promoted endogenous remyelination alone (shiverer NSPC) or both endogenous and exogenous remyelination (WT NSPC).

Two studies explored the capacity of embryonic GRPs, transplanted 8–9 days post T9 contusion, to promote remyelination and functional recovery in the rat. Cao et al. (2005) demonstrated localization of transplanted, GRP-derived mature oligodendrocytes with newly formed central myelin sheaths via immuno-EM. Quantitation of APC/GFP colocalization allowed them to conclude that transfection of D15A, a bi-functional neurotrophic factor that has both BDNF and NT-3 activities, promoted oligodendrocyte differentiation, and this correlated positively with improved hindlimb motor function post-injury, though changes in overall white matter were not different from control. Hu et al. (2013) indicate that co-transplantation of GRPs with Schwann cells improves overall spared white matter post-injury over either individual cell type alone. Co-localization of GFP/MBP-positive cells closely wrapped around neurofilament-positive axons supported the hypothesis that at least some of this remyelination is due to exogenous GRP remyelination. Consistently, co-transplanted rats exhibited modest improvements in hindlimb motor function over single-cell transplants alone.

Least represented among the studies that passed our exclusion criteria were two cell types: embryonic stem cells and induced pluripotent stem cell-derived neural stem cells (iPS-NSCs). McDonald et al. (1999) provided minimal evidence of differentiation of transplanted ESCs into mature, APC-positive oligodendrocytes, among other cell types. This correlated with improved functional recovery in the hindlimb relative to transplantation of control adult mouse neocortical cells. Salewski et al. (2015a) found that transplantation of iPS-derived NSCs promoted exogenous remyelination. Immuno-EM confirmed ensheathment and wrapping of these cells around axons. This improvement in remyelination correlated with improvements in BBB score and gait performance over shiverer iPS-NSC controls.

These studies underscore the fact that there is no consistency in the preclinical literature to suggest that a single myelinating CNS cell type shows more therapeutic promise than any of the other cell types tested. In addition, while these data suggest that providing supplemental growth factors, transcription factors, immunosuppressants, and/or inflammatory mediators potentiates functional remyelination, there is a similar lack of consistency as to which is optimal. Lastly, none of these studies has been fully replicated, either by the original laboratory that reported them or independently. Collectively, these data do not engender confidence to move forward clinically with any of these approaches. We next consider what has been done clinically with myelinating stem/precursor cell grafts.

Clinical trials transplanting CNS cells

Private biotechnology firms have contributed to the development of stem cell-based therapies. The durability of most stem cell types makes laboratory manipulation, standardization, and scaling up of manufacturing relatively straightforward and thus commercially viable. Granger et al. (2014) recently highlighted many of the methodological issues preventing preclinical laboratory results from being duplicated in human patients and translated into the clinic, including: sample size, allocation concealment, randomization of

allocation to treatment arms, blinded assessment of outcome, pre-specified outcome measures, and publication regardless of outcome. Despite these and other concerns, including ethical considerations and potential cell teratogenicity and cyst formation (Sahni and Kessler, 2010), limited cell transplantation studies have proceeded to clinical trials.

Geron Corporation received FDA approval to run the first clinical phase I trial to test the safety of human embryonic stem cell-derived OPCs (GRNOPC1) in patients with complete thoracic paraplegia with the loss of motor and sensory function (Lebkowski, 2011). These cells have the potential to myelinate demyelinated axons as well as either induce intrinsic cell reparative responses or provide direct neuroprotection. While there were no serious adverse events reported in the immediate follow-up of patients receiving transplantation, Geron terminated its SCI stem cell research program due to financial reasons in November 2011 (Frantz, 2012). The biotechnology firm Asterias Biotherapeutics, who acquired Geron's stem cell assets in 2013, has since reported no indications for safety concerns in transplanted patients (Hayden, 2014).

Neuralstem (Rockville, MD) has completed the final surgery in a Phase I safety trial of its NSI-566 neural stem cells for chronic SCI at the University of California, San Diego School of Medicine. These are pluripotent, allogeneic, fetal human NSPCs which can differentiate into all 3 neural cell lineages. These cells were previously shown to improve recovery of motor function and motor evoked potentials in a rat model of spinal cord ischemia when transplanted 21 days after injury (Cizkova et al., 2007). However, there was not an appropriate cellular control in that study, only medium. In trials of amyotrophic lateral sclerosis (ALS) patients, these cells showed extensive neuronal differentiation (Chen et al., 2016) and could also function as a neuroprotective cell therapy. The six-month post-surgery observation period of the SCI trial ended in December of 2015. As of October 2015, no serious adverse events and anecdotal evidence of limited functional recovery were reported (<http://www.neuralstem.com/cell-therapy-for-sci>).

StemCells, Inc. (Newark, California) is currently overseeing a Phase II trial of patients with chronic cervical SCI transplanted with their neural stem cells product (HuCNS-SC). These cells are also pluripotent, allogeneic, fetal human NSCs. In a mouse model of moderate SCI at T10, these cells were shown to differentiate into neurons and oligodendrocytes, and cell transplantation was correlated with improvements in hindlimb functional recovery and increases in myelination (Cummings et al., 2005), although NSC grafts in that study did not significantly improve functional recovery beyond that seen with fibroblast grafts. In November 2015, these clinicians reported an overall pattern of improvement in both muscle strength and motor function in four of six patients six months post-transplant (<http://investor.stemcellsinc.com/phoenix.zhtml?c=86230&p=irol-newsArticle&ID=2113751>). Q Therapeutics (Salt Lake City, UT) has recently received FDA approval for the initiation of Phase 1/2a clinical trials of its GRP "Q-Cell" product, though for patients with ALS not SCI.

