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Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Weidner N, Abel R, Maier D, et al. Safety and efficacy of intrathecal antibodies to Nogo-A in patients with acute cervical spinal cord injury: a randomised, double-blind, multicentre, placebo-controlled, phase 2b trial. *Lancet Neurol* 2025; **24:** 42–53.

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Weidner et al., Safety and efficacy of intrathecal antibodies to Nogo-A in patients with acute cervical spinal cord injury: a randomised, double-blind, multicentre, placebo-controlled, phase 2b trial

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SECTION S1: Study Personnel

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SECTION S2: Methods Details

Dose rationale

Clinical dose estimates for the preceding phase-I clinical trial were calculated from the results obtained in the monkey (macaque) pharmacodynamic studies, where internalization of the NG-101/Nogo-A complex is demonstrated after four weeks of continuous intrathecal infusion of 1.08 mg/day. Considering the interspecies difference in NG-101's affinity to human and monkey Nogo-A protein, the estimated effective dose in man is 5 mg/day. Since a dose of 60 mg/day is well tolerated by monkeys in the 28-day i.t. infusion toxicity study it provided the basis for estimating the safe starting dose for the completed first-in-man study in acute paraplegic SCI patients. The human dose equivalent, 300 mg/day, is based on interspecies differences in compartmental volumes (CSF volume) and antibody affinity. Division by the default safety factor of 10 would result in a maximum safe starting dose of 30 mg/day. However, this dose level is already well above the estimated effective dose in humans of 5 mg/day, which is derived from the 1 mg/day dose level used in the above mentioned proof of efficacy study in monkeys. Therefore, a starting dose of 5 mg/day has been selected for the first into man clinical trial. NG-101 in the first-in-man study (NCT00406016) was safe and well tolerated in spinal cord injured subjects at doses up to 15 mg/day for a maximum of 28 days using continuous i.t. infusion and at doses up to 6 × 45 mg over four weeks using repeated i.t. bolus injection. Repeated i.t. bolus injections appeared to be safer and less prone to technical complications compared to the continuous infusion mode of administration, and appear to meet tolerability and pharmacokinetics expectations. Based on the data from the first-in-man study (NCT00406016), the treatment regimen in the phase II study in spinal cord injury tetraplegic patients will be repeated i.t. bolus injection of 6 × 45 mg NG-101 over four weeks.

Trial Design

We used a recently developed stratification algorithm that is based on the individual prediction of the patients with acute cervical SCI. Using Unbiased Recursive Partitioning (URP) the distribution of UEMS outcomes at 6 months was revealed and different cohorts of outcome of UEMS recovery (nodes in the interference tree) were distinguished This enabled to exclude those patients who are expected to recovery in mean UEMS > 41 regardless of treatment group (mean UEMS above 41/50). These cohorts (node 20 and 21) clearly reach ceiling effects that will impact to reveal treatment effects as the spontaneous recovery is already that good that further improvements cannot be detected by measuring the UEMS. Furthermore, outcome cohorts (node 4, 5, 8, 9, 10, 13, 16, 17, 18) with limited outcome can be predicted and further improvements can be discerned in the treatment group compared to the control group. This approach allows to enroll about 73% of the patients with cervical AIS A - D which represents a reasonable high inclusiveness while applying this predictive

stratification that will avoid enrolling patients that might less likely benefit from the intervention. The analysis is based on the assessment of 575 patients with acute cervical SCI scored as AIS A – D (assessment within the first 2 weeks following acute SCI). The inclusiveness (i.e. the percentage of patients that might be recruited) is about 73% when excluding node 20 and 21.

Sample size calculation

The power calculation is based on the mean delta changes in the EMSCI data of the control group (nodes 4, 5, 8, 9, 10, 13, 16, 17, and 18 have a mean delta UEMS of 14.3 +/-SD of 10.8 motor scores) and a 42% treatment effect (mean delta change of 20.3 motor scores). Using t-tests means with an allocation 3:1, an estimated two-sided α error probability of 0.05 and a power (1-β err prob) of 0.8 a total of about 106 patients would be required. For an adequate powering of the study, we assume that 20% of patients will drop out of follow-up, which means that at approximately 114 patients will be needed to compensate. The protocol was amended in order to get more subjects exposed to NG101. After approval of the amendment the randomization ratio will be changed from 1:1 to 3:1 (NG101:placebo). If the randomisation ratio is altered after 30 subjects have been included at 15:15 and the remaining subjects will be recruited at 63:21, the resulting number of subjects is expected to be 78:36. The power of the test is expected to go down to 66%. The power calculation based on a t-test is a reasonable (and conservative given that the variance of the estimate should be lower with explanatory variables explaining some of the variance) approximation to parameter tests from the model outlined there. The analysis of delta UEMS changes between 2 weeks- 6 months compared to 2 weeks-12 months reveals that the UEMS scores have reached about 90% of their recovery within the first 6 months after injury and late changes are rather minor. These findings allow for a total study duration of 5.5- 6 months for each patient to reveal the effectiveness of NG101.

Enrolment details

Inclusion Criteria

1. Male or female, 18 through 70 years of age
2. Acute cervical spinal cord injury (SCI) (Neurological level of injury C1 ≤ lesion ≤ C8) with confirmed classification of ASIA impairment scale (AIS) A-D at screening and predicted upper extremities motor score (UEMS) recovery of less than 41/50 (according to the URP prediction model)
3. 4-28 days post-injury (i.e. initiation of bolus injection within 4-28 days post-injury)
4. Tetraplegic patients who are allowed to start treatment are those who either do not require mechanical ventilation or who do not completely depend on mechanical ventilation but show

some degree of spontaneous ventilation. Only those modes of ventilation where the patient show active initiation of breathing are allowed (e.g. continuous positive airway pressure (CPAP))

5. Hemodynamically and clinically stable according to the acute SCI condition at baseline
6. For patients of childbearing potential, use of reliable means of contraception as described below during the treatment period and for at least six months after the last dose of study drug: Males and Females of child bearing potential, who are willing to use a highly effective method of contraception [either combined hormonal contraception associated with inhibition of ovulation (oral, intravaginal, transdermal), progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable, implantable), intrauterine device, intrauterine hormone-releasing system, bilateral tubal occlusion, vasectomized partner or sexual abstinence)], or women not of child bearing potential, defined as women who have been surgically sterilized (total hysterectomy or bilateral oophorectomy, bilateral tubal ligation, staples, or another type of sterilization) or are postmenopausal for at least 2 years. Individuals who are convincingly sexually abstinent are also eligible. Sexual inactivity by abstinence must be consistent with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g., calendar ovulation, symptothermal, or post-ovulation methods) and withdrawal are not acceptable methods of contraception.
7. Written informed consent by patient before any study assessment is performed. If the patient is only able to consent orally a witness signs and confirms the patient's consent,
8. Cooperation and willingness to complete all aspects of the study
9. Ability of subject to understand character and individual consequences of the study

<u>Exclusion Criteria</u>

1. Complete anatomical transection confirmed by magnetic resonance imaging (MRI).
2. Trauma caused by ballistic or other injury that directly penetrates the spinal cord including gunshot and knife wounds.
3. Multiple levels of clinically relevant spinal cord lesions.
4. Major brachial or lumbar plexus damage/trauma.
5. Significant head trauma (e.g. cortical damage/lesion), or other injury that was, in the opinion of the investigator, sufficient to interfere with the assessment of the spinal cord function or otherwise compromise the validity of the patient's data.
6. Other significant pre-existing or current severe systemic disease such as lung, liver (exception: history of uncomplicated Hepatitis A), gastrointestinal, cardiac, immunodeficiency (including anamnestic known HIV) or kidney disease; or active malignancy or any other condition as

determined by history or laboratory investigation that could cause a neurological deficit including syphilis, myelopathy, clinically relevant polyneuropathy, etc.

7. History of or an acute episode of Guillain-Barre syndrome.
8. History of recent (6 months) meningitis or meningoencephalitis.
9. History of refractory epilepsy.
10. Patients with uncontrolled bleeding diathesis and/or who require uninterrupted concomitant therapeutic anticoagulation (e.g. phenoprocoumon (Marcumar®), heparin/heparinoids and new oral anticoagulants) at a higher dose than for the prophylaxis of venous thromboembolism
11. Presence of any unstable medical or psychiatric condition (defined by the Diagnostic and Statistical Manual of Mental Disorders, Edition 4 (DSM-IV)) that could reasonably have been expected to subject the patient to unwarranted risk from participation in the study or result in a significant deterioration of the patient's clinical course.
12. Drug dependence (as defined by DSM-IV) any time during the 6 month's preceding study entry.
13. Pregnant or nursing (lactating) women, where pregnancy is defined as the state of a female after conception and until the termination of gestation, confirmed by a positive human chorionic gonadotropin (hCG) laboratory test (> 5 mIU/mL).
14. History of a life-threatening allergic or immune mediated reaction.
15. Patients with the presence of infection around the location where the spinal needle insertions are planned for applying the intrathecal injections.
16. Inability to communicate effectively with the neurological examiner such that the validity of the patient's data could be compromised.
17. Participation in any clinical investigation within 4 weeks prior to dosing or longer if required by local regulations, and for any other limitation of participation based on local regulations.
18. Patients who are unconscious, including those patients who are unconscious due to medication causing marked sedation.
19. History of hypersensitivity to the investigational medicinal product or
20. to any drug with similar chemical structure.

Prespecified subgroup analysis

The following variables will be used to identify relevant subgroups and will be analyzed.

- _Age of the subject at baseline
- _Sex
- _Nodes used for randomization
- _ASIA impairment scale at baseline
- _Neurological level of injury at baseline

• _Bilateral pin prick score at baseline
• _Bilateral light touch score at baseline
• _Level of completeness of SCI at baseline
• _Level of intact motor function at baseline (right and left) at baseline
• _Level of sensory function at baseline (right and left) at baseline
• _Level (right and left) of dermatomal SSEP (dSSEP) C6 – Response at baseline
• _Level (right and left) of dermatomal SSEP (dSSEP) C8 – Response at baseline
• _Level (right and left) of somatosensory evoked potentials (SSEP) of tibial nerve – Response at baseline
• _Level (right and left) of nerve conduction velocity (NCV) of ulnaris nerve - Compound motor action potential at baseline

Randomisation

After a patients' eligibility according to inclusion and exclusion criteria has been confirmed, the patient was registered at the randomisation server via https://randomizer.at.

The randomisation server provided the number of a package available at the site. Neither package number nor study medication, even if unopened, was reassigned after an erroneous randomisation. The allocation of treatment used a balancing algorithm (Big stick allowing for an imbalance of up to 3 patients per cohort) stratified according to the cohorts obtained by the URP based stratification algorithm.

The cohorts (nodes) were derived from the screening (not baseline) measurements because the model has been developed on data obtained about 2 weeks after injury.

<u>Included nodes:</u>

Node 4: UEMS score ≤ 3, AIS = A

Node 5: 3 < UEMS score ≤ 11, AIS = A

Node 8: UEMS score ≤ 11, AIS > A, LEMS score = 0, light touch total score ≤ 62

Node 9: UEMS score ≤ 11, AIS > A, LEMS score = 0, light touch total score > 62

Node 10: UEMS score ≤ 11, AIS > A, LEMS score > 0

Node 13: 11 < UEMS total score ≤ 28, AIS = A or B

Node 16: 11 < UEMS score ≤ 17, AIS > B, LEMS score ≤ 17

Node 17: 17 < UEMS score ≤ 28, AIS > B, LEMS score ≤ 17

Node 18: 11 < UEMS score ≤ 28, AIS > B, LEMS score > 17

<u>Excluded nodes:</u>

Node 20:28 < UEMS score ≤ 38

Node 21: UEMS score > 38

Concomitant therapy

The treatment of accompanying illnesses not subject to the exclusion criteria was permissible if there was no effect on the outcome measures used in this study and no interference with the trial medication. In particular, the following drug groups were not permitted as concomitant medication:

- concomitant therapeutic anticoagulation (e.g. phenoprocoumon (Marcumar®), heparin/heparinoids and new oral anticoagulants) at a higher dose than for the prophylaxis of venous thromboembolism,
- Other investigational therapies (4 weeks prior to enrolment throughout the study period)
- vaccinations with live viruses (e.g. Measles, Mumps and Rubella, Varicella)

The following drug groups were permitted under restriction as concomitant medication:

- Metamizole (non-steroidal anti-inflammatory drug; frequent brandnames: Novalgin, Analgin, Berlosin, Metalgin, Metamizol-Puren, Nolotil, Novaminsulfon)

Attention was paid to patients under treatment with Metamizole where adjusted control of leucocytes was recommended (agranulocytosis). In the phase I study, one case of agranulocytosis/leucopenia (unrelated to NG-101) was seen, which most probably was related to the use of concomitant treatment of Metamizole.

