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·Review·

# Combination treatment with chondroitinase ABC in spinal cord injury—breaking the barrier

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After spinal cord injury (SCI), re-establishing functional circuitry in the damaged central nervous system (CNS) faces multiple challenges including lost tissue volume, insufficient intrinsic growth capacity of adult neurons, and the inhibitory environment in the damaged CNS. Several treatment strategies have been developed over the past three decades, but successful restoration of sensory and motor functions will probably require a combination of approaches to address different aspects of the problem. Degradation of the chondroitin sulfate proteoglycans with the chondroitinase ABC (ChABC) enzyme removes a regeneration barrier from the glial scar and increases plasticity in the CNS by removing perineuronal nets. Its mechanism of action does not clash or overlap with most of the other treatment strategies, making ChABC an attractive candidate as a combinational partner with other methods. In this article, we review studies in rat SCI models using ChABC combined with other treatments including cell implantation, growth factors, myelin-inhibitory molecule blockers, and ion channel expression. We discuss possible ways to optimize treatment protocols for future combinational studies. To date, combinational therapies with ChABC have shown synergistic effects with several other strategies in enhancing functional recovery after SCI. These combinatorial approaches can now be developed for clinical application.

**Keywords:** spinal cord injury; combination treatment; chondroitinase ABC; chondroitin sulfate proteoglycan; rehabilitation; plasticity; axon regeneration

## Introduction

Spinal cord injury (SCI) patients are usually left disabled for life. The two primary causes of SCI are vehicle accidents and falling from heights. Along with the rapid economic growth in China, the rate of SCI occurrence in Beijing has rapidly increased 10 times from 6.8 per million in the 1980s to 60.6 per million in 2002[1].

Following SCI, the challenge of restoring central nervous system (CNS) function faces difficulties in multiple areas. Research in the past three decades has provided several potential solutions by addressing different aspects of the problem. The strategies developed have included cell implantation, neurotrophin supplementation, and treatments to increase CNS plasticity. However, due to the complexity of the injured spinal cord, a single-treatment approach will probably not be sufficient to restore full functionality. In this review, we focus on studies of treatment with chondroitinase ABC (ChABC) combined with cell implantation, neurotrophic factors, neuroprotective agents, and myelin inhibitor antibodies. We first describe the effect and mechanism of ChABC treatment alone, and the viral vector delivery methods. Then we focus on combinational studies, especially the problems arising from the variability in SCI lesion models and their anatomical and behavioral readouts. Furthermore, in order to make clinically-relevant treatment designs, rehabilitation must be incorporated into the combination paradigm. In the last section, we discuss the topics of combination of rehabilitation with ChABC, and the possibility of using a consistent model for future combinational treatment experimental design.

## Chondroitinase ABC: Mechanism of Action

Chondroitin sulfate proteoglycans (CSPGs) are a major component of the normal CNS extracellular matrix (ECM) with diverse functional roles. After lesions, CSPGs are upregulated several-fold, peaking at between 10 to 14 days post-lesion[2]. Together with activated glial cells, the CSPGs form a dense layer of glial scar inhibitory to axon growth, and much of this inhibition is due to the activity of the glycosaminoglycan (GAG) chains[3]. In addition, CSPGs are major components of the perineuronal nets (PNNs), which are dense ECM structures that form around many neuronal cell bodies and dendrites late in development[4, 5].

In the spinal cord, PNNs surround ~30% of motoneurons in the ventral horn, 50% of large interneurons in the intermediate grey, and 20% of neurons in the dorsal horn[4], while in the brain they are particularly associated with inhibitory GABAergic interneurons. The PNNs in the visual cortex form in the second week of postnatal development, which coincides with closure of the critical period for visual functions[6]. Formation of these structures and the turning off of plasticity are triggered by impulse activity in neurons. Treatment with ChABC removes PNNs and reopens a window of CNS plasticity by promoting axon sprouting and the formation of new connections[7].

