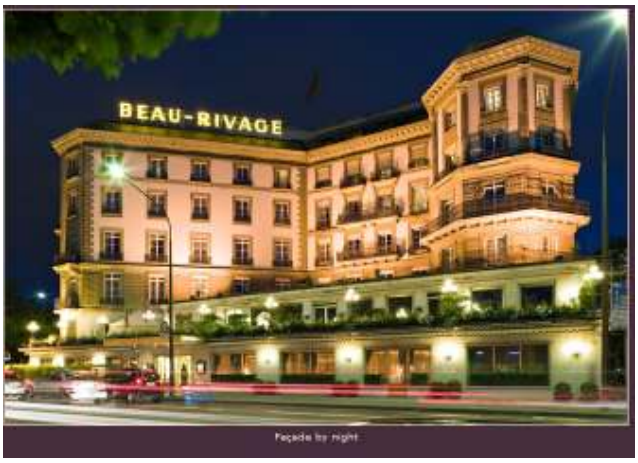
# Spinal Cord Repair 1

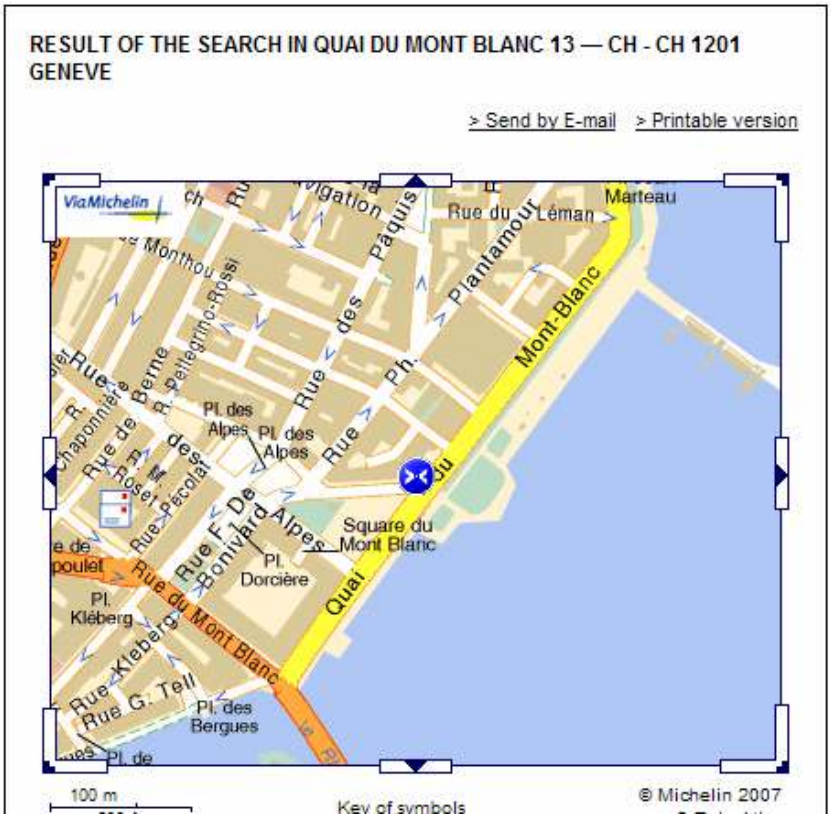
April 2008- Contents

## Meeting information for Geneva –

### 13th of July from 16:00 to 20:00 (Agenda will be provided prior to the meeting)

Location:
**Hotel BEAU-RIVAGE,** QUAI DU MONT BLANC 13, Geneva http://www.beau-rivage.ch



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## Literature updates

***Pubmed Search from last 30 days based on key words:***

***Spinal Cord; Spinal Cord Injury; Spinal Cord Development; Motor Neuron***

**1)The Interaction Between Developing Spinal Locomotor Networks in the Neonatal Mouse**

Gordon IT, Dunbar MJ, Vanneste KJ, Whelan PJ. **J Neurophysiol**. 2008 Apr 24;.

At birth, thoracosacral spinal cord networks in mouse can produce a coordinated locomotor-like pattern. In contrast, less is known about the cervicothoracic networks that generate forelimb locomotion. Here we show that cervical networks can produce coordinated rhythmic patterns in the brainstem-spinal cord preparation of the mouse. Segmentally the C5 and C8 neurograms were each found to be alternating left-right, and the ipsilateral C5 and C8 neurograms also alternated. Collectively these patterns were suggestive of locomotor-like activity. This pattern was not dependent on the presence of thoracosacral segments since they could be evoked following a complete transection of the spinal cord at T5. We next demonstrated that activation of thoracosacral networks either pharmacologically or by stimulation of sacrocaudal afferents could produce rhythmic activity within the C5 and C8 neurograms. On the other hand, pharmacological activation of cervical networks did not evoke alternating cervical rhythmic activity either in isolated cervicothoracic or cervicosacral preparations. Under these conditions, we found that activation of cervicothoracic networks could alter the timing of thoracosacral locomotor-like patterns. When thoracosacral networks were not activated pharmacologically, but received rhythmic drive from cervicothoracic networks, a pattern of slow bursts with superimposed fast synchronous oscillations became the dominant lumbar neurogram pattern. Our data suggest that in neonatal mice the cervical CPG is capable of producing coordinated rhythmic patterns in the absence of input from lumbar segments, but caudorostral drive contributes to cervical patterns and rhythm stability.

**2) A Score Predicting Posttreatment Ambulatory Status in Patients Irradiated for Metastatic Spinal Cord Compression.**

Rades D, Rudat V, Veninga T, Stalpers LJ, Basic H, Karstens JH, Hoskin PJ, Schild **Int J Radiat Oncol Biol Phys**. 2008 Apr 22; SE.

PURPOSE: To create a scoring system to predict ambulatory status after radiotherapy (RT) for metastatic spinal cord compression (MSCC). METHODS AND MATERIALS: On the basis of a multivariate analysis of 2096 MSCC patients, a scoring system was developed. This included the five prognostic factors significantly associated with post-RT ambulatory status: primary tumor type, interval between tumor diagnosis and MSCC, visceral metastases, motor function before RT, and time

developing motor deficits before RT. The score for each factor was determined by dividing the post-RT ambulatory rate (as a percentage) by 10. Total scores represented the sum of the scores for each factor and ranged between 21 and 44 points. Patients were divided into five groups according to this score. RESULTS: The post-RT ambulatory rates were 6% (24 of 389) for patients with scores of </=28 points, 44% (121 of 278) for those with 29-31 points, 70% (212 of 303) for those with 32-34 points, 86% (315 of 266) for those with 35-37 points, and 99% (750 of 760) for those with >/=38 points. The 3-month survival rates were 29%, 62%, 77%, 84%, and 98%, respectively. The 6-months survival rates were 6%, 31%, 42%, 61%, and 93%, respectively. CONCLUSIONS: Because patients with scores of </=28 points had poor functional outcome after RT and extraordinarily poor survival rates, short-course RT to decrease pain or best supportive care may be considered. Patients with scores of 29-37 points should be considered surgical candidates, because RT-alone results were not optimal. Patients with scores of >/=38 points seem to have excellent results with RT alone.

**3) Vgf is a novel biomarker associated with muscle weakness in amyotrophic lateral sclerosis (ALS), with a potential role in disease pathogenesis**

Zhao Z, Lange DJ, Ho L, Bonini S, Shao B, Salton SR, Thomas S, Pasinetti GM. **Int J Med Sci**. 2008 Apr 15;5(2):92-9.

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that affects nerve cells in the brain and the spinal cord. Previous proteomic evidence revealed that the content of certain peptide fragments including Vgf-derived peptide aa 398-411 (Vgf(398-411)) of the precursor Vgf protein in the cerebral spinal fluid (CSF) correctly identified patients with ALS from normal and disease controls. Using quantitative ELISA immunoassay we found that the CSF levels of Vgf decreases with muscle weakness in patients with ALS. In SOD1 G93A transgenic mice, loss of full-length Vgf content in CSF, serum and in SMI-32 immunopositive spinal cord motor neurons is noted in asymptomatic animals (approximately 75 days old) and continues to show a progressive decline as animals weaken. In vitro studies show that viral-mediated exogenous Vgf expression in primary mixed spinal cord neuron cultures attenuates excitotoxic injury. Thus, while Vgf may be a reliable biomarker of progression of muscle weakness in patients with ALS, restoration of Vgf expression in spinal cord motor neurons may therapeutically rescue spinal cord motorneurons against excitotoxic injury.

**4) High b-value q-space diffusion-weighted MRI of the human cervical spinal cord in vivo: Feasibility and application to multiple sclerosis.**

Farrell JA, Smith SA, Gordon-Lipkin EM, Reich DS, Calabresi PA, van Zijl PC.**Magn Reson Med**. 2008 Apr 21;59(5):1079-1089 [Epub ahead of print]

Q-space analysis is an alternative analysis technique for diffusion-weighted imaging (DWI) data in which the probability density function (PDF) for molecular diffusion is estimated without the need to assume a Gaussian shape. Although used in the human brain, q-space DWI has not yet been applied to study the human spinal cord in vivo. Here we demonstrate the feasibility of performing q-space imaging in the cervical spinal cord of eight healthy volunteers and four patients with multiple sclerosis. The PDF was computed and water displacement and zero-displacement probability maps were calculated from the width and height of the PDF, respectively. In the dorsal column white matter, q-space contrasts showed a significant ($P < 0.01$) increase in the width and a decrease in the height of the PDF in lesions, the result of increased diffusion. These q-space contrasts, which are sensitive to the slow diffusion component, exhibited improved detection of abnormal diffusion compared to perpendicular apparent diffusion constant measurements. The conspicuity of lesions compared favorably with magnetization transfer (MT)-weighted images and quantitative CSF-normalized MT measurements. Thus, q-space DWI can be used to study water diffusion in the human spinal cord in vivo and is well suited to assess white matter damage.

**5) The effects of inflammatory response associated with traumatic spinal cord injury in cutaneous wound healing and on expression of transforming growth factor-beta1 (TGF-beta(1)) and platelet-derived growth factor (PDGF)-A at the wound site in rats.**

Konya D, Gercek A, Akakin A, Akakin D, Tural S, Cetinel S, Ozgen S, Pamir MN. **Growth Factors**. 2008 Apr;26(2):74-9.

At the cellular level, spinal cord injury (SCI) provokes an inflammatory response that generates substantial secondary damage within the cord, but also may contribute to its repair. The aim of this study was to investigate the effects of inflammatory response associated with SCI in cutaneous wound healing and on expression of transforming growth factor-beta1 (TGF-beta(1)) and platelet-derived growth factor (PDGF)-A at the wound site in rats. At the 14th day analysis, the mean TGF-beta(1) score in trauma group (I) was significantly lower than that in control group (C) (2.60 +/- 0.90 vs. 3.64 +/- 0.37, respectively; $p < 0.05$). The mean score for PDGF-A expression in group I was similar to the corresponding value in group C (2.42 +/- 0.74 vs. 2.94 +/- 0.72, respectively). Compared to group C, group I had significantly lower mean scores for epidermal and dermal regeneration, but higher mean scores for granulation tissue thickness and similar scores for angiogenesis. The dermal layer contains diffuse deposition of collagen fibers that are not organised as in control rat skin, and intraepidermal and subepidermal vasocongestion is distinct. Based on the results on the parameters evaluated in the study, experimental SCI in rats results in delay in wound healing and low intensity of TGF-beta(1) in the dorsal wound-tissue specimens.

**6) Selective activation of muscle and skin nociceptors does not trigger exaggerated sympathetic responses in spinal-injured subjects.**

Burton AR, Brown R, Macefield VG. **Spinal Cord**. 2008 Apr 22 [Epub ahead of print]

Study design:Measurement of sympathetic effector organ responses to selective activation of muscle and skin nociceptors below lesion in spinal cord-injured (SCI) subjects.Objectives:To test whether selective noxious stimulation below lesion causes exaggerated sympathetic responses in human SCI.Setting:Prince of Wales Medical Research Institute, Australia.Methods:Twelve subjects (C5-T10, ASIA A-C), none of whom had sensation below the lesion, were included in the study. Selective stimulation of muscle or cutaneous nociceptors was produced by bolus injection of hypertonic (5%) saline into the tibialis anterior muscle or overlying skin and compared with non-noxious electrical stimulation of the abdominal wall. Cutaneous vasoconstrictor (photoelectric plethysmography) and sudomotor (skin conductance) responses, in addition to respiration, heart rate and continuous arterial pressure were monitored.Results:Electrical stimulation of the abdominal wall caused a significant increase in arterial pressure (31.8+/-6.1%). Conversely, intramuscular or subcutaneous injection of hypertonic saline caused no significant changes in blood pressure (-3.0+/-2.4%; -1.4+/-3.4%) heart rate, skin blood flow or sweat release.Conclusions:While hypertonic saline injected into muscle or skin induces strong pain, cutaneous vasoconstriction and sweat release in able-bodied subjects, we saw no evidence of exaggerated sympathoexcitation when these same noxious stimuli were delivered below lesion in subjects with SCI. This suggests that certain types of somatic noxious input may not trigger autonomic dysreflexia, and questions the concept that any painful stimuli originating below lesion can reliably trigger dysreflexia.

**7) Locomotor training for walking after spinal cord injury.**

Mehrholz J, Kugler J, Pohl M. **Cochrane Database Syst Rev**. 2008 Apr 16;(2):CD006676.

BACKGROUND: Locomotor training for walking is used in rehabilitation after spinal cord injury (SCI) and might help to improve walking. OBJECTIVES: To assess the effects of locomotor training on improvement in walking for people with traumatic SCI. SEARCH STRATEGY: We searched the Cochrane Injuries Group Specialised Register (last searched June 2007); the Cochrane Central Register of Controlled Trials (CENTRAL) (The Cochrane Library 2007, Issue 2); MEDLINE (1966 to June 2007); EMBASE (1980 to June 2007); National Research Register (2007, Issue 2); CINAHL (1982 to June 2007); AMED (Allied and Complementary Medicine Database) (1985 to June 2007); SPORTDiscus (1949 to June 2007); PEDro (the Physiotherapy Evidence database) (searched June

2007); COMPENDEX (engineering databases) (1972 to June 2007); INSPEC (1969 to June 2007); and the National Research Register, Zetoc, and Current Controlled Trials research and trials registers. We also handsearched relevant conference proceedings, checked reference lists and contacted study authors in an effort to identify published, unpublished and ongoing trials. SELECTION CRITERIA: We included randomised controlled trials (RCT) that compared locomotor training to any other exercise provided with the goal of improving walking function after SCI or to a no-treatment control group. DATA COLLECTION AND ANALYSIS: Two review authors independently selected trials for inclusion, assessed trial quality and extracted the data. The primary outcomes were the speed of walking and walking capacity at follow up. MAIN RESULTS: Four RCTs involving 222 patients were included in this review. Overall, the results were inconclusive. There was no statistically significant effect of locomotor training on walking function after SCI comparing bodyweight supported treadmill training with or without functional electrical stimulation or robotic-assisted locomotor training. AUTHORS' CONCLUSIONS: There is insufficient evidence from RCTs to conclude that any one locomotor training strategy improves walking function more than another for people with SCI. Research in the form of large RCTs is needed to address specific questions about the type of locomotor training which might be most effective in improving walking function of people with SCI.

**8) Repair effect of Wnt3a protein on the contused adult rat spinal cord.**

Yin ZS, Zu B, Chang J, Zhang H. **Neurol Res**. 2008 Apr 18 [Epub ahead of print]

OBJECTIVE: To explore the repair effect of Wnt3a on injured spinal cord in rats. METHODS: Moderate spinal cord contusion injurywasmade in 40 adult Sprague-Dawley rats at T10. Fifteen rats served as contusion controls (Group 1). Fifteen rats were treated withWnt3a 3 days after injury (Group 2). Ten additional rats received only T10 laminectomies to serve as non-injured controls (Group 0). The functional recovery of the rats was observed through Basso-Beattie-Bresnahan (BBB) open fieldlocomotor score.Rats were killed at 14 or 28 days after injury, then spinal cordswere removed for histopathologic examinations, and the expression of the bromodeoxyuridine (BrdU) plus neural cell markers was stained with immunohistochemical method. RESULTS: After an initial complete hindlimb paralysis, rats of all groups receiving a contusive injury recovered substantial function within 1 week. By 28 days, the BBB score for rats in Group 2 is better than that for rats in Group 1 by 7 points (Group 2=16.94, after 28 days versus Group 1=9.89 points; $p<0.05$). Light and electron microscopic works showed that the Wnt3a-treated group had moderate repair effect of myelin and axons. Immunohistochemical analysis showed a significant increase in the fnumber of the inducing differentiated neurons in Wnt3a-treated rats compared with control rats 2 weeks after injury. CONCLUSIONS: Exogenous Wnt3a administration can improve axonal conduction and spinal cord function in the injured spinal cord, and the administration of Wnt3a result in the increase in the populations of neurons, suggesting that these cells may be derived from neural precursors and stem cells.

**9) Selective activation of microglia in spinal cord but not higher cortical regions following nerve injury in adult mouse.**

Zhang F, Vadakkan KI, Kim SS, Wu LJ, Shang Y, Zhuo M. **Mol Pain**. 2008 Apr 18;4(1):15 [Epub ahead of print]

ABSTRACT: Neuronal plasticity along the pathway for sensory transmission including the spinal cord and cortex plays an important role in chronic pain, including inflammatory and neuropathic pain. While recent studies indicate that microglia in the spinal cord are involved in neuropathic pain, a systematic study has not been performed in other regions of the central nervous system (CNS). In the present study, we used heterozygous Cx3cr1GFP/+ mice to characterize the morphological phenotypes of microglia following common peroneal nerve (CPN) ligation. We found that microglia showed a uniform distribution throughout the CNS, and peripheral nerve injury selectively activated microglia in the spinal cord dorsal horn and related ventral horn. In contrast, microglia was not activated in supraspinal regions of the CNS, including the anterior cingulate cortex (ACC), prefrontal cortex (PFC), primary and secondary somatosensory cortex (S1 and S2), insular cortex (IC), amygdala, hippocampus, periaqueductal gray (PAG) and rostral ventromedial medulla (RVM). Our results provide strong

evidence that nerve injury primarily activates microglia in the spinal cord of adult mice, and pain-related cortical plasticity is likely mediated by neurons but not microglia.

**10) Ways of coping and perceived stress in women with spinal cord injury.**

Lequerica AH, Forschheimer M, Tate DG, Roller S, Toussaint L. **J Health Psychol**. 2008 Apr;13(3):348-54.

Using a cross-sectional design, this research aimed to assess whether a three-factor model of Positive Reappraisal, Escape-Avoidance, and Seeking Social Support based on the Ways of Coping Questionnaire (WOCQ) appropriately depicts coping within a sample of women with spinal cord injury (SCI). Forty-four community-dwelling women with spinal cord injury were interviewed from two urban rehabilitation facilities in the Midwestern United States. The main outcome measures used were the Perceived Stress Scale (PSS) and the WOCQ. The Positive Reappraisal, Escape-Avoidance, and Seeking Social Support scales of the WOCQ significantly accounted for variance in perceived stress. These three scales appear to be most relevant to perceived stress in women with SCI. Implications for coping research in this population are discussed.

**11) Changes in Corticospinal Function and Ankle Motor Control during Recovery from Incomplete Spinal Cord Injury.**

Wirth B, Van Hedel HJ, Curt A. **J Neurotrauma**. 2008 Apr 17 [Epub ahead of print]

ABSTRACT Little is known about the mechanisms that underlie motor recovery after incomplete spinal cord injury (iSCI) in humans. This study assessed changes in corticospinal tract (CST) function by measuring motor-evoked potentials (MEPs) and ankle motor control at 1, 3, and 6 months after acute iSCI. In 12 iSCI patients and matched controls, MEPs (evoked at 20% of maximal voluntary contraction [MVC]) were combined with a comprehensive ankle motor assessment protocol that measured ankle dorsiflexor strength (MVC, manual muscle testing, maximal movement velocity [MMV]), dexterity (the ability to accurately time ankle dorsiflexion movements) and gait (speed, walking aids). In the first 6 months after iSCI, all measures of muscle strength, gait and the MEP amplitudes significantly increased. The level of background electromyography (EMG) at 20% MVC remained stable, although absolute MVC increased. The MEP latencies were significantly delayed and remained unchanged during the first 6 months after iSCI. In addition, dexterity was preserved throughout rehabilitation. The percentage increase in MEP amplitude was significantly related only to the percentage improvement in MMV. The finding of unchanged CST conductivity, as assessed by MEP latencies in acute iSCI patients recovering motor function, is in accordance with previous studies in human SCI on this issue. The increased MEP facilitation at stable background EMG might indicate improved synchronization of the descending volley and/or responsiveness of motoneurons to supra-spinal input. The absence of a relationship between MEP amplitudes and recovery of ambulation and muscle strength implies that plastic changes in spinal neural circuits and preserved motor units might have contributed to the functional improvement.