Medication (or diagnostics) taken prior to first dosing: All prescription medications and over-the-counter drugs (including vitamins) taken during the screening phase and throughout the study were recorded in the patient's file and on the Concomitant Medications / Non-Drug Therapies page of the eCRF. New medications administered to the patients (e.g. to treat an AE) were recorded accordingly. Medication entries were documented to generic name, the start and
end date, and the reason for therapy.

Pharmacokinetics

CSF ELISA for NG101

For the detection of NG101 in the CSF a murine type 2 anti-idiotypic monoclonal antibody against NG101 (clone 1D2, Agro-Bio, La Ferté Saint-Aubin, France) was developed. The antibody was labeled with biotin or HRP, respectively. 0.25 µg/ml of 1D2biotin mouse anti-NG101 capture antibody dissolved in 1% BSA (Sigma) in PBS-0.1% Tween20 (Sigma) was bound on pre-coated neutravidin plates (Thermo Fisher #15507). Each plate contained a serial dilution of NG101 as internal standard and CSF samples 5- and 10-fold diluted. Detection was performed with a second monoclonal mouse anti-NG101 HRP antibody (clone 1D2 HRP). The plates were developed with TMB substrate (Pierce) and stopped with 1M HCl. The readouts were acquired on a Tecan Sparc plate reader at 450nm with 620nm correction. The ELISA had the following detection limits: LLOD <4.15 ng/ml, LLOQ 14.6 ng/ml and ULOQ 1000 ng/ml, (Precision (%CV)=25% and Accuracy (%RE)=25%).

Serum ELISA

For the detection of NG101 in serum, 2 µg/ml of a synthetic peptide (16 aa of Nogo-A corresponding to the NG101 epitope, biotin-labelled; JPT Peptide Technologies, Berlin, Germany) in PBS (Gibco) were coated on a 96 well costar plate (Corning #3690) for 2 h at 37°C. Plates were washed three times with TBS-0.1% Tween20 and blocked with SeraSub (CST Technologies) for 1.5 h at 37°C. Each plate contained a serial dilution of NG101 as internal standard. Serum samples were diluted in the blocking solution 5-fold and incubated on the plates for 2 h at 37°. Plates were then washed three times with TBS-0.1%Tween20 and incubated with a mouse anti-human IgG4 antibody (Bio-Rad #919001) diluted 1000-fold in SeraSub for 1 h at 37°C. Plates were again washed three times and incubated with a goat anti-mouse HRP-coupled antibody (Invitrogen) diluted 40,000-fold in SeraSub for 1 h at 37°C. Finally, the plates were washed six times and developed with TMB substrate (Pierce). The readouts were acquired on a Tecan Sparc plate reader at 450 nm and 620 nm correction. The ELISA had the following detection limits: LLOD <0.244 ng/ml, LLOQ 1.7 ng/ml and ULOQ 1000 ng/ml (Precision (%CV) =25% and Accuracy (%RE) =25%).

MRI scanning

In an exploratory post-hoc analysis, neuronal tissue preservation was assessed in every sagittal slice by an expert rater (LF) using Jim software (version 7.0, Xinapse Systems, Aldwincle, UK), focusing on the lesion site. The shortest distances between the hyperintense intramedullary cyst and the spinal canal were measured perpendicular to the alignment of the cord in the head-to-foot direction.

The following scanner were used at the different sites: a 1.5 T Toshiba scanner (Canon Medical Systems Cooperation, Otawara, Tochigi, Japan) in Hessisch-Lichtenau, a 3T Philips scanner (Philips Healthcare, Best, The Netherlands) in Murnau, Nottwil, Halle and Berlin or a 3T Siemens scanner (Siemens Healthcare, Erlangen, Germany) in Zurich, Prague, Barcelona, Bayreuth, Basel, Bochum, and Heidelberg. Throughout the assessment process, raters were blinded to treatment arms and time points.

Mass spectrometry-based proteome analysis

Spectra were searched against the human reference proteome (UP000005640) from the UniProtKB database (release 2021_11), with trypsin as the enzyme, allowing for a maximum of three missed cleavages, and variable modifications including methionine oxidation and cysteine carbamidomethylation as fixed. Peptide spectrum match identification used a scrambled decoy-based false discovery rate of 0.01. Quantification was set to "only proteotypic" with the protein LFQ method and cross-run normalisation set to "local normalisation". Quantitative protein data for NfL were exported from Spectronaut and reported as normalised protein expression values with intensity on a log2 scaleSamples were analysed using an Evosep One system (Evosep, Odense, Denmark) coupled to a timsTOF Pro2 mass spectrometer (Bruker Daltonics, Bremen, Germany) for liquid chromatography-mass spectrometry analysis. Raw data were further processed with Spectronaut (version 16.5; Biognosys AG, Schlieren) utilizing the protein label-free quantitation (LFQ) method and cross-run normalisation. Quantitative protein data for NfL were reported as normalized and log2 transformed LFQ values representing the relative protein abundance.

SECTION S3: Statistical Analysis Details

R code implementing the primary analysis

```
                                        ### R version 4.4.1 (2024-06-14)
library("lme4")                         ### version lme4_1.1-35.5
library("multcomp")             ### multcomp_1.4-26
### uems:       upper extremity motor score (UEMS, 0...50)
### node:       URP tree node (4-5, 8-9, 10, 13, 16-18)
### trt:treatment (Placebo, Nogo_A_Inhibitor)
### tmstd:      time (standardised such that tmstd = 1 means 168 days)
### id: patient id

### primary analysis: baseline fixed main effects of
### node and trt, fixed effect of time, time x trt interation
### within patient correlations by random-intercept / random slope
### model
m <- lmer(uems ~                        ### primary outcome UEMS
                                ### fixed main effects of
          node +                ### URP tree node
          trt +             ### treatment
          tmstd +               ### time
          tmstd:trt +       ### fixed interaction effect =
                                        ### treatment effect
          (tmstd | id),     ### within patient correlations by
                                        ### random-intercept / random slope
        data = ...)
### 95% confidence interval for treatment effect:
### group difference in UEMS change from baseline
### to day 168 post baseline
confint(
  glht(m, linfct = "trtNogo_A_Inhibitor:tmstd = 0"),
  calpha = univariate_calpha())         ### no corrections
```

Sensitivity Analysis

The treatment effect parameter in normal linear mixed-effects models was defined as a linear fixed-effect interaction of treatment and time (in days, scaled such that value zero corresponds to start of treatment at baseline and value one corresponds to six months follow-up), allowing an interpretation as difference in mean change in UEMS and SCIM self-care endpoints attributable to anti-Nogo-A treatment. Assumptions inherent in these normal linear mixed-effects models, such as conditional normality of the endpoint and linearity of the recovery profiles, were assessed in a sensitivity analysis. Mixed-effects proportional odds models for ordinal outcomes (with URP node-specific baseline thresholds using a smooth transformation for UEMS [1]) neither assume normality nor any other parametric outcome distribution and respect the ordinal scale of both endpoints. Potentially non-linear node-specific recovery profiles replace the linear node-specific recovery, and correlated patient-specific random intercepts-random slopes capture unexplained heterogeneity. Treatment effects are

not expressed as differences in mean changes but are interpretable on the log-odds ratio scale for the odds of staying below a certain outcome value at six months, comparing patients within the same node and given random effects. Subgroup analyses for proportional odds mixed-effects models were implemented by the addition of subgroup x time x treatment interaction parameters for estimating subgroup-specific treatment effects. Structurally equivalent models were estimated for URP nodes and subgroups of complete and incomplete SCIs. These additional analyses were performed using the add-on package tramME [2].

Treatment effects estimated using non-normal mixed-effects proportional odds models showed the same direction and order of magnitude compared to effect estimates obtained under conditional normality of the endpoints. Due to the more complex model structure and thus increased variability of parameter estimates, P-values obtained from these models are on average larger than P-values obtained from normal linear mixed effects models. In general, results discussed in the main text hold also under relaxed model assumptions and therefore the main results are not sensitive to restrictive model assumptions. Treatment effects, corresponding confidence intervals, and P-values obtained from both models are given in Table S2.

SECTION S4: Supplementary Results

Pharmacokinetics

Levels of NG101 in CSF and serum, and CSF half-life estimation

Lumbar NG101 CSF levels were determined before every injection, i.e. five days after each previous injection. Over all verum patients, the NG101 CSF trough levels showed a median NG101 concentration of 120 ng/ml (95% CI: 70, 240). at visit 4, i.e. 5 days after the first injection, with a slow increase to 199ng/ml (95% CI: 134, 287.8) at visit 8, i.e. 5 days after the fifth injection (Fig. 3A, Table S4). The CSF trough concentrations in individual patients varied. The reasons for these variations are not known; local conditions at the injection site and variations in the dynamics of the CSF flow or differences in antibody elimination or metabolism may have played important roles. From the CSF trough concentrations, half-life calculations were performed using the median NG101 concentration of each visit and the starting concentration for each injection (C0= 45mg/130ml CSF =0.35 mg/ml) in the linear half-life equation (LnC = LnC0 -k*t). The half-lives for patients with trough concentrations of 20 – 2000 ng/ml were10 – 16 h, whereas the highest patient group (>2000 ng/ml) had a half-life of 30 h. The values are in line with earlier published CSF half-life values for other antibodies [3]. The abnormally long half-life of the few patients of the group >2000 ng/ml may be related to impaired CSF flow or an impaired outflow from the injection site. The calculated PK enabled us to estimate the time point at which the antibody concentration would have dropped below the critical concentration of 10 µg/ml shown to be efficacious in in-vitro neurite outgrowth experiments (Fig. S9A) [4]. For the lowest CSF concentrations (5-100 ng/ml), we calculated an underdosing period (’dosing holiday’) of 86.7–67.8 h. For CSF concentrations in the range of 101-500 ng/ml, the dosing holiday range would be 67.7 – 55 h, and for concentrations of 501-2000 ng/ml, 55 – 37.5 h. The shorter dosing holidays with higher antibody doses will instruct the choice of dosing schemes of future clinical trials. Retention of antibodies by the CNS tissue and the targets are unknown, however, but may be expected to prolong tissue exposure beyond the values seen in the CSF.

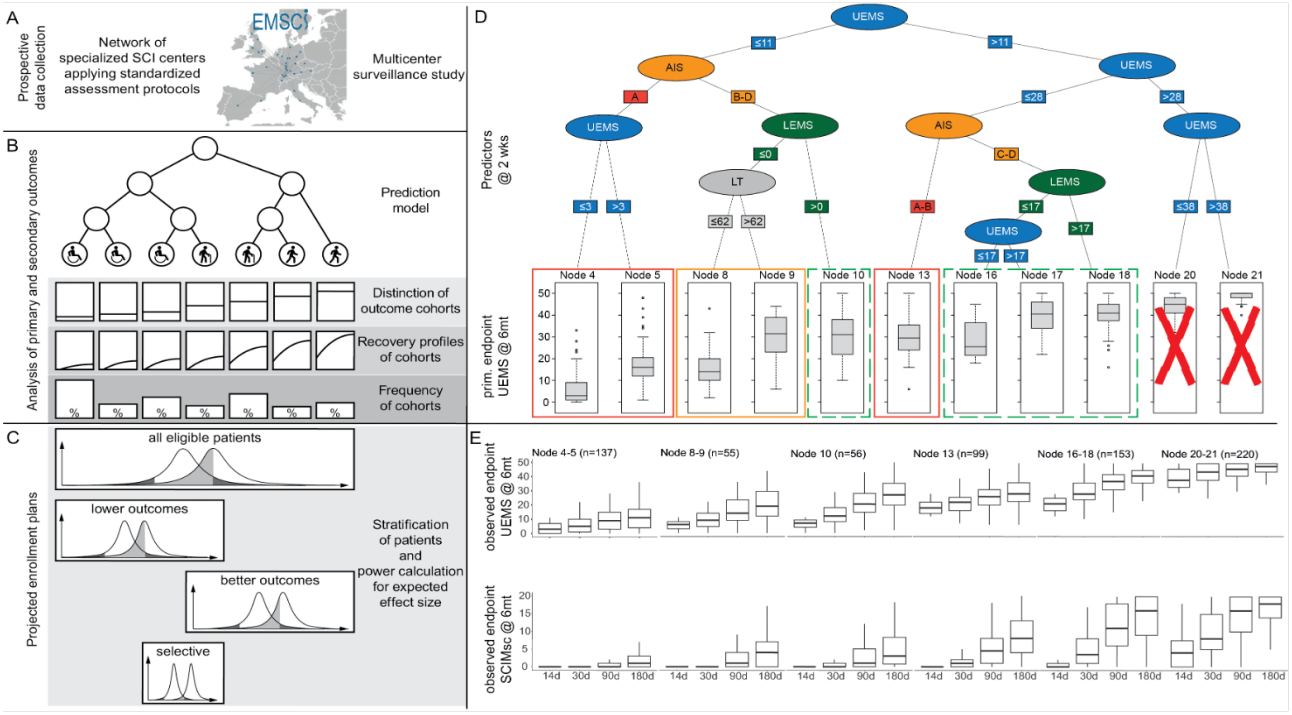
In a post-hoc analysis, the NG101 CSF concentrations were then related to the delta UEMS recovery (change from baseline to 168 days post baseline; Fig. S9B). The analysis showed a trend for a higher UEMS recovery in patients with higher NG101 CSF levels (except for the highest CSF concentrations (data not shown) which might be reflective of impaired CSF flow and/or antibody distribution).