Schwann Cells

The ability of SCs, the myelinating glial cells of the peripheral nervous system (PNS), to create an environment in the peripheral nervous system (PNS) that is permissive for axon regeneration led to subsequent experiments testing the role of SCs in fostering regenerative

conditions after CNS injury (David and Aguayo, 1981; Oudega and Xu, 2006; Pearse and Barakat, 2006; Pearse and Bunge, 2006; Richardson et al., 1980). Accumulating evidence of the repair potential of SCs in the injured CNS has come from studies in rodent and primate models of SCI [for reviews see (Bunge, 2002; Lavdas et al., 2008; Zujovic et al., 2007)]. Up to half of axons are remyelinated by transplanted SCs in lesioned spinal cords in monkeys with associated functional improvement (Bachelin et al., 2005). The development of *in vitro* protocols to generate human SC cultures has made autologous transplantation in the clinic feasible (Levi et al., 1995; Rutkowski et al., 1995). After injury, myelination also appears to occur by endogenous SCs recruited to the lesion site from the periphery through the dorsal root (Beattie et al., 1997; Perez-Bouza et al., 1998). The recruitment signals to endogenous SCs are unknown but proposed sources include angiogenic (Gilmore et al., 1993; Raine et al., 1978), immune (Fukaya et al., 2003), axonal (Raine et al., 1978) or extracellular matrix (King et al., 2006). Reports that cell grafting enhances endogenous SC invasion into the injured spinal cord suggest chemotactic factors could stimulate SC migration (Fukaya et al., 2003; Rabinovich et al., 1999). However, transplanted SCs seem to only partially remyelinate the injured spinal cord, leading to the search for additional combinatorial strategies to enhance their repair potential (Fortun et al., 2009; Lu and Tuszynski, 2008).

Our literature search identified 21 studies that met our initial inclusion criteria (Table 3). For most of these studies, SCs were isolated from nerves of neonatal or adult rats. Additional studies used SC or skin-derived progenitors from neonatal mice. These SCs were transplanted alone or with additional treatments to test whether factors to improve cell survival or migration/axon growth would enhance remyelination and functional recovery. Of the 21 papers considered, 16 reported some functional change with SC transplant after SCI, as assessed by locomotor tests (Fig. 2). Eleven papers reporting behavioral changes after SC transplantation used appropriate controls, and 9 of those papers also used proper statistical analysis. Filtering the papers based on appropriate statistics and controls, 56% of the papers reporting functional changes remained. SCs can support recovery from SCI through remyelination or by enhancing host cellular repair. However, only 6 papers reporting functional recovery also evaluated myelin (Table 4). The hope in transplanting SCs into the spinal cord after SCI is that enhanced remyelination will lead to greater functional recovery. In these final 6 papers that met the latter inclusion criteria, SC grafting alone typically did not change the number of myelinated axons in the spinal cord after injury (Flora et al., 2013; Lavdas et al., 2010; Pearse et al., 2004; Pearse et al., 2007; Sparling et al., 2015; Takami et al., 2002), with the exception of two papers (Pearse et al., 2004; Takami et al., 2002). The source of SCs did not appear to have a consistent effect on overall remyelination.

Within these same studies, the effect of additives on remyelination and functional recovery after SC transplant was tested. Two studies co-transplanted olfactory ensheathing glia (OEG) with SCs (Pearse et al., 2007; Takami et al., 2002). OEGs promote axon regeneration in the injured spinal cord and improve functional recovery. It has been proposed that the beneficial effects of OEGs are mediated by the transplant expression of growth factors such as NT3 or BDNF, expression of adhesion molecules such as L1, or ensheathment and remyelination of spinal cord axons (Barnett et al., 2000; Bartolomei and Greer, 2000; Lipson et al., 2003; Ramón-Cueto et al., 2000; Woodhall et al., 2001). In both studies, cells were transplanted into the injury epicenter after rat T9 contusion. Inclusion of OEGs in the SC transplant did

not improve remyelination or axon ingrowth/survival. In fact, remyelination in OEG/SC co-transplant groups was reduced compared to SC alone transplant in both studies. This is likely due to two reasons: 1) OEGs showed reduced viability compared to SCs after transplant into the injury epicenter and 2) half as many SCs are transplanted in the OEG/SC co-transplant group than the SC alone group. There was a discrepancy in the effect of OEG/SC co-transplant on functional recovery. Pearse et al. (2007) reported that OEG/SC co-transplant was the only group with significantly higher BBB scores than fibroblast transplant controls. However, Takami et al. (2002) report that SC alone transplant was the only group with a significant increase in BBB score compared to control, although the OEG/SC co-transplant group trended toward scores similar to SC alone. A separate study by Pearse and colleagues saw no significant change in functional recovery with SC alone or OEG/SC co-transplant (Pearse et al., 2004). However, myelin was not assessed and the source of variability between these studies is unclear. Three additional studies, not from within the final 6, compared transplantation of OEGs and SCs (Barakat et al., 2005; Barbour et al., 2013; Li et al., 2012). Significant improvement in BBB scores was seen for both groups, but media was the only control used. Without additional cell controls, it is unclear whether the functional improvement is due to the transplanted cells or infiltration of endogenous cells, which is known to occur after cell transplantation (Biernaskie et al., 2007; Hill et al., 2006).