**12) Extramedullary Chitosan Channels Promote Survival of Transplanted Neural Stem and Progenitor Cells and Create a Tissue Bridge After Complete Spinal Cord Transection.**

Nomura H, Zahir T, Kim H, Katayama Y, Kulbatski I, Morshead CM, Shoichet MS, Tator CH. **Tissue Eng Part A**. 2008 Apr 17 [Epub ahead of print]

Transplantation of neural stem and progenitor cells (NSPCs) is a promising strategy for repair after spinal cord injury. However, the epicenter of the severely damaged spinal cord is a hostile environment that results in poor survival of the transplanted NSPCs. We examined implantation of extramedullary chitosan channels seeded with NSPCs derived from transgenic green fluorescent protein (GFP) rats after spinal cord transection (SCT). At 14 weeks, we assessed the survival, maturation, and functional results using NSPCs harvested from the brain (brain group) or spinal cord (SC group) and seeded into chitosan channels implanted between the cord stumps after complete SCT. Control SCT animals had empty chitosan channels or no channels implanted. Channels seeded

with brain or spinal cord-derived NSPCs showed a tissue bridge, although the bridges were thicker in the brain group. Both cell types showed long-term survival, but the number of surviving cells in the brain group was approximately five times as great as in the SC group. In both the brain and SC groups at 14 weeks after transplantation, many host axons were present in the center of the bridge in association with the transplanted cells. At 14 weeks astrocytic and oligodendrocytic differentiation in the channels was 24.8% and 17.3%, respectively, in the brain group, and 31.8% and 9.7%, respectively, in the SC group. The channels caused minimal tissue reaction in the adjacent spinal cord. There was no improvement in locomotor function. Thus, implantation of chitosan channels seeded with NSPCs after SCT created a tissue bridge containing many surviving transplanted cells and host axons, although there was no functional improvement.

**13) Adrenomedullin-2/Intermedin Induces cAMP Accumulation in Dissociated Rat Spinal Cord Cells: Evidence for the Existence of a Distinct Class of Sites of Action.**

Owji AA, Chabot JG, Dumont Y, Quirion R. **J Mol Neurosci**. 2008 Apr 17 [Epub ahead of print]

Adrenomedullin-2/intermedin is structurally related to the calcitonin family of peptides, which includes calcitonin gene-related peptide (CGRP), adrenomedullin, and amylin. We recently reported that CGRP and adrenomedullin act through distinct receptors to induce cyclic adenosine monophosphate (cAMP) accumulation in dispersed cells from embryonic rat spinal cord. Here, we investigated the apparent affinity and efficacy of adrenomedullin-2/intermedin for these receptors. Adrenomedullin-2/intermedin competed with [(125)I]-CGRP for binding to specific embryonic spinal cord cells with a pIC(50) of 9.73 +/- 0.06. Interestingly, adrenomedullin-2/intermedin competed for specific [(125)I]-adrenomedullin binding in a biphasic manner with pIC(50) of 9.03 +/- 0.22 and 6.45 +/- 0.24, respectively. Cellular levels of cAMP were increased by adrenomedullin-2/intermedin (pEC(50) 7.84 +/- 0.08) when cells were exposed to this peptide for 10 min at 37 degrees C. This effect was partially inhibited by the non-peptide antagonist BIBN4096BS (pA(2) 6.56 +/- 0.12), the adrenomedullin antagonist hAM(22-52) (pA(2) 6.36 +/- 0.30), and the adrenomedullin/CGRP antagonist CGRP(8-37) (pA(2) 7.24 +/- 0.60). More interestingly, a highly significant effect of adrenomedullin-2/intermedin on cAMP accumulation (pEC(50) 7.3 +/- 0.14) was still observed even in the presence of a mixture of saturating concentrations of BIBN4096BS, hAM(22-52), and the amylin antagonist AC187. Taken together, these data provide evidence for the possible existence of a distinct class of receptor sites for adrenomedullin-2/intermedin in embryonic rat spinal cord cells.

**14) Spinal cord mechanisms of pain.**

D'Mello R, Dickenson AH. **Br J Anaesth**. 2008 Apr 15 [Epub ahead of print]

The spinal cord is the first relay site in the transmission of nociceptive information from the periphery to the brain. Sensory signals are transmitted from the periphery by primary afferent fibres into the dorsal horn of the spinal cord, where these afferents synapse with intrinsic spinal dorsal horn neurones. Spinal projection neurones then convey this information to higher centres in the brain, where non-noxious and noxious signals can be perceived. During nociceptive transmission, the output of the spinal cord is dependent on various spinal mechanisms which can either increase or decrease the activity of dorsal horn neurones. Such mechanisms include local excitatory and inhibitory interneurones, N-methyl-d-aspartate receptor activation, and descending influences from the brainstem, which can be both inhibitory and excitatory in nature. After nerve injury or conditions of inflammation, shifts can occur in these excitatory and inhibitory mechanisms which modulate spinal excitability, often resulting in the heightened response of dorsal neurones to incoming afferent signals, and increased output to the brain, a phenomenon known as central sensitization. In this review, we consider the ways in which spinal cord activity may be altered in chronic pain states. In addition, we discuss the spinal mechanisms which are targeted by current analgesics used in the management of chronic pain.

**15) Locomotor Dysfunction and Pain: The Scylla and Charybdis of Fiber Sprouting After Spinal Cord Injury.**

Deumens R, Joosten EA, Waxman SG, Hains BC. **Mol Neurobiol**. 2008 Apr 15 [Epub ahead of print]

Injury to the spinal cord (SCI) can produce a constellation of problems including chronic pain, autonomic dysreflexia, and motor dysfunction. Neuroplasticity in the form of fiber sprouting or the lack thereof is an important phenomenon that can contribute to the deleterious effects of SCI. Aberrant sprouting of primary afferent fibers and synaptogenesis within incorrect dorsal horn laminae leads to the development and maintenance of chronic pain as well as autonomic dysreflexia. At the same time, interruption of connections between supraspinal motor control centers and spinal cord output cells, due to lack of successful regenerative sprouting of injured descending fiber tracts, contributes to motor deficits. Similarities in the molecular control of axonal growth of motor and sensory fibers have made the development of cogent therapies difficult. In this study, we discuss recent findings related to the degradation of inhibitory barriers and promotion of sprouting of motor fibers as a strategy for the restoration of motor function and note that this may induce primary afferent fiber sprouting that can contribute to chronic pain. We highlight the importance of careful attentiveness to off-target molecular- and circuit-level modulation of nociceptive processing while moving forward with the development of therapies that will restore motor function after SCI.

**16) C-Reactive protein in adults with chronic spinal cord injury: increased chronic inflammation in tetraplegia vs paraplegia.**

Gibson AE, Buchholz AC, Martin Ginis KA. **Spinal Cord**. 2008 Apr 15 [Epub ahead of print]

Study design:Cross-sectional.Objectives:In community-dwelling adults with chronic spinal cord injury (SCI), to (1) quantify C-reactive protein (CRP), a marker of inflammation and cardiovascular disease (CVD) risk; (2) determine factors associated with CRP.Setting:Hamilton, Ontario, Canada.Methods:We examined CVD risk factors in 69 participants. Measurements included length, weight, waist circumference, blood pressure, percent fat mass (bioelectrical impedance analysis) and fasting blood parameters (high-sensitivity CRP, lipids, insulin, glucose, insulin resistance by homeostasis model assessment (HOMA)).Results:Mean CRP of the group was 3.37+/-2.86 mg-l(-1), consistent with the American Heart Association (AHA) definition of high risk of CVD. CRP was 74% higher in persons with tetraplegia (4.31+/-2.97) than those with paraplegia (2.47+/-2.47 mg l(-1), P=0.002), consistent with high CVD risk. Participants with high CRP (3.1-9.9 mg l(-1)) had greater waist circumference, BMI, percent fat mass and HOMA values than those with lower CRP (</=3.0 mg l(-1), all P<0.05). LogCRP was independently correlated with waist circumference (r=0.612), logTriglycerides (r=0.342), logInsulin (r=0.309) and logHOMA (r=0.316, all P<0.05). Only level of lesion and waist circumference remained significantly associated with logCRP when variables with significant bivariate correlations were included in multiple regression analysis.Conclusion:Mean CRP values in this sample of adults with chronic SCI were consistent with the AHA classification of high CVD risk, especially those of persons with tetraplegia. Level of lesion and waist circumference are independently associated with CRP in this population.

**17) Expression and Regulation of the Vitamin D Receptor in the Zebrafish, Danio rerio.**

Craig TA, Sommer S, Sussman CR, Grande JP, Kumar R. **J Bone Miner Res**. 2008 Apr 14 [Epub ahead of print]

Abstract Vitamin D and vitamin D metabolites such as 25-hydroxyvitamin D and 1alpha, 25-dihydroxyvitamin D (1alpha, 25(OH)(2)D(3)) circulate in the serum of fish. The receptor for 1alpha, 25(OH)(2)D(3) (VDR) has previously been cloned from fish intestine, and ligand binding assays have demonstrated the presence of the VDR in the gills, intestine and liver of fish. Using immunohistochemical methods with specific antibodies against the VDR, we now report that the VDR is widely expressed in tissues of the adult male and female zebrafish, Danio rerio, specifically in epithelial cells of gills, tubular cells of the kidney, and in absorptive cells in the intestine. Additionally, the VDR is expressed in the skin, the olfactory organ, in the retina, brain, and spinal cord. Sertoli cells of the testis, oocytes, acinar cells of the pancreas, hepatocytes and bile duct epithelial cells express

substantial amounts of the receptor. Osteoblast-like cells and chondrocytes also express the VDR. Pre-immune serum and anti-serum pre-adsorbed with Danio VDR protein fails to detect the VDR in the same tissues. The VDR is also present in the developing eye, brain, and otic vesicle of 48 h and 96h post-fertilization zebrafish embryos. Parenteral administration of 1alpha, 25(OH)(2)D(3) increases concentrations of the VDR in intestinal epithelial cells but not in epithelial cells of the gills. Lithocholic acid, however, does not alter concentrations of the VDR following parenteral administration. The data suggest that the VDR is widely distributed in tissues of the zebrafish, Danio rerio, and is likely to play important roles in epithelial transport, bone and endocrine function. Furthermore, concentrations of the receptor appear to be regulated by its ligand, 1alpha, 25-dihydroxyvitamin D, but not by lithocholic acid. Zebrafish may serve as a useful model in which to assess the function of the VDR in diverse tissues.

**18) The development of spinal cord anatomy.**

Pearce JM. **Eur Neurol.** 2008;59(6):286-91. Epub 2008 Apr 11.

A panel illustrating spinal cord injury in The Dying Lioness in the British Museum dates to 650 BC. This paper outlines the subsequent progression of knowledge of the anatomy of the spinal cord. The animal dissections of Galen are considered because his deductions persisted through the Dark Ages until the late 18th century. Anatomy advanced gradually to yield discoveries of the complex tracts and grey matter elements of the cord and their functions. Amongst many distinguished exponents, the works of Blasius, Huber, Vicq d'Azyr and Stilling are emphasised. (c) 2008 S. Karger AG, Basel

**19) Comparison of the effects of octreotide and melatonin in preventing nerve injury in rats with experimental spinal cord injury.**

Erol FS, Kaplan M, Tiftikci M, Yakar H, Ozercan I, Ilhan N, Topsakal C. **J Clin Neurosci**. 2008 Apr 11 [Epub ahead of print]

In this study, we aimed to investigate the biochemical and histopathological protective effects of octreotide and melatonin in an experimental model of spinal cord injury. Fifty- six male albino Wistar rats were divided into four groups. Rats in the G1 group (n=7; control group) did not undergo any treatment except for anesthesia prior to being killed. Rats in the G2 group (n=7) underwent laminectomy and aneurysmal clip application at the T4-5 level. G3 group rats (n=14) were either treated with a 7.5 mg/kg intraperitoneal dose of melatonin (Sigma, St. Louis, MO, USA) immediately after laminectomy, then the same dose again on the day following injury (G3a), or given three equal doses over 10 days to achieve a total dose of 7.5 mg/kg/day (G3b). G4 group rats (n=14) were either treated with a 30mug/kg intraperitoneal dose of octreotide (Sandostatin; Novartis, Istanbul, Turkey) immediately after laminectomy, then the same dose again on the day following injury (G4a), or given three equal doses over 10 days to achieve a total dose of 30mug/kg/day (G4b). Rats in the G3 and G4 groups were sacrificed on days 1 and 10 after spinal cord injury (n=7 at each time point) and spinal cord samples were obtained. Tissue malonyldialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GSH-Px) levels were assayed. G3a, G3b and G4b had significantly lower levels of MDA than G2 ($p<0.01$). G3b had significantly higher SOD and GSH-Px levels than G2 ($p<0.01$). Histopathologically, melatonin significantly reduced necrosis and degeneration in both the initial and late stages ($p<0.01$). Octreotide had significant effects on necrosis and degeneration during the late stages, and on edema and congestion in both the initial and the late stages of injury ($p<0.01$). Melatonin was found to be superior to octreotide with respect to the prevention of congestion, edema, axonal degeneration and necrosis.

**20) Enhanced regeneration in spinal cord injury by concomitant treatment with granulocyte colony-stimulating factor and neuronal stem cells.**

Pan HC, Cheng FC, Lai SZ, Yang DY, Wang YC, Lee MS. **J Clin Neurosci**. 2008 Apr 9 [Epub ahead of print]

Granulocyte colony-stimulating factor (G-CSF) inhibits programmed cell death and stimulates neuronal progenitor differentiation. Neuronal stem cells transplanted into injured spinal cord can survive, differentiating into astroglia and oligodendroglia, and supporting axon growth and myelination. Herein, we evaluate the combined effects of G-CSF and neuronal stem cells on spinal cord injury. For 40 Sprague-Dawley rats (n=10 in each group) transverse spinal cord resections at the T8-9 level were carried out, leaving an approximately 2-mm gap between the distal and proximal ends of the cord. Neuronal stem cells embedded in fibrin glue treated with or without G-CSF (50 mug/kgx5 days) (groups III and IV) or fibrin glue with or without G-CSF (50 mug/kgx5 days) (groups I and II) were transplanted into the gap in the injured spinal cord. Spinal cord regeneration was assessed using a clinical locomotor rating scale scores and electrophysiological, histological and immunohistochemical analysis 3 months after injury. Regeneration was more advanced in group IV than in groups III or II according to the clinical motor score, motor evoked potential, and conduction latency. Most advanced cord regeneration across the gap was observed in group IV rats. Higher densities of bromodeoxyuridine in the injured area and higher expression levels of Neu-N and MAP-2 over the distal end of the injured spinal cord were observed in group IV compared with groups II or III, but there was no significant difference in expression of glial fibrillary acid protein. This synergy between G-CSF and neuronal stem cells may be due to increased proliferation of progenitor cells in the injured area and increased expression of neuronal stem cell markers extrinsically or intrinsically in the distal end of injured cord.

**21) Enhancing neurite outgrowth from primary neurones and neural stem cells using thermoresponsive hydrogel scaffolds for the repair of spinal cord injury.**

Nisbet DR, Moses D, Gengenbach TR, Forsythe JS, Finkelstein DI, Horne MK. **J Biomed Mater Res A**. 2008 Apr 10 [Epub ahead of print]

In this study, thermoresponsive xyloglucan hydrogel scaffolds were investigated as candidates for neural tissue engineering of the spinal cord. The hydrogels were optimized to provide similar mechanical properties to that of native spinal cord, although also being functionalized through the immobilization of poly-D-lysine to promote neurone adhesion and neurite outgrowth. Under 2D and 3D culture conditions, xyloglucan scaffolds supported the differentiation of primary cortical neurones. Furthermore, functionalization provided a means of controlling and optimizing the cell diameter, number, migration and the neurite density, and the direction of growth. The interaction of neural stem cells (NSCs) was also investigated on the xyloglucan scaffolds in vitro. The survival of the NSCs and the axonal extensions on the scaffolds were similar to that of the primary cortical neurones. These findings suggest that xyloglucan-based materials are suitable for providing a neurotrophic milieu.

**22) Gene-Modified Mesenchymal Stem Cells Express Functionally Active Nerve Growth Factor on an Engineered Poly Lactic Glycolic Acid (PLGA) Substrate.**

Rooney GE, Moran C, McMahon SS, Ritter T, Maenz M, Flügel A, Dockery P, O'Brien T, Howard L, Windebank AJ, Barry FP. **Tissue Eng Part A**. 2008 Apr 11 [Epub ahead of print]

Delivery of cellular and/or trophic factors to the site of injury may promote neural repair or regeneration and return of function after peripheral nerve or spinal cord injury. Engineered scaffolds provide a platform to deliver therapeutic cells and neurotrophic molecules. We have genetically engineered mesenchymal stem cells (MSCs) from the green rat (CZ-004 [SD TgN(act-EGFP)OsbCZ-004]) to express nerve growth factor (NGF) using an adenoviral vector. Cells maintained their stem cell phenotype as judged by expression of CD71 and CD172 markers, and absence of the hematopoietic marker CD45. Cells continued to express green fluorescent protein (GFP) on a long-term basis. Morphology, viability, and growth kinetics were maintained when cells were grown on a poly-lactic-co-

glycolic acid (PLGA) polymer scaffold. Under appropriate growth conditions, they differentiated into chondrogenic, osteogenic, and adipogenic phenotypes, demonstrating that they retained their characteristics as MSCs. NGF was secreted from transduced MSCs at physiologically relevant levels (approximately 25 ng/mL) measured by enzyme-linked immunoabsorbent assay (ELISA). Secreted NGF was functionally active in a neurite growth assay with PC12 cells. We conclude that MSCs are a good candidate for delivery of therapeutic factors into the injured nervous system. They are autologous, may be genetically modified to express neurotrophins, and are compatible with polymer surfaces that may be used as a potential delivery system.

**23) Prominent role of the spinal central pattern generator in the recovery of locomotion after partial spinal cord injuries.**

Barrière G, Leblond H, Provencher J, Rossignol S. **J Neurosci**. 2008 Apr 9;28(15):3976-87.

The re-expression of hindlimb locomotion after complete spinal cord injuries (SCIs) is caused by the presence of a spinal central pattern generator (CPG) for locomotion. After partial SCI, however, the role of this spinal CPG in the recovery of hindlimb locomotion in the cat remains mostly unknown. In the present work, we devised a dual-lesion paradigm to determine its possible contribution after partial SCI. After a partial section of the left thoracic segment T10 or T11, cats gradually recovered voluntary quadrupedal locomotion. Then, a complete transection was performed two to three segments more caudally (T13-L1) several weeks after the first partial lesion. Cats that received intensive treadmill training after the partial lesion expressed bilateral hindlimb locomotion within hours of the complete lesion. Untrained cats however showed asymmetrical hindlimb locomotion with the limb on the side of the partial lesion walking well before the other hindlimb. Thus, the complete spinalization revealed that the spinal CPG underwent plastic changes after the partial lesions, which were shaped by locomotor training. Over time, with further treadmill training, the asymmetry disappeared and a bilateral locomotion was reinstated. Therefore, although remnant intact descending pathways must contribute to voluntary goal-oriented locomotion after partial SCI, the recovery and re-expression of the hindlimb locomotor pattern mostly results from intrinsic changes below the lesion in the CPG and afferent inputs.

**24) Developmental changes in the fidelity and short term plasticity of GABAergic synapses in the neonatal rat dorsal horn.**

Ingram RA, Fitzgerald M, Baccei ML. **J Neurophysiol**. 2008 Apr 9 [Epub ahead of print]

The lower thresholds and increased excitability of dorsal horn neurones in the neonatal rat suggest that inhibitory processing is less efficient in the immature spinal cord. This is unlikely to be explained by an absence of functional GABAergic inhibition as antagonism of GABAARs augments neuronal firing in vivo from the first days of life (Bremner et al. 2006). However, it is possible that more subtle deficits in GABAergic signaling exist in the neonate, such as decreased reliability of transmission or greater depression during repetitive stimulation, both of which could influence the relative excitability of the immature spinal cord. To address this issue we examined monosynaptic GABAergic inputs onto superficial dorsal horn neurones using whole cell patch clamp recordings made in spinal cord slices at a range of postnatal ages (P3, P10 and P21). The amplitudes of evoked IPSCs were significantly lower and showed greater variability in younger animals, suggesting a lower fidelity of GABAergic signaling at early postnatal ages. Paired-pulse ratios were similar throughout the postnatal period, while trains of stimuli (1, 5, 10 and 20 Hz) revealed frequency-dependent short-term depression (STD) of IPSCs at all ages. Although the magnitude of STD did not differ between ages, the recovery from depression was significantly slower at immature GABAergic synapses. These properties may affect the integration of synaptic inputs within developing superficial dorsal horn neurones and thus contribute to their larger receptive fields and enhanced afterdischarge.