NG101 serum concentrations and PK

NG101 serum concentrations showed a slow build-up over the time of the repeated injections and were quite homogenous across all verum patients (Fig. 3B). Mean and median concentrations were very similar (Table S5). The serum half-life of NG101 was calculated as 23.8 ± 10 days, which is well in line with known values of human IgG4 antibodies [5]. No decrease in serum levels, which could be indicative of active anti-drug antibodies, was observed during the treatment period.

SECTION S5: Supplementary Figures

Supplementary Figure 1



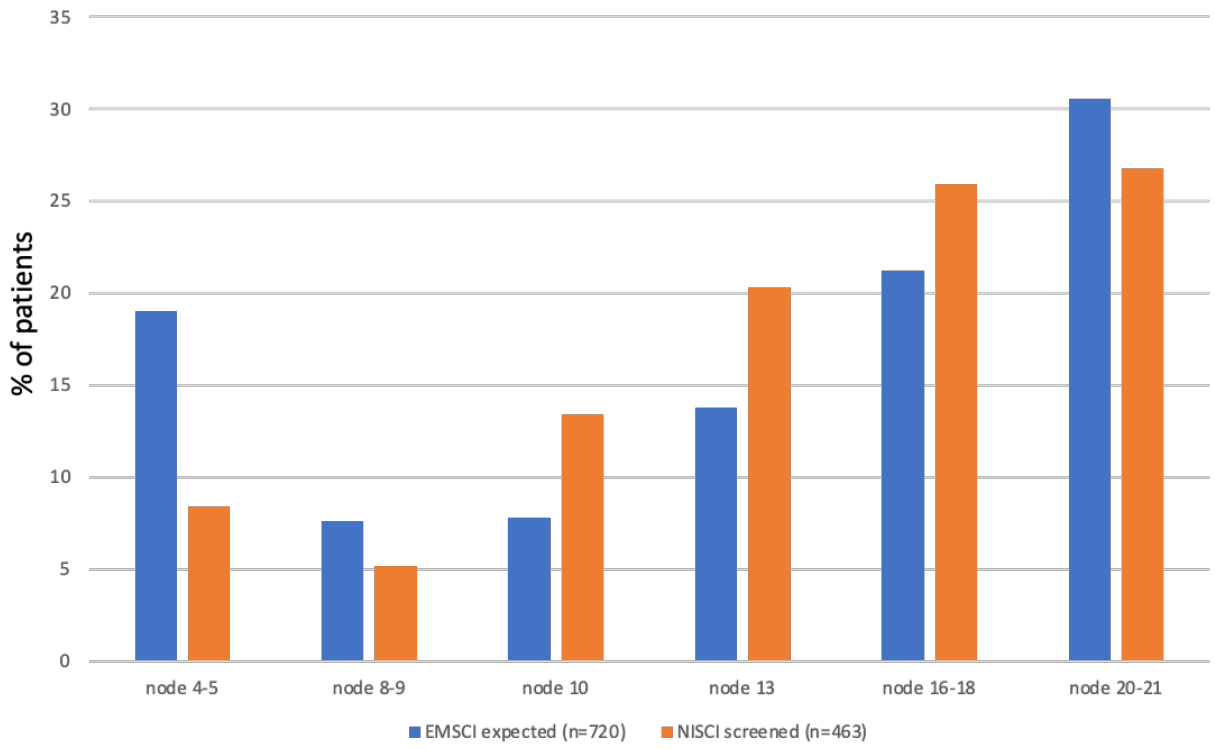
Supplementary Figure 1: Development of trial design. **(A)** Prospectively collected multicenter, multinational clinical data were obtained in acute cervical SCI (www.EMSCI.org) to improve the understanding of clinical outcomes and to inform the trial design. **(B)** URP-based prediction models, which contain information from clinical assessments, allow the stratification of patients into distinct outcome cohorts (nodes). Respective nodes support better balancing between treatment arms and provide information regarding expected frequencies in distinct outcome cohorts and allow the identification of SCI patients likely to be subject to a ceiling effect. **(C)** Information regarding expected frequencies in distinct outcome cohorts enables the informed planning of patient enrolment according to the envisioned patient groups and power calculations based on the distribution of outcomes within cohorts. **(D)** The URP tree utilizes information regarding the different injury severities (AIS grades), motor/sensory function and neurological levels of injury early after injury according to the ISNCSCI protocol to stratify patients into distinct predefined UEMS recovery (primary endpoint) nodes. Patients with predicted ceiling effects in respect to the selected primary endpoint – in this study all SCI patients in node 20-21 – were excluded. **(E)** The predefined outcome cohorts show distinct and reliable UEMS recovery profiles over time (14 to 180 days post injury), which are reflected in the corresponding SCIM-III self-care recovery profiles.

Supplementary Figure 2

Phase	SC	BL	Treatment						Follow-up		
Visit	1	2	3	4	5	6	7	8	9	10	11
Days	-28 to -2	-1	0	5	10	15	20	25	30	84	168
Treatment			x	x	x	x	x	x			
Assessments											
ISNCSCI	x	x							x	x	x
SCIM-III		x							x	x	x

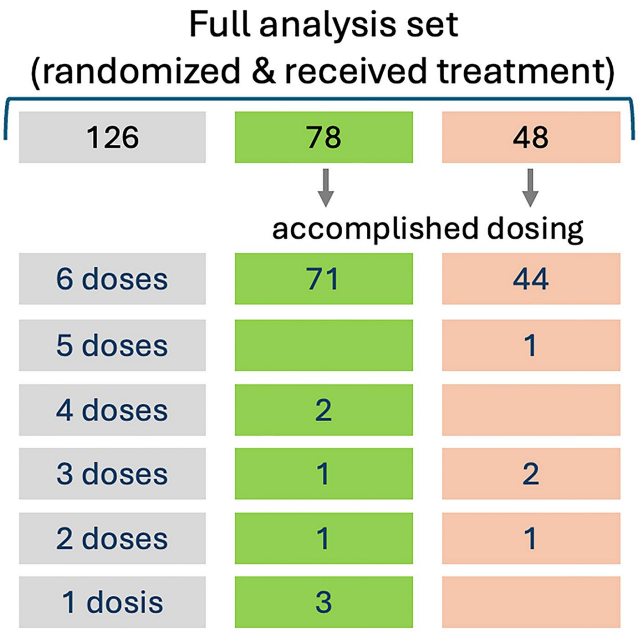
Supplementary Figure 2. Overview of treatment and key assessment timelines. In total, 11 visits were scheduled with 2 visits from screening to baseline (1-2), 6 visits comprising the intrathecal drug applications (3-8), and 3 follow up visits (9-11) until 168 days post baseline. Day -28 is equivalent to the date of injury. Treatment consisted of 6 intrathecal bolus injections of 45mg NG101 in 3ml vehicle (verum) or vehicle only (placebo) administered over 60 seconds each. Time intervals between injections were set to a minimum of 3 days and a maximum of 7 days (5±2 days). Abbreviations: *SC screening visit, BL baseline visit, ISNCSCI International Standards for Neurological Classification of Spinal Cord Injury, SCIM-III Spinal Cord Independence Measure.*

Supplementary Figure 3



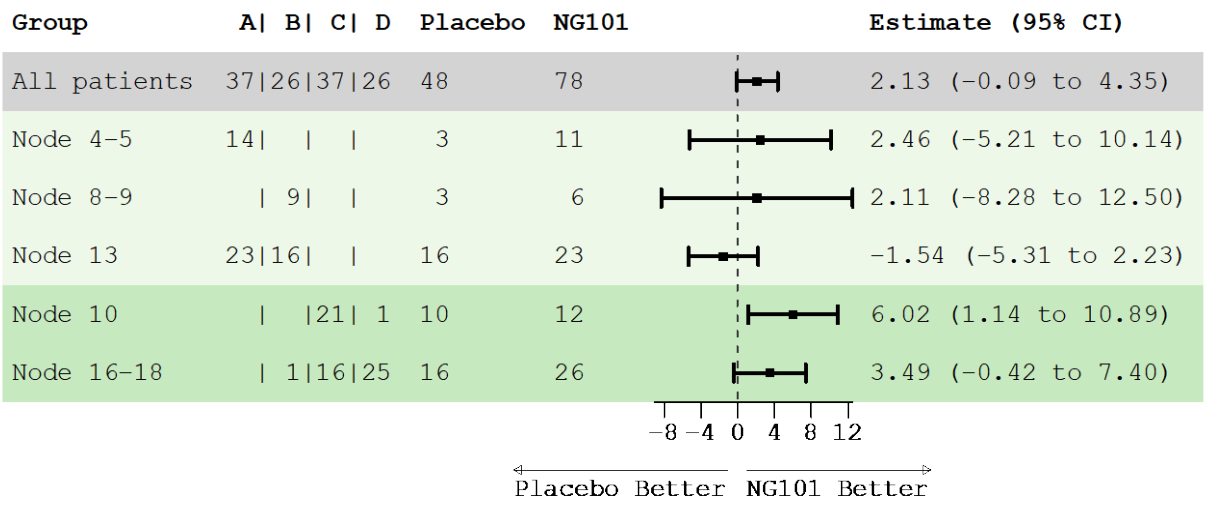
Supplementary Figure 3. Distribution of cervical SCI patients across UEMS outcome cohorts (nodes). The prediction of URP predefined UEMS outcome cohorts based on EMSCI observational data (n=720) confirmed an uneven distribution of frequencies across the different outcome cohorts of acute cervical SCI. This distribution of predicted patient outcomes in the NISCI study evolved as expected but with a lower frequency for nodes 4-5, where the EMSCI data contain relatively more severely affected SCI patients. Overall, the screened study population can be considered a representative study population in respect to neurological outcomes.

Supplementary Figure 4



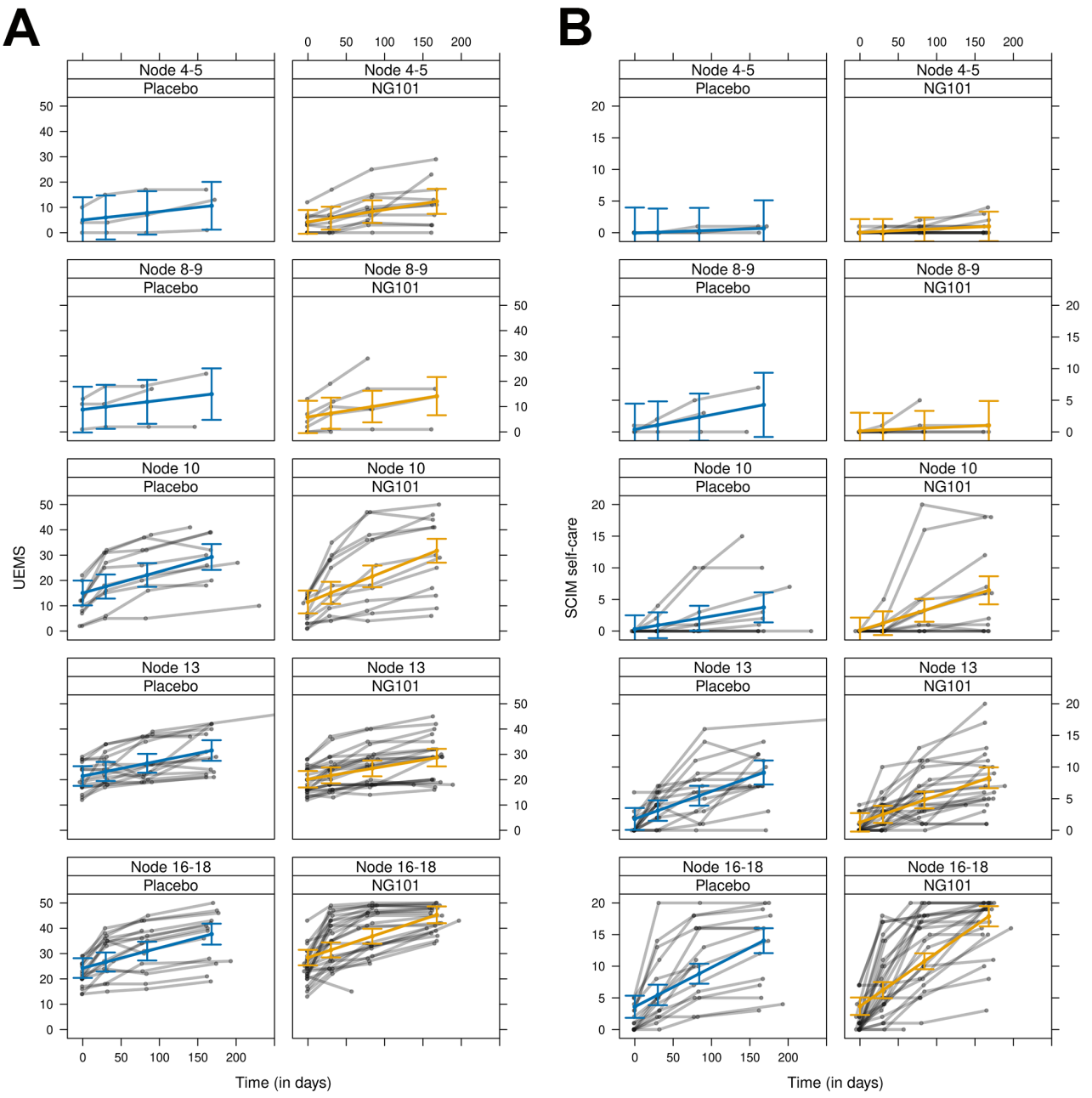
Supplementary Figure 4. Overview of accomplished dosing in the full analysis set.