A few studies examined the influence of adhesion molecules on functional recovery when administered in combination with SC transplant. One paper from our final 6 papers tested the effect of the adhesion protein L1 on remyelination and functional recovery after SC transplant (Lavdas et al., 2010). L1 has been shown to promote axon regeneration and remyelination (Chen et al., 2005a; Chen et al., 2005b; Haney et al., 1999; Wood et al., 1990). A mixture of L1- and L1-Fc-expressing SCs was transplanted into mice after T8 compression injury. Transplant of the L1/L1-Fc SCs significantly increased remyelination and axon regeneration. Mice receiving the L1/L1-Fc SC transplant had significantly higher locomotor rating scores on the Basso Mouse Scale (BMS) as well. Two additional studies used elevated PSA-NCAM levels to improve functional recovery with the SC transplant after SCI in mouse and rat (Ghosh et al., 2012; Papastefanaki et al., 2007). PSA-NCAM has previously demonstrated benefit for axon growth across lesioned tissue (El Maarouf et al., 2006; Luo et al., 2011; Oumesmar et al., 1995). Both studies saw improved scores in BBB and BMS assessment as well as increased axon regeneration. SCs harboring increased PSA-NCAM levels also migrated to areas of the spinal cord beyond the lesion site, whereas unaltered SC transplants were largely restricted to the lesion area. The mouse study by Papastefanaki et al. (2012) also reported increased myelination, which was not assessed in the rat study (Ghosh et al., 2007). It is not clear whether the changes seen were significant due to errors in statistical analysis. However, neural adhesion molecules seem to be promising targets for improved axon growth across the lesioned spinal cord.

Improved tissue sparing after injury is another ongoing focus for functional recovery after SCI. Two strategies for tissue sparing are to increase cAMP levels, which decline after injury, and to provide neurotrophic support. Pearse et al. (2004) reported that combining administration of rolipram and the cAMP analog db-cAMP to elevate cAMP levels led to improved functional recovery after T8 contusion in the rat. Increases of myelination and axon regeneration were also seen. While db-cAMP treatment trended toward increased

myelin and axon regeneration, treatment with rolipram significantly improved remyelination and axon regeneration more consistently. Furthermore, the effects on myelin and axon regeneration and behavior were greatest when rolipram and db-cAMP were used together compared to either intervention alone.

Sharp et al. (2012) attempted to replicate the Pearse study, but did not observe any difference in functional recovery with any of the treatments. These authors proposed that the discrepancy is due to differences in experimental procedure. These included differences in whether experimental groups were matched according to BBB scores prior to treatment, whether the experimental groups were run at the same time, whether the severity of the contusion was consistent, and the quality of the SC transplant. Recommendations from Scott et al. (2008) for improving preclinical study design may aid translatability and reproducibility in SCI studies. They recommend that: 1) each cohort should have a sufficient number of matched/balanced animals (for SCI, this would mean matching according to pre-treatment BBB scores); 2) exclusion of animals that die for reasons outside the scope of the study, and exclusion of the matched animals from other groups; 3) when possible, investigators should be blinded to the experimental groups; 4) verifying injury severity; and 5) appropriate statistics. Controlling these variables is especially important when the effect size is not large or when the number of animals being tested is small or groups are too large to be run simultaneously. Sharp et al. (2012) raise a valid point worth discussion: If a treatment or intervention is not effective enough to be detected without highly controlled conditions, should it be advanced for clinical application?

Flora and colleagues examined the effect of D15A combined with rolipram and SC transplant on myelination and functional recovery (Flora et al., 2013). Combining rolipram and D15A administration with SC transplant led to improved functional recovery after T8 contusion in rat. Increases of myelination and axon regeneration across the lesion were also seen. In contrast to Pearse et al. (2004), treatment with rolipram alone also improved functional recovery, in addition to myelin sparing, remyelination, and axon regeneration. The reason for this discrepancy may be that this study administered rolipram for 4 weeks, rather than 2, and assessed BBB scores for 5 additional weeks compared to the Pearse study. D15A treatment alone increased serotonergic axon length, as did rolipram and the combined treatment. The effects on myelin and axon regeneration and behavior were greatest when a combination of rolipram and D15A was used (Flora et al., 2013). Golden et al., (2007) reported a similar positive effect of D15A on axon regeneration. Increased SC number and myelinated axons were also reported. No change in BBB score was observed.

Sparling et al. (2015) took a different approach to SC transplantation. Unlike other studies in this group that waited one week after injury, they transplanted SCs immediately after C4/5 crush injury of the left dorsal longitudinal fasciculus. The Sparling study also compared the effectiveness of nerve-derived SCs (N-SCs) to SCs generated from skin-derived precursors (SKP-SCs), both isolated from neonatal GFP+ Sprague Dawley rats. Although graft survival was limited, transplantation of SCs from either source led to functional recovery over transplantation of neonatal dermal fibroblasts (Fb) as measured using a catwalk. Rats receiving N-SCs or SKP-SCs transplants also displayed increased spared tissue and increased RST axon counts in the gray matter compared to Fb transplants. In a separate

study, the same group transplanted SKP-SCs one week after T9 contusion (Biernaskie et al., 2007). BBB assessment found functional improvement in rats receiving SKP-SCs compared to neurosphere or SKPs. They also reported increased axonal sparing and myelination with SKP-SCs transplant. However, the statistical analysis used prevented accurate interpretation of some results, as Student's t-tests are an inappropriate *post hoc* t-test procedure for multiple comparisons since they increase the likelihood of causing Type 1 errors when used in this manner.