**25) No evidence for chronic demyelination in spared axons after spinal cord injury in a mouse.**

Lasiene J, Shupe L, Perlmutter S, Horner P. **J Neurosci**. 2008 Apr 9;28(15):3887-96.

The pattern of remyelination after traumatic spinal cord injury remains elusive, with animal and human studies reporting partial to complete demyelination followed by incomplete remyelination. In the present study, we found that spared rubrospinal tract (RST) axons of passage traced with actively transported dextrans and examined caudally to the lesion 12 weeks after mouse spinal cord contusion injury were fully remyelinated. Spared axons exhibited a marginally reduced myelin thickness and significantly shorter internodes. CASPR (contactin-associated protein) and K(v)1.2 channels were used to identify internodes and paranodal protein distribution properties were used as an index of myelin integrity. This is the first time the CNS myelin internode length was measured in a mouse. To better understand the significance of shortened internodes and thinner myelin in spared axons, we modeled conduction properties using McIntyre's et al. model of myelinated axons. Mathematical modeling predicted a 21% decrease in the conduction velocity of remyelinated RST axons attributable to shortened internodes. To determine whether demyelination could be present on axons exhibiting a pathological transport system, we used the retroviral reporter system. Virally delivered green fluorescent protein unveiled a small population of dystrophic RST axons that persist chronically with evident demyelination or abnormal remyelination. Collectively, these data show that lasting demyelination in spared axons is rare and that remyelination of axons of passage occurs in the chronically injured mouse spinal cord.

**26) Axonal growth therapeutics: regeneration or sprouting or plasticity?**

Cafferty WB, McGee AW, Strittmatter SM. **Trends Neurosci**. 2008 Apr 4 [Epub ahead of print]

Loss of function after neurological injury frequently occurs through the interruption of axonal connectivity, rather than through cell loss. Functional deficits persist because a multitude of inhibitory factors in degenerating myelin and astroglial scar prevent axonal growth in the adult brain and spinal cord. Given the high clinical significance of achieving functional recovery through axonal growth, substantial research effort has been, and will be, devoted toward this desirable goal. Unfortunately, the labels commonly used in the literature to categorize post-injury axonal anatomy might hinder advancement. In this article, we present an argument for the importance of developing precise terms that describe axonal growth in terms of the inciting event, the distance of axonal extension and the timing of axonal growth. The phenotypes produced by molecular interventions that overcome astroglial scar or myelin-associated inhibitors are reframed and discussed in this context.

**27) Assessing the capacity of the sympathetic nervous system to respond to a cardiovascular challenge in human spinal cord injury.**

Brown R, Macefield VG. **Spinal Cord**. 2008 Apr 8 [Epub ahead of print]

Study design:Measurement of haemodynamic responses and cutaneous blood flow during an inspiratory-capacity apnoea following spinal cord injury (SCI).Objective:To assess the capacity of the sympathetic nervous system to respond to a cardiovascular challenge following SCI.Setting:Prince of Wales Medical Research Institute, Australia.Subjects:Thirteen spinal cord injured subjects with injuries ranging from C5-T8 and eight able-bodied control subjects.Methods:Continuous blood pressure, an electrocardiogram, respiration and cutaneous blood flow were recorded during a static maximum inspiratory breath-hold for 40 s.Results:On average, systolic blood pressure decreased 26% from baseline in the spinal group during the breath-hold and remained below baseline throughout the entire apnoeic period. Heart rate in this group had an initial decrease from baseline but quickly increased throughout the breath-hold, being 17% above baseline in the recovery period. Systolic pressure in the control group decreased 12% from baseline at the beginning of the breath-hold but quickly stabilized for the remainder of the apnoea, with heart rate initially decreasing 22% and remaining below baseline throughout the breath-hold.Conclusion:A maximal inspiratory breath-hold, which is known to cause a sustained increase in muscle sympathetic nerve activity, is a simple test to perform in supine spinal cord-injured subjects, and provides information on the capacity of muscle and splanchnic vasoconstrictor activity to increase blood pressure in SCI. A sustained decrease in blood pressure,

coupled with an increase in heart rate, infers interruption of sympathetic vasoconstrictor pathways to muscle and splanchnic vascular beds.

**28) Delivery of AAV-IGF-1 to the CNS Extends Survival in ALS Mice Through Modification of Aberrant Glial Cell Activity.**

Dodge JC, Haidet AM, Yang W, Passini MA, Hester M, Clarke J, Roskelley EM, Treleaven CM, Rizo L, Martin H, Kim SH, Kaspar R, Taksir TV, Griffiths DA, Cheng SH, Shihabuddin LS, Kaspar BK. **Mol Ther**. 2008 Apr 1 [Epub ahead of print]

Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease of the motor system. Recent work in rodent models of ALS has shown that insulin-like growth factor-1 (IGF-1) slows disease progression when delivered at disease onset. However, IGF-1's mechanism of action along the neuromuscular axis remains unclear. In this study, symptomatic ALS mice received IGF-1 through stereotaxic injection of an IGF-1-expressing viral vector to the deep cerebellar nuclei (DCNs), a region of the cerebellum with extensive brain stem and spinal cord connections. We found that delivery of IGF-1 to the central nervous system (CNS) reduced ALS neuropathology, improved muscle strength, and significantly extended life span in ALS mice. To explore the mechanism of action of IGF-1, we used a newly developed in vitro model of ALS. We demonstrate that IGF-1 is potently neuroprotective and attenuates glial cell-mediated release of tumor necrosis factor-alpha (TNF-alpha) and nitric oxide (NO). Our results show that delivering IGF-1 to the CNS is sufficient to delay disease progression in a mouse model of familial ALS and demonstrate for the first time that IGF-1 attenuates the pathological activity of non-neuronal cells that contribute to disease progression. Our findings highlight an innovative approach for delivering IGF-1 to the CNS.

**29) Self-assembling nanofibers inhibit glial scar formation and promote axon elongation after spinal cord injury.**

Tysseling-Mattiace VM, Sahni V, Niece KL, Birch D, Czeisler C, Fehlings MG, Stupp SI, Kessler JA. **J Neurosci**. 2008 Apr 2;28(14):3814-23.

Peptide amphiphile (PA) molecules that self-assemble in vivo into supramolecular nanofibers were used as a therapy in a mouse model of spinal cord injury (SCI). Because self-assembly of these molecules is triggered by the ionic strength of the in vivo environment, nanoscale structures can be created within the extracellular spaces of the spinal cord by simply injecting a liquid. The molecules are designed to form cylindrical nanofibers that display to cells in the spinal cord the laminin epitope IKVAV at nearly van der Waals density. IKVAV PA nanofibers are known to inhibit glial differentiation of cultured neural stem cells and to promote neurite outgrowth from cultured neurons. In this work, in vivo treatment with the PA after SCI reduced astrogliosis, reduced cell death, and increased the number of oligodendroglia at the site of injury. Furthermore, the nanofibers promoted regeneration of both descending motor fibers and ascending sensory fibers through the lesion site. Treatment with the PA also resulted in significant behavioral improvement. These observations demonstrate that it is possible to inhibit glial scar formation and to facilitate regeneration after SCI using bioactive three-dimensional nanostructures displaying high densities of neuroactive epitopes on their surfaces.

**30) Single-Dose Application of CNTF and BDNF Improves Remyelination of Regenerating Nerve Fibers after C7 Ventral Root Avulsion and Replantation.**

Lang EM, Schlegel N, Reiners K, Hofmann GO, Sendtner M, Asan E. **J Neurotrauma**. 2008 Apr;25(4):384-400.

ABSTRACT Although axonal regeneration has been observed after replantation of avulsed ventral roots (VR) into the spinal cord, the functional outcome of this treatment in terms of motor reinnervation is unsatisfactory. In the present study, effects of single-dose ciliary and/or brain-derived neurotrophic factor (CNTF, BDNF) application on axon regeneration after C7 VR avulsion and replantation in adult rabbits were morphologically assessed by analysis of numbers, calibers, and myelination of axons in replanted VRs. Electromyography (EMG) was carried out to document the time course of de- and

reinnervation in individual animals. After 3 weeks, replanted C7 VRs were almost devoid of myelinated axons. At week 8, active EMG-denervation was confirmed in affected muscles, but was less pronounced in neurotrophic factor (NF)-treated animals than in controls. Reinnervation potentials were identified in paraspinal muscles in more NF-treated animals than in controls. After 6 months, the number of myelinated axons in replanted VRs was approximately 45% of that in unlesioned roots in all groups, with small-sized axons constituting the majority of axons. At this time, more NF-treated animals than controls featured reinnervation. Moreover, myelination deficits of regenerated axons in controls were less pronounced in NF-treated animals. Especially in CNTF + BDNF-treated animals, myelination of regenerated axons of specific sizes was significantly increased compared to regenerated controls. In summary, NFs stimulated reinnervation early after the lesion and, for the first time, our morphological data quantitatively indicate positive effects of CNTF + BDNF on remyelination.

**31) A graded forceps crush spinal cord injury model in mice.**

Plemel JR, Duncan G, Chen KW, Shannon C, Park S, Sparling JS, Tetzlaff W. **J Neurotrauma**. 2008 Apr;25(4):350-70.

ABSTRACT Given the rising availability and use of genetically modified animals in basic science research, it has become increasingly important to develop clinically relevant models for spinal cord injury (SCI) for use in mice. We developed a graded forceps crush model of SCI in mice that uses three different forceps with spacers of 0.25, 0.4, and 0.55 mm, to produce severe, moderate, and mild injuries, respectively. Briefly, each mouse was subjected to laminectomy of T5-T7, 15-second spinal cord crush using one of those forceps, behavioral assessments, and post-mortem neuroanatomical analyses. There were significant differences among the three injury severity groups on behavioral measures (Basso Mouse Score, footprint, and ladder analyses), demonstrating an increase in neurological deficits for groups with greater injury severity. Quantitative analysis of the lesion demonstrated that as injury severity increased, lesion size and GFAP negative area increased, and spared tissue, spinal cord cross-sectional area, spared grey matter and spared white matter decreased. These measures strongly correlated with the behavioral outcomes. Similar to other studies of SCI in mice, we report a dense laminin and fibronectin positive extracellular matrix in the lesion sites of injured mice, but unlike those previous studies, we also report the presence of numerous p75 positive Schwann cells in and around the lesion epicenter. These results provide evidence that the graded forceps crush model is an attractive alternative for the study of SCI and related therapeutic interventions. Because of its demonstrated consistency, ease of use, low cost, and clinical relevance, this graded forceps crush is an attractive alternative to the other mouse models of SCI currently available.

**32) Single, high-dose intraspinal injection of chondroitinase reduces glycosaminoglycans in injured spinal cord and promotes corticospinal axonal regrowth after hemisection but not contusion.**

Iseda T, Okuda T, Kane-Goldsmith N, Mathew M, Ahmed S, Chang YW, Young W, Grumet M. **J Neurotrauma**. 2008 Apr;25(4):334-49.

ABSTRACT Chondroitin sulfate proteoglycans (CSPGs) inhibit axonal growth, and treatment with chondroitinase ABC promotes axonal regeneration in some models of central nervous system (CNS) injury. The aims of this study were (1) to compare the spatiotemporal appearance of CSPG expression between spinal cord contusion and hemisection models, and (2) to evaluate chondroitinase treatment effects on axonal regrowth in the two injury models. After hemisection, CSPG-immunoreactivity (IR) in the injury site rose to peak levels at 18 days but then decreased dramatically by 49 days; in contrast, CSPG-IR remained high for at least 49 days after contusion. After hemisection, many anterogradely labeled corticospinal tract (CST) axons remained close to CSPG-rich lesion sites, but after contusion, most CST axons retracted by approximately 1 mm rostral from the rostral-most CSPG-rich cyst. Intraspinal injection of chondroitinase at 0, 1, 2, and 4 weeks following injury dramatically reduced CSPG-IR in both injury models within 4 days, and CSPG-IR remained low for at least 3 weeks. After the chondroitinase treatment, many axons grew around the lesion site in hemisected spinal cords but not in contused spinal cords. We propose that improved axonal

growth in hemisected spinal cords is due to decreased inhibition resulting from degradation of CSPGs located adjacent to severed CST axons. However, in spinal cord contusions, retracted CST axons fail to grow across gliotic regions that surround CSPG-rich injury sites despite efficient degradation with chondroitinase, suggesting that other inhibitors of axonal growth persist in the gliotic regions.

**33) Toll-like receptor 3 contributes to spinal glial activation and tactile allodynia after nerve injury.**

Obata K, Katsura H, Miyoshi K, Kondo T, Yamanaka H, Kobayashi K, Dai Y, FukuokaT, Akira S, Noguchi K. **J Neurochem**. 2008 Apr 9 [Epub ahead of print]

Toll-like receptors (TLRs) play an essential role in innate immune responses and in the initiation of adaptive immune responses. Microglia, the resident innate immune cells in the CNS, express TLRs. In this study, we show that TLR3 is crucial for spinal cord glial activation and tactile allodynia after peripheral nerve injury. Intrathecal administration of TLR3 antisense oligodeoxynucleotide suppressed nerve injury-induced tactile allodynia, and decreased the phosphorylation of p38 mitogen-activated protein kinase, but not extracellular signal-regulated protein kinases 1/2, in spinal glial cells. Antisense knockdown of TLR3 also attenuated the activation of spinal microglia, but not astrocytes, caused by nerve injury. Furthermore, down-regulation of TLR3 inhibited nerve injury-induced up-regulation of spinal pro-inflammatory cytokines, such as interleukin-1beta, interleukin-6, and tumor necrosis factor-alpha. Conversely, intrathecal injection of the TLR3 agonist polyinosine-polycytidylic acid induced behavioral, morphological, and biochemical changes similar to those observed after nerve injury. Indeed, TLR3-deficient mice did not develop tactile allodynia after nerve injury or polyinosine-polycytidylic acid injection. Our results indicate that TLR3 has a substantial role in the activation of spinal glial cells and the development of tactile allodynia after nerve injury. Thus, blocking TLR3 in the spinal glial cells might provide a fruitful strategy for treating neuropathic pain.

**34) Prox1 regulates a transitory state for interneuron neurogenesis in the spinal cord.**

Misra K, Gui H, Matise MP. **Dev Dyn**. 2008 Apr;237(4):1214.

No abstract

**35) ALS-causing SOD1 mutants generate vascular changes prior to motor neuron degeneration.**

Zhong Z, Deane R, Ali Z, Parisi M, Shapovalov Y, O'Banion MK, Stojanovic K, Sagare A, Boillee S, Cleveland DW, Zlokovic BV. **Nat Neurosci**. 2008 Apr;11(4):420-2. Epub 2008 Mar 16.

We report here that amyotrophic lateral sclerosis-linked superoxide dismutase 1 (SOD1) mutants with different biochemical characteristics disrupted the blood-spinal cord barrier in mice by reducing the levels of the tight junction proteins ZO-1, occludin and claudin-5 between endothelial cells. This resulted in microhemorrhages with release of neurotoxic hemoglobin-derived products, reductions in microcirculation and hypoperfusion. SOD1 mutant-mediated endothelial damage accumulated before motor neuron degeneration and the neurovascular inflammatory response occurred, indicating that it was a central contributor to disease initiation.

**36) Transforming growth factor-beta1 regulates the fate of cultured spinal cord-derived neural progenitor cells.**

Park SM, Jung JS, Jang MS, Kang KS, Kang SK. **Cell Prolif**. 2008 Apr;41(2):248-64.

OBJECTIVES: We have evaluated the physiological roles of transforming growth factor-beta1 (TGF-beta1) on differentiation, migration, proliferation and anti-apoptosis characteristics of cultured spinal cord-derived neural progenitor cells. METHODS: We have used neural progenitor cells that had been isolated and cultured from mouse spinal cord tissue, and we also assessed the relevant reaction mechanisms using an activin-like kinase (ALK)-specific inhibitory system including an inhibitory RNA,

and found that it involved potential signalling molecules such as phosphatidylinositol-3-OH kinase (PI3K)/Akt and mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK1/2). RESULTS AND CONCLUSIONS: Transforming growth factor-beta1-mediated cell population growth was activated after treatment and was also effectively blocked by an ALK41517-synthetic inhibitor (4-(5-benzo(1,3) dioxol-5-yl-4-pyridine-2-yl-1H-imidazole-2-yl) benzamide (SB431542) and ALK siRNA, thereby indicating the involvement of SMAD2 in the TGF-beta1-mediated growth and migration of these neural progenitors cells (NPC). In the present study, TGF-beta1 actively induced NPC migration in vitro. Furthermore, TGF-beta1 demonstrated extreme anti-apoptotic behaviour against hydrogen peroxide-mediated apoptotic cell death. At low dosages, TGF-beta1 enhanced (by approximately 76%) cell survival against hydrogen peroxide treatment via inactivation of caspase-3 and -9. TGF-beta1-treated NPCs down-regulated Bax expression and cytochrome c release; in addition, the cells showed up-regulated Bcl-2 and thioredoxin reductase 1. They also had increased p38, Akt and ERK1/2 phosphorylation, showing the involvement of both the PI3K/Akt and MAPK/ERK1/2 pathways in the neuroprotective effects of TGF-beta1. Interestingly, these effects operate on specific subtypes of cells, including neurones, neural progenitor cells and astrocytes in cultured spinal cord tissue-derived cells. Lesion sites of spinal cord-overexpressing TGF-beta1-mediated prevention of cell death, cell growth and migration enhancement activity have been introduced as a possible new basis for therapeutic strategy in treatment of neurodegenerative disorders, including spinal cord injuries.

**37) Effect of 17beta-estradiol on functional outcome, release of cytokines, astrocyte reactivity and inflammatory spreading after spinal cord injury in male rats.**

Ritz MF, Hausmann ON. **Brain Res**. 2008 Apr 8;1203:177-88. Epub 2008 Feb 13.

The effect of 17beta-estradiol on the secondary damage following spinal cord injury (SCI) was examined in male rats subjected to moderate compression. Two doses of 17beta-estradiol (0.1 or 4 mg/kg) were injected i.p. immediately after spinal cord compression. Functional outcome was observed during 4 weeks following injury with two different tests. Release of cytokines (IL-1alpha, IL-1beta and IL-6) was assessed 6 h, 3 days and 1 week post-injury. Reactive astrocytes expressing the glial fibrillary acidic protein GFAP and vimentin, and diffusion of CD68-positive inflammatory cells were examined from 3 days to 4 weeks following SCI. Treatment with 17beta-estradiol significantly increased locomotor function from the first week until 4 weeks post-SCI. The injured spinal cord of 17beta-estradiol-treated rats expressed more IL-1alpha, IL-1beta and IL-6 than controls 6 h after injury. Moreover, 17beta-estradiol-treated rats showed reactive astrocytes as soon as 3 days following SCI, with increased GFAP expression, smaller lesion areas and more limited diffusion of CD68-positive cells after 1 week post-injury compared to controls. The number of CD68-positive cells was also reduced in 17beta-estradiol-treated rats one week post-SCI. However, these differences between 17beta-estradiol-treated and control rats disappeared after 4 weeks. These results suggest that 17beta-estradiol protects the spinal cord by stimulating early cytokines release and astroglial responses. These stimulations may prevent the area of damage from expanding and inflammatory cells to spread in the surrounding tissue during the critical first week following SCI. Although transient, these effects improved the locomotor recovery that was sustained over 4 weeks after injury.

**38) Detrimental effects of antiapoptotic treatments in spinal cord injury.**

Cittelly DM, Nesic O, Johnson K, Hulsebosch C, Perez-Polo JR. **Exp Neurol**. 2008 Apr;210(2):295-307. Epub 2007 Mar 7.

Long-term functional impairments due to spinal cord injury (SCI) in the rat result from secondary apoptotic death regulated, in part, by SCI-induced decreases in protein levels of the antiapoptotic protein Bcl-x(L). We have shown that exogenous administration of Bcl-x(L) spares neurons 24 h after SCI. However, long-term effects of chronic application of Bcl-x(L) have not been characterized. To counteract SCI-induced decreases in Bcl-x(L) and resulting apoptosis, we used the TAT protein transduction domain fused to the Bcl-x(L) protein (Tat-Bcl-x(L)), or its antiapoptotic domain BH4 (Tat-BH4). We used intrathecal delivery of Tat-Bcl-x(L), or Tat-BH4, into injured spinal cords for 24 h or 7 days, and apoptosis, neuronal death and locomotor recovery were assessed up to 2 months after

injury. Both, Tat-Bcl-x(L) and Tat-BH4, significantly decreased SCI-induced apoptosis in thoracic segments containing the site of injury (T10) at 24 h or 7 days after SCI. However, the 7-day delivery of Tat-Bcl-x(L), or Tat-BH4, also induced a significant impairment of locomotor recovery that lasted beyond the drug delivery time. We found that the 7-day administration of Tat-Bcl-x(L), or Tat-BH4, significantly increased non-apoptotic neuronal loss and robustly augmented microglia/macrophage activation. These results indicate that the antiapoptotic treatment targeting Bcl-x(L) shifts neuronal apoptosis to necrosis, increases the inflammatory response and impairs locomotor recovery. Our results suggest that a combinatorial treatment consisting of antiapoptotic and anti-inflammatory agents may be necessary to achieve tissue preservation and significant improvement in functional recovery after SCI.