Supplementary Figure 5



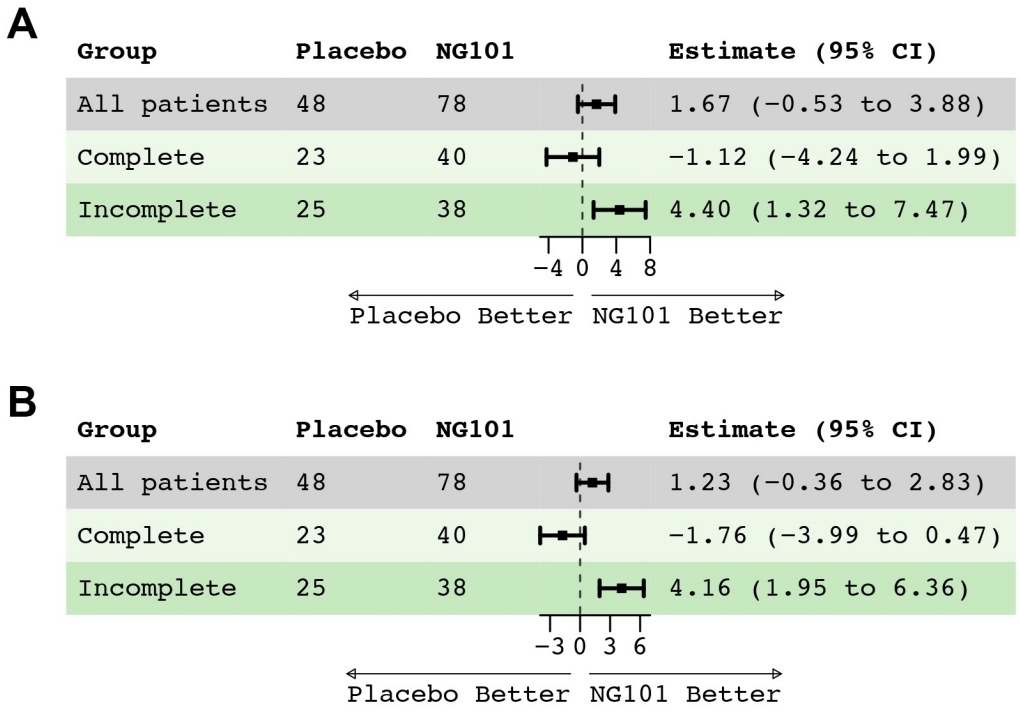
Supplementary Figure 5. Primary endpoint analysis of the full analysis set and predefined subgroups. Linear mixed-effects models addressed the change in UEMS from baseline to 168 days post baseline. Model assuming non-uniform recovery profiles including all URP-predefined UEMS outcome cohorts (nodes) for all patients (highlighted in grey). Estimates for subgroup-specific treatment effects are shown for each outcome cohort. URP nodes 4-5, 8-9, 13 contain only motor-complete SCI patients (AIS A and B; highlighted in light green), whereas nodes 10 and 16-18 contain - with one exception (AIS B) - only motor-incomplete SCI patients (AIS C and D; highlighted in dark green). The small sample sizes in some of the individual nodes necessitated the combining of nodes 4-5, 8-9 and 16-18, respectively. Each of these combined nodes belong to the same final URP-CTREE branch (appendix p 17). The prespecified nodes 20 and 21 (not shown in the figure) include all patients expected to ceil in respect to UEMS at 6 months (N=171, 37%).

Supplementary Figure 6



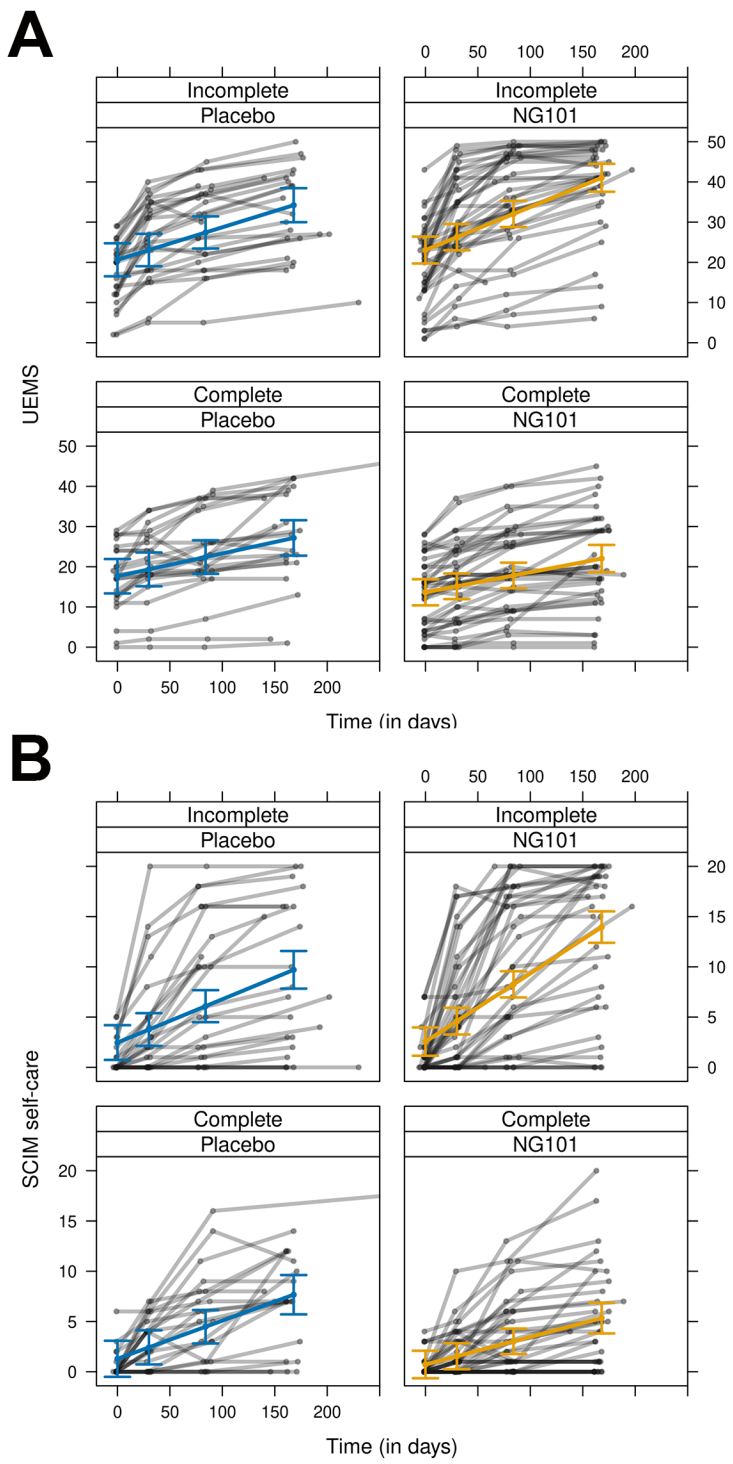
Supplementary Figure 6. UEMS and SCIM self-care patient profiles – nodes. **(A)** UEMS and **(B)** SCIM self-care treatment profiles by treatment group and node overlaid with model-based estimates of the mean UEMS and SCIM-III self-care, respectively. Confidence intervals are shown at baseline, 30, 85 and 168 days post baseline. Estimated slopes and 95% confidence intervals for UEMS delta changes (colored lines in A) are provided in Supplementary Table 1. *Abbreviations: UEMS upper extremity motor score, SCIM spinal cord independence measure.*

Supplementary Figure 7



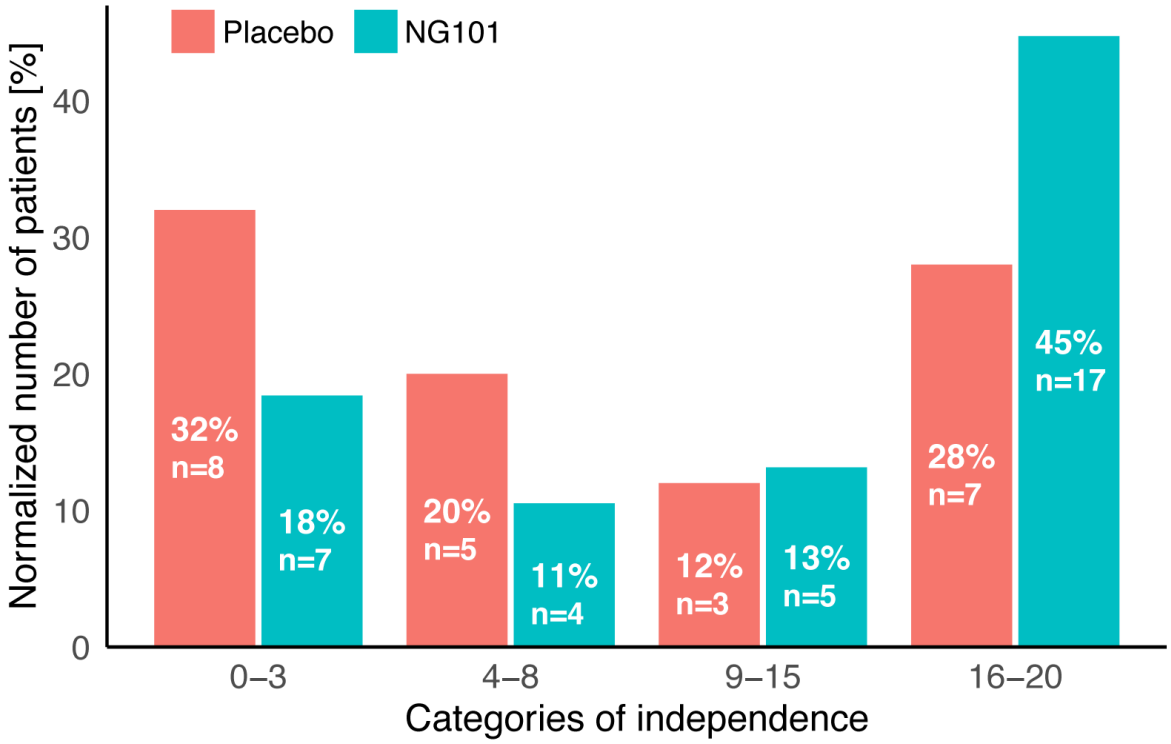
Supplementary Figure 7. UEMS and SCIM self-care forest plot – motor completeness. Post-hoc analysis. Linear mixed-effects models addressed the change in **(A)** UEMS and **(B)** SCIM self-care from baseline to 168 days post baseline. Model accounting for non-uniform recovery profiles in motor-complete (AIS A and B) and motor-incomplete (AIS C and D) SCI (global treatment effect estimate, *All patients:* subgroup specific treatment effect estimate, highlighted in grey; *Complete: highlighted in light green; Incomplete: highlighted in dark green*).