In some cases, there appeared to be a mix of myelination by grafted SCs and endogenous SCs. Three papers from the final 6 showed clear evidence that remyelination by the transplanted cells was occurring (Lavdas et al., 2010; Pearse et al., 2007; Sparling et al., 2015). This is an important distinction as endogenous SCs are also recruited into the spinal cord after injury (Beattie et al., 1997; Perez-Bouza et al., 1998). In all 3 papers, GFP was used to label the transplanted cells, either through isolation from GFP+ transgenic animals or viral transduction prior to transplantation. However, the Lavdas study was the only one to quantify the contribution of transplanted and endogenous SCs to remyelination. They found that while L1/L1-Fc expression increased myelination 12-fold over alkaline phosphatase (AP)-expressing controls, the majority of myelin was GFP-, indicating endogenous SC myelination. In fact, L1/L1-Fc SC transplant increased myelination by endogenous SCs 8-fold compared to AP-expressing SC. It appeared that the greatest benefit provided by grafts of L1/L1-Fc SCs was the recruitment of endogenous SCs. Sparing of oligodendrocyte-derived myelin was not affected with SC grafts alone.

Although studies of SC transplant were more consistent in the cell source used than CNS cell transplant studies, no true replication of any studies occurred to validate promising results. Replication is a key step in ensuring the design of effective clinical trials based on preclinical data. The vast majority of studies meeting our inclusion criteria transplanted adult SCs into T8-T10 contusion, while the remaining studies transplanted neonatal SCs. However, little other overlap exists among the included studies. In the majority of studies, transplant of SCs alone did not improve myelination or functional recovery after SCI, regardless of the source of SC. The treatments tested in conjunction with SC transplant can be broadly categorized into elevating cAMP levels, administering neurotrophic factors, and modifying cell adhesion. In the core of 6 well-controlled, statistically-sound papers, all three groups showed promise in improving tissue sparing, myelination, and functional recovery after SCI. Unfortunately, none of these studies have been completely replicated in all experimental aspects. As was pointed out in a response to the one attempted replication study by Sharp et al., many experimental details were changed that could underlie the failure to replicate the key findings of the original study (Bunge et al., 2012). The Sharp study used a different anesthetic and exposed more of the spinal cord during the laminectomy, potentially affecting both the functional outcome after injury and injury severity. The levels of cAMP were not validated in the Sharp study, so it is not clear whether their treatment was effective. Finally, the exclusion criteria used by the two studies were different, which would raise the probability of observing different results. Clearly, replication of promising preclinical studies is needed. However, more extensive, detailed, and ongoing communication between the original investigators and the replicating group is required to ensure true experimental replication. As of now, it remains uncertain whether any of the

additional treatments tested are reliable and potent enough to warrant translation to the clinic.

Clinical trials transplanting SC

In the last decade, clinical investigation of the potential of SC transplants for repair of SCI has moved from pre-clinical studies into Phase 1 clinical studies. Currently SC transplant studies are in the safety assessment and dose-escalation phase of development. Additionally, consistent preparation of autologous human SC cultures is achievable. This will provide an advantage over use of allogenic SC transplants as it should not require immune suppression and should avoid possible transmission of allograft donor abnormalities. Although no studies under FDA oversight have been completed, two international studies have suggested that no serious adverse effects are associated with the SC transplants. Two studies conducted in Iran have reported on autologous SC transplant in SCI patients (Hooshang Saberi et al., 2011; Saberi et al., 2008). In the first study, patients with chronic mid-thoracic SCI were transplanted with autologous SCs and followed for one year. SC cultures were purified from the sural nerve of each patient, initially grown under “starvation” conditions without growth factors, followed by exposure to autologous serum to avoid use of artificial mitogens. In the second study, 33 patients were enrolled with an average duration after injury of 4.1 years. Twenty-four patients had thoracic and 9 patients had cervical injuries. Sixteen patients were ASIA Grade A and seventeen were categorized as ASIA Grade B. SC cultures were again harvested from autologous sural nerve and cultured under the same conditions as the first study. Patients were followed for 2 years after transplantation. While no significant clinical improvements were seen in either study, no major concerns such as neurological worsening, deep infection, deformity, or tumorigenesis were observed. One study in China involving 6 patients with chronic SCI followed the patients for 5 years after transplantation (Zhou et al., 2012). SC cultures were derived from sural nerve that was pre-degenerated by cutting it within the patient one week prior to removal for cell culture. No severe adverse effect was observed, indicating the potential safety and feasibility of autologous SC transplant into chronic SCI patients.

Currently, 2 studies are listed on [Clinicaltrials.gov](https://clinicaltrials.gov) involving SC transplant into SCI patients, NCT01739023 and NCT02354625, both involving The Miami Project to Cure Paralysis. The first Phase 1 study, which is no longer enrolling patients, will evaluate the safety of autologous SC transplant into subacute SCI patients. Safety and efficacy assessments including International Standards of Neurological Classification for SCI (ISNCSCI), MRI, and neuropathic pain will be performed 1 week, 2 weeks, and 2, 6, and 12 months post-transplantation. Although no results have been reported, the study is estimated to be completed in the next 6 months. In the second Phase 1 study, safety of autologous SC transplant into chronic SCI patients will be assessed by measuring ISNCSCI, MRI, neuropathic pain, ISCI Basic Pain dataset v2, Pain Diagram, and Quantitative Sensory Testing at 1 week, 2 weeks, and 2, and 6 months post-transplantation. The estimated completion date of the study, which is currently enrolling patients, is January 2018.