ChABC is a bacterial enzyme isolated from *Proteus vulgaris*[8]. It degrades CSPGs by cleaving the GAG chains into soluble disaccharides or tetrasaccharides, and leaves behind the core protein. This enzymatic digestion results in the release of bound growth factors from chondroitin sulfate GAG chains[9], and reduction of the glial scar neoepitope-induced immune response[10]. Also, one of the digestion products, the chondroitin sulfate E-disaccharide, is growth-promoting[11]. The enzyme remains active *in vivo* for 10 days after injection. In injured spinal cord, intraspinal injection of ChABC 1 mm above and 1 mm below the lesion causes extensive digestion by ChABC covering >5 mm around the lesion site rostro-caudally and 1.5 mm dorso-ventrally[12].

ChABC has been shown in many *in vitro* and *in vivo* models to be an effective treatment for improving axonal regeneration and sprouting, and for promoting functional recovery in acute and chronic SCI in various animal models[13-17]. In animal experiments, ChABC treatment allows some axons to regenerate through the lesion, and leads to an increased sprouting response in both lesioned and spared systems after SCI[18]. ChABC also digests the PNNs[19], and opens a window of plasticity which allows effective acquisition of motor skills with rehabilitation[12, 16]. Importantly, none of the sprouting responses seen after ChABC treatment have led to increased pain sensitivity.

## Viral Vector-mediated ChABC Delivery

Both lentiviral (LV) and adeno-associated virus (AAV) vector tools have been developed to deliver ChABC into the mammalian CNS[20]. The ChABC gene of bacterial origin has been modified to enable the production of active chondroitinase by mammalian cells. Injection of LV-ChABC into the cortex or spinal cord results in the secretion of active ChABC both locally and from long-distance axon projections, with activity persisting for >4 weeks, and AAV-ChABC has an even longer active period. In SCI models, the same beneficial effects on damaged corticospinal axons as ChABC were observed in animals which received LV-ChABC injection directly into the vicinity of a spinal cord lesion. Due to the advantages of a longer-lasting effect and ease of administration, LV- or AAV-ChABC may be considered as a substitute for ChABC in combinational studies.

## Combinatorial Treatments with Cells, Growth Factors, Ion Channels, Myelin Inhibitor Blockers and ChABC

In moderate or severe SCI, a cavity is formed due to tissue loss. Implants used to refill the space include tissue bridges or cells such as Schwann cells, olfactory ensheathing cells (OECs), neural progenitor cells, umbilical cord blood-derived cells, mesenchymal stem cells, and induced pluripotent stem cells[21]. The main issues with this strategy are cell survival, differentiation, incorporation, and exit of regenerated axons from the caudal end of the transplant and re-enter into host tissue. Various experiments, summarized below and in Fig. 1B, have combined ChABC with cell implants and with neurotrophic factors to overcome these problems.

### *Schwann Cells, OECs and ChABC*

First, in an *in vitro* model with Schwann cell/astrocyte co-culture, degradation or inhibition of CSPGs by ChABC or xylosyltransferase-1, a DNA enzyme against GAG-chain-initiating enzyme, allowed mixing of the two cell types[22], and indicated that ChABC treatment improves the