**39) The biological activity of 3alpha-hydroxysteroid oxido-reductase in the spinal cord regulates thermal and mechanical pain thresholds after sciatic nerve injury.**

Meyer L, Venard C, Schaeffer V, Patte-Mensah C, Mensah-Nyagan AG. **Neurobiol Dis**. 2008 Apr;30(1):30-41. Epub 2007 Dec 14.

Identification of cellular targets pertinent for the development of effective therapies against pathological pain constitutes a difficult challenge. We combined several approaches to show that 3alpha-hydroxysteroid oxido-reductase (3alpha-HSOR), abundantly expressed in the spinal cord (SC), is a key target, the modulation of which markedly affects nociception. 3alpha-HSOR catalyzes the biosynthesis and oxidation of 3alpha,5alpha-reduced neurosteroids as allopregnanolone (3alpha,5alpha-THP), which stimulates GABA(A) receptors. Intrathecal injection of Provera (pharmacological inhibitor of 3alpha-HSOR activity) in naive rat SC decreased thermal and mechanical nociceptive thresholds assessed with behavioral methods. In contrast, pain thresholds were dose-dependently increased by 3alpha,5alpha-THP. In animals subjected to sciatic nerve injury-evoked neuropathic pain, molecular and biochemical experiments revealed an up-regulation of 3alpha-HSOR reductive activity in the SC. Enhancement of 3alpha,5alpha-THP concentration in the SC induced analgesia in neuropathic rats while Provera exacerbated their pathological state. Possibilities are opened for chronic pain control with drugs modulating 3alpha-HSOR activity in nerve cells.

**40) Administration of phosphodiesterase inhibitors and an adenosine A1 receptor antagonist induces phrenic nerve recovery in high cervical spinal cord injured rats.**

Kajana S, Goshgarian HG. **Exp Neurol**. 2008 Apr;210(2):671-80. Epub 2008 Jan 5.

High cervical spinal cord hemisection interrupts the descending respiratory drive from the medulla to the ipsilateral phrenic motoneurons, consequently leading to the paralysis of the ipsilateral hemidiaphragm. Previous studies have shown that chronic oral administration of theophylline, a phosphodiesterase inhibitor and an adenosine receptor antagonist, can restore function to the quiescent phrenic nerve and hemidiaphragm ipsilateral to hemisection. Both of these actions of theophylline result in an increase in 3'-5'-cyclic adenosine monophosphate (cAMP). Furthermore, the chronic theophylline-mediated respiratory recovery persists long after the animals have been weaned from the drug. To date, the precise cellular mechanisms underlying the recovery induced by theophylline are still not known. Since theophylline has two modes of action, in the present study we tested whether chronic administration of pentoxifylline, a non-selective phosphodiesterase inhibitor, rolipram, a phosphodiesterase-4 specific inhibitor, and 8-cyclopentyl-1,3-dipropylxanthine (DPCPX), an adenosine A1 receptor antagonist, would induce recovery similar to that induced by theophylline in male Sprague-Dawley rats following a left C2 spinal cord lesion. Recovery of left phrenic nerve activity was assessed at 5 or 10 days after the last drug administrations to assess the persistent nature of the recovery. Pentoxifylline, rolipram and DPCPX, all capable of modulating 3',5'-cyclic monophosphate (cAMP) levels, brought about long-term respiratory recovery in the phrenic nerve ipsilateral to the left C2 lesion at 5 and 10 days after the last drug administration. Therefore, these results suggest that compounds capable of regulating cAMP levels may be therapeutically useful in promoting functional recovery following spinal cord injury.

**41) Greatly improved neurological outcome after spinal cord compression injury in AQP4-deficient mice.**

Saadoun S, Bell BA, Verkman AS, Papadopoulos MC. **Brain**. 2008 Apr;131(Pt 4):1087-98. Epub 2008 Feb 11.

Aquaporin-4 (AQP4) is a water channel protein expressed in astrocytes throughout the CNS. In brain, AQP4 facilitates water balance and glial scar formation, which are important determinants of outcome after injury. Here, we provide evidence for AQP4-dependent spinal cord swelling following compression injury, resulting in remarkably improved outcome in AQP4-null mice. Two days after transient T6 spinal cord compression injury, wild-type mice developed more severe hindlimb weakness than AQP4-null mice, as assayed by the Basso open-field motor score, inclined plane method and footprint analysis. Basso motor scores were 1.3 +/- 0.5 (wild-type) versus 4.9 +/- 0.6 (AQP4-null) (SE, $P < 0.001$). Improved motor outcome in AQP4-null mice was independent of mouse strain and persisted at least 4 weeks. AQP4-null mice also had improved sensory outcome at 2 days, as assessed by spinal somatosensory evoked responses, with signal amplitudes approximately 10 microV (uninjured), 1.7 +/- 0.7 microV (wild-type) and 6.4 +/- 1.3 microV (AQP4-null) ($P < 0.01$). The improved motor and sensory indices in AQP4-null mice corresponded to remarkably less neuronal death and myelin vacuolation, as well as reduced spinal cord swelling and intraparenchymal spinal cord pressure measured at T6 at 2 days after injury. AQP4 immunoreactivity at the injury site was increased in grey and white matter at 48 h. Taken together, our findings indicate that AQP4 provides a major route for excess water entry into the injured spinal cord, which in turn causes spinal cord swelling and elevated spinal cord pressure. Our data suggest AQP4 inhibition or downregulation as novel early neuroprotective manoeuvres in spinal cord injury.

**42) Interplay between neuromodulator-induced switching of short-term plasticity at sensorimotor synapses in the neonatal rat spinal cord.**

Barrière G, Tartas M, Cazalets JR, Bertrand SS. **J Physiol**. 2008 Apr 1;586(7):1903-20. Epub 2008 Feb 7.

In the present study, we investigated the modulation of short-term depression (STD) at synapses between sensory afferents and rat motoneurons by serotonin, dopamine and noradrenaline. STD was elicited with trains of 15 stimuli at 1, 5 and 10 Hz and investigated using whole-cell voltage-clamp recordings from identified motoneurons in the neonatal rat spinal cord in vitro. STD was differentially modulated by the amines. Dopamine was effective at all stimulation frequencies, whereas serotonin affected STD only during 5 and 10 Hz stimulus trains and noradrenaline during 1 and 5 Hz trains. Dopamine and serotonin homogenized the degree of depression observed with the different stimulation modalities, in contrast to noradrenaline, which amplified the rate differences. The different modulatory profiles observed with the amines were partly due to GABAergic interneuron activity. In the presence of GABA(A) and GABA(B) receptor antagonists, the rate and/or kinetics of STD did not vary with the stimulation frequency in contrast to the control condition, and noradrenaline failed to alter either synaptic amplitude or STD, suggesting indirect actions. Dopamine and serotonin strongly decreased STD and converted depression to facilitation at 5 and 10 Hz during the blockade of the GABAergic receptors in 50% of the neurons tested. Altogether, these results show that STD expressed at sensorimotor synapses in the neonatal rat not only is a function of the frequency of afferent firing but also closely depends on the neuromodulatory state of these connections, with a major contribution from GABAergic transmission.

**43) Spatial distribution and acute anti-inflammatory effects of Methylprednisolone after sustained local delivery to the contused spinal cord.**

Chvatal SA, Kim YT, Bratt-Leal AM, Lee H, Bellamkonda RV. **Biomaterials**. 2008 Apr;29(12):1967-75. Epub 2008 Feb 5.

Methylprednisolone (MP) has been shown to reduce acute inflammation resulting from a secondary damage cascade initiated by the primary physical injury to the spinal cord. The current clinical practice for delivering systemic MP is inefficient, and high doses are required, resulting in adverse, undesired,

dose-related side effects in patients. Here, we report a novel, minimally invasive, localized drug delivery system for delivering MP to the contused adult rat spinal cord that potentially side-steps the deleterious consequences of systemic cortico-steroid therapy. MP was encapsulated in biodegradable PLGA based nanoparticles (NP), and these nanoparticles were embedded in an agarose hydrogel for localization to the site of contusion injury. To visualize and quantify its spatial distribution within the injured spinal cord, MP was conjugated to Texas-red cadaverine prior to encapsulation in nanoparticles. When delivered via the hydrogel-nanoparticle system, MP entered the injured spinal cord and diffused up to 1.5mm deep and up to 3mm laterally into the injured spinal cord within 2 days. Furthermore, topically delivered MP significantly decreased early inflammation inside the contusion injured spinal cord as evidenced by a significant reduction in the number of ED-1(+) macrophages/activated microglia. This decreased early inflammation was accompanied by a significantly diminished expression of pro-inflammatory proteins including Calpain and iNOS. Additionally, topically delivered MP significantly reduced lesion volume 7 days after contusion injury. The minimally invasive MP delivery system reported in this study has the potential to enhance the effectiveness of MP therapy after contusion injury to the spinal cord and avoid the side effects arising from high dose cortico-steroid therapy.

**44) Fibronectin/integrin system is involved in P2X(4) receptor upregulation in the spinal cord and neuropathic pain after nerve injury.**

Tsuda M, Toyomitsu E, Komatsu T, Masuda T, Kunifusa E, Nasu-Tada K, Koizumi S, Yamamoto K, Ando J, Inoue K. **Glia**. 2008 Apr;56(5):579-85.

We have previously shown that activation of the ATP-gated ion channel subtype P2X(4) receptors (P2X(4)Rs) in the spinal cord, the expression of which is upregulated in microglia after nerve injury, is necessary for producing neuropathic pain. The upregulation of P2X(4)Rs in microglia is, therefore, a key process in neuropathic pain, but the mechanism remains unknown. Here, we find a fibronectin/integrin-dependent mechanism in the upregulation of P2X(4)Rs. Microglia cultured on dishes coated with fibronectin, an extracellular matrix molecule, expressed a higher level of P2X(4)R protein when compared with those cultured on control dishes. The increase was suppressed by echistatin, a peptide that selectively blocks beta(1) and beta(3)-containing integrins, and with a function-blocking antibody of beta(1) integrin. In in vivo studies, the upregulation of P2X(4)Rs in the spinal cord after spinal nerve injury was significantly suppressed by intrathecal administration of echistatin. Tactile allodynia in response to nerve injury and intrathecal administration of ATP- and fibronectin-stimulated microglia was inhibited by echistatin. Furthermore, intrathecal administration of fibronectin in normal rats increased the level of P2X(4)R protein in the spinal cord and produced tactile allodynia. Moreover, the fibronectin-induced allodynia was not observed in mice lacking P2X(4)R. Taken together with the results of our previous study showing an increase in the spinal fibronectin level after nerve injury, the present results suggest that the fibronectin/integrin system participates in the upregulation of P2X(4)R expression after nerve injury and subsequent neuropathic pain.

**45) Inflammatory myelopathies and traumatic spinal cord lesions: comparison of functional and neurological outcomes.**

Scivoletto G, Cosentino E, Mammone A, Molinari M. **Phys Ther**. 2008 Apr;88(4):471-84. Epub 2008 Jan 24.

BACKGROUND AND PURPOSE: OUTCOMES: knowledge is essential to answer patients' questions regarding function, to plan the use of resources, and to evaluate treatments to enhance recovery. The purpose of this study was to compare the outcomes of patients with traumatic spinal cord injury (SCI) with those of patients with inflammatory spinal cord lesions (ISCLs). SUBJECTS AND METHODS: The authors evaluated 181 subjects with traumatic SCI and 67 subjects with ISCLs. Using a matching cohorts procedure, 38 subjects were selected from each group. The measures used were the American Spinal Injury Association (ASIA) Impairment Scale (motor function), the Barthel Index (BI), the Rivermead Mobility Index (RMI), and the Walking Index for Spinal Cord Injury (WISCI). RESULTS: The subjects in the ISCL group were older than those in the SCI group, with a longer interval from onset of lesion to rehabilitation admission and more incomplete lesions. In the matching cohorts, at admission, the traumatic SCI group had RMI and WISCI scores comparable to those of the ISCL

group, but the traumatic SCI group had lower scores on the BI (greater dependence on assistance for activities of daily living). At discharge, the 2 groups had comparable functional outcomes. The neurological status of the 2 groups was comparable at admission and discharge. DISCUSSION AND CONCLUSION: The results indicate that, at admission, patients with SCI have a greater physical dependence for assistance with activities of daily living than patients with ISCLs who have comparable neurological status. Such a difference depends on factors not related to the spinal cord lesion, such as the presence of associated lesions, the need to wear an orthotic device, or the sequelae of surgery. The outcomes of patients with SCI are determined more by factors such as lesion level and severity and age than by etiology. This finding could have implications for health care planning and rehabilitation research.

**46) Evidence for the role of mitogen-activated protein kinase signaling pathways in the development of spinal cord injury.**

Genovese T, Esposito E, Mazzon E, Muià C, Di Paola R, Meli R, Bramanti P, Cuzzocrea S. **J Pharmacol Exp Ther**. 2008 Apr;325(1):100-14. Epub 2008 Jan 7.

Mitogen-activated protein kinase (MAPK) signaling pathways involve two closely related MAPKs, known as extracellular signal-regulated kinase (ERK)1 and ERK2. The aim of the present study was to evaluate the contribution of MAPK3/MAPK1 in the secondary damage in experimental spinal cord injury (SCI) in mice. To this purpose, we used 2-(2-amino-3-methoxyphenyl)-4H-1-benzopyran-4-one (PD98059), which is an inhibitor of MAPK3/MAPK1. Spinal cord trauma was induced by the application of vascular clips (force of 24 g) to the dura via a four-level T5-T8 laminectomy. SCI in mice resulted in severe trauma characterized by edema, neutrophil infiltration, and production of inflammatory mediators, tissue damage, and apoptosis. PD98059 treatment (10 mg/kg i.p.) at 1 and 6 h after the SCI significantly reduced 1) the degree of spinal cord inflammation and tissue injury (histological score), 2) neutrophil infiltration (myeloperoxidase activity), 3) nitrotyrosine formation, 4) proinflammatory cytokines expression, 5) nuclear factor-kappaB activation, 6) phospho-ERK1/2 expression, and 6) apoptosis (terminal deoxynucleotidyl transferase dUTP nick-end labeling staining, Fas ligand, Bax, and Bcl-2 expression). Moreover, PD98059 significantly ameliorated the recovery of limb function (evaluated by motor recovery score) in a dose-dependent manner. Taken together, our results clearly demonstrate that PD98059 treatment reduces the development of inflammation and tissue injury associated with spinal cord trauma.

**47) Therapeutic time window for the application of chondroitinase ABC after spinal cord injury.**

García-Alías G, Lin R, Akrimi SF, Story D, Bradbury EJ, Fawcett JW. **Exp Neurol**. 2008 Apr;210(2):331-8. Epub 2007 Nov 21.

Rats with a crush in the dorsal funiculi of the C4 segment of the spinal cord were treated with chondroitinase ABC delivered to the lateral ventricle, receiving 6 intraventricular injections on alternate days. In order to investigate the time window of efficacy of chondroitinase, treatment was begun at the time of injury or after a 2, 4 or 7 days delay. Behavioural testing over 6 weeks showed that acutely treated animals showed improved skilled forelimb reaching compared to penicillinase controls. Forelimb contact placing recovered in treated animals but not controls, and gait analysis showed recovery towards normal forelimb stride length in treated animals but not controls. Chondroitinase-treated animals showed greater axon regeneration than controls. The treatment effect on contact placing, stride length and axon regeneration was not dependent on the timing of the start of treatment, but in skilled paw reaching acutely treated animals recovered better function. The area of chondroitinase ABC digestion visualized by stub antibody staining included widespread digestion around the lateral ventricles and partial digestion of cervical spinal cord white matter, but not grey matter.

**48) A systematic review of functional ambulation outcome measures in spinal cord injury.**

Lam T, Noonan VK, Eng JJ; the SCIRE Research Team. **Spinal Cord**. 2008 Apr;46(4):246-254. Epub 2007 Oct 9.

Study design:Systematic review.Objectives:To systematically review the psychometric properties of outcome measures used to assess ambulation in people with spinal cord injury (SCI).Setting:Vancouver, BC, Canada.Methods:A keyword literature search of original articles that evaluated the psychometric properties of ambulation outcome measures in the SCI population was conducted using multiple databases. Multidimensional scales of function were included if specific data were available on ambulation-related subscales. Reliability, validity and responsiveness values were extracted and conclusions drawn about the psychometric quality of each measure.Results:Seven outcome measures were identified and were broadly categorized into timed and categorical measures of ambulation. Timed measures included timed walking tests that showed excellent reliability, construct validity and responsiveness to change. The psychometric properties of the categorical scales were more variable, but those that were developed specifically for the SCI population had excellent reliability and validity. Categorical scales also exhibited some floor or ceiling effects.Conclusion:Excellent tools are available for measuring functional ambulation capacity. Further work is required to develop and evaluate outcome measures to include environmental factors that contribute to the ability to achieve safe, functional ambulation in everyday settings.Sponsorship:Rick Hansen Man-in-Motion Foundation and Ontario Neurotrauma Fund.

**49) Functional electrical stimulation after spinal cord injury: current use, therapeutic effects and future directions.**

Ragnarsson KT. **Spinal Cord**. 2008 Apr;46(4):255-74. Epub 2007 Sep 11.

Repair of the injured spinal cord by regeneration therapy remains an elusive goal. In contrast, progress in medical care and rehabilitation has resulted in improved health and function of persons with spinal cord injury (SCI). In the absence of a cure, raising the level of achievable function in mobility and self-care will first and foremost depend on creative use of the rapidly advancing technology that has been so widely applied in our society. Building on achievements in microelectronics, microprocessing and neuroscience, rehabilitation medicine scientists have succeeded in developing functional electrical stimulation (FES) systems that enable certain individuals with SCI to use their paralyzed hands, arms, trunk, legs and diaphragm for functional purposes and gain a degree of control over bladder and bowel evacuation. This review presents an overview of the progress made, describes the current challenges and suggests ways to improve further FES systems and make these more widely available.

**50) Spinal cord injury and mental health.**

Migliorini C, Tonge B, Taleporos G. **Aust N Z J Psychiatry**. 2008 Apr;42(4):309-14.

Objectives: The aim of the study was to examine the mental health of adults with spinal cord injury living in the community Methods: The study was a representative community cross-sectional cohort self-report survey, carried out in adults with traumatic spinal cord injury registered on the Victorian Spinal Cord Injury Register and adults with non-traumatic spinal cord injury attending a specialist non-traumatic spinal cord injury rehabilitation clinic. Participants (n=443) completed a self-report survey by internet, telephone or hard copy, which used reliable and valid measures of depression, anxiety and stress (Depression, Anxiety and Stress Scale) and post-traumatic stress disorder (Impact of Events Scale-Revised). Results: Nearly half (48.5%) of the population with spinal cord injury suffered mental health problems of depression (37%), anxiety (30%), clinical-level stress (25%) or post-traumatic stress disorder (8.4%). Overall, there was a twofold or more increase in the probability of emotional disorders compared to the general population. Of those with one mental health disorder, 60% also had at least one other emotional disorder, representing a substantial 56% increase over the general population in the probability of comorbidity of psychopathology. Better health and time since injury were associated with decreasing the risk of psychopathology. Conclusion: The results of the present study underscore the vulnerability of the population with spinal cord injury to emotional disorders. This

study highlights the complexity of mental health problems experienced by many individuals with spinal cord injury living in the community. The delivery of mental health services to this vulnerable population requires recognition of comorbidity and problems of mobility, access and stigma.

**51) GlialCAM, an immunoglobulin-like cell adhesion molecule is expressed in glial cells of the central nervous system.**

Favre-Kontula L, Rolland A, Bernasconi L, Karmirantzou M, Power C, Antonsson B, Boschert U. **Glia**. 2008 Apr 15;56(6):633-45.