Conclusions

Improving functional recovery following SCI remains an important challenge. While much has been learned regarding the underlying pathology of demyelination and remyelination following contusion, the potential for myelinating cell transplantation, with or without pharmacological/genetic manipulation, to conclusively and significantly remyelinate axons and improve functional recovery has yet to be realized. Due to incomplete evaluation and lack of replication, full consensus regarding the potential for cell therapy in the field of SCI has yet to be achieved. Significant heterogeneity exists around the nature of the cell types being transplanted and what is optimal. Additionally, the extrapolation of rodent data to complicated human injury is problematic. In the context of criteria discussed by a large number of SCI clinicians and researchers (Kwon et al., 2010), where independent replication was overwhelmingly deemed essential, no myelinating cell transplant type meets all the criteria for translation to the clinic. Combinatorial strategies that target both the preservation of endogenous myelin and the facilitation of early remyelination may yield better outcomes.

In the context of remyelination, none of these trials are fully aligned with the preclinical data, for as indicated in Tables 2 and 4, optimal remyelination requires additional trophic and/or immunomodulatory therapy. A further complicating issue is the balance between cellular and molecular modification of myelinating cell grafts needed to optimize functional remyelination (e.g. efficacy) and the regulatory concerns of the FDA to ensure safety. Currently, only unmodified cell grafts have been approved, and this is at odds with the preclinical biology. Given the widely varied preclinical data, this conservative approach by the FDA is clearly warranted.

The Neural Stem and Stem Cells Inc. cells are pluripotent and have the capacity to differentiate with oligodendrocytic, astrocytic, or neuronal phenotypes. The Geron/Asterias and Q Therapeutics cells are presumably restricted to oligodendrocyte and oligodendrocyte/astrocyte lineage fates, respectively. Preclinical data have been unable to discriminate the mechanism(s) by which these cells are predicted to enhance functional recovery. The subacute clinical trials with SCs may better reflect preclinical data as their biology is well characterized and the mechanism underlying potential recovery are likely similar, though there currently is no preclinical data supporting SC transplantation in chronic (over 1 year) injuries. Importantly, no preclinical studies have unequivocally demonstrated that cell-specific differentiation is responsible for any behavioral improvement reported. If these cells ultimately prove to be clinically effective, cell-specific mechanisms could be involved. However, it is entirely possible that these grafted cells could act to enhance host reparative responses. While there is obvious discord between the preclinical data and how these respective clinical trials have been structured, they are nonetheless ongoing and will continue to be so. That barn door has not only been left open, it has been obliterated. It is now paramount to definitively answer the question as to whether current clinical trials will be therapeutically beneficial. Even conclusive negative data would be important as we would know how not to proceed. It is imperative that all results once completed, both positive and negative, be released for scrutiny by the SCI scientific community.

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HIGHLIGHTS

- Preclinical studies generally suffer from a lack of consistency and replication
- Preclinical data is inconclusive regarding optimal cell type/manipulation for clinical trials
- Use of improper controls and inappropriate statistics prevents interpretation of many studies
- Future clinical trials should release negative results to the SCI research field

Figure 1. Venn diagram illustrating the sequential exclusion of CNS-derived myelinating cell transplantation studies using the indicated criteria.

Figure 2. Venn diagram illustrating the sequential exclusion of Schwann cell transplantation studies using the indicated criteria.

Table 1

All Studies Using CNS-Derived Myelinating Cells That Met the Initial Inclusion Criteria.

Injury	Cells	Additions	Reported Behaviour	Myelin Sparing	Other Sparing	Reference
Rat, T9-10 NYU 10g 12.5 mm	Rat P0 spinal cord OPCs	+/- Shh	BBB: +	IHC: + WMS: + Ephys: +		Bambakidis et al., 2004
Mice, T8 IH 50 kdyne	Mouse adult NPCs or mouse fibroblasts		BBB: +		Increased titionine-stained spared tissue	Bottai et al., 2008
Rat, T8 NYU 12.5 g /cm	Rat adult SVZ NPCs or rat E14 NSPCs			IHC: - for GFP/mAb328 colocalization		Cao et al., 2001
Rat, T8 NYU 12.5g /cm	Rat E14 spinal cord NRPs with GFP or BrdU labeling			IHC: - for GFP/MBP colocalization		Cao et al., 2002
Rat, T9 IH 150 kdyn	Rat E14 spinal cord GFP- GRPs	D15A	BBB: +	iEM: + Ephys: + WMS: - APC ⁺ OLs: +		Cao et al., 2005
Rat, T9 IH 150 kdyn	Rat Adult OPCs, Fibroblasts	Retroviral GFP or CNTF	BBB: +	IHC: + iEM: + Ephys: + OL- remyelinated axons: +		Cao et al., 2010
Mouse, T7-10 electro-magnetic compression	Mouse ESC-derived NPCs	+/- L1 over-expression		IHC: -		Chen et al., 2005
Rat, T9 IH 150 kdyne	Rat spinal cord E14 NRPs or OPCs or NSPCs	Retroviral infection with GFP or GFP-noggin		IHC: - for GFP/RIP colocalization		Enzmann et al., 2005
Rat, T7 25g static compression	Rat E15 GFP- NSPCs			IHC: +		Fujiwara et al., 2004
Rat, T7 clip compression	Mouse adult YFP NPC, shiverer NPC	EGF/FGF, and PDGF +/- minocycline, cyclosporine	BBB: +	IHC: + iEM: +	Increased gray matter, + ED ⁺ cells	Hawryluk et al., 2014