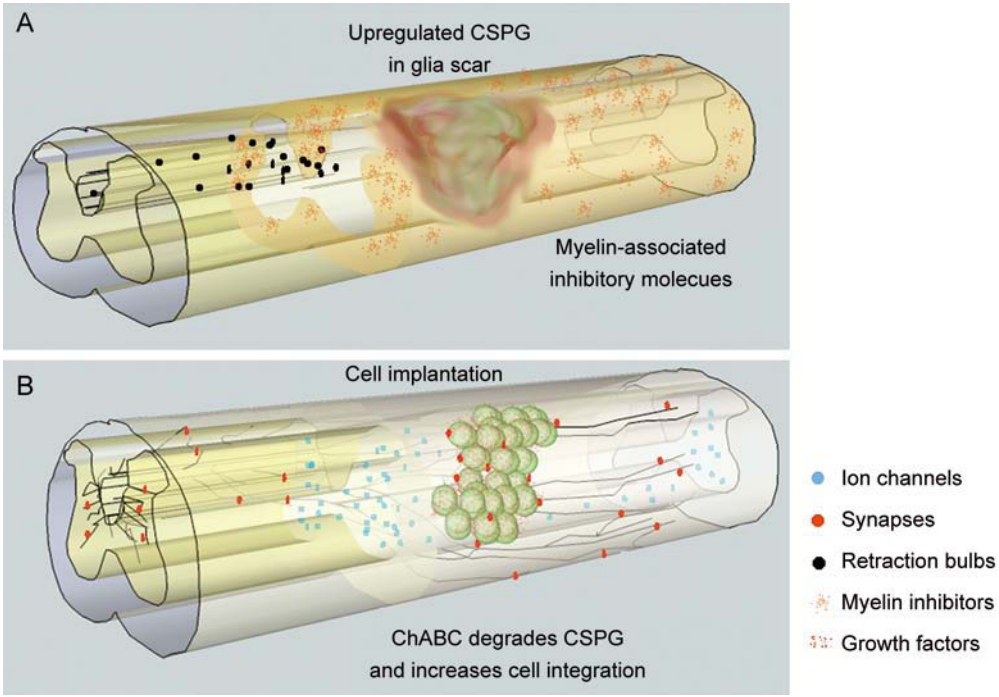


**Fig. 1. Schematic depicting the conditions in the spinal cord after injury and strategies developed for treating SCI. A: Typical lesion site of a dorsal column crush injury. Axons retract a few hundred micrometers from the site and form retraction bulbs (black dots); in white mater, myelin inhibitory molecules (orange dots) prevent axons from sprouting or regeneration; around the lesion, activated astrocytes and oligodendrocyte precursors upregulate the CSPG level and form a dense layer of glial scar, which inhibits the grow of regenerating axons through, or for implanted cells to integrate into host tissue. B: Several strategies improve spinal cord repair, including chondroitinase ABC (ChABC) treatment to degrade CSPGs in the glial scar, cell implantation to reconnect the circuitry or provide a tissue bridge for axons to grow through (green spheres), ion channel expression to re-establish neuronal activity to that of the juvenile state (blue dots), and anti-Nogo treatment to block myelin inhibitors. Combinations of these strategies increase axon regeneration and plasticity, as well as re-connection of the circuitry (functional synapses represented by red dots), and improve cell implant survival and integration into host tissue.**

integration of cells implanted into the surrounding tissue by removing the CSPG barrier. This was followed by an *in vivo* SCI experiment with complete T8 transection, after which OECs or Schwann cells were implanted around or into the lesion, together with prolonged ChABC infusion for four weeks. These combinations significantly improved graft integration and axon regeneration across the lesion, as well as locomotor recovery compared to control or graft alone[21]. This finding was reproduced and additional histological investigation with retrograde tracing showed that axons regenerate across the cell bridge into the caudal spinal cord[23]. Bladder function was also studied in a thoracic transection model, using a similar treatment with Schwann cells, OECs and ChABC[24]. In the combined-treatment group, increased bladder size and decreased amount of disorganized smooth muscle fibers and collagen deposit were found in comparison to the control group.

### *Neurotrophins, Neural Progenitor Cells, and ChABC*

In a clip-compression SCI model, a triple-combination study was performed using implanted neural precursor cells, ChABC, and a cocktail of epidermal growth factor, fibroblast growth factor, and platelet-derived growth factor[25]. This study showed successful differentiation, migration and integration of neural precursor cells (principally into oligodendrocytes), increased GAP43 levels, and increased plasticity of the corticospinal tract (CST) and serotonergic axons. The Basso, Beattie, and Bresnahan (BBB) score was slightly improved two months after treatment, and a significant improvement in ladder-walking

was only found in the group receiving the combination treatment. A later study combined precursor cells, polymer scaffolds, neurotrophin-3 (NT3), and ChABC in a rat hemisection model. Locomotor recovery and motor-evoked potentials following transcranial magnetic stimulation were recorded only in the combination group[26]. Sensitivity tests showed that the treatment did not lead to aberrant sensory responses or post-traumatic neuropathic pain in these experiments.

