# Translational research on spinal cord injury and cell-based therapies : a focus on pain and sensorimotor disturbances

# Dissertation summary

Current SCI treatment options are limited. Promising treatments to promote regrowth and regeneration in the injured spinal cord carry the risk of worsening outcomes, such as pain, which is likely due to further stimulating aberrant neural plasticity. However, little is known on the mechanisms of SCI pain or aberrant neural plasticity. Clues to its etiology might be found by analyzing differences between studies on different SCI populations, as widely varying pain prevalences have been reported. On the other hand, one can also accept the limited understanding of aberrant neural plasticity etiology and take a perhaps more pragmatic path by screening the currently most promising regenerative treatments, such as cell-based-therapies, in an adequate preclinical *in vivo* model. However, translation of positive preclinical results into the clinical setting has thus far been disappointing, which calls for a reevaluation of our preclinical tools.

Therefore, this dissertation 1) explores causes for the heterogeneity in SCI pain prevalences and searches for pain determinants, 2) explores tools (sensory and motor assays, and immunosuppression protocols) for the preclinical and translational evaluation of SCI treatments, and 3) investigates the relevance and feasibility of promising candidate cell-based therapies (olfactory ensheathing cells, OEC, and human fetal spinal cord-derived neural stem cells, HSSC) for SCI treatment in preclinical models. The research questions asked are:

1. Is it possible to identify which study and/or population characteristics show a relationship with the differences in reported pain prevalence rates in SCI patient populations? (chapter 2)

2. Can we differentiate between spinal reflex mediated functionality and supraspinally mediated functionality in rodent SCI models? (chapter 3 and 4)

3. Does transplantation of OEC or HSSC into the lesion site provide functional improvement in an experimental SCI model? (chapter 5 and 6)

4. Is long-term HSSC engraftment safe for clinical use and achievable with the use of slow-release immunosuppressive drug formulas? (chapter 7 and 8)

Ad 1) The answer is yes. Eighty-two studies reporting on SCI pain prevalence were examined. Study design related determinants of SCI pain prevalence reports were: pain definition strictness (mild, moderate, or high), primary study goal (pain study or not), data source (retrospective or not), and, in

a limited number of cases, response/attrition rates. While correcting for latter items, populated characteristics found to determine pain prevalence rates were both the proportion of patients with a depression and the average time after injury (positive correlations). Between-study heterogeneity may remain even after the identification/correction of abovementioned causes of heterogeneity. Hence, pain after SCI seems to relate to the duration of the injury and depression, yet, major causes of bias in reported pain prevalence are found to be related to the primary study goal (pain study or not), choice of pain definition, and the use of retrospective data.

Ad 2) The answer is yes. Reduced hindpaw withdrawal thresholds (after rat thoracic SCI), which are often believed to model below-level pain states in human Spinal cord Injury patients do not reflect any decrease in thresholds for pain perception (i.e. hyperalgesia), but instead, coincide with a decrease in below-level perception (i.e. hypoesthesia; which, indeed, is also more the rule than the exception in human SCI patients, and a limiting factor in recovery of locomotion). Yet, increased hindpaw withdrawal responses might still relate to another clinical problem in SCI patients; the "spastic"- or "upper motor neuron"-syndrome (which is thus far largely ignored in translational research). Hence, it was tested whether spasticity/muscle stretch hyperreflexia co-occurred with the increased hindpaw responses, but no such spasticity was observed. Therefore we suggest/discuss that hindpaw withdrawal responses are best interpreted as either 1) a different subset of clinical problems related to the spastic syndrome (i.e. spasms and/or clonus), or 2) as mostly a species-specific phenomenon. Also, widely-used existing locomotion tests can be questioned for their translational value, as, unlike human SCI patients, rats 1) easily recovers hindpaw walking ability after severe SCI, 2) can even do this without sensory perception (see above), 3) have a vastly easier (more stable) bodily position during walking (quadrupedalism vs erect bipedalism), 4) require merely simple 'stepping patterns' for normal/daily-life function, and 5) learn to walk much faster in the postnatal period. This indicates that rat locomotion has a low dependence on spinal integrity or supra-spinal input (i.e. depend on their "Central Pattern Generator" or "Spinal" walking), while humans are much more dependent on supra-spinal control. During preclinical therapy evaluations this difference should be kept in mind and an adequate/translatable testing paradigm which is sensitive for supra-spinal mediated motor function must be used. A novel locomotion test is proposed in this dissertation, i.e. backward walking on a rotating rod, and is shown to be more sensitive to SCI than existing tests, even in the chronic phase post SCI, and is likely to be more dependent on supra-spinal input.

Ad 3) The answer is 'possibly not' and 'yes', respectively. OEC are a promising graftable cell-based therapy, but only few studies have focused on experimental models with large cavitations (as occurs

in humans), which will require bridging substrates to transfer and maintain OEC within the lesion site. A state-of-the-art collagen-based multi-channeled three dimensional scaffold is used to deliver olfactory ensheathing cells to 2 mm long unilateral low-thoracic hemisection cavities in rats. Hyperreflexia of the hindpaws was monitored using the Von Frey hair filament test, while an extensive analysis of motor ability was performed. No substantial improvement or deterioration of motor functions was induced and there was no effect on lesion-induced hyperreflexia. On the basis of these data, it can be concluded that relatively large spinal cord lesions with cavitation may present additional hurdles to the therapeutic effect of OEC. Also, intraspinal grafting of clinical grade HSSC in a rat model of acute lumbar (L3) SCI was tested. Spasticity, lesion volume, various motor and sensory dysfunctions, and neural integration was assessed. Treatment led to a progressive improvement in lower extremity paw placement, amelioration of spasticity and thermal and tactile pain/escape thresholds. Near complete injury-cavity-filling by grafted cells and development of putative GABA-ergic synapses between grafted and host neurons was observed.

Ad 4) The answer is very likely ‘yes’. Achievement of effective, safe and long-term immunosuppression represents one of the challenges in experimental allogeneic and xenogeneic cell and organ transplantation. A reliable, long-term immunosuppression protocol in rats was developed by: 1) comparing the pharmacokinetics of four different subcutaneously delivered tacrolimus formulations (tacrolimus is a widely clinically used immunosuppressive agent which inhibits T-lymphocyte signal transduction and IL-2 transcription), and 2) defining the survival and immune response in animals receiving spinal injections of human neural precursors. It is demonstrated that use of TAC pellets can represent an effective, long-lasting immunosuppressive drug delivery system that is safe, simple to implement and is associated with a long-term spinal graft survival in SCI or SOD+ rats. Furthermore, especially the potential tumorigenic property of many contemporary experimental stem cell therapies requires special preclinical safety assessments, but no standard guidelines or any stem cell safety studies have yet been published. A preclinical assessment of the safety of HSSC therapy for a phase 1 clinical trial application is described. The design of the reported safety study is the result of a dialogue between researchers in this field and the national drug authority (the Food and Drug Administration). The negative results found for tumorigenicity, toxicity, and worsening outcomes, in conjunction with data from HSSC efficacy/therapeutic studies have resulted in the approval of the currently ongoing clinical trial of spinally applied HSSC in human patients.