Supplementary Figure 8



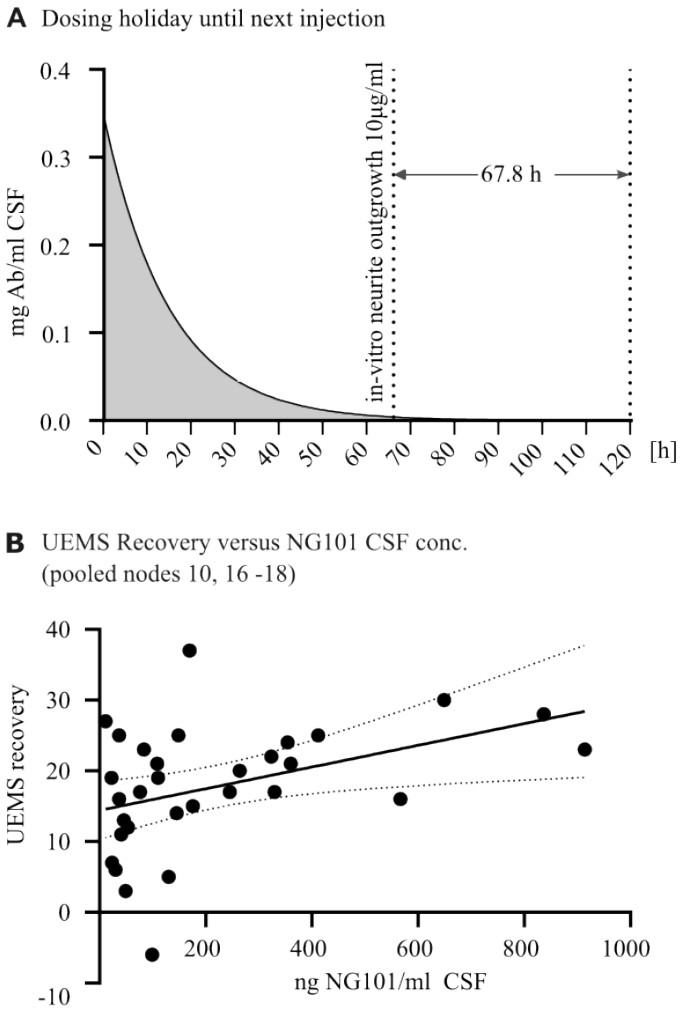
Supplementary Figure 8. UEMS and SCIM self-care patient profiles – motor completeness. **(A)** UEMS and **(B)** SCIM-III self-care treatment profiles by treatment group and motor completeness overlaid with model-based estimates of the mean UEMS and SCIM self-care, respectively. Confidence intervals are shown at baseline, 30, 85 and 168 days post baseline. *Abbreviations: UEMS upper extremity motor score, SCIM spinal cord independence measure.*

Supplementary Figure 9:



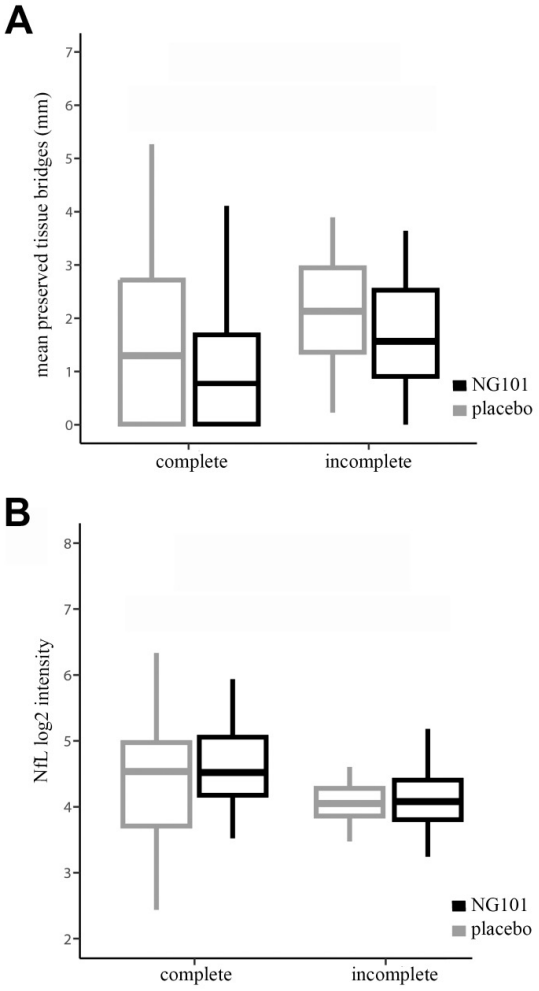
Supplementary Figure 9. Illustration of SCIM self-care recovery. SCIM self-care was divided into 4 categories representing increasing (from left to right) levels of independence in activities of daily living. At 168 days post baseline, participants with motor-incomplete SCI were assigned to their respective SCIM self-care category. Percentages represent the number of participants in each treatment arm within each SCIM self-care category relative to the total number of participants with motor-incomplete SCI in the respective treatment arm (placebo n=25, NG101 n=38). SCIM self-care data was missing in 2 participants in the placebo and 5 in the NG101 treatment arm at 168 days post baseline.

Supplementary Figure 10



Supplementary Figure 10. NG101 in CSF, pharmacokinetic-related underdosing and correlation with UEMS recovery. **(A)** The fast decline of the antibody concentration in the CSF after each injection leads to a dosing holiday when related to the concentration of 10 µg/ml required for *in vitro* neurite outgrowth on a spinal cord extract substrate [4]. The dotted line indicates the time point after which the concentration of NG101 drops below 10 µg/ml. Arrow indicates the time patients might have been underdosed until the next injection was applied. Calculation shown is for the participants with the lowest CSF concentrations of 5-100 ng/ml and a T1/2 of 10.2 h. **(B)** UEMS recovery in motor-incomplete participants (nodes 10, 16-18) related to the respective median NG101 trough CSF concentrations over all visits 4-8 for each patient. Dotted lines indicate 95% confidence intervals, slope of the linear regression is 0.01533, Spearman two-tailed correlation for the difference to zero shows p=0.0184.

Supplementary Figure 11



Supplementary Figure 11. Biomarkers of structural damage. (A) The assessment of tissue bridges (TB) by means of MRI obtained at screening and (**B**) markers of axonal damage by means of CSF NfL levels measured at visit 3 (before first intrathecal injection), Boxplots depict TB extent and NfL levels in motor-complete and motor-incomplete SCI cohorts, with the median represented by a horizontal line and whiskers extending from the minimum to maximum values. Both TB and NFL levels are statistically significantly different in motor-complete versus motor-incomplete SCI (TB P=0.003; NfL P=0.008), while remaining well balanced between respective treatment arms without statistical difference. *Sample sizes: n=90 for MRI based assessment of TB (n=40 motor-complete participants with n=14 in the placebo and n=26 in the verum group; n=50 motor-incomplete participants with n=22 in the placebo and n=28 in the verum group). n=106 for CSF NfL analysis (n=58 motor-complete participants with n=20 in the placebo and n=38 in the verum group; n=58 motor-incomplete participants with n=22 in the placebo and n=36 in the verum group)*

SECTION S6: Supplementary Tables

Supplementary Table 1

	UEMS		SCIM self-care	
	delta 95% CI	P/P_{sens}	delta 95% CI	P/P_{sens}
by URP node				
all patients	2.13 [-0.09, 4.35]	0.060/0.079	**1.58** [0.13, 3.03]	0.033/0.169
node 4-5	2.46 [-5.21, 10.14]	0.529/0.473	0.21 [-4.80, 5.23]	0.933/0.581
node 8-9	2.11 [-8.28, 12.50]	0.690/0.399	-3 [-9.74, 3.75]	0.384/0.495
node 10	**6.02** [1.14, 10.89]	0.016/0.043	2.89 [-0.29, 6.08]	0.075/0.273
node 13	-1.54 [-5.31, 2.23]	0.422/0.599	-0.29 [-2.75, 2.16]	0.816/0.942
node 16-18	3.49 [-0.42, 7.40]	0.080/0.169	**3.77** [1.22, 6.32]	0.004/0.053
by motor completeness				
all patients	1.67 [-0.53, 3.88]	0.137/0.176	1.23 [-0.36, 2.83]	0.130/0.337
complete	-1.12 [-4.24, 1.99]	0.480/0.743	-1.76 [-3.99, 0.47]	0.123/0.311
incomplete	**4.4** [1.32, 7.47]	0.005/0.026	**4.16** [1.95, 6.36]	<0.001/0.022

Supplementary Table 1. Efficacy analysis. Estimated treatment effects (differences in mean change, Delta) for UEMS and SCIM self-care obtained from normal linear mixed effects models with corresponding 95% confidence intervals and P-values. Positive delta values favor the anti-Nogo-A antibody treatment. Global effects for all participants were obtained from models with subgroup-specific recovery profiles (defined by URP nodes and motor completeness) captured by a time x subgroup interaction. Subgroup-specific treatment effects (for each URP node and for motor-incomplete and motor-complete SCI) were estimated from the same models with an additional time x treatment x subgroup interaction. In addition, all models were refitted under relaxed assumptions (non-normality of ordinal outcomes, non-linear time recovery profiles) and corresponding P-values are given as P_{sens}. Treatment effect estimates significant at the unadjusted 5% level are printed in bold font.

Supplementary Table 2

	Placebo			NG101		
	Delta	95% CI	P	Delta	95% CI	P
Node 4-5	5.63	[-4.08, 15.34]	0.668	8.09	[3.00, 13.19]	<0.001
Node 8-9	6.11	[-5.71, 17.93]	0.798	8.22	[-0.75, 17.19]	0.098
Node 10	14.21	[9.21, 19.21]	<0.001	20.23	[15.39, 25.08]	<0.001
Node 13	10.08	[6.08, 14.07]	<0.001	8.53	[4.93, 12.14]	<0.001
Node 16-18	13.40	[9.08, 17.73]	<0.001	16.89	[13.36, 20.43]	<0.001

Supplementary Table 2. Estimated UEMS delta-changes per node.

Supplementary Table 3

SCIM self-care upper limb function	1 feeding	2A bathing	2B bathing	3A dressing	3B dressing	4 grooming	total score
no hand function no arm function	0	0	0	0	0	0	0
no hand function limited proximal arm function	0-1	0-1	0	0	0	1	0-3
passive tendodesis for grasp function proximal arm function assisted wheelchair transfer	2	1	0-1	0-2	0-1	1-2	4-8
active tenodesis for grasp function distal & proximal arm function active wheelchair transfer	2	1-2	1-2	2-3	1-3	2-3	9-15
intrinsic hand function good upper limb function active wheelchair transfer independent dressing	3	2-3	2-3	3-4	3-4	3	16-20

Supplementary Table 3. Categories of independence. The SCIM self-care items of feeding (1), bathing upper body (2A), bathing lower body (2B), dressing upper body (3A), dressing lower body (3B), and grooming (4) cover major aspects relevant for independence in activities of daily living, in particular in patients with cervical SCI. The ability to accomplish these activities with increasing independence - indicated by the need for technical aids or caregiver assistance - is reflected by higher SCIM scores, which critically depend on the degree of UEMS recovery.

Supplementary Table 4

	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 10
patients (n)	77	73	71	69	67	69	64
25% percentile	0	36.05	62.1	55.6	49.2	82.5	0
median	0	120.7	162.8	178.4	183.5	199	0
75% percentile	0	462	772	821.6	636.1	892.8	0
95% CI of median							
confidence level (%)	96.05	96.56	96.81	97.05	95.02	97.05	96.72
lower confidence limit	0	70.5	96.1	100.6	132	134	0
upper confidence limit	0	240.1	329.6	280.4	329	287.8	0
mean	0	4505	1471	4052	1130	9070	0.6672
std. deviation	0	32978	4448	17803	3548	59648	4.679
std. error of mean	0	3860	527.9	2143	433.5	7181	0.5849

Supplementary Table 4. Trough concentrations (ng/ml) of NG101 in CSF.

Supplementary Table 5

	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10
patients (n)	70	74	73	69	70	70	71	72
25% percentile	0	319	515	593.5	668	742.3	685	54.75
median	0	474.5	719	814	861.5	894.5	916	186
75% percentile	0	646	856	989	1079	1097	1241	305.5
95% CI of median								
confidence level (%)	95.86	95.26	96.56	97.05	95.86	95.86	96.81	95.56
lower confidence limit	0	423	616	710	777	818	808	143
upper confidence limit	0	539	779	887	918	994	1037	227
mean	0	476.6	695.8	821.4	894.3	963.1	979.5	224.8
std. deviation	0	209.8	266.6	314.3	336.7	385.2	455.8	204.2
std. error of mean	0	24.39	31.21	37.83	40.24	46.03	54.1	24.06

Supplementary Table 5. Trough concentrations (ng/ml) of NG101 in serum.

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CLINICAL TRIAL PROTOCOL

EudraCT No. 2016-001227-31

NISCI
NOGO INHIBITION IN SPINAL CORD INJURY

Antibodies against Nogo-A to enhance plasticity, regeneration and functional recovery after acute spinal cord injury

A multicenter international randomized double blinded placebo controlled phase II clinical proof of concept trial

Short title: **NISCI** (Nogo Inhibition in Spinal Cord Injury)

Acknowledgment

This work is part of the project “NISCI - Antibodies against Nogo-A to enhance plasticity, regeneration and functional recovery after acute spinal cord injury, a multicenter European clinical proof of concept trial"

Supported by the **European Commission (EC) HORIZON 2020**, contract no. 681094, and by the Swiss State Secretary for Education, Research and Innovation (SERI) under contract number 15.0255. The opinions expressed and arguments employed herein do not necessarily reflect the official views of the EC and the Swiss government.

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Scientific Advisory Board (SAB)

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PARTICIPATING SITES

Refer to an extra document listing all participating sites and countries.