Using structure based genome mining targeting vascular endothelial and platelet derived growth factor immunoglobulin (Ig) like folds, we have identified a sequence corresponding to a single transmembrane protein with two Ig domains, which we cloned from a human brain cDNA library. The cDNA is identical to hepatocyte cell adhesion molecule (hepaCAM), which was originally described as a tumor suppressor gene in liver. Here, we show that the protein is predominantly expressed in the mouse and human nervous system. In liver, the expression is very low in humans, and is not detected in mice. To identify the central nervous system (CNS) regions and cell types expressing the protein, we performed a LacZ reporter gene assay on heterozygous mice in which one copy of the gene encoding the novel protein had been replaced with beta-galactosidase. beta-galactosidase expression was prominent in white matter tracts of the CNS. Furthermore, expression was detected in ependymal cells of the brain ventricular zones and the central canal of the spinal cord. Double labeling experiments showed expression mainly in CNPase positive oligodendrocytes (OL). Since the protein is predominantly expressed in the CNS glial cells, we named the molecule glial cell adhesion molecule (GlialCAM). A potential role for GlialCAM in myelination was supported by its up-regulation during postnatal mouse brain development, where it was concomitantly expressed with myelin basic protein (MBP). In addition, in vitro, GlialCAM was observed in various developmental stages of OL and in astrocytes in processes and at cell contact sites. In A2B5 positive OL, GlialCAM colocalizes with GAP43 in OL growth cone like structures. Overall, the data presented here indicate a potential function for GlialCAM in glial cell biology. (c) 2008 Wiley-Liss, Inc.

**52) Ascl1 is required for oligodendrocyte development in the spinal cord.**

Sugimori M, Nagao M, Parras CM, Nakatani H, Lebel M, Guillemot F, Nakafuku M. **Development.** 2008 Apr;135(7):1271-81. Epub 2008 Feb 20.

Development of oligodendrocytes, myelin-forming glia in the central nervous system (CNS), proceeds on a protracted schedule. Specification of oligodendrocyte progenitors (OLPs) begins early in development, whereas their terminal differentiation occurs at late embryonic and postnatal periods. How these distinct steps are controlled remains unclear. Our previous study demonstrated an important role of the helix-loop-helix (HLH) transcription factor Ascl1 in early generation of OLPs in the developing spinal cord. Here, we show that Ascl1 is also involved in terminal differentiation of oligodendrocytes late in development. Ascl1(-/-) mutant mice showed a deficiency in differentiation of myelin-expressing oligodendrocytes at birth. In vitro culture studies demonstrate that the induction and maintenance of co-expression of Olig2 and Nkx2-2 in OLPs, and thyroid hormone-responsive induction of myelin proteins are impaired in Ascl1(-/-) mutants. Gain-of-function studies further showed that Ascl1 collaborates with Olig2 and Nkx2-2 in promoting differentiation of OLPs into oligodendrocytes in vitro. Overexpression of Ascl1, Olig2 and Nkx2-2 alone stimulated the specification of OLPs, but the combinatorial action of Ascl1 and Olig2 or Nkx2-2 was required for further promoting their differentiation into oligodendrocytes. Thus, Ascl1 regulates multiple aspects of oligodendrocyte development in the spinal cord.

**53) Activated Notch1 maintains the phenotype of radial glial cells and promotes their adhesion to laminin by upregulating nidogen.**

Li H, Chang YW, Mohan K, Su HW, Ricupero CL, Baridi A, Hart RP, Grumet M. **Glia.** 2008 Apr 15;56(6):646-58.

Radial glia are neural stem cells that exist only transiently during central nervous system (CNS) development, where they serve as scaffolds for neuronal migration. Their instability makes them difficult to study, and therefore we have isolated stabilized radial glial clones from E14.5 cortical progenitors (e.g., L2.3) after expression of v-myc. Activated Notch1 intracellular region (actNotch1) promotes radial glia in the embryonic mouse forebrain (Gaiano et al., (2000), and when it was introduced into E14.5 cortical progenitors or radial glial clone L2.3, the cells exhibited enhanced radial morphology and increased expression of the radial glial marker BLBP. A representative clone of L2.3 cells expressing actNotch1 called NL2.3-4 migrated more extensively than L2.3 cells in culture and in white matter of the adult rat spinal cord. Microarray and RT-PCR comparisons of mRNAs expressed in these closely related clones showed extensive similarities, but differed significantly for certain mRNAs including several cell adhesion molecules. Cell adhesion assays demonstrated significantly enhanced adhesion to laminin of NL2.3-4 by comparison to L2.3 cells. The laminin binding protein nidogen was the most highly induced adhesion molecule in NL2.3-4, and immunological analyses indicated that radial glia synthesize and secrete nidogen. Adhesion of NL2.3-4 cells to laminin was inhibited by anti-nidogen antibodies and required the nidogen binding region in laminin, indicating that nidogen promotes cell adhesion to laminin. The combined results indicate that persistent expression of activated Notch1 maintains the phenotype of radial glial cells, inhibits their differentiation, and promotes their adhesion and migration on a laminin/nidogen complex. (c) 2008 Wiley-Liss, Inc.

**54) Gemals, a new drug candidate, extends lifespan and improves electromyographic parameters in a rat model of amyotrophic lateral sclerosis.**

Nicaise C, Coupier J, Dabadie MP, De Decker R, Mangas A, Bodet D, Poncelet L, Geffard M, Pochet R. **Amyotroph Lateral Scler.** 2008 Apr;9(2):85-90.

Amyotrophic lateral sclerosis (ALS) is a fatal disease involving selective and progressive degeneration and death of motor neurons. ALS is a multifactorial disease in which oxidative stress, glutamate excitotoxicity, intracellular aggregates, neurofilamentous disorganization, zinc excitotoxicity, mitochondrial damage, neuroinflammation, abnormalities in growth factors and apoptosis play a role. Any therapeutic approach to delay or stop the evolution of ALS should therefore ideally target these multiple pathways leading to motor neuron death. We have developed a combination therapy (Gemals) composed of functional polypeptides (fatty acids, free radical scavengers and amino acids linked to poly-L-lysine), chosen according to their known potentiality for regeneration or protection of neuronal components such as myelin, axon transport and mitochondria. We found that Gemals significantly extended lifespan and improved electromyographic parameters in a SOD1(G93A) rat model. The use of two drug concentrations indicated a possible dose dependence. These initial findings open the way to further investigation necessary to validate this new drug as a candidate for ALS treatment.

**55) Suppression of interneuron programs and maintenance of selected spinal motor neuron fates by the transcription factor AML1/Runx1.**

Stifani N, Freitas AR, Liakhovitskaia A, Medvinsky A, Kania A, Stifani S. **Proc Natl Acad Sci U S A.** 2008 Apr 21 [Epub ahead of print]

Individual spinal motor neuron identities are specified in large part by the intrinsic repertoire of transcription factors expressed by undifferentiated progenitors and maturing neurons. It is shown here that the transcription factor AML1/Runx1 (Runx1) is expressed in selected spinal motor neuron subtypes after the onset of differentiation and is both necessary and sufficient to suppress interneuron-specific developmental programs and promote maintenance of motor neuron characteristics. These findings show an important role for Runx1 during the consolidation of selected

spinal motor neuron identities. Moreover, they suggest a requirement for a persistent suppression of interneuron genes within maturing motor neurons.

**56) Ubiquitin-Mediated Stress Response in the Spinal Cord After Transient Ischemia.**

Yamauchi T, Sakurai M, Abe K, Matsumiya G, Sawa Y. **Stroke.** 2008 Apr 3 [Epub ahead of print]

BACKGROUND AND PURPOSE: Vulnerability of motor neurons in the spinal cord against ischemia is considered to play an important role in the development of delayed paraplegia after surgery of the thoracic aorta. However, the reasons for such vulnerability are not fully understood. Recently, the ubiquitin system has been reported to participate in neuronal cell death. In the present study, we investigated the expression of ubiquitin system molecules and discussed the relationship between the vulnerability and the ubiquitin system after transient ischemia in the spinal cord. METHODS: Fifteen minutes of spinal cord ischemia in rabbits was applied with the use of a balloon catheter. In this model, the spinal motor neuron shows selectively delayed neuronal death, whereas other spinal neurons such as interneurons survive. Immunohistochemical analysis and Western blotting for ubiquitin system molecules, ubiquitin, deubiquitylating enzyme (ubiquitin carboxy-terminal hydrolase 1), and ubiquitin-ligase parkin were examined. RESULTS: In cytoplasm, ubiquitin and ubiquitin carboxy-terminal hydrolase 1 were strongly induced both in interneuron and motor neuron at the early stage of reperfusion, but the sustained expression was observed only in motor neuron. Parkin was induced strongly at 3 hours after the reperfusion, but the immunoreactivity returned to the sham control level at 6 hours in both neurons. In the nuclei, ubiquitin, ubiquitin carboxy-terminal hydrolase 1, and parkin were strongly induced in interneuron, whereas no upregulation of these proteins was observed in motor neuron. CONCLUSIONS: These results indicate that the vulnerability of motor neuron of the spinal cord might be partially attributed to the different response in ubiquitin-mediated stress response after transient ischemia.

**57) Progranulin functions as a neurotrophic factor to regulate neurite outgrowth and enhance neuronal survival.**

Van Damme P, Van Hoecke A, Lambrechts D, Vanacker P, Bogaert E, van Swieten J, Carmeliet P, Van Den Bosch L, Robberecht W. **J Cell Biol**. 2008 Apr 7;181(1):37-41. Epub 2008 Mar 31.

Recently, mutations in the progranulin (PGRN) gene were found to cause familial and apparently sporadic frontotemporal lobe dementia (FTLD). Moreover, missense changes in PGRN were identified in patients with motor neuron degeneration, a condition that is related to FTLD. Most mutations identified in patients with FTLD until now have been null mutations. However, it remains unknown whether PGRN protein levels are reduced in the central nervous system from such patients. The effects of PGRN on neurons also remain to be established. We report that PGRN levels are reduced in the cerebrospinal fluid from FTLD patients carrying a PGRN mutation. We observe that PGRN and GRN E (one of the proteolytic fragments of PGRN) promote neuronal survival and enhance neurite outgrowth in cultured neurons. These results demonstrate that PGRN/GRN is a neurotrophic factor with activities that may be involved in the development of the nervous system and in neurodegeneration.

**58) Facilitated sprouting in a peripheral nerve injury.**

Xu QG, Midha R, Martinez JA, Guo GF, Zochodne DW. **Neuroscience**. 2008 Apr 9;152(4):877-87. Epub 2008 Feb 15.

During regeneration of injured peripheral nerves, local conditions may influence how regenerative axon sprouts emerge from parent axons. More extensive lesions might be expected to disrupt such growth. In this work, we discovered instead that long segmental crush injuries facilitate the growth and maturation of substantially more axon sprouts than do classical short crush injuries (20 mm length vs. 2 mm). At identical distances from the proximal site of axon interruption there was a 45% rise in the numbers of neurofilament labeled axons extending through a long segmental crush zone by 1 week. By 2 weeks, there was a 35% greater density of regenerating myelinated axons in long compared with

short crush injuries just beyond (5 mm) the proximal injury site. Moreover, despite the larger numbers of axons, their maturity was identical and they were regular, parallel, associated with Schwann cells (SCs) and essentially indistinguishable between the injuries. Backlabeling with Fluorogold indicated that despite these differences, the axons arose from similar numbers of parent motor and sensory neurons. Neither injury was associated with ischemia. Both injuries were associated with rises in GFAP (glial acidic fibrillary protein) and p75 mRNAs, markers of SC plasticity but p75, GFAP and brain-derived neurotrophic factor mRNAs did not differ between the injuries. There was a higher local mRNA level of GAP43/B50 at 7 days following injury and a higher sonic hedgehog protein (Shh) mRNA at 24 h in long crush zones. GAP43/B50 protein and SHH protein both had prominent localization within regenerating axons. Long segmental nerve trunk crush injuries do not impair regeneration but instead generate greater axon plasticity that results in larger numbers of mature myelinated axons. The changes occur without apparent change in SC activation, overall nerve architecture or nerve blood flow. While the mechanism is uncertain, the findings indicate that manipulation of the nerve microenvironment can induce substantial changes in regenerative sprouting.

**59) Intrathecally injected granulocyte colony-stimulating factor produced neuroprotective effects in spinal cord ischemia via the mitogen-activated protein kinase and Akt pathways.**

Chen WF, Jean YH, Sung CS, Wu GJ, Huang SY, Ho JT, Su TM, Wen ZH. **Neuroscience**. 2008 Apr 22;153(1):31-43. Epub 2008 Feb 15.

Granulocyte colony-stimulating factor (G-CSF) is a potent hematopoietic factor. Recently, this factor has been shown to exhibit neuroprotective effects on many CNS injuries. Spinal cord ischemic injury that frequently results in paraplegia is a major cause of morbidity after thoracic aorta operations. In the present study, we examined the neuroprotective role of G-CSF on spinal cord ischemia-induced neurological dysfunctions and changes in the mitogen-activated protein kinase (MAPK) and Akt signaling pathways in the spinal cord. Spinal cord ischemia was induced in male Wistar rats by occluding the descending aorta with a 2F Fogarty catheter for 12 min 30 s. Immediately after ischemia surgery, the rats were administered G-CSF (10 mug) or saline by intrathecal (i.t.) injection. The rats were divided into four groups: control, ischemia plus saline, ischemia plus G-CSF and G-CSF alone. The neurological dysfunctions were assessed by calculating the motor deficit index after ischemia surgery. The expressions of MAPK and Akt were studied using Western blotting and double immunohistochemistry. First, we observed that ischemia plus i.t. G-CSF can significantly reduce the motor function defects and downregulate phospho-p38 and phospho-c-Jun N-terminal kinase protein expressions-this can be compared with the ischemia plus saline group. In addition, G-CSF inhibited the ischemia-induced activation of p38 in the astrocytes. Furthermore, we concluded that i.t. G-CSF produced a significant increase in phospho-Akt and phospho-ERK in the motor neurons and exhibited beneficial effects on the spinal cord ischemia-induced neurological defects.

**60) BDNF increases homotypic olivocerebellar reinnervation and associated fine motor and cognitive skill.**

Willson ML, McElnea C, Mariani J, Lohof AM, Sherrard RM. **Brain**. 2008 Apr;131(Pt 4):1099-112. Epub 2008 Feb 25.

Recovery of complex neural function after injury to the adult CNS is limited by minimal spontaneous axonal regeneration and/or sprouting from remaining pathways. In contrast, the developing CNS displays spontaneous reorganization following lesion, in which uninjured axons can develop new projections to appropriate target neurons and provide partial recovery of complex behaviours. Similar pathways can be induced in the mature CNS, providing models to optimize post-injury recovery of complex neural functions. After unilateral transection of a developing olivocerebellar path (pedunculotomy), remaining inferior olivary axons topographically reinnervate the denervated hemicerebellum and compensate functional deficits. Brain-derived neurotrophic factor (BDNF) partly recreates such reinnervation in the mature cerebellum. However the function of this incomplete reinnervation and any unwanted behavioural effects of BDNF remain unknown. We measured olivocerebellar reinnervation and tested rotarod and navigation skills in Wistar rats treated with BDNF/vehicle and pedunculotomized on day 3 (Px3; with reinnervation) or 11 (Px11; without spontaneous reinnervation). BDNF treatment did not affect motor or spatial behaviour in normal

(control) animals. Px11-BDNF animals equalled controls on the rotarod, outperforming Px11-vehicle animals. Moreover, Px3-BDNF and Px11-BDNF animals achieved spatial learning and memory tasks as well as controls, with Px11-BDNF animals showing better spatial orientation than Px11-vehicle counterparts. BDNF slightly increased olivocerebellar reinnervation in Px3 animals and induced sparse (22% Purkinje cells) yet widespread reinnervation in Px11 animals. As reinnervation correlated with spatial function, these data imply that after injury even a small amount of reinnervation that is homotypic to correct target neurons compensates deficits in appropriate complex motor and spatial skills. As there was no effect in control animals, BDNF effectively induces this axon collateralisation without interfering with normal neuronal circuits.

**61) Inactivation of zebrafish mrf4 leads to myofibril misalignment and motor axon growth disorganization.**

Wang YH, Li CK, Lee GH, Tsay HJ, Tsai HJ, Chen YH. **Dev Dyn**. 2008 Apr;237(4):1043-50.

Mrf4 is a basic helix-loop-helix (bHLH) transcription factor associated with myogenesis. Two mrf4 transcripts, mrf4_tv1 and mrf4_tv2, were identified in zebrafish generated by alternative splicing. To study their biological functions, we separately injected the Mrf4-morpholinos, including MO1 (mrf4_tv1:mrf4_tv2 knockdown), MO2+MO3 (mrf4_tv1:mrf4_tv2 knockdown), MO3 (mrf4_tv1 knockdown), and MO4 (mrf4_tv2 knockdown), into zebrafish embryos to observe mrf4 gene knockdown phenotypes. No phenotypic abnormalities were observed following injection with 0.5 ng of MO1 but those injected with 4.5, 9, or 13.5 ng displayed curved-body phenotypes, such as indistinct somite boundaries, and a lack of uniformly sized cell blocks. Similar results were also observed in the (MO2+MO3)-, MO3-, and MO4-injected groups. To further investigate the molecular mechanisms that lead to curved-body phenotypes, we stained embryos with alpha-bungrotoxin and specific monoclonal antibodies F59, Znp1, and Zn5 to detect morphological changes in acetyl-choline receptor (AChR) clusters, muscle fibers, common path of the primary neurons, and secondary neurons axonal projections, respectively. Our results show that the muscle fibers of mrf4_(tv1:tv2)-morphant aligned disorderly and lost their integrity and attachment, while the defects became milder in either mrf4_tv1-morphant or mrf4_tv2-morphant. On the other hand, reduced axonal projections and AChR clusters were found in both mrf4_tv2-morphant and mrf4_(tv1:tv2)-morphant but distributed normally in the mrf4_tv1-morphant. We conclude that Mrf4_tv2 is involved in alignment of muscle fibers, and Mrf4_tv1 might have cooperative function with Mrf4_tv2 in muscle fiber alignment, without affecting the muscle-nerve connection.

# Funding - Open calls

**2008 Marie Curie ITN** (Initial training network – PhD students and post docs).

- Closes: 02 September 2008 at 17:00:00
- One stage submission only
- Must be industrial partners active in the application
- Can be a twinning between centres in Europe or a consortium composed of at least three different entities from three diverse European member states
- Funds support salary based on Marie Curie slary grid for European countries, consumables, and training courses

**Christopher and Dana Reeve Foundation – Individual Research Grants**

**FUNDING PRIORITIES**

 Studying strategies that may promote neuronal growth and survival, encourage the formation of synapses, enhance the production of myelin, restore conduction capabilities, or may otherwise lead to restoration of the compromised circuitry in the acutely and chronically injured spinal cord.

 Evaluating the efficacy of drugs or other interventions that protect against secondary neuronal injury or provide insight into the mechanisms causing such damage.

 Defining anatomical characteristics of spinal cord injury in well-defined animal models and in the human spinal cord, specifically documenting the neuronal systems that are most vulnerable to spinal cord injury and the functional losses occurring as a result.

 Elucidating the biological mechanisms underlying approaches to improve concomitant functions affected by spinal cord injury, (e.g., bladder function, sexual function) and alleviate chronic pain and spasticity.

The development of treatments for chronic injury is a high priority for the organization; however, funding will also be provided for studies more relevant to the acute phase of injury. Basic research will be supported if it has clear potential to accelerate progress at the applied end of the continuum and/or if it reflects a research “change of direction.”

Application and Guidelines

STEP 1: Be sure you have read our guidelines. Applications that do not adhere to the guidelines will be rejected without review. Online forms must be submitted by June 16, 2008 and hard copies mailed no later than June 17, 2008.

** Applicants resubmitting applications (originally submitted in June 2007) should submit their revisions no later than Jan.17, 2008 (unless you have made other arrangements with Dr. Landsman), and follow the guidelines as if submitting a new application. You are encouraged to include a cover letter responding to reviewer comments.

To gain a better idea about whether your research is a priority for the SCI population, click here to read a new paper about SCI research priorities by Dr. Kim Anderson of the Reeve-Irvine Research Center at the University of California, Irvine.

If you are submitting a proposal focused on stem cells, please also consult the IOM guidelines

( http://dels.nas.edu/bls/stemcells ) on stem cell research before applying.

STEP 2: Download application forms and complete and mail five hard copies of the research grant applications to CDRF. The application is downloadable in Word format.

Click here to download (48 KB) or open the file in your browser.

STEP 3: Use the information contained in your completed hardcopy application form to fill out the online application, which can be completed in multiple sessions. You will also need to attach a PDF version of your proposal. Applicants can visit Adobe(r) to learn more about PDF files. To fill out an online application, you must log in to:
https://www.grantrequest.com/SID_290/?SA=SNA&FID=35004

**If you have already started an online application, you can access your application by visiting https://www.grantrequest.com/sid_290.