In addition, the combination of NT3 and cell implantation with ChABC improved ascending sensory tract regeneration to the targets in the dorsal column nuclei (DCN) after SCI. Sensory neurons implanted between the SCI site and DCN two weeks after injury were able to grow in the white matter tract, but unable to enter the DCN. This problem was partially resolved by ChABC or NT3 alone, but the combination significantly increased the number of axons entering the target region in the brain stem[27].

### *Neuroprotective/conditioning Agents and ChABC*

Another approach combined the inflammation-inducing preparation Zymosan and ChABC. In a dorsal root injury model, introducing the conditioning agent to the ganglia before injury, combined with ChABC-induced modification of CSPGs in the dorsal root entry zone (DREZ), resulted in robust regeneration of sensory axons through the DREZ. Functional connectivity was confirmed by electrophysiology. However, the conditioning strategy only worked when applied before, but not after injury[28]. In the same lesion model, the neural regeneration effect of oncomodulin, a $Ca^{2+}$-binding protein was tested in combination with cAMP and ChABC. The combination resulted in increased regeneration of sensory nerves into the DREZ, but the effect was extremely limited compared to Zymosan[29].

Another approach combined ChABC and clenbuterol, a beta2-adrenoceptor agonist that has neuroprotective effects, conducted by Bai *et al.*[30]. In adult rats with complete transection at T10, ChABC was applied acutely and clenbuterol was supplied in the drinking water. ChABC treatment decreased both CSPG and collagen deposition and reduced the gap between intact regions. Activated cAMP response element binding protein (CREB) was found in retrograde tracing-labeled neurons, which regenerated axons through the lesion site. Recovery of locomotor function was observed 2 to 3 months after the lesion and was enhanced in the combined group[30].

### *ChABC and N-methyl-D-aspartate (NMDA) Receptor Type*

Previous work has shown that plasticity in motoneurons is increased in the presence of NT3, and the NMDA receptor reverts to an immature type by expressing the NR2D subunit. In a lateral T8 hemisection SCI model, elevating the spinal levels of NT3 while increasing expression of the NR2D subunit of the NMDA receptor was combined with ChABC. The most axonal sprouting and best behavioral recovery in the BBB locomotor test was seen in the full combination group, and electrical conductivity across the lesion was only reestablished in rats receiving the combination. This treatment did not cause hypersensitivity in any group[31, 32].

### *ChABC and Blocking Myelin Inhibitors*

Myelin and myelin debris contain several myelin-associated molecules that inhibit regeneration[33-35], among which the Nogo-A N-terminal region (amino-Nogo-A) is one of the most potent inhibitors. Inhibition of Nogo-A increases axon plasticity and regeneration and improves functional recovery[36-38]. In a recent study, the effectiveness of a combination of *α*-Nogo-A, ChABC and rehabilitation was tested in a cervical dorsal column crush model[39]. The combination treatment applied *α*-Nogo-A antibody acutely, followed by delayed ChABC treatment starting at 3 weeks after injury, and rehabilitation starting at 4 weeks, to accommodate the requirement that *α*-Nogo-A be applied acutely, and that rehabilitation be given after the cessation of *α*-Nogo-A treatment. Using a CST-dependent skill (paw-reaching staircase task) as the main readout, single treatment with *α*-Nogo-A or ChABC and rehabilitation training improved functional recovery to a similar extent. The combination treatment was more effective; after treatment and 3 months of training, the combination group recovered to 80% of the level before the lesion. Anatomically, single treatments increased sprouting and axon regeneration, but combination treatment produced greater increases. *α*-Nogo-A stimulated growth of a greater number of axons with diameters >3 μm, while ChABC treatment stimulated increased growth of finer axons with varicosities. These results point to different functions of Nogo-A and CSPGs for axonal regeneration.

Due to its scar-degrading and plasticity-promoting properties, a combination of chABC and other treatment

strategies including Nogo-A blocking, increasing cAMP, inserting ion channels, and cell implantation have synergistic effects in both acute and delayed SCI models. The combination tends to increase cell survival and incorporation into host tissue, and reestablishes circuit connectivity and functional recovery without causing abnormal sensitivity.