Injury	Cells	Additions	Reported Behaviour	Myelin Sparing	Other Sparing	Reference
Rat, T9-10 NYU/MASCIS 10g 12.5mm	Rat E13.5 spinal cord PLAP-GRPs	+/- cyclosporine A, MP		IHC: +		Hill et al., 2004
Rat, T8 contusion, weight drop	Rat adult neural stem cells	Neurogenin-2	BBB: + Gridwalking: +	IHC: + WMS: + MRI: +	Decreased allodynia	Hofstetter et al., 2005
Rat, T9 NYU 25 g/cm	Rat E14.5 spinal cord GFP-NSPCs	+/- Olig2 over-expression and +/- MBP-activated T cells	BBB: +	IHC: + WMS: + iEM: +	+ IL10, IL13, neurotrophin, increased M2 macrophage, decreased lesion volume	Hu et al., 2012
Rat, T9 NYU 25 g/cm	Rat E14 GFP-GRPs +/- SCs		BBB: + Gridwalking: +	IHC: + WMS: +	Increased 5HT fibers	Hu et al., 2013
Rat, T10 NYU/MASCIS 10g 25 mm	Rat E14 GFP-NSPCs	Inactive or active ChABC			ChABC increased integrated NSPCs	Ikegami et al., 2005
Rat, C6 bilateral clip compression	Mouse adult YFP-NPCs	+/- QL6 (self assembling peptides)	BBB: - Grip strength: + Inclined plane: - Catwalk: +		Motor neurons spared, Less glial scar	Iwasaki et al., 2014
Rat, T7 clip compression	Mouse adult YFP-NPCs	Growth factors, minocycline, cyclosporine A or none	BBB: + Grid walk: +	IHC: + iEM: +		Karimi-Abdolrezaee et al., 2006
Rat, T7 clip compression	Mouse adult YFP-NPCs	ChABC, EGF, FGF, PDGF	BBB: + Footfall: +	IHC: +	Increased 5HT fibers	Karimi-Abdolrezaee et al., 2010
Rat, T8 IH 200 kdyn	Rat E14.5 NSPCs	Control or S1P ₁ shRNAi				Kimura et al., 2007
Rat, T9 NYU 10 g 25 mm	Rat P2-O2A cells (OPCs)		BBB: +	Ephys: + IHC: +		Lee et al., 2005
Rat, T9 150 g, 5 min compression	Rat E16 spinal cord NSCs, Rat E16 spinal cord SCs		BBB: -			Li et al., 2007
Rat, T9 NYU 10g 12.5 mm	Rat spinal cord E14.5 GFP-NPC derived OPCs	+/- cyclosporine A	BBB: +	WMS: + OLs: -	Increased spared motoneurons, + effect on inflammation	Lu et al., 2010

Injury	Cells	Additions	Reported Behaviour	Myelin Sparing	Other Sparing	Reference
Rat, T9 NYU 10g 12.5 mm	Rat E14.5 GFP OPCs					Lu et al., 2010
Rat, T9-10 NYU 10g 25mm	Mouse D3 ESCs, β -gal expressing ESCs, or neocortical cell control		BBB: +	IHC: +		McDonald et al., 1999
Rat, T8-9 MASCIS 25 mm	Rat(AP)-E13.5 NRP GRP (25-75%)		BBB: + Micturition: + Thermal withdrawal latency: +	IHC: +	Modification in lumbosacral circuitry	Mitsui et al., 2005
Rat, T2 clip compression	Rat adult GFP NSPC	HAMC-rPDGF matrix or media	BBB: - Ladder walk: +	IHC: +		Mothe et al., 2013
Rat, T8-9 NYU/MASCIS 25 mm	Rat spinal cord eNRPs and eGRPs		BBB: +		Increased spared tissue	Neuhuber et al., 2008
Rat, T10-11 NYU 25g-cm	Rat PLAP-E13.5-spinal cord GRPs	cAMP	BBB: - Micturition: +	WMS: -		Nout et al., 2011
Rat, C4-5 clip compression (35 g for 15 min)	Rat E14.5 WT or Ta1-YFP NSPCs		Skilled reaching task: +	IHC: + iEM: -		Ogawa et al., 2002
Mouse, T10 IH 60 kdyne	Mouse E14 NSPC	Lentiviral luciferase and GFP	BBB: +	IHC: +		Okada et al., 2005
Rat, T8-9 clip compression (35g)	Rat GFP-NPCs		BBB: -	IHC: +		Parr et al., 2007
Rat, T8-9 Clip compression	Rat spinal cord GFP-NSPC +/- adult Rat BMSC		BBB: + Footfalls: +	iEM: -		Parr et al., 2008
Rat, T9 OSU 1.5 mm	Mouse GFP-E14 PRP (PDGF-responsive neural precursors)	FGF, PDGF	BBB: - Catwalk: - Thermal allodynia: -	IHC: + EM: +		Plenel et al., 2011

Injury	Cells	Additions	Reported Behaviour	Myelin Sparing	Other Sparing	Reference
Mouse, T6 clip compression	Mouse adult GFP-ESC- clonally derived NSC or media		BMS: + Catwalk: +	IHC: + WMS: + iEM: +		Salewski et al., 2015
Mouse, T6 clip compression	Mouse WT and Shiverer-IPSC NSCs		BMS: +	WMS: + iEM: + Ephys: +		Salewski et al., 2015
Mouse, T9 30g static compression	Mouse E14 NPSCs	Noggin or control-transfected	BBB: +	IHC: +		Setoguchi et al., 2004
Rat, T9 OSU 10 g weight drop	Mouse P2 GFP OPCs	Lentiviral TROY or TROY-RNAi or BLV (blank virus)	BBB: + Inclined plane: +	IHC: + EM: + Ephys: +		Sun et al., 2014
Mouse, T9-10 NYU 10g-25mm	Mouse ESC derived NG2+ progenitors					Vadivelu et al., 2015
Rat, C6 bilateral clip compression	Mouse adult YFP NPCs	EGFP, FGF, PDGF	Grip strength: +	WMS: + Ephys: +	Reduced astrogliosis and glial scar	Wilcox et al., 2014
Rat, T8-9 glass rod (25g, 90s) contusion	Rat E16 GFP- NSPCs					Wu et al., 2002
Rat, T10 NYU 2.5 gm/cm	Rat P2 cortical OPCs		BBB: +	EM: + Ephys: +		Wu et al., 2012
Rat, T9 NYU 10 g 12.5 mm	Rat GFP-E15 NSCs	+/- NgR antibody	BBB: + Grid Walk: +	IHC: +	Increased spared VH motor neurons	Xu et al., 2011
Mouse, T10 IH 60 kdyn	Mouse E14 WT shiverer striatal NSPCs + GFP		BMS: + Rotarod: +	IHC: + iEM: + Ephys: +		Yasuda et al., 2011
Mouse, T9 IH 50, 70, 90 kdyn	Mouse E13 GFP-NSPC		BMS: +			Yokota et al., 2015
Mouse, T12 IH 200 kdyn	Mouse adult GFP-NPCs	pMOG (35-55), pMOG (altered)	BMS: +		improved neurogenesis	Ziv et al., 2006