Please contact Dr. Landsman at 1-973-379-2690, or dlandsman(at)christopherreeve.org, as soon as possible with any questions or if you experience technical difficulties with the online application. Remember to mail five (5) hard copies of your completed application to:

Douglas S. Landsman, Ph.D.
Director, Individual Research Grants Program
Christopher and Dana Reeve Foundation
636 Morris Turnpike
Suite 3A
Short Hills, NJ 07078

For more info click **HERE**

# In the news

## UK drug industry needs shot in the arm

**Ailing companies are being forced to look at new ways of financing, reports Jonathan Russell**

The UK biotech sector is badly in need of some of its own medicine. One of the great hopes for value creation in the UK economy is seeing its vital signs grow weaker, almost by the day, as it runs out of the capital that is its lifeblood.

Research by analysts at investment bank Cannacord Adams for The Daily Telegraph shows that of the 66 listed biotech companies that report in sterling, only 10 made a profit in their last reported accounts.

More worryingly, of the 56 loss-making companies, less than half have more than 12 months of cash left to fund their research programmes. For a market that is effectively closed for fundraising this means there are serious changes ahead.

Karl Keegan, Canaccord Adams' head of life science research, said: "You are already seeing casualties. Companies are having a very tough time.

Some have put themselves up for sale or have been forced to look for alternative sources of financing." Although Mr Keegan is reticent to name companies it is not hard to identify them.

**More from the pharmaceutical and healthcare sectors**

In February, reproductive healthcare company Ardana put itself up for sale. According to its last reported accounts the company had little more than £10m in the bank and was losing £14m per year.

The company is not on its own. The UK biotech sector is a cash-hungry machine. Over the last year, the 56 loss-making companies in our survey lost a combined £439m. This was balanced by the 10 profitable companies reporting a £40m profit.

The figures may be large but they are not unusual. Drug research is expensive and long term. When companies start approaching profitability they are often bought out by their larger pharmaceutical cousins meaning the sector is less able to bask in their success. But this research needs cash, £400m per annum according to last year's figures - and apart from a few companies, it isn't available.

Aisling Burnand, chief executive of the BioIndustry Association, said: "For a couple of years there has been a strong feeling that the normal way of raising finance has run its course. If you are looking at the classic sequence of raising money from family and friends, angel investors, venture capitalists and flotation, it is gone."

Although the credit crunch has made matters worse, the problems run deeper. A combination of over-promising and under-delivering from companies, and an increasingly short-term outlook from investors, have left the sector more and more isolated and vulnerable to bad news.

Even companies that are well financed are not immune to the ills prevailing in the industry. Renovo, the developer of scar tissue treatment, saw the value of its shares halved after it reported poor clinical results for its lead product Juvista last month.

While the bad news merited a correction in the share price, what is extraordinary is the extent. In its last reported account Renovo had cash reserves of just over £100m, enough to fund it through four years of development. But with a current market cap of £75m, investors are not just discounting the company's cash holdings, they appear to be factoring in value destruction rather than creation by the management.

Not everyone shares this gloomy outlook. Investors such as the biotech entrepreneur Sir Christopher Evans, chairman of the recently renamed investment house Excalibur, are repositioning themselves to take advantage of cheap companies.

Sir Christopher said: "There are companies that are worth less than the cash they hold. I understand why it has happened but I think it is overcooked. However it does present opportunities. Some companies will deservedly go down, but there are those that will come back up. The test will be whether these companies are prepared to sell or consolidate before it is too late. I hope they will have the guts to consolidate, grow and ditch poor management. We will definitely be having a look at some of them."

But while times are tough and some companies have lost their way, others are finding new ways to navigate out of their current problems.

This month, Plethora, a specialist sexual health pharmaceutical company, raised $28m (£14m) through a "revenue interest financing agreement" with investment house Paul Capital.

The deal avoided the problem of diluting the existing shareholders' equity base by securitising future income, primarily from the company's erectile dysfunction drug, ErecAid. The deal proved that for a limited number of companies prepared to look at alternative financing mechanisms there are possibilities for raising new money.

There are also signs that consolidation within the industry, to create a smaller number of larger, more sustainable companies, could be under way. This week's acquisition of minnow CeNeS by German group Paion for £10.9m could be the start of a wave of just deals.

For long-suffering investors, it won't be before time. A decade ago the indie pop band The Verve topped the charts with their hit The Drugs Don't Work. It's about time at least some UK biotech companies proved them wrong.

## A new form of cloning - 'Now we have the technology that can make a cloned child'

A new form of cloning has been developed that is easier to carry out than the technique used to create Dolly the sheep, raising fears that it may one day be used on human embryos to produce "designer" babies.

Scientists who used the procedure to create baby mice from the skin cells of adult animals have found it to be far more efficient than the Dolly technique, with fewer side effects, which makes it more acceptable for human use.

The mice were made by inserting skin cells of an adult animal into early embryos produced by in-vitro fertilisation (IVF). Some of the resulting offspring were partial clones but some were full clones – just like Dolly.

Unlike the Dolly technique, however, the procedure is so simple and efficient that it has raised fears that it will be seized on by IVF doctors to help infertile couples who are eager to have their own biological children.

One scientist said this weekend that a maverick attempt to perform the technique on humans is now too real to ignore. "It's unethical and unsafe, but someone may be doing it today," said Robert Lanza, chief scientific officer of American biotechnology company Advanced Cell Technology.

"Cloning isn't here now, but with this new technique we have the technology that can actually produce a child. If this was applied to humans it would be enormously important and troublesome," said Dr Lanza, whose company has pioneered developments in stem cells and cell reprogramming.

"It raises the same issues as reproductive cloning and although the technology for reproductive cloning in humans doesn't exist, with this breakthrough we now have a working technology whereby anyone, young or old, fertile or infertile, straight or gay can pass on their genes to a child by using just a few skin cells," he said.

The technique involves the genetic reprogramming of skin cells so they revert to an embryonic-like state. Last year, when the breakthrough was used on human skin cells for the first time, it was lauded by the Catholic Church and President George Bush as a morally acceptable way of producing embryonic stem cells without having to create or destroy human embryos.

However, the same technique has already been used in another way to reproduce offspring of laboratory mice that are either full clones or genetic "chimeras" of the adult mouse whose skin cells were reprogrammed.

The experiments on mice demonstrated that it is now possible in principle to take a human skin cell, reprogramme it back to its embryonic state and then insert it into an early human embryo. The resulting child would share some of the genes of the person who supplied the skin tissue, as well as the genes of the embryo's two parents.

These offspring are chimeras – a genetic mix of two or more individuals – because some of their cells derive from the embryo and some from the skin cell. Technically, such a child would have three biological parents. Human chimeras occur naturally when two embryos fuse in the womb and such people are often normal and healthy. Dr Lanza says there is no reason to believe that a human chimera created by the new technique would be unhealthy.

Furthermore, studies on mice have shown that it is possible to produce fully cloned offspring that are 100 per cent genetically identical to the adult. This was achieved by using a type of defective mouse embryo with four sets of chromosomes instead of the normal two.

This "tetraploid" embryo only developed into the placenta of the foetus and when it was injected with a reprogrammed skin cell, the rest of the foetus developed from this single cell to become a full clone of the adult animal whose skin was used.

None of the scientists working on cell reprogramming to produce induced pluripotent stem (iPS) cells – as the embryonic cells are known – plan to use it for human reproductive medicine. Their main aim is to produce stem cells for the therapeutic treatment of conditions such as Parkinson's, Alzheimer's and stroke.

However, Dr Lanza said that the mouse experiments his company had done demonstrated how easily the technology could be used to produce cloned or chimeric babies by inserting iPS cells into early human embryos. This is not banned in many countries, where legislation has not kept pace with scientific developments.

In Britain, the Human Tissue and Embryos Bill going through Parliament does not mention the iPS technique, although experts believe that the new law should make it illegal because it involves genetic modification of cells that become part of the embryo.

"In addition to the great therapeutic promise demonstrated by this technology, the same technology opens a whole new can of worms," Dr Lanza said.

"At this point there are no laws or regulations for this kind of thing and the bizarre thing is that the Catholic Church and other traditional stem-cell opponents think this technology is great when in reality it could in the end become one of their biggest nightmares," he said. "It is quite possible that the real

legacy of this whole new programming technology is that it will be introducing the era of designer babies.

"So for instance if we had a few skin cells from Albert Einstein, or anyone else in the world, you could have a child that is say 10 per cent or 70 per cent Albert Einstein by just injecting a few of their cells into an embryo," he said.

## Can micro-scaffolding help stem cells rebuild the brain after stroke?

Inserting tiny scaffolding into the brain could dramatically reduce damage caused by strokes the UK National Stem Cell Network Annual Science Meeting will hear today. With funding from the Biotechnology and Biological Sciences Research Council (BBSRC) neurobiologists from the Institute of Psychiatry and tissue engineers from The University of Nottingham have joined forces to tackle the challenge of tissue loss as a result of stroke.

Speaking at the conference in Edinburgh, Dr Mike Modo from the Institute of Psychiatry will explain how combining scaffold microparticles with neural stem cells (NSCs) could regenerate lost brain tissue.

Strokes cause temporary loss of blood supply to the brain which results in areas of brain tissue dying — causing loss of bodily functions such as speech and movement. Neural Stem Cells offer exciting possibilities for tissue regeneration, but there are currently major limitations in delivering these cells to the brain. And while NSC transplantation has been proven to improve functional outcomes in rats with stroke damage little reduction in lesion volume has been observed.

The research is being carried out by Dr Mike Modo and Professor Jack Price from the Institute of Psychiatry and Professor Kevin Shakesheff from The University of Nottingham.

Their findings are being presented at the UK National Stem Cell Network Inaugural Science Meeting at the Edinburgh Conference Centre on 10 April 2008. The conference is a showcase of the best and latest UK stem cell science across all stem cell disciplines.

Working with rats, Dr Modo and his team are developing cell-scaffold combinations that could be injected into the brain to provide a framework inside the cavities caused by stroke so that the cells are held there until they can work their way to connect with surrounding healthy tissue.

Dr Modo explains: "We propose that using scaffold particles could support NSCs in the cavity to re-form the lost tissue and provide a more complete functional repair. The ultimate aim is to establish if this approach can provide a more efficient and effective repair process in stroke."

Kevin Shakesheff, Director of The University of Nottingham's new £25m Centre for Biomolecular Sciences and Professor of Tissue Engineering in the School of Pharmacy, said: “Within the body our cells function within tightly controlled 3D architectures. Our scaffolds can recreate some of the architectural features and thereby protect the cells and help them to integrate and function."

The team hope their work will pave the way for NSCs to be successfully used in clinical settings to re-develop parts of the brain damaged by stroke and neurodegenerative diseases.

## Self-Assembled Materials Form Mini Stem Cell Lab

Imagine having one polymer and one small molecule that instantly assemble into a flexible but strong sac in which you can grow human stem cells, creating a sort of miniature laboratory. And that sac, if used for cell therapy, could cloak the stem cells from the human body’s immune system and biodegrade upon arriving at its destination, releasing the stem cells to do their work.

Futuristic? Only in part. A research team from Northwestern University’s Institute for

BioNanotechnology in Medicine has created such sacs and demonstrated that human stem cells will grow in them. The researchers also report that the sacs can survive for weeks in culture and that their membranes are permeable to proteins. Proteins, even large ones, can travel freely across the membrane.

This new and unexpected mode of self-assembly, to be published March 28 in the journal Science, also can produce thin films whose size and shape can be tailored. The method holds promise for use in cell therapy and other biological applications as well as in the design of electronic devices by self-assembly, such as solar cells, and the design of new materials.

"We started with two molecules of interest, dissolved in water, and brought the two solutions together," said Samuel I. Stupp, Board of Trustees Professor of Materials Science and Engineering, Chemistry and Medicine, who led the research.

"We expected them to mix, but, much to our surprise, they formed a solid membrane instantly on contact. This was an exciting discovery, and we then proceeded to investigate why it happened. Understanding the surprising molecular mechanism was even more exciting."

One of the molecules is a peptide amphiphile (PA), small synthetic molecules that Stupp first developed seven years ago, which have been essential in his work on regenerative medicine. The other molecule is the biopolymer hyaluronic acid (HA), which is readily found in the human body, in places like joints and cartilage. Stupp recently had started a new research project on the regenerative medicine of cartilage, which drew him to hyaluronic acid.

"This is a clear example of informed discovery," said Stupp, director of the Institute for BioNanotechnology in Medicine. "We knew there was something interesting about the interaction between peptide amphiphiles and biopolymers from our previous work on nanostructures that can cause blood vessels to grow. And we were particularly interested in hyaluronic acid because of its role in cartilage, a tissue that adults cannot regenerate and, when damaged in joints, causes grief to humans."

Using just these two molecules, Stupp and his team can make many different structures, the two most important being sacs, which have a solid membrane on the outside and liquid inside, and flat membranes of any shape. The researchers can make the structures large or small, pick up the material with tweezers, stretch it and even easily repair the sacs through self-assembly should the material tear or have some other defect. The sacs also are robust enough to be sutured by surgeons to biological tissues.

The large (hyaluronic acid) and small (peptide amphiphile) molecules come together through supramolecular interactions, not by chemical reaction, in which covalent bonds are formed.

In the case of the flat membrane, the researchers put the peptide amphiphile solution at the bottom of a shallow mold and added on top the hyaluronic acid solution. The two interacted on contact, creating a solid. By varying the mold, the researchers produced a variety of shapes, including stars, triangles and hexagons, each having two chemically different surfaces. When dry, the materials are stiff and strong, like plastic.

In creating a sac, the researchers took advantage of the fact that hyaluronic acid (HA) molecules are larger and heavier than the smaller peptide amphiphile (PA) molecules. In a deep vial, they poured the PA solution and into that poured the HA solution. As the heavier molecules sank, the lighter molecules engulfed them, creating a closed sac with the HA solution trapped inside the membrane.

Having formed the sacs, Stupp and his team next studied human stem cells engulfed by the self-assembly process inside sacs that they placed in culture. The researchers found that the cells remained viable for up to four weeks, that a large protein -- a growth factor important in the signaling of stem cells -- could cross the membrane, and that the stem cells were able to differentiate.

"We expect that genes, siRNAs and antibodies will cross the membranes as well, making this mini cell biology lab a powerful device for research or therapies," said Stupp. "For the development of cancer

therapies, we will be able to confine cells within the sacs and study their reaction to different types of therapies as well as to signaling by different cells in neighboring sacs."

In a clever demonstration of self-repair, if the sac's membrane had a hole (from a needle injection, for example), the researchers simply placed a drop of the PA solution on the tear, which interacted with the HA inside, resulting in self-assembly and a sealed hole.

"The membrane is a fascinating and unusual structure with a high degree of hierarchical order," said Stupp. "The membrane grows through a dynamic self-assembly process which generates hybrid nanofibers made up of both molecules and oriented perpendicular to the plane of the membrane. This architecture is very difficult to get spontaneously in materials. Using the right chemistry, the thick membrane structure could be designed to get conduits of charge in solar cells or nanoscale columns of catalytic nanostructures that would extend over arbitrary macroscopic dimensions."

While the underlying, highly ordered structure of the sacs and membranes has dimensions on the nanoscale, the sacs and membranes themselves can be of any dimension and are visible to the naked eye.

The Science paper is titled "Self-Assembly of Large and Small Molecules into Hierarchically Ordered Sacs and Membranes." In addition to Stupp, other authors are Ramille M. Capito (lead author), Yuri S. Velichko and Alvaro Mata, all of Northwestern's Institute for BioNanotechnology in Medicine (IBNAM); and Helena S. Azevedo, of IBNAM and the University of Minho, Portugal.

The research was supported by the U.S. Department of Energy, the National Institutes of Health and the National Science Foundation

## New Nanotechnology For Spinal Cord Injury Shows Potential

A spinal cord injury often leads to permanent paralysis and loss of sensation below the site of the injury because the damaged nerve fibers can't regenerate. The nerve fibers or axons have the capacity to grow again, but don't because they're blocked by scar tissue that develops around the injury.

Northwestern University researchers have shown that a new nano-engineered gel inhibits the formation of scar tissue at the injury site and enables the severed spinal cord fibers to regenerate and grow. The gel is injected as a liquid into the spinal cord and self -assembles into a scaffold that supports the new nerve fibers as they grow up and down the spinal cord, penetrating the site of the injury.

When the gel was injected into mice with a spinal cord injury, after six weeks the animals had a greatly enhanced ability to use their hind legs and walk.

The research is published today in the April 2 issue of the *Journal of Neuroscience.*

"We are very excited about this," said lead author John Kessler, M.D., Davee Professor of Stem Cell Biology at Northwestern University's Feinberg School of Medicine. "We can inject this without damaging the tissue. It has great potential for treating human beings."

Kessler stressed caution, however, in interpreting the results. "It's important to understand that something that works in mice will not necessarily work in human beings. At this point in time we have no information about whether this would work in human beings."

"There is no magic bullet or one single thing that solves the spinal cord injury, but this gives us a brand new technology to be able to think about treating this disorder," said Kessler, also the chair of the Davee Department of Neurology at the Feinberg School. "It could be used in combination with other technologies including stem cells, drugs or other kinds of interventions."

"We designed our self-assembling nanostructures -- the building blocks of the gel -- to promote neuron growth," said co-author Samuel I. Stupp, Board of Trustees Professor of Materials Science and

Engineering, Chemistry, and Medicine and director of Northwestern's Institute for BioNanotechnology in Medicine. "To actually see the regeneration of axons in the spinal cord after injury is a fascinating outcome."

The nano-engineered gel works in several ways to support the regeneration of spinal cord nerve fibers. In addition to reducing the formation of scar tissue, it also instructs the stem cells --which would normally form scar tissue -- to instead to produce a helpful new cell that makes myelin. Myelin is a substance that sheaths the axons of the spinal cord to permit the rapid transmission of nerve impulses.

The gel's scaffolding also supports the growth of the axons in two critical directions -- up the spinal cord to the brain (the sensory axons) and down to the legs (the motor axons.) "Not everybody realizes you have to grow the fibers up the spinal cord so you can feel where the floor is. If you can't feel where the floor is with your feet, you can't walk," Kessler said.

Now Northwestern researchers are working on developing the nano-engineered gel to be acceptable as a pharmaceutical for the Food & Drug Administration.

If the gel is approved for humans, a clinical trial could begin in several years. "It's a long way from helping a rodent to walk again and helping a human being walk again," Kessler stressed again. "People should never lose sight of that. But this is still exciting because it gives us a new technology for treating spinal cord injury."

## STEMCELL Technologies Introduces a New Method for Identifying and Quantifying Neural Stem and Progenitor Cells In Vitro

The NeuroCult® Neural Colony-Forming Cell (NCFC) Assay increases the accuracy of studying neural stem cells *in vitro* and therefore, the evaluation of the therapeutic potential of neural stem cells.

"The **NeuroCult® Neural Colony-Forming Cell Assay** is an **improvement over** the widely used **Neurosphere Assay** because it provides researchers with a reliable way to detect alterations in neural stem cell frequency, which is necessary for evaluating the cells' therapeutic potential," states Dr. Sharon A. Louis, Senior Scientist at STEMCELL Technologies. "Our findings imply that while the Neurosphere Assay provides a simple means to isolate and expand neural stem cells harvested from the embryonic and adult mammalian CNS, its application as a quantitative *in vitro* assay for measuring NSC frequency is limited. Presently, the Neurosphere Assay is the most frequently adopted method for isolating, expanding and calculating the frequency of neural stem cells, so our findings will impact highly cited research performed using this method."
The new NeuroCult® Neural Colony-Forming Cell Assay is a **significant advancement** in the neural stem cell field because it **discriminates between neural stem cells and neural progenitor cells** based on the size of the colonies they form (proliferative potential), allowing researchers to easily and accurately distinguish and quantify these cell populations *in vitro*.
The results of this study are published in the article "Enumeration of Neural Stem and Progenitor Cells in the Neural Colony-Forming Cell Assay" in the April 2008 issue of Stem Cells.

The NeuroCult® Neural Colony-Forming Cell Assay was developed by Dr. Sharon A. Louis and Dr. Brent A. Reynolds at STEMCELL Technologies with further work performed in collaboration with Dr. Brent A. Reynolds and researchers at the Queensland Brain Institute, University of Queensland, Australia. Partial support was also provided from the National Health and Medical Research Council of Australia project grant and a Pfizer Australia Senior Research Fellowship to Rietze R. L.

## U of M researchers identify process that may help treat Parkinson's, spinal cord injuries

A new discovery by University of Minnesota researchers may lead to a better understanding of how the spinal cord controls how people walk. These insights could help lead to treatments for central nervous system maladies such as Parkinson's disease and spinal cord injuries.

The study, headed by Joshua Puhl, Ph.D., and Karen Mesce, Ph.D., in the Departments of Entomology and Neuroscience, discovered its possible that the human nervous system within each segment or region of spinal cord may have its own unit burst generator to control rhythmic movements such as walking.