## Combination of Regenerative Treatments with Rehabilitation

Rehabilitation is an integral part of SCI management; any potential future interventions will almost certainly have to be compatible with rehabilitation. It is therefore important to study the treatment effects of regenerative treatments in combination with rehabilitation. ChABC opens a window of plasticity and has proven effective when specific rehabilitation is given during the period of drug administration, in both acute and delayed stages and up to one year post-injury in two SCI models[16, 31]. When combined with other treatments however, the timing between treatments and rehabilitation may influence the functional recovery. For example, *α*-Nogo-A treatment gives better results when the rehabilitation starts after the treatment period, rather than simultaneously[40, 41]. When combined with ChABC and rehabilitation immediately after the lesion, although significantly increased axonal plasticity occurs, functional recovery is insignificant (unpublished data). Instead, when the ChABC is delayed for one week, and rehabilitation is started two weeks after completion of *α*-Nogo-A treatment, the treatment paradigm has a significant synergistic effect[39], indicating a significant role of timing in treatment planning.

## Discussion and Perspective

After injury, tissue repair in the spinal cord faces challenges from both a limited ability for intrinsic growth and the presence of multiple inhibitory molecules in the surrounding tissue. In order to reach the goal of functional recovery, SCI treatment must address all these issues by combining multiple interventions. Research in this direction has provided promising results. However, due to the complexity and highly-variable nature of *in vivo* experiments, they normally require a large input of resources, so it is especially important for researchers to carefully design experiment taking into consideration the lesion model, types of readout, and the timing of medical intervention in relation to the lesion, to rehabilitation training, and to each other.

The outcome of studies depends greatly on the animal model used. Many studies use the thoracic SCI model, and the BBB test as the readout for functional locomotor recovery. Locomotion is one of the most important functions, and the BBB score is widely accepted as a good measure. However, as the therapeutic power grows, more 'demanding' behavioral tests will be necessary to assess the level of recovery in a way that might predict therapeutic efficacy in humans. A battery of behavioral tests has been developed in the past decades to study movement function after SCI. Kinematic analysis gives detailed information about locomotor control, and software tools are available to quantify limb movements[42, 43].

However, humans depend heavily on supraspinal input, particularly from the CST for motor control. Animal models which test supraspinal inputs may therefore be more clinically relevant. The CST has been a focus for both axon regeneration, and more recently for behavioral tests. In rodents, the main function of the CST is skilled paw function, which can provide a high-resolution model. Tests include the skilled paw-reaching staircase task[44-46], pasta handling[47], single-pellet retrieval[48, 49], ladder walking[50, 51], skilled walking and swimming[52, 53].

*In vivo* combinational studies are major undertakings. Introducing each additional treatment into a combinational paradigm adds an extra parameter, which in theory doubles the number of experimental groups and total number of animals. As a result, the scale of an experiment quickly becomes impractical unless a large group of researchers is involved. In order for the results from these large experiments to be comparable, it is important that standardized protocols for behavioral analysis, histological analysis and data processing are used.

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## REFERENCES

[1] Hua R, Shi J, Wang X, Yang J, Zheng P, Cheng H, *et al.* Analysis of the causes and types of traumatic spinal cord

injury based on 561 cases in China from 2001 to 2010. Spinal Cord 2013, 51: 218–221.

[2] Lin R, Kwok JC, Crespo D, Fawcett JW. Chondroitinase ABC has a long-lasting effect on chondroitin sulphate glycosaminoglycan content in the injured rat brain. J Neurochem 2008, 104: 400–408.

[3] Gilbert RJ, McKeon RJ, Darr A, Calabro A, Hascall VC, Bellamkonda RV. CS-4,6 is differentially upregulated in glial scar and is a potent inhibitor of neurite extension. Mol Cell Neurosci 2005, 29: 545–558.

[4] Kwok JC, Carulli D, Fawcett JW. *In vitro* modeling of perineuronal nets: hyaluronan synthase and link protein are necessary for their formation and integrity. J Neurochem 2010, 114: 1447–1459.