The study will be conducted in conjunction with the EMSCI network (www.emsci.org). The EMSCI network may provide additional backup sites and they may become involved if recruitment falls behind schedule.

TABLE OF CONTENTS

PROTOCOL SYNOPSIS

TITLE	Antibodies against Nogo-A to enhance plasticity, regeneration and functional recovery after acute spinal cord injury, a multicenter international randomized double blinded placebo-controlled phase II clinical proof of concept trial
SHORT TITLE	NISCI (**N**ogo **I**nhibition in **S**pinal **C**ord **I**njury)
EUDRACT NO.	2016-001227-31
INDICATION	complete to incomplete acute cervical spinal cord injury (SCI)
OBJECTIVES and ENDPOINTS	**Primary objective:** To evaluate the efficacy of acute treatment (initiation of drug treatment within 4 - 28 days post-injury) with NG-101 by repeated intrathecal (i.t.) bolus injections (6 injections of 45 mg each over 4 weeks) Primary efficacy endpoint: Upper extremity motor scores (UEMS) according to the International Standards for the Neurological Classification of Spinal Cord Injury (ISNCSCI) **Secondary objectives and endpoints:** • Effect on motor and sensory function according to the ISNCSCI protocol (ASIA impairment scale, ASIA lower extremities motor score (LEMS) and sensory scores (light touch (LT), pin prick (PP)) • Effect on autonomic dysfunction (i.e. bladder function as measured by bladder diary, Qualiveen questionnaire and bladder function assessment) • Effect on functioning evaluated by the Spinal Cord Independence Measure (SCIM-III) • Effect on hand/upper limb function as assessed by the Graded and Redefined Assessment of Strength, Sensibility and Prehension (GRASSP) subscales • Effect on the Walking Index for Spinal Cord Injury (WISCI), 10 meter walk test (10mWT) and the 6-minute walking test (6MWT) • Effect on neurophysiological parameters (nerve conducting velocity, Somatosensory evoked potentials) • To evaluate the pharmacokinetics (PK) and immunogenicity of NG-101 **Safety objectives and endpoints:** To evaluate the safety of acute treatment (initiation of drug treatment within 4 - 28 days post-injury) with NG-101 by repeated intrathecal bolus injections (6 injections of 45 mg each over 4 weeks) • Adverse Events (Frequency, type, duration and intensity of AEs and SAEs) • Relationship of AE/SAE frequency and time and duration of study medication administration • Documented reasons for any unplanned study medication interruptions and/or withdrawal from the study • Vital signs (blood pressure, heart frequency, body temperature) • Muscle spasticity measured by the Modified Ashworth Scale • Effect on pain (neuropathic pain and non-neuropathic pain) assessed by SCI pain data set, allodynia questionnaire and SCIPI

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	Exploratory objectives and endpoints: • effect on outcome of the Spinal Cord Ability Ruler (SCAR) • activity counts (sensors) • Mapping of rehab training (MART)
PHASE	II
INVESTIGATIONAL MEDICINAL PRODUCT(S)	**NG-101 (also known as ATI355):** anti-Nogo-A antibody (recombinant human monoclonal antibody directed against the human Nogo-A protein) Route of administration: repeated intrathecal bolus injections: 6 injections of 45 mg [in 3 ml] each pharmaceutical formulation: solution for injection Treatment duration: 4 weeks **Placebo:** same composition and configuration as the investigational drug product but does not contain NG-101 Route of administration: repeated intrathecal bolus injections pharmaceutical formulation: solution for injection Treatment duration: 4 weeks

Phase	SCR	BL	Treatment						Follow-up
Visit	1	2	3 to 8						9 to 11
Days	-28* to -2	-1	Days 0 to 25						Days 30 to 168
			0	**5**	**10**	**15**	**20**	**25**	
			6 bolus injections**						

*SCR: Screening – The duration of the Screening Phase cannot exceed the protocol requirement that treatment must be initiated from 4–28 days post-injury; BL: Baseline
**6 intrathecal (i.t.) bolus injections (each administered over 60 seconds) over 4 weeks, each injection containing 45 mg NG-101 [in 3 mL] or placebo; bolus injection time intervals must not fall below 3 days and must not exceed 7 days (5 ± 2 days)

STUDY DESIGN	multi-center, international, placebo controlled, double blind; randomized phase II (2 parallel treatment groups) trial
STUDY POPULATION	**Inclusion Criteria** 1. Male or female, 18 through 70 years of age 2. Acute cervical spinal cord injury (SCI) (Neurological level of injury C1 ≤ lesion ≤ C8) with confirmed classification of ASIA impairment scale (AIS) A-D at screening and predicted upper extremities motor score (UEMS) recovery of less than 41/50 (according to the URP prediction model) 3. 4-28 days post-injury (i.e. initiation of bolus injection within 4-28 days post-injury) 4. Tetraplegic patients who are allowed to start treatment are those who either do not require mechanical ventilation or who do not completely depend on mechanical ventilation but show some degree of spontaneous ventilation. Only those modes of ventilation where the patient show active initiation of breathing are allowed (e.g. continuous positive airway pressure (CPAP)) 5. Hemodynamically and clinically stable according to the acute SCI condition at baseline

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6. **For patients of childbearing potential**, use of reliable means of contraception as described below during the treatment period and for at least six months after the last dose of study drug:

   Males and Females of child bearing potential, who are willing to use a highly effective method of contraception [either combined hormonal contraception associated with inhibition of ovulation (oral, intravaginal, transdermal), progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable, implantable), intrauterine device, intrauterine hormone-releasing system, bilateral tubal occlusion, vasectomized partner or sexual abstinence)], or women not of child bearing potential, defined as women who have been surgically sterilized (total hysterectomy or bilateral oophorectomy, bilateral tubal ligation, staples, or another type of sterilization) or are postmenopausal for at least 2 years. Individuals who are convincingly sexually abstinent are also eligible.

   Sexual inactivity by abstinence must be consistent with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g., calendar ovulation, symptothermal, or post-ovulation methods) and withdrawal are not acceptable methods of contraception.

7. Written informed consent by patient before any study assessment is performed. If the patient is only able to consent orally a witness signs and confirms the patient's consent,
8. Cooperation and willingness to complete all aspects of the study
9. Ability of subject to understand character and individual consequences of the study

Exclusion Criteria

1. Complete anatomical transection confirmed by magnetic resonance imaging (MRI).
2. Trauma caused by ballistic or other injury that directly penetrates the spinal cord including gunshot and knife wounds.
3. Multiple levels of clinically relevant spinal cord lesions.
4. Major brachial or lumbar plexus damage/trauma.
5. Significant head trauma (e.g. cortical damage/lesion), or other injury that was, in the opinion of the investigator, sufficient to interfere with the assessment of the spinal cord function or otherwise compromise the validity of the patient's data.
6. Other significant pre-existing or current severe systemic disease such as lung, liver (exception: history of uncomplicated Hepatitis A), gastrointestinal, cardiac, immunodeficiency (including anamnestic known HIV) or kidney disease; or active malignancy or any other condition as determined by history or laboratory investigation that could cause a neurological deficit including syphilis, myelopathy, clinically relevant polyneuropathy, etc.
7. History of or an acute episode of Guillain-Barre syndrome.
8. History of recent (6 months) meningitis or meningoencephalitis.
9. History of refractory epilepsy.
10. Patients with uncontrolled bleeding diathesis and/or who require uninterrupted concomitant therapeutic anticoagulation (e.g. phenoprocoumon (Marcumar®), heparin/heparinoids and new oral anticoagulants at a higher dose than for the prophylaxis of venous thromboembolism)
11. Presence of any unstable medical or psychiatric condition (defined by the Diagnostic and Statistical Manual of Mental Disorders, Edition 4 (DSM-IV)) that could reasonably have been expected to subject the

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	patient to unwarranted risk from participation in the study or result in a significant deterioration of the patient's clinical course. 12. Drug dependence (as defined by DSM-IV) any time during the 6 month's preceding study entry. 13. Pregnant or nursing (lactating) women, where pregnancy is defined as the state of a female after conception and until the termination of gestation, confirmed by a positive human chorionic gonadotropin (hCG) laboratory test (> 5 mIU/mL). 14. History of a life-threatening allergic or immune mediated reaction. 15. Patients with the presence of infection around the location where the spinal needle insertions are planned for applying the intrathecal injections. 16. Inability to communicate effectively with the neurological examiner such that the validity of the patient's data could be compromised. 17. Participation in any clinical investigation within 4 weeks prior to dosing or longer if required by local regulations, and for any other limitation of participation based on local regulations. 18. Patients who are unconscious, including those patients who are unconscious due to medication causing marked sedation. 19. History of hypersensitivity to the investigational medicinal product or to any drug with similar chemical structure.
SAMPLE SIZE	An expected total number of 114 subjects (78 subjects per NG-101 group and 36 per Placebo group) will be enrolled. This estimate is based on finding a treatment effect with 80 per cent probability (power) using a level-0.05 test if the true effect is 6 scale points on the UEMS with a standard deviation of 10.8 scale points. The dropout rate is estimated at 20 per cent. After approval of the current amendment, subjects will be randomized in a 3:1 (NG-101 vs placebo) instead of a 1:1 ratio in order to aim for an overall 2:1 ratio. Depending on the overall recruitment at the time of amendment implementation, the total number of subjects and placebo treated patient may vary, to achieve the treatment of 78 patients with NG-101.
TRIAL DURATION	Total trial duration: 108 months Duration of clinical phase (FSI-LSO): 48 months Beginning of the preparation phase: Q1 2016 FSI (first subject in): Q2 2019 LSI (last subject in): Q2 2022 LSO (last subject out): Q4 2022 DBL (database lock): Q4 2022 Statistical analyses completed: Q2 2023 Trial report completed: Q4 2023 The actual overall study duration and/or subject recruitment may vary.
STATISTICAL ANALYSIS	114 patients providing a power (given a 2:1 ratio) of 1-β=66 per cent to reject the null hypothesis of no effect of treatment on mean change in UEMS motor score at a level of α=.05 if the true effect is 6 with a standard deviation of 10.8 score points. The assumptions of standard deviations are based on EMSCI register data.

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	The UEMS level 168 days after randomization, as the primary response, will be estimated using a linear mixed model with 1 month, 12 weeks and 24 weeks measurements as response. All analyses will be on the full analysis set using all randomized patients (with exceptions under strict conditions), while primary analysis will be repeated for the per-protocol set with patients receiving randomized treatment according to protocol. Analyses on secondary endpoints will be carried out in a similar fashion (using Generalized Linear Mixed Models (GLMM) for longitudinal data) as for the primary endpoint. Safety endpoints: Adverse events by grading/relatedness and treatment group, by System Organ Class (SOC)/Preferred Term (PT) and treatment group. Detailed instructions for analysis are to be found in the Statistical Analysis plan finalized before the blind is removed.
NUMBER OF TRIAL SITES	14 sites in 5 countries (planned countries: Germany, Switzerland, Italy, Spain, Czech Republic; number of sites and countries may be subject to change)
FINANCING	• Funded by the European Union's program Horizon 2020, Swiss State Secretary for Education, Research and Innovation (SERI), Swiss Paraplegic Foundation and Wings for Life Spinal Cord Research Foundation • anti-Nogo-A antibody is provided free of charge by Novartis Pharma AG and Wyss Zurich/ University of Zurich
SUBSTUDIES	Sub studies (work packages (WP 2 - 4)): **Biostatistics (sub study WP2)** Objective: develop a statistical model for the conditional distribution of ordinal measurements in two-armed randomized clinical trials. Based on this model, minimal clinically important differences shall be discussed, and methods for sample size estimation, statistical inference and subgroup analyses shall be developed. We plan to set-up a statistical framework allowing future clinical trials using SCIM self- care & mobility or UEMS as primary endpoints to be planned and analyzed with procedures taking the specific properties of these endpoints into account. Results shall be used to improve the power to discern treatment effects in future studies. **Proteomics (sub study WP3)** Important part of the current trial is the collection of high-quality biological samples (Serum derived from blood samples and CSF collected during diagnostic and therapeutic interventions, respectively) of patients suffering from acute SCI. Serum and CSF samples obtained in the course of the study will undergo proteomics analyses in order to identify proteomics-based biomarkers to enable or support prognosis and outcome of the patients. Afterwards, a systematic categorization of the proteomes will be performed with comprehensive bioinformatic tools. **Neuroimaging (sub study WP4)** Baseline MRI data acquired at the cervical level and brain will be used to characterize the extent of the lesion and the subsequent de- and regenerative processes occurring remote from the cervical cord injury both treatment groups. For the longitudinal data acquired at three distinct time points (0, 1 and 6 months), routine post processing pipelines will be applied to assess differences between the rates of change of

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	neurodegeneration between both groups and explore possible treatment effects. Specifically, these quantitative images will provide information on the spontaneous and potentially treatment altered rates of changes of atrophy (volumetry), de- and remyelination and iron accumulation in both groups.