By studying a simpler model of locomotion, in the medicinal leech, the research shows where these unit burst generators reside and that each nerve cord segment has a complete generator. When a neuron fires, it sets off a chain reaction that gives rise to rhythmic movement. Once those circuits are turned on, the body essentially goes on autopilot.

Mesce and her research group targeted the segmented leech for study because they have fewer and larger neurons making them easier to study.

The study was published today online in the Journal of Neuroscience.

For most of us, we can chew gum and walk at the same time, Mesce said. We do not have to remind ourselves to place the right leg out first, bring it back and do the same for the other leg. So how does the nervous system control rhythmic behaviors like walking or crawling"

Furthermore, and perhaps just as important, the study found that dopamine a common human hormone can turn each of these complete generator units on.

Since dopamine regulates movements and activates those unit burst generators, the next step will be figuring out how dopamine makes individual neurons more or less active.

Because dopamine affects movement in many different animals, including humans, our studies may help to identify treatments for Parkinsons patients and those with spinal cord injury, Mesce said.

## Behind Novartis' Eye-Popping Buyout

***The Swiss drug giant's $39 billion deal for Alcon will give it fresh avenues for growth -- and further expansion into ophthalmic care***

With sales of prescription drugs expected to slow, Swiss pharmaceutical giant Novartis (NVS) announced on Apr. 7 it will acquire Alcon (ACL), the world's largest and most profitable eye-care company, in a deal potentially worth $39 billion. Novartis will pay $143.18 per share—a price based on Alcon's volume-weighted average share price between Jan. 7 and Apr. 4—about 3.5% less than Alcon's $148.44 closing price on Apr. 4.

It's the culmination of several years of discussions off and on between Novartis and Alcon's majority owner, Nestlé (NESN.DE). Novartis, which had 2007 revenues of $39.8 billion, initially will buy a 25% stake in Alcon for $11 billion, with exclusive rights to acquire Nestlé's remaining 52% stake for $28 billion between January, 2010, and July, 2011. Alcon's revenues amounted to $5.6 billion last year. "This enables us to take advantage of a major growth opportunity while diversifying risk," Novartis Chairman and Chief Executive Officer Daniel Vasella told *BusinessWeek*.

The deal and its eye-popping price tag indicate the intensity of the challenges facing the pharmaceutical business worldwide. Drugmakers are under intense pressure from governments,

insurers, and consumers to curb soaring prices. At the same time, industry productivity is waning as the cost of research and development soars.

**Patent Crunch**

Complicating matters, generic drug companies are successfully challenging patents on the pharmaceutical industry's biggest money-spinners. And regulators around the world, led by the U.S. Food & Drug Administration, are becoming much more cautious about new drug approvals. "In this environment, you have to ask yourself: How do I want to position the company?" Vasella says.

The answer, he says, is to be a diversified health-care business not overly reliant on cutting-edge pharmaceuticals. It's a strategy Vasella has been pursuing for nearly a decade. Recognizing consumer demand for cheaper drugs, he splashed out $13 billion to acquire German generic drugmakers Hexal and Eon Labs in 2005. Today, the Swiss company's generic business, Sandoz, is No. 2 worldwide, behind Israel's Teva (TEVA). In 2006, Novartis paid $5.7 billion for U.S. vaccine maker Chiron. And all along, the company has continued to build its over-the-counter and Ciba Vision optical units (the latter overlaps with Alcon in contact lens solutions). "We were never a pharma pure play," Vasella notes. "And it's getting harder to find good opportunities for acquisitions in pharmaceuticals."

Nevertheless, Novartis faced a number of setbacks last year. At the request of the FDA, the company withdrew Zelnorm, a drug for irritable bowel syndrome, from the market because of links to an increased risk of heart attack. U.S. approval was delayed on Novartis' new diabetes drug Galvus. Painkiller Prexige was once viewed by the company and analysts as a potential blockbuster. But as part of the same COX-2 class of drugs as Merck's (MRK) ill-fated Vioxx, it is now unlikely to gain U.S. approval.

Vasella knows Novartis needs to find new sources of growth. Within the next four years, approximately one-third of the company's revenue sources will face generic competition, according to analysts at Morgan Stanley (MS). Novartis' best-selling drug, blood pressure medication Diovan, with sales of more than $5 billion, will be the biggest casualty when it loses patent protection in 2012.

**Greener Pastures**

What Alcon offers Novartis is access to a fast-growing specialty health-care business, whose business spans prescription drugs for eye diseases such as glaucoma, over-the-counter products such as Opti-Free contact lens solutions, and medical devices and products for ophthalmic surgery. Analysts say it meshes well with Novartis' Ciba Vision business and with the company's Lucentis, a fast-growing drug for age-related macular degeneration, a leading cause of blindness in the elderly. Novartis acquired the rights to sell Lucentis outside the U.S. from Genenetech (DNA). The drug is expected to generate revenues for Novartis of just under $1 billion in 2008.

An even bigger attraction for Novartis is that Alcon's sales come from diversified sources, ranging from consumers to managed-care providers, making it less vulnerable to price regulation and a potential slowdown in consumer spending. Instead of depending mainly on managed-care providers or governments for sales, as is the case with prescription drugs, Alcon gets a sizable chunk of its business from customers who pay out of pocket for over-the-counter products or procedures such as laser eye surgery.

Alcon also should help Novartis in developing economies, where the eye-care company is growing fast. It's the lead player in the $2.5 billion global cataract surgery market—a franchise set to boom in emerging markets, where the procedure is only now starting to become popular. Last year, Alcon's sales in developing markets grew 21%.

Still, the reaction to the deal was muted. Novartis' shares, already down 18% year to date, dropped a further 1.4% in Swiss trading by day's end and were off 3% by midday in New York. "With Alcon, Novartis is buying a quality business at attractive terms," says Karl Heinz Koch, a pharmaceuticals

analyst at Bank Vontobel (VONN.DE) in Zurich. "But Novartis would have been better served by addressing some of the issues in its core pharmaceuticals business before it started diversifying."

## Gene test offers hope of sight to patients

Hundreds of thousands of people with failing eyesight have been given fresh hope of a cure after gene therapy techniques were used to treat a teenager.

- **Gene therapy is a light in the dark for blind student**
- **Telegraph TV: How the eye gene therapy works**

The treatment transformed the life of a severely visually impaired 18-year-old. Healthy genes were injected into one eye, leading to a significant improvement

The technique could be ready for use within two years to treat people suffering from some inherited diseases of the retina, which affect 20,000 people in Britain.

Within five years it could be ready for testing on people who suffer age related macular degeneration, a condition that affects 500,000 Britons.

In the trial carried out by a team at the University College of London Institute of Ophthalmology and Moorfields Eye Hospital, the world's first gene transplant for blindness produced an unprecedented improvement in Steven Howarth's sight.

Currently, there is no treatment for the condition. Born with no peripheral or night vision, he noticed a marked improvement after the two-hour operation.

"Now, my sight when it's getting dark or it's badly lit is definitely better," he says. "It's a small change - but it makes a big difference."

Prof Robin Ali, the head of the team, said the evidence of his improvement was "compelling".

The doctors injected genes only into Mr Howarth's worst affected eye and used the lowest dose in what was strictly a safety trial.

"It is more than we could have expected at this stage," said Prof Ali, who has been racing to perform the transplants against a rival team at the Children's Hospital of Philadelphia, where three young adults have also been successfully treated.

The treatment, described in the New England Journal of Medicine, should be available for other patients with this rare condition in two years.

But Prof Ali believes that this is a proof of principle for treating a vast range of blinding conditions, from 100 other inherited kinds to the most common of all, age-related macular degeneration.

"It is a major boost for the whole field," he said.

The first trials to use gene therapy to treat macular degeneration could start in three to five years, said Prof Ali.

The blindness trial was partly funded by £1 million from the Department of Health.

The public health minister, Dawn Primarolo, said: "This is a major achievement for British science and the NHS."

However, a leading British gene therapist said the level of Government support was inadequate.

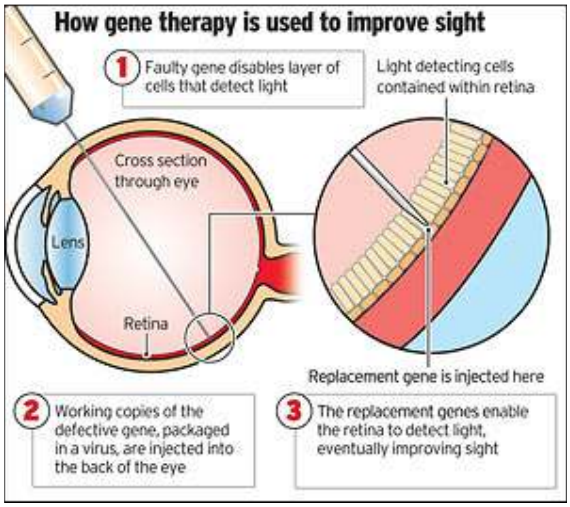
"This is good stuff and we have the potential to broaden these technologies to many other areas," he told The Daily Telegraph.

"But this needs major investment from the Department of Health."

Success with three more patients was reported in the same journal by a second team - The University of Pennsylvania, The Children's Hospital of Philadelphia, the Second University of Naples and the Telethon Institute of Genetics and Medicine, both in Italy.

Dr Albert Maguire led the study with his wife, Prof Jean Bennett, and the gene therapist Prof Katherine High.

Speaking to The Daily Telegraph, Profs High and Bennett conceded that Prof Ali was the first to perform the treatment.




**How gene therapy is used to improve sight: click to enlarge**

"Our own study was facilitated by the safety data that he generated when his first subject was injected."

More than one third of a million people in the UK are registered blind or partially sighted and, overall, the RNIB estimates two million people have significant sight loss.

## Alzheimer's Market Set to Grow Substantially

**Geriatric Bubble Will Lead to 13 Million**

You have heard plenty about the surge of aging baby boomers and the impending disastrous financial impact caused by an explosive growth in government entitlements. Medicare and Medicaid costs were $627 billion last year, and the Congressional Budget Office says that costs will double in 10 years. Now, add the epidemic proportions of individuals with dementia or some form of Alzheimer's disease (AD). There are currently about five million people in the U.S. living with AD. Worldwide, there are more than 100 million people suffering from diseases of the brain characterized by loss of neurons.

The Lewin Group's 2004 report estimates that Medicare spending for AD will be $189 billion by 2015. Currently, about $5 billion annually is being spent worldwide on drugs to treat AD. These therapies, however, do not cure AD, they simply alleviate the symptoms. The current economic burden for AD patient care in the U.S. is about $100 billion.

There is a lot of new information on risk factors and how interventions in life style, such as diet, exercise, and intellectual stimulation, can slow down the disease process, however, scientists do not fully understand the cause of AD. Recently there has been a step-up in R&D, and there is a growing clinical pipeline of new drugs. Diagnostic tests, particularly biomarkers, are at an early stage but are urgently needed to show the effectiveness of drugs in development.

***Investment Opportunities***

The market potential for Alzheimer's disease and related cognitive disorder therapies is huge due to the numbers of patients affected and the cost of care. The R&D investment and knowledge base is growing each year with positive data for several new therapies expected in 2009. Products currently on the market are sold by larger companies such as **Pfizer** and **Novartis** as well as generic firms, so their stock prices are not very sensitive to sales growth.

Many top-tier drug companies also have products in clinical development for AD. Investing in small- to mid-cap biotech companies offer the best growth potential in spite of the clinical trial risk involved. As always, an investment portfolio comprising a range of companies is the best approach with an overweighting on specific firms as they put out positive clinical data in the later stage. Another indicator of lower risk would be the establishment of an ETF (exchange-traded funds) for companies in this sector, such as the HHN Neuroscience ETF created by Xshares.

Emerging companies that have encouraging Phase II data are mentioned in this article. The first next-generation product expected to come to market hopefully by the 2010–11 time frame will have multibillion dollar revenue potential.

***Current and Predicted Market***

Glutamate is a key neurotransmitter affecting memory and learning and is a target for drug discovery. It is implicated not only in AD but also in other neurodegenerative diseases such as schizophrenia and depression. Memantine is currently approved for AD and appears to block the glutamate pathway (NMDA receptor modulator) but also boosts the acetycholine (AChEI's) pathway.

Recent studies based on cognitive tests and feedback from caregivers have shown the drug to be beneficial. Memantine, marketed under the brand names Namenda by **Forest Laboratories**, Axura by **Merz**, and Ebixa by **Lundbeck** is thought to have advantages over more established AD drugs such as **Eisai** and Pfizer's Aricept and Novartis' Exelon, which boost AChEI-related signaling. More clinical studies are required, though.

The current U.S. market for all these products is about $2.5 billion and growing at over 10% per year. The global market should reach $5 billion within a few years. Pharmaceutical audit data from Wolters Kluwer shows 2007 sales of $1.4 billion for Aricept, $705 million for Namenda, and $191 million for Exelon.

According to a 2007 Decision Resources report, this AChEI market is expected to eventually slow or decline as generics take over and new-generation products hit the market. This may take a few years, however. Frost and Sullivan forecasts the U.S. market to grow to about $4 billion by 2010 then briefly decline followed by renewed growth as disease-modifying drugs are introduced in 2011.

A MedaCorp and Leerink Swann & Co. White Paper states, "Treatment that could delay the onset of Alzheimer's disease could reduce the number of patients by 50%, thus saving $50 billion in annual healthcare costs."

**Elan** has a broad pipeline of products in clinical development in Phases I through III. Bill Tanner, Ph.D., of Leerink Swann has an Outperform rating on the firm. CSFB has also added Elan to its outperform list. Decision Resources projects that its lead candidate, bapineuzumab, will launch by 2011 with blockbuster potential of $5 billion by maturity if there are no safety issues.

Elan's immunotherapies consist of an immunoconjugate in Phase II and two humanized mAbs. While one mAb is in preclinical development, bapineuzumab is in a pivotal Phase III trial. The study is using a new cognitive-function endpoint called NTB (neuropsychological test battery) instead of the ADAS-cog gold standard. Bapineuzumab is engineered to clear the neurotoxic beta-amyloid peptide, which accumulates in the brains of AD patients.

Elan is also developing beta and gamma secretase inhibitors with a Phase II product in a partnership with **Eli Lilly**. These enzyme inhibitors clip the amyloid precursor protein thus preventing the formation of amyloid plaques.

Another company faring well with some analysts is **Epix Pharmaceuticals**. Alan Carr of Needham and Co. has a Buy on Epix considering that the company has four mid-stage clinical programs and new drivers are expected over the next six months. RBC Capital also recently initiated an Outperform on the stock.

Epix Phase II portfolio consists of one AD candidate, while the other two target pulmonary hypertension and depression. The firm also has a Phase I AD candidate.

The mid-stage small molecule, 5HT-4, is a selective agonist of a specific GPCR. It is being evaluated in a Phase IIa trial as a single agent and in combination with Aricept. A Phase IIb trial is expected to begin by mid-year with partner **GlaxoSmithKline**. The mechanism of action is the stimulation of the alpha-secretase pathway and Ach production in the brain, which should improve cognition.

**Myriad Genetics**' Phase III compound, on the other hand, is a selective amyloid (amyloid beta 42) lowering agent that acts through a gamma-secretase pathway. A recent report by Decision Resources regarding Flurizan's effect in delaying progression from mild cognitive impairment to AD, touted the drug's potential to become the gold standard of treatment.

The launch of Flurizan is expected in 2010 with a 23% market share by 2016. Ian Sanderson of Cowen and Co. forecasts sales of $600 million by 2012 and he expects it to be the first next-generation drug to be approved by the FDA. Annabel Samimy of UBS has a Buy on the stock after considering the firm's pipeline as well as its diagnostic business. Geoffrey Meacham, Ph.D., of JPMorgan has an Overweight rating on the stock.

**Targacept** is focused on a class of molecular targets called neuronal nicotinic receptors (NNR) that are involved in neurotransmitter activity potentially strengthening the nerve signal. Six analysts currently have a Buy rating on the company: CIBC, Deutsche Securities, Leerink Swann, Lazard, Nataxis, and Pacific Growth.

Targacept's lead compound, which is partnered with **AstraZeneca**, is in a Phase IIb study. AZD-3480 is a small molecule drug being investigated as a treatment for cognitive impairment in both AD and schizophrenia. Results are expected by the end of 2008.

AZD-3480 has already been tested in 12 trials involving 540 subjects, it was well tolerated and had positive effects in cognition. The company has extensive IP for NNR and is also partnered with GlaxoSmithKline for other NNR drug targets. Additional IND's are expected in Q2.

Other companies with Phase II drugs are **Prana Biotechnology** and **Medivation**. Recently, Prana reported a successful Phase IIa trial for PBT2 that showed reduced Abeta 42, a biomarker associated with AD. PBT2 is an MPAC (metal protein-attenuating compound) 8-hydroxyquinoline that reduces the impact of naturally occurring metals such as copper, thus attenuating the damaging effects of amyloid beta.

Medivation plans to initiate a pivotal confirmatory Phase III trial with Dimebon in Q2. The successful Phase II study was done in Russia, but the late-stage evaluation will be in the U.S. Dimebon appears to block a new target that involves mitochondrial pores, which are believed to play a role in cell death. Dimebon was previously approved and is used in Russia as an antihistamine.

Alzheimer's disease is a huge market, with drugs in development that can be very cost effective. Phase II results from the aforementioned companies are encouraging, and winners should emerge as early as the first quarter of 2009. Review company websites, analysts' reports, and technicals before investing.

## Renowned Stem Cell Researcher Jeanne F. Loring Heads New Center at The Scripps Research Institute

LA JOLLA, CA, March 26, 2008—Professor Jeanne F. Loring, Ph.D., has been named founding director of the newly created Center for Regenerative Medicine at The Scripps Research Institute in La Jolla, California.

Loring is an internationally recognized authority in the emerging field of stem cell research, which explores the potential of these cells to differentiate into various cell types that may be used to treat diseases and conditions such as Parkinson's and Alzheimer's, spinal cord injury, stroke, burns, heart disease, diabetes, osteoarthritis, and rheumatoid arthritis.

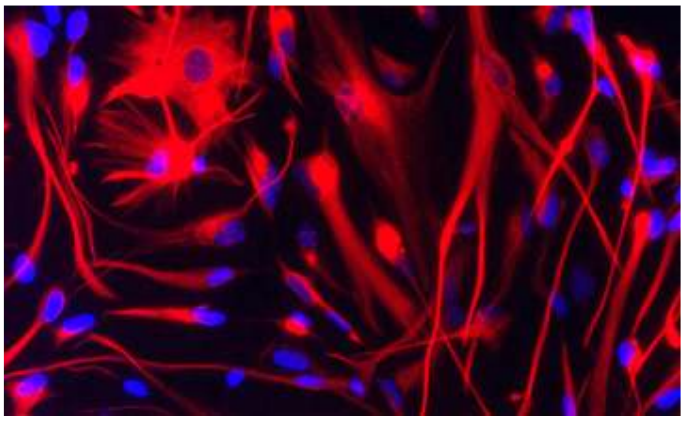
"The potential of stem cell research is vast," said Scripps Research President Richard A. Lerner, M.D., in announcing the creation of the new center. "It takes a scientist of Professor Loring's foresight, knowledge, and experience in basic and applied research to lead the institute's team to new discoveries that will significantly benefit human health."

"I am excited about the potential of this new center to push research in the field forward," said Loring. "I'm looking forward to growing the center in the Scripps Research traditions of cross-disciplinary collaboration and cutting-edge science."

Researchers at the Scripps Research Center for Regenerative Medicine will explore many aspects of stem cells, including embryonic, adult, and malignant cancer stem cells, from their basic biology to potential clinical applications in drug discovery, drug delivery, and cell therapy.

The new center's major mission is to provide infrastructure to support collaboration and strategic partnerships in human stem cell research and train the next generation of stem cell scientists. An intensive NIH-sponsored human embryonic stem cell laboratory course will be offered this fall, and the center will be the site of the San Diego area training course supported by the California Institute for Regenerative Medicine.

## Stemming the tumorous tide



**Cancers grow from stem cells. That discovery should translate into better treatment for tumours of all types**

STEM cells have a controversial reputation, but in truth they are what makes human life possible. Each tissue in the body grows from a particular sort of stem cell. When it divides, one of its daughters remains a stem cell while the other eventually turns into whatever tissue its mother was designed to produce—be it blood, muscle, nerve or whatever. That is how healthy tissues are renewed, and it is

now looking likely that it is how unhealthy tissues are renewed, too. Indeed, many researchers think that the underlying cause of cancer is the brakes coming off the regulatory system that stops normal stem cells from reproducing too much. For one of the most important medical discoveries made in recent years is that cancers, too, have stem cells and that these appear to be the source of the rest of the tumour.