[5] Kwok JC, Dick G, Wang D, Fawcett JW. Extracellular matrix and perineuronal nets in CNS repair. Dev Neurobiol 2011, 71: 1073–1089.

[6] Galtrey CM, Kwok JC, Carulli D, Rhodes KE, Fawcett JW. Distribution and synthesis of extracellular matrix proteoglycans, hyaluronan, link proteins and tenascin-R in the rat spinal cord. Eur J Neurosci 2008, 27: 1373–1390.

[7] Fawcett J. Molecular control of brain plasticity and repair. Prog Brain Res 2009, 175: 501–509.

[8] Suzuki S, Saito H, Yamagata T, Anno K, Seno N, Kawai Y*, et al.* Formation of three types of disulfated disaccharides from chondroitin sulfates by chondroitinase digestion. J Biol Chem 1968, 243: 1543–1550.

[9] Crespo D, Asher RA, Lin R, Rhodes KE, JW F. How does chondroitinase promote functional recovery in the damaged CNS? Exp Neurol 2007, 206: 12.

[10] Glant TT, Buzas EI, Finnegan A, Negroiu G, Cs-Szabo G, Mikecz K. Critical roles of glycosaminoglycan side chains of cartilage proteoglycan (aggrecan) in antigen recognition and presentation. J Immunol 1998, 160: 3812–3819.

[11] Rolls A, Avidan H, Cahalon L, Schori H, Bakalash S, Litvak V*, et al.* A disaccharide derived from chondroitin sulphate proteoglycan promotes central nervous system repair in rats and mice. Eur J Neurosci 2004, 20: 1973–1983.

[12] Garcia-Alias G, Barkhuysen S, Buckle M, Fawcett JW. Chondroitinase ABC treatment opens a window of opportunity for task-specific rehabilitation. Nat Neurosci 2009, 12: 1145–1151.

[13] Bradbury EJ, Moon LD, Popat RJ, King VR, Bennett GS, Patel PN*, et al.* Chondroitinase ABC promotes functional recovery after spinal cord injury. Nature 2002, 416: 636–640.

[14] Garcia-Alias G, Lin R, Akrimi SF, Story D, Bradbury EJ, Fawcett JW. Therapeutic time window for the application of chondroitinase ABC after spinal cord injury. Exp Neurol 2008, 210: 331–338.

[15] Moon LD, Asher RA, Rhodes KE, Fawcett JW. Regeneration of CNS axons back to their target following treatment of adult rat brain with chondroitinase ABC. Nat Neurosci 2001, 4: 465–466.

[16] Wang D, Ichiyama RM, Zhao R, Andrews MR, Fawcett JW. Chondroitinase combined with rehabilitation promotes recovery of forelimb function in rats with chronic spinal cord injury. J Neurosci 2011, 31: 9332–9344.

[17] Zuo J, Neubauer D, Dyess K, Ferguson TA, Muir D. Degradation of chondroitin sulfate proteoglycan enhances the neurite-promoting potential of spinal cord tissue. Exp Neurol 1998, 154: 654–662.

[18] Barritt AW, Davies M, Marchand F, Hartley R, Grist J, Yip P*, et al.* Chondroitinase ABC promotes sprouting of intact and injured spinal systems after spinal cord injury. J Neurosci 2006, 26: 10856–10867.

[19] Kwok JC, Afshari F, Garcia-Alias G, Fawcett JW. Proteoglycans in the central nervous system: plasticity, regeneration and their stimulation with chondroitinase ABC. Restor Neurol Neurosci 2008, 26: 131–145.

[20] Zhao RR, Muir EM, Alves JN, Rickman H, Allan AY, Kwok JC*, et al.* Lentiviral vectors express chondroitinase ABC in cortical projections and promote sprouting of injured corticospinal axons. J Neurosci Methods 2011, 201: 228–238.

[21] Bunge MB. Novel combination strategies to repair the injured mammalian spinal cord. J Spinal Cord Med 2008, 31: 262–269.