ASSESSMENT SCHEDULE

Scheduler	**Screening**	**Baseline**	**Treatment** *bolus injection time intervals must not fall below 3 days and should not exceed 7 days (5 ± 2 days)*						**Follow Up[15]**			**Early termination**
Visit	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Day	**Day -28* to -2**	**Day -1**	**Day 0**	**Day 5**	**Day 10**	**Day 15**	**Day 20**	**Day 25**	**Day 30**	**Day 84**	**Day 168**	
time window (days)			**± 0**	**± 2**	**± 2**	**± 2**	**± 2**	**± 2**	**±2**	**±7**	**±7**	
Screening/ Inclusion												
Informed Consent	x											
In/ exclusion criteria	x	x[1]										
Medical History	x	x										
Randomization		x										
Medical Assessment												
Physical/neurological examination[8]		x	x	x	x	x	x	x	x	x	x	x
Vital signs[2]	x	x	x	x	x	x	x	x	x	x	x	
Height/weight[13]	x											
Electrocardiogram		x										
Concomitant med/therapy	x	x	x	x	x	x	x	x	x	x	x	x
Adverse events[11]			x	x	x	x	x	x	x	x	x	x
MRI (spinal/brain)	x								x		x	x
Intervention												
IMP administration (60 seconds i.t. bolus injection)			x In 1	x In 2	x In 3	x In 4	x In 5	x In 6				
Neurological												
ISNCSCI protocol	x	x			x (between In3 and In4)				x	x	x	x
Pain assessments (SCI pain data set, allodynia questionnaire & SCIPI)	x								x	x	x	x
Modified Ashworth Scale		x							x	x	x	
SCIM-III, GRASSP		x(p)							x(c)	x(c)	x(c)	x(c)
WISCI II, 6mWT, 10MWT[14]		x							x	x	x	X
MART & activity counts[9]		Consecutively three to five days per week during in-patient rehabilitation										
Urological												
Bladder assessments[6]	x									x	x	
Electrophysiological Assessments												
dSSEP = C6 & C8, SSEP = tibialis, NCV = ulnaris	x								x	x	x	

ASSESSMENT SCHEDULE – PAGE 2

Scheduler	**Screening**	**Baseline**	**Treatment** *bolus injection time intervals must not fall below 3 days and should not exceed 7 days (5 ± 2 days)*						**Follow Up[15]**			**Early termination**
Visit	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**
Day	**Day -28* to -2**	**Day -1**	**Day 0**	**Day 5**	**Day 10**	**Day 15**	**Day 20**	**Day 25**	**Day 30**	**Day 84**	**Day 168**	
time window (days)			**± 0**	**± 2**	**± 2**	**± 2**	**± 2**	**± 2**	**±2**	**±7**	**±7**	
Local Laboratory												
Pregnancy test (serum)	x											
Laboratory parameters – blood and urine[3/4]	x[3]	x[3]	x[4]	x[4]	x[4]	x[4]	x[4]	x[4]	x[3]	x[3]		
Laboratory parameters – CSF[5]			x	x	x	x	x	x		x		
Central Laboratory (mandatory for all subjects)[7]												
PK and Immunogenicity samples – serum[10]			x	x	x	x	x	x	x	x		
PK samples – CSF[10]			x	x	x	x	x	x		x		
Optional: Proteomics and Future Research (sub study)[7]												
Serum sample[10]			x	x	x	x	x	x	x	x		

* SCR: Screening – The duration of the Screening Phase cannot exceed the protocol requirement that treatment must be initiated from 4–28 days post-injury

**Future research refers to analysis of serum and CSF not directly related to the IMP, which will be covered by a separate study protocol and a separate patient informed consent form.

Abbreviations: In= injection; IMP= investigational medicinal product; i.t.= intrathecal, MRI= Magnetic Resonance Imaging, CSF= cerebrospinal fluid, LP= lumbar puncture; PK=pharmacokinetic; SOP= standard operating procedure
p= partial; c= complete (related to GRASSP)

[1] (**re-)evaluation of inclusion** criterion No 5, exclusion criterion No 11, 15, 18 at baseline

[2] **blood pressure**, respiratory rate, pulse rate, body temperature will be retrieved from the hospital patient chart when being an in-patient, after discharge it will be retrieved during the physical examination as an out-patient. Vital signs will be measured prior to IMP administration.

[3] **laboratory** parameters screening, baseline, day 30 & day 84:

- serum: albumin, alkaline phosphatase, total bilirubin**, calcium, cholesterol, chlorine, creatinine, CK, glucose, y-GT, LDH, lipase, amylase, potassium, total protein, AST, ALT, sodium, triglycerides, uric acid and CRP
  **If the total bilirubin concentration is increased above 1.5 times the upper limit of normal, direct and indirect bilirubin should be differentiated.
- Complete Blood Count (CBC): hemoglobin, hematocrit, WBC (count with differential if medical indicated), RBC (absolute value), platelet count (absolute value).
- Clotting analysis: Quick/INR and aPTT
- Urine analysis: Specific gravity, pH, glucose, protein, bilirubin, ketones, leucocytes, blood. (If screening and baseline visits are scheduled within 3 days, only the screening lab has to be done)

For day 84: Results should be obtained and checked before lumbar puncture (LP)

[4] **laboratory** parameters day 0, 5, 10, 15, 20 & 25:

- hemoglobin, hematocrit, WBC, RBC count, platelet count
- Clotting analysis: Quick/INR and aPTT
- chlorine, potassium, creatinine, CRP, glucose
- urine analysis only if required by PI: Specific gravity, pH, glucose, protein, bilirubin, ketones, leucocytes, blood

Results should be obtained and checked before lumbar puncture (LP)

If day 0 is scheduled within 3 days to baseline or screening and there is no history of coagulation disorder or recent infection no lab is required on day 0.

[5] **laboratory** parameters day 0, 5, 10, 15, 20, 25 & 84:

– CSF (routine, local laboratory) during study drug application: cell count, glucose, lactate, protein.
Immediately prior to each intrathecal bolus injection CSF sample will be taken (i.e. days 0, 5, 10, 15, 20, 25). On day 84 CSF will be taken without IMP application. CSF collection should be done the day prior to bladder assessments.

[6] bladder function: Qualiveen questionnaire and bladder function assessment will be completed during screening and repeated within days 84-168. The bladder diary will cover three days and must be completed during 3 days before the FU-visits on day 84 & 168

[7] for sampling details please refer to chapter 7, 17.6 and 17.7
CSF and serum collection immediately before each IMP administration. Storage in a ≤-70C freezer should occur as soon as possible after processing of samples. As soon as all samples from 3 subsequent patients have been collected they will be shipped to the central Biobank (CENTRAL LABORATORY BIOBANKING OF SERUM AND CSF in Heidelberg, Dr. Weis).

[8] Physical/neurological examinations: see appendix; examination prior to IMP administration

[9] MART & activity counts: assessments will be done -for in-patients only- consecutively three to five days per week over the whole study period.

[10] On injection visits, samples should be taken before IMP administration. At other visits, samples may be taken at any time during the visit. Refer to page 83 and 84.

[11] from the first administration of IMP until study completion or early termination visit

[12] Pseudonymized data of MRI will also be used for the sub study of the EU work package 4

[13] If determination of weight is not possible, the following procedure should be applied: first patient should be asked for weight, if patient answer is not sufficient study personnel should estimate weight

[14] 6mWT and 10MWT assessments must be actually performed if the subject is able to walk, while should be rated as "0" if walking is not feasible.

[15] The follow up visits can be timed according to the condition of the patient lasting up to 48 hrs.

ABBREVIATIONS

10mWT	10-meter walk test
6MWT	6-minute walk test
AE	Adverse Event
AIS	ASIA Impairment scale
ALS	Amyotrophic lateral sclerosis
ALT	Alanine aminotransferase
AMG	German Drug Law (Deutsches Arzneimittelgesetz)
ANS	Autonomic nerve system
aPTT	activated partial thromboplastin time
ASAF	Autonomic Standard Assessment Form
ASIA	American Spinal Injury Association
AST	Aspartate aminotransferase
ATC	Anatomical-Therapeutic-Chemical Code, part of WHO-DRL (Drug Reference List)
b.i.d.	twice a day
BL	base line
BM	Biomarker
BUN	blood urea nitrogen
CI	confidence interval
CK	Creatinine kinase
CMAP	Compound muscle action potential
Cmax	The observed maximum serum concentration following drug administration (ng/ml)
CNS	Central nervous system
CPAP	Continuous positive airway pressure
CRA	Clinical Research Associate
CRO	Contract research organization
CRP	C - reactive protein
CSF	Cerebrospinal fluid
CV	Curriculum vitae
DBL	Data Base Lock
DSM IV	Diagnostic and Statistical Manual of mental disorders Edition 4
DSMB	Data Safety Monitoring Board
dSSEP	dermatomal somatosensory evoked potential
DSUR	Development Safety Update Report
DTI	Diffusion Tensor Imaging
EAU	European Association of Urology
EC	Ethics Committee
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
ECRIN	European Clinical Research Infrastructure Network
EMSCI	European multicenter study about spinal cord injury
EPAF	EMSCI Pain Assessment Form
EPT	Electrical perception threshold
FACS	Flow cytometry (Fluorescence-activated cell sorting)
FD	Financial Disclosure
FEV_1	Forced expiratory volume in one second
FSI	First Subject In

GCP	Good clinical practice
GGT/ γ-GTf	Gamma glutamyl transferase
GLMM	Generalized Linear Mixed Models
GMP	Good manufacturing practice
GRASSP	Graded and redefined assessment of strength, sensibility and prehension
HCB	Heidelberg Cardio Biobank
hCG	human chorionic gonadotropin
HEENT	Head, Eye, Ear, Nose, Throat
HIV	Human immunodeficiency virus
HR	Heart rate
i.t.	Intrathecal
i.v.	Intravenous
IB	Investigator´s Brochure
ICCP	International Campaign for Cures of spinal cord injury Paralysis
ICH GCP	ICH harmonized tripartite guideline on GCP
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICMJE	International Committee of Medical Journal Editors
IgG	Immunoglobulin G
IMP	Investigational Medicinal Product
INR	International normalized ratio
ISCIPDS	International Spinal Cord Injury Pain Data Set
ISF	Investigator Site File
ISNCSCI	International Standards for the Neurological Classification of Spinal Cord Injury
ISRCTN	International Standard Randomized Controlled Trial Number
ITT	Intention To Treat
KKS	Coordination Centre for Clinical Trials (Koordinierungszentrum für Klinische Studien) Heidelberg
LDH	Lactate Dehydrogenase
LEMS	Lower extremities motor score
LKP	National / Coordinating Investigator in Germany (Leiter der Klinischen Prüfung)
LLOQ	Lower limit of quantification
LOI	Level of injury
LRR	Leucine rich repeat
LSI	Last Subject In
LSO	Last Subject Out
LT	light touch
MACS	Magnetic cell separation
MART	Mapping of rehab training
MAS	Modified Ashworth Scale
MCID	Minimal clinical important differences
MedDRA	Medical Dictionary for Regulatory Activities
MMP9	Matrix metalloproteinase-9
MRI	Magnetic resonance imaging
MRM	Multiple reaction monitoring
MS	Multiple sclerosis
MT	magnetization transfer
n.a.	not applicable
NC	National Coordinator
NCS	Nerve Conduction Studies

NCV	Nerve Conduction Velocity
NG-101	Recombinant human monoclonal antibody directed against the human Nogo-A protein
NgR	Nogo receptor
NgR1	Nogo receptor 1
NISCI	Nogo Inhibition in Spinal Cord Injury
p.o.	Per os (oral)
PD	Pharmacodynamics
pH	negative log hydrogen ion concentration
PI	Principal Investigator
PK	Pharmacokinetics
PP	Per-Protocol
PP	pin prick
PT	Preferred Term
PTT	Partial thromboplastin time
Q	quarter (time span)
RBC	Red blood cell
RhoA	Rhokinase A
RNA	Ribonucleic acide
RR	Riva-Rocci
S1PR	Sphingosine 1-phosphate receptor
SAB	Scientific Advisory Board
SAE	Serious adverse event
SC	Steering Committee
SCA	Spinal Cord Area
SCAR	Spinal Cord Ability Ruler
SCI	Spinal cord injury
SCIM	Spinal cord independence measure
SCIPI	Spinal Cord Injury Pain Instrument
SCR	screening
sFRP2	Secreted frizzled-related protein 2
sFRP4	Secreted frizzled-related protein 4
SmPC	Summary of Product Characteristics
SMQ	Standardized MedDRA query
SOC	System Organ Class
SOP	Standard Operating Procedure
SSEP	Somatosensory-evoked potential
SUSAR	Suspected Unexpected Serious Adverse Reaction
Tmax	Time to reach the maximum concentration after drug administration (h)
TMF	Trial Master File
TNF	Tumor necrosis factor
TRL	Technology readiness level
UE	Upper extremity
UEMS	Upper extremities motor score
URP	unbiased recursive partitioning
VC	Vital capacity
WBC	White blood cell (leukocytes)
WHO	World Health Organization
WISCI	Walking index for spinal cord injury
WP	Work package

1 Introduction

1.1 Scientific Background

Spinal cord injuries (SCI) result in life-long para- and tetraplegia and a big loss in quality of life. Health and social costs are enormous. There is no cure for SCI at present. Spontaneous regeneration of the interrupted nerve fiber tracts in the injured spinal cord is essentially absent, which represents the main reason for the low degree of recovery following SCI or brain injury (1) (2). Important progress has been made in the last 20 years in the scientific understanding of the processes regulating nerve fiber growth and regeneration. Most importantly, molecular impediments that form the basis of the lacking fiber tract regeneration in the adult mammalian and human central nervous system (CNS) were identified. One of the most potent neurite growth inhibitory molecules is the membrane and myelin protein Nogo-A, a protein comprising multiple inhibitory domains that activate independent receptors.