This helps to explain why cancers are so hard to deal with. Treatments that kill the bulk of a tumour, but leave the stem cells alive, are only buying time. On the other hand, if all of a tumour's stem cells could be killed then it would torpedo the old wisdom that no patient is ever cured of cancer, but merely goes into remission. True cures for cancer would be possible.

The cancer-stem-cell theory, though plausible, was based on animal experiments and its relevance to humans was untested. But a series of studies reported this week at a meeting of the American Association for Cancer Research, in San Diego, has changed that. They suggest both that cancer stem cells are very relevant indeed to survival, and that going after them is an excellent idea.

The relevance of cancer stem cells to survival was shown by William Matsui of the Johns Hopkins Sidney Kimmel Cancer Centre in Baltimore. He looked at samples from 268 people with pancreatic cancer and found that the pattern of stem cells in their tumours predicted how long they would live. Those whose tumours had stem cells at their edges (the “invasive margin” in the militaristic jargon of the cancer-warriors) lived on for an average of 14 months. Those who did not lived an average of 18 months. Not a huge difference, but confirmation that cancer stem cells have an impact on the outcome of disease.

**Bombs away**

Such stem cells, then, are as bad as theory suggests they should be. The question is, can they be eradicated?

Animal tests suggest this is hard. For reasons as yet unknown, stem cells are resistant to standard cancer chemotherapies. With this in mind, Jeffrey Rosen and his colleagues at Baylor College of Medicine in Houston, Texas, compared samples from breast-cancer patients taken before and after 12 weeks of chemotherapy. They reasoned that if stem cells were resistant in people as well as mice, then the proportion of stem cells within a tumour would increase as more vulnerable cells were killed off in disproportionate numbers.

And that is exactly what happened. Among women treated with old-fashioned chemotherapy, the share of stem cells within their tumours rose from 5% before treatment to 14% afterwards. Dr Rosen, however, went further. In a parallel experiment he looked at a group of women being treated with a new drug called lapatinib. In these people, the proportion of cancer stem cells decreased from 10% before therapy to 7.5% after they were treated.

Lapatinib is a product of the growing field of molecular medicine—the design of drugs to attack specific protein molecules associated with particular diseases. In this case the protein attacked is HER2, a molecule often found on the surface of breast-tumour cells. Ironically, however, it was not lapatinib's effect on HER2 that made it potent against stem cells. Lapatinib, it turns out, also inhibits the activity of a protein called the epidermal growth factor receptor, which has been found to be important for stem-cell proliferation. When its activity is blocked, a stem cell's daughters both lack stem-cell qualities and the chain of “stem-cellness” that the system depends on is broken. Hence the proportion of stem cells in the tumour falls.

It is too early to tell if Dr Rosen's discovery is a life-prolonging one. But Dr Matsui and his colleague, Carol Ann Huff, are thinking along similar lines.

Alongside Dr Matsui's pancreatic-cancer work, they have been looking at treating multiple myeloma, a type of blood cancer, with a combination of heavy artillery and guided missiles: high-dose cytotoxic chemotherapy to kill the bulk of the cancer cells and antibodies called rituximab. These bind to a protein called CD20 that sits on the surface of cancer stem cells. It was expected to kill them.

Not every experiment works, and this one did not. As expected, patients left with the fewest cancer stem cells after the therapy lived longest. But this was the luck of the draw, for the rituximab failed to kill the cancer stem cells. Indeed, Dr Huff and Dr Matsui could see stem cells coated with the antibodies alive and well in their samples.

The next step, Dr Huff and Dr Matsui agree, is to bring in even more powerful missiles. Another proprietary antibody that binds to CD20, called tositumomab, is radioactively labelled. If this coats the myeloma stem cells in the way that rituximab does, the radiation should kill them.

Researchers at the University of Michigan, where tositumomab was developed, have already begun such an experiment. Andrzej Jakubowiak, who is leading the trial, says four patients have been on the treatment long enough to be evaluated, and three of them have already been taken off other drugs and appear clinically stable—which for advanced myeloma is an unusually good success rate. The laboratory data also look promising. The radiological bombs seem to be destroying the cancerous stem cells in exactly the predicted manner—although Dr Jakubowiak rightly cautions that too few patients have been treated to allow firm conclusions.

The upshot of all this is that the stem-cell hypothesis of cancer growth looks a good one. It explains a lot of things, and allows biologists to look at tumours in a new way—almost akin to developing organs, albeit ones with no function and growth that is out of control. That insight, and a better understanding of stem-cell biology, may be the chink in cancer's armour that people have long been searching for. And that is a truly optimistic thought.

## Biotech Isn't in the DNA

***Despite Chicago's efforts to foster a local life-science community, the metro area trails second-tier genetics centers—and is way behind the leaders***

At the end of a daylong conference of the Illinois Biotechnology Industry Organization, Norbert Riedel, chairman of the nonprofit outfit and Baxter International's (BAX) chief scientific officer, summed up the high points of the previous 12 hours. He segued from the new Institute for Genomic Biology at the University of Illinois in Urbana-Champaign to startups spinning out of Northwestern University and its $350 million nanotechnology institute, to efforts by Monsanto (MON) and Tate & Lyle to develop crop-based alternatives in Illinois for fuels and petrochemicals.

"A lot of fantastic research is going on here in Illinois," he reminded the dozens of executives and academics in the audience, citing as an example a 340-acre farm that U of I is offering near its flagship campus for a BP (BP) biofuels project. "I haven't seen anything like that on the East Coast or the West Coast."

Riedel, a tall man with cropped blond hair, a light German accent, and seemingly boundless energy, concluded with a rallying cry: "Let's keep going. Make it happen."

Outside the February symposium at the Hyatt Regency Chicago, however, it's hard to see what all the excitement is about. Despite years of work by iBIO, as Riedel's group is known, and its corporate, university, and government allies, Chicago remains a biotech backwater. In 2007, eight Illinois biotechnology startups received a total of $115.8 million of private investment, according to a report by PricewaterhouseCoopers and the National Venture Capital Assn. That compares with 477 biotech deals worth $5 billion nationwide. That means Illinois as a whole is snagging just 2% of all venture funding in biotech, while Chicago, which received $1 million for one company, isn't even a blip.

As a partner at Clarus Ventures, Dr. Jeffrey M. Leiden sees similar figures. Leiden, who stepped down as president and chief operating officer at Abbott Laboratories (ABT) in 2006, says the Cambridge (Mass.) partnership probably reviews 1,000 proposals a year from life-science startups seeking cash. Last year, pitches came from up and down both seaboards, as well as Canada and Europe. Chicago

didn't submit one. In fact, he says, metro Chicago ranks behind such second-tier biotech sites as Minneapolis, Austin, Tex., and Madison, Wis.

Chicago's underachiever status comes down to a half-dozen interrelated deficits. The area lacks early-stage money, business-savvy researchers, serial entrepreneurs, public-sector financial and regulatory support, cooperation among institutions, and practical infrastructure. Any one could prevent an idea from ever becoming more than that. Combined, they seem to doom all hope. Notes William O'Neill, who has been a professor of bioengineering at the University of Illinois at Chicago for 42 years: "It's the biggest city with the fewest things going on."

One by one, say Riedel and other biotech boosters, they're filling in Chicago's gaps—by opening a business incubator with rentable lab space, say, and linking up scientists and executives. They also point to some successes. Nanosphere (NSPH), for instance, a Northbrook company that has combined biotech and nanotech to come up with a supersensitive diagnostic test, raised $102 million last fall in an IPO. "If I were living in some other place in the U.S., I would probably say it would be nice, but it's naive," Riedel says of creating a biotechnology cluster. "But here it's very real; it's very doable."

Chicago also can take comfort that other cities have built themselves into biotech hubs. But these same role models also work against Chicago. Up-and-coming researchers, entrepreneurs, venture capitalists—they all tend to flock where their kind are already established. Thus, Chicago is no longer battling only San Francisco and Boston, the nation's biotech capitals; it's up against San Diego and North Carolina's Research Triangle, to name a couple of the newer centers, as well as dozens of other wannabes. "I like the city," says Arnold Oronsky, a veteran biotech investor and general partner of InterWest Partners in Menlo Park, Calif. "But this is a daunting task for Chicago. It's sort of a vicious circle."

**Amgen Defection**

The fever to become the next biotech hot spot is understandable. Jobs in biotechnology pay some 75% to 100% more than average private-sector positions, and the highly educated folks who do this work are the types that cities and states crave. While older industries fade, the sector is growing, as aging and wealthier populations around the world demand new treatments for disease. The technology is also a marvel: the manipulation of genes and cells to create new pharmaceuticals, engineered organisms, and medical devices.

Had only a few events unfolded differently, Chicago might have been bubbling with these businesses today, including heavyweight Amgen (AMGN). The company is the biotech industry's largest, with a market cap of more than $43 billion and annual sales of $14.77 billion. Its first blockbuster was Epogen, an anemia drug derived from research at the University of Chicago. The company's founding chief executive was George Rathmann, a former Abbott vice-president for research and a Northwestern grad, and Abbott itself was an early investor. But Rathmann left Chicago to help start Amgen in 1980 in Thousand Oaks, Calif., because that's where the biophiles in venture capital were.

Why were the VCs there? Four years earlier, the world's first biotech company, Genentech (DNA), opened in South San Francisco. The Genentech story has a what-if element, too: Its president from 1985 to 1995 was G. Kirk Raab, who also decamped from Abbott's North Chicago campus, where he had been president and chief operating officer.

Baxter itself has been seeding biotech startups with management talent—but almost always far from Chicago. In her 2005 book, *Career Imprints*, Monica Higgins, a professor at Harvard's Graduate School of Education, traced a quarter of U.S. biotech startups to executives who had left the Deerfield-based medical-products maker. "They trained the best managers in the biotech industry, and they all left," says Alicia I. Löffler, director of the Center for Biotechnology at Northwestern's Kellogg School of Management. "That was a missed opportunity."

**No Local Money**

Despite Riedel's exhortations, it may be too late for Chicago to catch up. Most private equity firms are rooted in coastal cities, and the early-stage investors who are here don't know much about biotechnology; they tend to focus on other industries, such as business services or financial technology. That forces life-science newbies to look elsewhere for seed investment, and many can't find it. "The lack of venture capital forces our companies to bootstrap themselves," says Peter Shagory, a partner at Baird Venture Partners in Chicago.

Metro Chicago's pharma companies have done little to encourage local entrepreneurs. Biotech innovator Millennium Pharmaceuticals (MLNM) of Cambridge, Mass., and cardiac-device maker Medtronic (MDT) of Minneapolis are celebrated for spawning biotech industries in their hometowns as their scientists leave to launch companies of their own. Indianapolis-based Eli Lilly (LLY), meantime, has its own venture fund that invests in startups. Abbott and Baxter both spend billions of dollars annually on research and development, and Abbott has state-of-the-art labs at its headquarters. But Abbott conducts its biotech research in Worcester, Mass., while Baxter's bioengineering facilities are in California and Austria. If their bioresearchers do go out on their own, they do it there, not here.

In an industry that's global, transforming Chicago into a center for biotech R&D isn't a priority for corporate chieftains. "It doesn't matter if the technology is created across the street or across the ocean, because it's so transportable," says Dr. John Leonard, Abbott's senior vice-president for pharmaceutical R&D. Indeed, two rising stars on the local biotech scene—Ovation Pharmaceuticals of Deerfield and Sagent Pharmaceuticals of Schaumburg—don't do any research at all; they just buy rights to compounds discovered by others and market them.

Chicago-area universities aren't any better at creating biotech entrepreneurs. Many in the industry blame academic cultures that have dissuaded researchers from commercializing their work. University rivalries have gotten in the way, too, forcing researchers to work in isolation. There's also a shortage of fully equipped labs. Illinois has 1.5 million square feet of wet lab space, thanks in part to Illinois Science & Technology Park, which fills a third of the former G.D. Searle site in Skokie. But with only 1/15th the population, Madison matches Chicago in lab space. And Chicago isn't even close to Boston, which has more than 14 million sq. ft., or San Francisco, with some 11.5 million sq. ft.

The state hasn't done much to encourage the industry, either. It doesn't offer tax credits to so-called angel investors, the way Wisconsin, Michigan, and Indiana do. Nor does Illinois allocate public money to support the industry directly. Ohio, on the other hand, has a fund that invests roughly $700 million in bioscience, and Governor Ted Strickland wants to plow another $300 million into the life sciences this year.

If anyone can put Chicago on the biotech map, it may be Riedel. Formerly the head of biotech at Hoechst Marion Roussel, he joined Baxter in 1998 as president of its recombinant therapeutic protein unit in Glendale, Calif. Riedel, who earned a PhD in biochemistry from the University of Frankfurt, transferred to Deerfield in 2001 when he took over as chief scientific officer. He has been chairman of iBIO for almost two years. He's certain that the conditions to create a thriving biotech industry are all here—it will just take perseverance. "It was the same on the East Coast and the West Coast," he insists. "It wasn't there to begin with."

**Fourth-Largest Hub**

IBIO, which started in late 2001, has a budget of $2 million and 125 corporate, university, and government agency members. It pushed the General Assembly to form the state's first biotechnology committee and is lobbying Springfield to pass angel tax credits and funnel state funds into biotech. Its latest effort: a one-year-old program that hooks up successful executives with those just starting out. Called Propel, it is modeled after a mentoring program in San Diego that is credited with greatly increasing the success rate of new biotech companies.

The advocacy group also argues that the state's bioscience industry—under iBIO's broad definition, the industry encompasses medical-device, diagnostic, and bioagricultural companies—is in fact quite

healthy. According to a report by the Battelle Memorial Institute, done on behalf of the Biotechnology Industry Organization (BIO), Chicagoland has more than 1,000 such companies, with 46,000 employees altogether, making it the fourth-largest concentration of biotechnology in the country.

David Miller, iBIO's president, points to the BIO 2006 conference in Chicago as a sign that things are turning up. The national meeting was the first BIO had ever held in an interior city, and it broke attendance and revenue records. Miller is also encouraged that Chicago will host the conference again in 2010. But out-of-towners don't necessarily see the return engagement as an endorsement of Chicago's biotech prowess. "You shouldn't be flattered," says San Diego venture capitalist Ivor Royston, who is credited with starting that city's first biotech company, Hybritech, in 1978. "There aren't that many places where you can hold a convention for 20,000 people."

So far, disappointments outnumber achievements. Richard Morimoto, a biology professor at Northwestern, helps run the Chicago Biomedical Consortium, which, with $50 million from the Searle family, is attempting to bring together academics from Northwestern, Chicago, and the University of Illinois at Chicago. Yet for his own startup, Proteostasis Therapeutics, which is developing drugs to treat Parkinson's and Alzheimer's, Morimoto and his partners chose Cambridge, Mass. "It wasn't even a discussion that I could form it in Chicago," he admits. "It's astonishingly easier to do things in the Bay Area, Cambridge, or Stanford."

Even Miller concedes that when it comes to spinning out startups and nourishing them with sufficient capital and business talent, Chicago is failing. "We're not great at this startup thing," he says, "but we're getting better."

## U.S. pharma market slows

***U.S. drug market growth matches sluggish Japan, but lags behind Mexico, Brazil, Russia, Turkey, China, says IMS.***

America is still the biggest drug market in the world, but its sluggish growth was blown away by most international markets last year, said IMS Health on Tuesday. Worldwide prescription drug sales grew 6.4% to $712 billion in 2007, said IMS Health, a market research firm. This includes the United States drug market, which grew a mere 3.8% in 2007 to $286.5 billion, its lowest rate since the early 1960s, said Connecticut-based IMS.

The drug market for the U.S. combined with Canada grew 4.2% in 2007 to $304 billion, said IMS. But while the U.S.-Canada market accounted for 46% of worldwide drug sales, its expansion slowed dramatically from 2006, when sales grew 8.3%.

This growth was outpaced by markets south of the border. Mexico's drug market grew 7.5% to $11.1 billion in 2007, said IMS, while the markets in Central and South America surged 11.6% to $42.4 billion. This includes the relatively large Brazilian market, which grew 9.7% to $15.7 billion.

The drug market in five major European countries -- France, Germany, the United Kingdom, Italy and Spain -- grew 4.8% to $140 billion in 2007. Without including these countries, the European market grew at a much faster 10.9% pace to $81.6 billion. This includes Russia, one of the fastest-growing markets with a 20.2% expansion, and Turkey, which grew 17.2%.

China was the most rapidly-expanding market in 2007, with a growth of 25.7%, said IMS, while South Korea grew 10.7% and India grew 13%. IMS did not release totals for these countries. But it said the Asian market, not including Japan, Australia and New Zealand, was 11% of the world total, so it would exceed $70 billion.

The Japanese market is one of the largest outside the U.S., but its growth matches America's glacial pace. Drug sales in Japan grew 3.8% in 2007 to $65.2 billion, said IMS. Growth in the so-called mature markets tends to be slower than in countries undergoing rapid modernization and development, which often results in better access to healthcare.

## Intellectual Property Protection - Joint Development Agreements can protect outsourced IP

With the rise in outsourcing new drug and biologic development to overseas laboratories, how are biopharmaceutical executives protecting their intellectual property (IP) from insider theft?

Drug and biotech co-development projects are increasingly common as firms seek to increase their product pipelines and speed time to market. Smaller firms are at a distinct dis-advantage when it comes to negotiating agreements that protect their current intellectual property and their long-term creative potential.

Outside of the FDA regulated marketplace, one successful strategy to place co-development partners on equal footing and protect both partners' IP has been the creation of a Joint Development Agreement (JDA). Such a document can be created either during the initial negotiations or as a follow-on guiding set of principles incorporated as a contract amendment.

**Intellectual Property Ownership**

If the contract between product development organizations does not specify ownership rights of various components of intellectual property, the JDA is the place to summarize these. There are three areas of IP ownership the joint development agreement must specify:

- Initial IP brought to the collaboration by each partner;
- Ownership of the new IP that results from the partnership; and
- Rules for negotiation around any IP outgrowths that are not necessarily part of the partnership's goals, but that develop as natural offshoots of the partnership's work.

As a best practice, one should consider creating an IP factsheet as an addendum to the JDA that reviews the basic types of intellectual property as well as the essentials of IP ownership and rights.

Even if the partnership contract specifies some components of IP ownership, consider using the JDA to further elaborate on ambiguous points. In such a case, the goal is to ensure that the scientists and engineers involved clearly understand the expectations of their executive teams.

**Knowledge Flow Management**

The flow of information and knowledge between collaborating organizations is one of the main thrusts of a joint development agreement. Key components to address include: communication, issue resolution, project reviews and stage gate responsibilities, roles of each team member, checkpoints to ensure tangents are minimized yet still allowed, storage of information and security protocols. The goal of such efforts is to ensure that information exchanges and decision-making expectations are aligned among the collaborating organizations.

**IP Storage and Security**

To achieve this, involve personnel from records management and information technology groups. Records management must clarify the appropriate records retention rules around information generated during the co-development project and may need to bring in legal counsel to provide advice on certain aspects to minimize risk and liability in the worst-case event of future litigation and information discovery motions.

Information technology (IT) professionals will be required to understand the necessary levels of security expected and provide recommendations. Consider having the project manager or other non-IT executive approve the various levels of electronic security; leaving the security of developing intellectual property in the hands of your computer department risks an imbalance of security versus access.

I frequently advise my clients to avoid situations wherein five or more individuals who are in constant contact with each other have the exact same levels of access to IP. Drawing on historical patterns seen in financial fraud, five is the trigger number of people required for theft of IP without alarm bells sounding until it is too late.

Without careful consideration of options and techniques, the five-person trigger point may seem an unnecessary burden by project managers and information technology personnel. Consider asking your outside counsel, an independent consultant or even your alliance partner for help in implementing this control.

Place all joint project documents, schedules, meeting summaries and so forth in a central repository where they become the property of all parties involved in the collaboration. Note that storing the information in a central location does not imply across-the-board access.

Many of the documents in the repository will be "read-only" (or may not even be visible), depending upon each individual's role within the collaboration. In addition, have the information technology department of each organization provide a regular electronic back-up, as well as periodic snapshots of the repository for long-term archival. This will provide each partner protection and proof for its claims of intellectual property ownership and project effort.

**Final Thoughts**

While the joint development agreement is not a legally binding document until it is made into a contract amendment or otherwise incorporated contractually, the JDA is a set of operating principles and guidelines agreed upon between the collaborating organizations' executives and project teams.

Review the JDA on an annual basis. Ask yourself the following questions:

- Are the controls still working as intended?
- Are there any further areas of work undertaken together that need to be incorporated into the JDA?
- Are there new intellectual property aspects that need to be captured?
- Have all team members and senior executives reviewed the agreement?
- Do they understand their roles and accountabilities?

If executives are uncomfortable asking these questions, recognize this as a warning flag. Consider bringing in an outside facilitator to help with an unbiased review and set of recommendations.