[22] Grimpe B, Pressman Y, Lupa MD, Horn KP, Bunge MB, Silver J. The role of proteoglycans in Schwann cell/astrocyte interactions and in regeneration failure at PNS/CNS interfaces. Mol Cell Neurosci 2005, 28: 18–29.

[23] Vavrek R, Pearse DD, Fouad K. Neuronal populations capable of regeneration following a combined treatment in rats with spinal cord transection. J Neurotrauma 2007, 24: 1667–1673.

[24] Fouad K, Pearse DD, Tetzlaff W, Vavrek R. Transplantation and repair: combined cell implantation and chondroitinase delivery prevents deterioration of bladder function in rats with complete spinal cord injury. Spinal Cord 2009, 47: 727–732.

[25] Karimi-Abdolrezaee S, Eftekharpour E, Wang J, Schut D, Fehlings MG. Synergistic effects of transplanted adult neural stem/progenitor cells, chondroitinase, and growth factors promote functional repair and plasticity of the chronically injured spinal cord. J Neurosci 2010, 30: 1657–1676.

[26] Hwang DH, Kim HM, Kang YM, Joo IS, Cho CS, Yoon BW*, et al.* Combination of multifaceted strategies to maximize the therapeutic benefits of neural stem cell transplant for spinal cord repair. Cell Transplant 2011, 20: 1361–1379.

[27] Massey JM, Amps J, Viapiano MS, Matthews RT, Wagoner MR, Whitaker CM*, et al.* Increased chondroitin sulfate proteoglycan expression in denervated brainstem targets following spinal cord injury creates a barrier to axonal regeneration overcome by chondroitinase ABC and neurotrophin-3. Exp Neurol 2008, 209: 426–445.

[28] Steinmetz MP, Horn KP, Tom VJ, Miller JH, Busch SA, Nair D*,*

*et al.* Chronic enhancement of the intrinsic growth capacity of sensory neurons combined with the degradation of inhibitory proteoglycans allows functional regeneration of sensory axons through the dorsal root entry zone in the mammalian spinal cord. J Neurosci 2005, 25: 8066–8076.

[29] Harel R, Iannotti CA, Hoh D, Clark M, Silver J, Steinmetz MP. Oncomodulin affords limited regeneration to injured sensory axons *in vitro* and *in vivo*. Exp Neurol 2012, 233: 708–716.

[30] Bai F, Peng H, Etlinger JD, Zeman RJ. Partial functional recovery after complete spinal cord transection by combined chondroitinase and clenbuterol treatment. Pflugers Arch 2010, 460: 657–666.

[31] Garcia-Alias G, Petrosyan HA, Schnell L, Horner PJ, Bowers WJ, Mendell LM*, et al.* Chondroitinase ABC combined with neurotrophin NT-3 secretion and NR2D expression promotes axonal plasticity and functional recovery in rats with lateral hemisection of the spinal cord. J Neurosci 2011, 31: 17788–17799.

[32] Garcia-Alias G, Fawcett JW. Training and anti-CSPG combination therapy for spinal cord injury. Exp Neurol 2012, 235: 26–32.

[33] Schwab ME. Nogo and axon regeneration. Curr Opin Neurobiol 2004, 14: 118–124.

[34] Yiu G, He Z. Glial inhibition of CNS axon regeneration. Nat Rev Neurosci 2006, 7: 617–627.

[35] Kopp MA, Liebscher T, Niedeggen A, Laufer S, Brommer B, Jungehulsing GJ*, et al*. Small-molecule-induced Rho-inhibition: NSAIDs after spinal cord injury. Cell Tissue Res 2012, 349: 119–132.

[36] Oertle T, van der Haar ME, Bandtlow CE, Robeva A, Burfeind P, Buss A*, et al.* Nogo-A inhibits neurite outgrowth and cell spreading with three discrete regions. J Neurosci 2003, 23: 5393–5406.

[37] GrandPre T, Nakamura F, Vartanian T, Strittmatter SM. Identification of the Nogo inhibitor of axon regeneration as a Reticulon protein. Nature 2000, 403: 439–444.