Columns with a "+," indicate positive evidence within the performed experiment reported. Columns with a "-," indicate no evidence found within the performed experiment reported. Blank columns indicate no respective experiment reported. Highlighted rows indicate studies that passed the latter exclusion criteria.

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Abbreviations: 5HT=5-hydroxytryptamine, pgal=beta-galactosidase, AP=Alkaline Phosphatase, APC=Adenomatous polyposis coli oligodendrocyte marker, BBB=Basso, Beattie, Bresnahan locomotor rating scale, BMS=Basso Mouse Scale, BrdU=Bromodeoxyuridine, cAMP=cyclic Adenosine Monophosphate, C=Cervical level of spinal cord injury, ChABC=Chondroitinase ABC, CNTF=Ciliary Neurotrophic Factor, EGF=Epidermal Growth Factor, Ephys=Electrophysiology, ESC=Embryonic Stem Cells, FGF=Fibroblast Growth Factor, GFP=Green Fluorescence Protein, GRP=Glial Restricted Precursor Cells, HAMC-rPDGF=Hyaluronan And Methyl Cellulose covalently modified with recombinant rat Platelet-Derived Growth Factor-A, iEM=immuno-Electron Microscopy, IH=Infinite Horizons spinal cord impactor, IHC=Immunohistochemistry, iPS-NSC=induced Pluripotent Stem derived-Neural Stem Cells, MBP=Myelin Basic Protein, MP=Methylprednisolone, MRI=Magnetic Resonance Imaging, NPC=Neural Precursor Cells, NRP=Neural Restricted Precursor cells, NSPC=Neural Stem Progenitor Cells, NYU=New York University spinal cord impactor, OL=oligodendrocytes, OPC=Oligodendrocyte Precursor Cells, OSU=Ohio State University spinal cord impactor, PDGF=Platelet-Derived Growth Factor, PLAP=Placental Alkaline Phosphatase, SC=Schwann Cells, Shh=Sonic hedgehog, SVZ=Sub-Ventricular Zone, T=thoracic level of spinal cord injury, WMS=White Matter Sparing, YFP=Yellow Fluorescent Protein.

Table 2

All Studies Using CNS-Derived Myelinating Cells That Met the Latter Inclusion Criteria.

Cell type:	OPCs	NRPs	adult NSPCs	embryonic NSPCs	embryonic GRPs	ESCs	IPS-NSCs
Study:	Bambakidis et al., 2004 Sun et al., 2014 Cao et al., 2010	Setoguchi et al., 2004	Hawryluk et al., 2014 Hofstetter et al., 2005 Karimi-Abdolrezaee et al., 2010 Mothe et al., 2013	Hu et al., 2012 Lu et al., 2010 Yasuda et al., 2011	Cao et al., 2005 Hu et al., 2013	McDonald et al., 1999	Salewski et al., 2015
Genetic / Drug Treatment:	Shh TROY inhibition CNTF	Noggin	minocycline+ cyclosporine, Neurogenin-2, ChABC, HAMC- rPDGF	Olig2/MBP activated T cells, cyclosporin A, No treatment	D15A, Schwann cell co- transplant	No treatment	No treatment

Studies that met inclusion criteria were grouped into 7 broad categories of cell types used for transplantation. Relevant genetic or pharmacologic additions are identified within each study.

Abbreviations: ChABC=Chondroitinase ABC, CNTF=Ciliary Neurotrophic Factor, ESC=Embryonic Stem Cells, GRP=Glial Restricted Precursor Cells, HAMC-rPDGF=Hyaluronan And Methyl Cellulose covalently modified with recombinant rat Platelet-Derived Growth Factor-A, IPS-NSC=induced Pluripotent Stem derived-Neural Stem Cells, NPC=Neural Precursor Cells, NRP=Neural Restricted Precursor cells, NSPC=Neural Stem Progenitor Cells, OPC=Oligodendrocyte Precursor Cells, Shh=Sonic hedgehog.

Table 3

All Studies Using Schwann Cells That Met the Initial Inclusion Criteria.