[38] Chen MS, Huber AB, van der Haar ME, Frank M, Schnell L, Spillmann AA*, et al.* Nogo-A is a myelin-associated neurite outgrowth inhibitor and an antigen for monoclonal antibody IN-1. Nature 2000, 403: 434–439.

[39] Zhao RR, Andrews MR, Wang D, Warren P, Gullo M, Schnell L, *et al*. Combination treatment with anti-Nogo-A and chondroitinase ABC is more effective than single treatments at enhancing functional recovery after spinal cord injury. Eur J Neurosci 2013. DOI: 10.1111/ejn.12276.

[40] Maier IC, Ichiyama RM, Courtine G, Schnell L, Lavrov I, Edgerton VR*, et al.* Differential effects of anti-Nogo-A antibody treatment and treadmill training in rats with incomplete spinal cord injury. Brain 2009, 132: 1426–1440.

[41] Marsh BC, Astill SL, Utley A, Ichiyama RM. Movement rehabilitation after spinal cord injuries: emerging concepts and future directions. Brain Res Bull 2011, 84: 327–336.

[42] Courtine G, Roy RR, Hodgson J, McKay H, Raven J, Zhong H*, et al*. Kinematic and EMG determinants in quadrupedal locomotion of a non-human primate (Rhesus). J Neurophysiol 2005, 93: 3127–3145.

[43] Ichiyama RM, Courtine G, Gerasimenko YP, Yang GJ, van den Brand R, Lavrov IA*, et al.* Step training reinforces specific spinal locomotor circuitry in adult spinal rats. J Neurosci 2008, 28: 7370–7375.

[44] Galtrey CM, Fawcett JW. Characterization of tests of functional recovery after median and ulnar nerve injury and repair in the rat forelimb. J Peripher Nerv Syst 2007, 12: 11–27.

[45] Montoya CP, Campbell-Hope LJ, Pemberton KD, Dunnett SB. The "staircase test": a measure of independent forelimb reaching and grasping abilities in rats. J Neurosci Methods 1991, 36: 219–228.

[46] Starkey ML, Barritt AW, Yip PK, Davies M, Hamers FP, McMahon SB*, et al.* Assessing behavioural function following a pyramidotomy lesion of the corticospinal tract in adult mice. Exp Neurol 2005, 195: 524–539.

[47] Khaing ZZ, Geissler SA, Jiang S, Milman BD, Aguilar SV, Schmidt CE*, et al.* Assessing forelimb function after unilateral cervical spinal cord injury: novel forelimb tasks predict lesion severity and recovery. J Neurotrauma 2012, 29: 488–498.

[48] Onifer SM, Rodriguez JF, Santiago DI, Benitez JC, Kim DT, Brunschwig JP*, et al.* Cervical spinal cord injury in the adult rat: assessment of forelimb dysfunction. Restor Neurol Neurosci 1997, 11: 211–223.

[49] Stackhouse SK, Murray M, Shumsky JS. Effect of cervical dorsolateral funiculotomy on reach-to-grasp function in the rat. J Neurotrauma 2008, 25: 1039–1047.

[50] Metz GA, Whishaw IQ. The ladder rung walking task: a scoring system and its practical application. J Vis Exp 2009, (28). pii: 1204. doi: 10.3791/1204.

[51] Whishaw IQ, Travis SG, Koppe SW, Sacrey LA, Gholamrezaei G, Gorny B. Hand shaping in the rat: conserved release and collection *vs.* flexible manipulation in overground walking, ladder rung walking, cylinder exploration, and skilled reaching. Behav Brain Res 2010, 206: 21–31.

[52] Gonzenbach RR, Gasser P, Zorner B, Hochreutener E, Dietz V, Schwab ME. Nogo-A antibodies and training reduce muscle spasms in spinal cord-injured rats. Ann Neurol 2010, 68: 48–57.

[53] Zorner B, Filli L, Starkey ML, Gonzenbach R, Kasper H, Rothlisberger M*, et al.* Profiling locomotor recovery: comprehensive quantification of impairments after CNS damage in rodents. Nat Methods 2010, 7: 701–708.