Injury	Cells	Additions	Reported Behavior	Myelin Sparing	Other Sparing	Reference
Rat T10 contusion	Neonatal rat SCs	Anti-GalC antibodies	BBB: -	IHC: +	Increased BDA+ axon regeneration	Azanchi et al, 2004
Rat T8 contusion	Adult rat OEGs or SCs	none	BBB: +			Barakat et al, 2005
Rat T10 contusion	Adult rat OEGs or SCs	none	BBB: + Ladder: -		Increased sparing/ growth of axons from the Reticular Formation and Raphe and Vestibular Nuclei	Barbour et al, 2013
Rat T9 1.5 mm displacement	SVZ neurosphere and neonatal SKPs or SKP-SCs	none	BBB: + Sensory threshold: -	IHC: +	Increased 5HT and TH fibers	Biernaskie et al, 2007
Rat T10 clip compression	Adult rat SCs	none	BBB: -	IHC: +		Chi et al, 2010
Rat T9 contusion	Neonatal rat SCs	Fetal spinal cord suspension, NGF or BDNF	BBB: +		Increased CST axon growth	Feng et al, 2005
Rat clip crush	Neonatal rat SCs	none	BBB: +			Firouzi et al, 2006
Rat T8 weight drop	Adult rat SCs	LV-D15A; rolipram	BBB: +	TEM: +	Increased 5HT fibers, Increased sparing/ growth of axons from the Reticular Formation and Raphe and Vestibular Nuclei	Flora et al, 2013
Rat T8 contusion	Adult rat SCs	PST	BBB: +		Increased 5HT and CST axon growth	Ghosh et al, 2012
Rat T8 weight drop	Adult rat SCs	AdV-D15A or LV-D15A	BBB: -	TEM: +	Increased 5HT, DbH, and CGRP fibers	Golden et al, 2007
Mouse T8 compression	GFP+ P5 mouse SCs	AP or L1/L1-Fc	BMS: +	IHC: +	Increased 5HT fibers	Lavdas et al, 2010
Rat T10 contusion	Adult rat OEGs or SCs	none	BBB: -	Ephys: -	Increased NF stain with OEGs	Li et al, 2012
Mouse T8 crush	GFP+ P5 mouse SCs	AP or STX	BMS: +	IHC: +	Increased 5HT fibers	Papastefanaki et al, 2007

Injury	Cells	Additions	Reported Behavior	Myelin Sparing	Other Sparing	Reference
Rat T8 contusion	Adult rat SCs	Rolipram or cAMP	BBB: +	IHC: + TEM: +		Pearse et al, 2004a
Rat T9 contusion	Adult rat OEGs or SCs	MP/IL-10	BBB: + Foot rotation: + Foot fall errors: +		Increased 5HT fibers, Increased graft volume, Less cavitation	Pearse et al, 2004b
Rat T9 contusion	Adult rat OEGs and SCs	none	BBB: + Foot rotation: +	IHC: +	Increased 5HT, CST, and CGRP fibers	Pearse et al, 2007
Rat T8 contusion	Adult rat SCs	Rolipram or cAMP	BBB: - Kinematics: -	IHC: + WMS: +	No change in lesion volume	Sharp et al, 2012
Rat C4/5 crush	Neonatal GFP+ rat fibroblasts, neonatal SCs, or SKP-SCs	none	Cylinder: + Catwalk: +	IHC: + Ephys: +	Increased spared axons	Sparling et al, 2015
Rat T9 contusion	Adult rat OEGs and SCs	none	BBB: +	TEM: +	Increased propriospinal, supraspinal, and corticospinal axons	Takami et al, 2002
Rat C6 contusion	GFP+ adult rat SCs	bpV	Forelimb: + Sensorimotor: +		Decreased lesion, Increased penumbra neurons and vascularization	Walker et al, 2015
Rat T9 contusion	Adult rat GFP- SCs	none	BBB: - Foot rotation: +	IHC: +	Increased blood vessels in graft	Wang and Xu, 2014

Columns with a "+," indicate positive evidence within the performed experiment reported. Columns with a "-," indicate no evidence found within the performed experiment reported. Blank columns indicate no respective experiment reported. Highlighted rows indicate studies that passed the latter exclusion criteria.

Abbreviations: 5HT=5-hydroxytryptamine, AP=Alkaline Phosphatase, BBB=Basso, Beattie, Bresnahan locomotor rating scale, BDA=Biotinylated Dextran Amines, BDNF=Brain-Derived Neurotrophic Factor, bpV=bisphosphonate, CGRP=Calcitonin Gene-Related Peptide, CST=Corticospinal Tract, DbH=Dopamine beta Hydroxylase, Ephys=Electrophysiology, GFP=Green Fluorescence Protein, MP=Methylprednisolone, NF=Neurofilament, NGF=Nerve Growth Factor, OEG=Olfactory Ensheathing Glia, TEM=Transmission Electron Microscopy, IHC=Immunohistochemistry, PST=Polysialyltransferase, SC=Schwann Cells, SKD=Skin Derived precursor cells, STX=Sialyl-transferase X, SVZ=Sub-Ventricular Zone, T=thoracic level of spinal cord injury, TH=Tyrosine Hydroxylase, WMS=White Matter Sparing.

Table 4

All Studies Using Schwann Cells That Met the Latter Inclusion Criteria.

Cell type:	Adult rat SCs	GFP+ P5 mouse SCs	Adult rat SCs	Adult rat OEGs or SCs	Neonatal GFP+ rat fibroblasts, neonatal SCs or SKP-SCs	Adult rat OEGs or SCs
Study:	Flora et al, 2013	Lavdas et al, 2010	Pearse et al, 2004a	Pearse et al, 2007	Sparling et al, 2015	Takami et al, 2002
Genetic/drug Treatment:	LV-D15A; rolipram	AP or L1/L1-Fc	Rolipram or cAMP	none	none	none

Relevant genetic or pharmacologic additions are identified within each study.

Abbreviations: AP=Alkaline Phosphatase, cAMP=cyclic Adenosine Monophosphate, GFP=Green Fluorescence Protein, OEG=Olfactory Ensheathing Glia, SC=Schwann Cells, SKP=Skin derived Precursor cells.