Monoclonal antibodies against Nogo-A have been shown to neutralize the inhibitory activity of purified or recombinant Nogo-A, of oligodendrocytes and of CNS myelin in vitro (3, 4). More importantly, a number of publications over more than 15 years have shown that function blocking anti-Nogo-A antibodies mediate significant improvements in functional recovery in rodent models of SCI (2, 5-7), non- traumatic brain injury (8, 9) and traumatic brain injury (10).

Anti-Nogo-A antibody treatment facilitates neuroregeneration at the anatomical level in rodents and two non-human primate models of SCI (5, 11, 12). Very similar results on the anatomical and functional level were obtained in Nogo-A knockout mice, in rodents treated with Nogo receptor-derived function blocking fusion proteins or antibodies against the Nogo receptor associated protein Lingo-1 (2, 13).

Based on these potent and highly reproducible effects in preclinical models, an approach was taken to develop a neutralizing human antibody against human Nogo-A. The antibody selected for development (NG-101) is a fully human monoclonal antibody generated from Medarex mice which are genetically reconstituted with human immunoglobulin genes, and is directed against a defined sequence of the human Nogo-A protein. The antibody is of the IgG4/κ class and is designed to treat acute injuries to the CNS with markedly reduced potential for antigenicity and immune cell and complement interactions. Detailed background information on the chemistry, pharmacology and toxicology of NG-101 is given in the Investigator's Brochure.

In animal models of SCI (both rodents and non-human primates), intrathecal delivery of anti-Nogo-A antibodies promotes neurite outgrowth, axonal regeneration, compensatory fiber growth and most importantly it mediates significant improvements in functional recovery (2, 5, 11, 12). The mechanism of action of anti-Nogo-A antibodies involves steric hindrance of the inhibitory domains of Nogo-A and internalization of the Nogo-A-antibody complex. In Cynomolgus monkeys, it has been demonstrated in vivo that there is accumulation of Nogo-A and the antibody within Cathepsin D positive structures most likely corresponding to lysosomes suggesting that antibody binding result in endocytosis of the antibody-Nogo-A complex and its subsequent degradation (14). This is supported by the finding that the amount of Nogo-A protein present in CNS tissue was down-regulated after anti-Nogo-A antibody treatment compared to control antibody treatment.

Macaque monkeys were subjected to a unilateral section of the spinal cord at the C7/C8 border and treated with a 4-week intrathecal infusion of NG-101 or control IgG from the time of lesion. Manual dexterity for the affected left hand was determined using the modified Brinkman board. The size of the lesion was determined histologically at the end of the experiment. NG-101 treated animals reached pre-lesion performance even when the lesion size was as high as 85% (5, 12).

The reported worldwide incidence of SCI lies between 10.4 and 83 per million inhabitants per year. The mean age of SCI patients is 33 years, and the sex distribution (men/women) is reported as 3.8/1 (15). Over 90% of affected individuals survive near-normal life spans and cost of care is very high. These injuries result from motor vehicle accidents (36%), violence (28.9%), or falls (21.2%). Tetraplegia is slightly more common than paraplegia. Currently there is no cure for SCI. Steroid drugs such as methylprednisolone reduce swelling, which is a common cause of secondary damage at the time of injury; efficacy of these steroids, however, have not been clearly established. Complications secondary to SCI are often treated with antimuscarincholinergics for bladder dysfunction, baclofen for spasticity, and opioids for pain.

Until now there are no medical treatment options for para- and tetraplegic patients after SCI. As summarized above, animal models showed a strong effectiveness of anti-Nogo-A antibody therapy in CNS-injured rodents and non-human primates. Thus, there is clear evidence for the therapeutic potential of anti-Nogo-A antibody therapy in acute SCI patients. To prove this assumption, we took a first translational step and initiated a clinical trial phase I/IIa for further drug development and evaluated the acute safety, tolerability, feasibility and pharmacokinetics of six dose regimens of anti-Nogo-A antibody in acute SCI patients (phase I/II safety study, NCT00406016). A total of three different countries were involved in this study (Germany, Switzerland and Canada) and all of them had the ethical approval of each country's ethical commission. In the phase I/IIa clinical study no clinically obvious, relevant adverse events (AE) or drug related severe adverse events (SAE) were observed. The NG-101 anti-Nogo-A antibody was proven to be safe, well tolerated and feasible, and the pharmacokinetics showed no abnormalities. Based on these promising results, we would like now with the present project to take the next necessary step and perform a double-blind, placebo-controlled proof-of-concept phase II study with a bigger patient group in order to show significant improvements in acute SCI patients. In the proposed clinical phase II study, the clinical protocol was amended to specifically address recovery of upper limb motor function (by means of Upper Extremities Motor Scores (UEMS)) as the primary efficacy endpoint. Dosing and route of application of the drug corresponds to study phase-I study cohort V where study drug was administrated as bolus during 60 seconds. According to the AE/SAE profile of the phase-I study no changes in the AE/SAE are expected in this follow-up study. Thus, we are convinced that there is an adequate and reasonable balance between the significance and importance and the risk of the study, and that this clinical phase II study is highly justified.

1.2 Trial Rationale/ Justification

The scientific rationale underlying this study has been established in several independent laboratories around the world in the last 2 decades, providing evidence that antibodies against Nogo-A or agents suppressing its activity play a critical role in the regeneration and repair of injuries to the CNS. The principal finding could be proven in several preclinical animal models (from mice to rats to non-primate monkeys) and triggered further international research to disentangle the complex effects of neurite growth inhibition in the CNS.

SCI are mainly caused by work, traffic and sports accidents and by violence. Paraplegia (leg and autonomic function affected) and tetraplegia (leg, arm and autonomic function affected; potentially combined with need for artificial respiration) impair the quality of life and the ability to work in the majority of patients in a severe and dramatic way. The social and economic burden of life-long care including frequent secondary complications (i.e. urinary tract infections, pressure sores, neuropathic pain, spasticity etc.) is enormous.

Regeneration of interrupted nerve fiber tracts and plastic "hardware" changes in the adult CNS of mammals and humans are extremely restricted, a phenomenon which represents a main reason for the low degree of recovery following SCI and brain injury (1). One of the most potent neurite growth inhibitory molecules is Nogo-A, a membrane protein comprising multiple inhibitory domains that activates independent receptors (2, 13).

Monoclonal antibodies against Nogo-A have been shown to neutralize the inhibitory activity of purified or recombinant Nogo-A, oligodendrocytes and CNS myelin in vitro (3, 4). More importantly, a number of publications over more than 15 years have shown that blocking anti-Nogo-A antibodies mediates significant improvements in functional recovery in rodent models of SCI (2, 5-7), non-- traumatic brain injury (8, 9) and traumatic brain injury (10).

1.3 Risk-benefit Assessment

1.3.1 General risk assessment

The expected impact for patients is considered to be tremendous if the novel intervention is able to increase neural plasticity and axonal regeneration in the human, injured spinal cord. In analogy to the preclinical data, significantly higher levels of recovery of neurological function can be expected. So far, patients with SCI are basically only benefiting from the rehabilitation programs that enable patients to compensate and adjust by maximizing their functional skills for the given neurological impairment due to the spinal cord injury (16).

Therefore, there is still a huge unmet need to develop treatment options for the damaged spinal cord itself to allow an improved neurological recovery that will have immediate consequences on the functional recovery and the quality of life of para- and tetraplegic patients (17).

In any case of increased axonal sprouting, regeneration or neuroplasticity, extensive effects on the functional recovery of patients are to be expected. Based on clinical knowledge in many hundreds of patients there is a clear relationship between the extent of neural damage and functional outcome (18). Therefore, any improvement of the neurological condition and reduction of the neurological damage (neural repair, neuroregeneration) will have a strong impact on patient's outcome and patient's life (19).

Most importantly in patients with cervical SCI, i.e. in the patient population targeted in the present trial, effects on improved upper limb and hand function, lower limb function but also on the autonomic nervous system (bladder/ bowel control) will allow patients to achieve a higher independence in activities of daily living and less dependency on care giver support.

Until now there are no medical treatment options for SCI patients. Fortunately, rodent and primate animal models showed strong efficacy of anti-Nogo-A therapy following CNS injury and, thus, there is clear evidence for the therapeutic potential of anti-Nogo-A antibody therapy in acute SCI patients. A phase I clinical trial for safety and tolerability of anti-Nogo-antibody NG-101 in acute SCI patients was successful and promising (NCT00406016).

Risk evaluation is based on non-clinical pharmacology and toxicology studies in rat and cynomolgus monkey as well as clinical experience with NG-101 from the first-in-man study in spinal cord injury paraplegic and tetraplegic subjects (NCT00406016). NG-101 (in the latter study called ATI355) was safe and well tolerated in spinal cord injured subjects at doses up to 15 mg/day for a maximum of 28 days using continuous i.t. infusion and at doses up to 6 × 45 mg over four weeks using repeated i.t. bolus injection. Repeated i.t. bolus injections appeared to be safer and less prone to technical complications compared to the continuous infusion mode of administration. Based on the data from the first-in-man study (NCT00406016), the treatment regimen in the phase II study in spinal cord injury tetraplegic patients will be repeated i.t. bolus injection of 6 × 45 mg NG-101 over four weeks.

In the present proposed clinical phase II study, the clinical phase-I-protocol was amended to specifically address clinical outcome/ benefit concerning recovery of upper limb motor function (by means of Upper Extremities Motor Scores (UEMS)) as the primary efficacy endpoint, while dosing and route of application of the drug will not be changed from the phase I study regarding bolus administration of cohort V, and therefore no changes in the occurrence of AEs/SAEs are expected in this follow-up study. Only a few AEs were reported for the two cohorts during bolus administration such as infections, blood and lymphatic system disorders, respiratory, thoracic and mediastinal disorders, vascular disorder, skin and subcutaneous tissue disorders. None of the SAE reported were related to NG-101. These were rather related to the injury itself, to concomitant medication or to the continuous intrathecal infusion mode of administration. Furthermore, we have improved the patients' stratification (that ultimately improves patients' safety) and introduce more sensitive clinical outcome measures in the current trial protocol. Thus, we are convinced that there is an adequate and reasonable balance between the significance and importance and the risk of the study, and that this clinical phase II study is highly justified.

A success of the NISCI trial by demonstrating enhanced motor outcome and a higher quality of life in tetraplegic patients after anti-Nogo-A antibody therapy would represent a breakthrough in the field of spinal cord and brain injury and repair. It could lead to phase III trials in SCI, but also in other indications, in particular for stroke, traumatic brain injury and multiple sclerosis.

1.3.2 Possible Measures in Case of Restrictions During a Pandemic

In view of the COVID-19 pandemic and its impact on the national health systems and public restrictions, the following mitigation measures are implemented in this clinical trial to ensure safety and well-being of the patients and health care staff at the trial centres as well as to ensure reliability of trial results. The following changes to the clinical trial protocol are only applicable in case conduction of the clinical trial in a protocol-conformal manner is not possible due to a new pandemic situation comparable to the COVID-19 pandemic in spring 2020. The principal investigator at each trial centre will judge the necessity as per current situation in the trial country, at the trial centre and the patients' ability to attend a personal patient visit at the trial centre.

Patient visits at home:

If a personal patient visit at the trial centre is not possible, the trial visit can take place at the patient's home. All assessments and procedures that can be performed for the specific visit may be conducted there. This includes questionnaires as well as respective explanations for proper use. Importantly, the investigator has to ask for the patient's well-being, i.e. AEs and concomitant medication as required per protocol (see above for a detailed description of the trial visits). These changes do not apply for the period of IMP application.

Patient visits by phone call:
If a personal meeting between investigator and patient is not possible, the clinical trial visit can be converted into a phone visit. CSF will not be collected in this case. Importantly, the investigator has to ask for the patient's well-being to ensure adequate medical supervision and explain the respective procedures of this visit in accordance with the trial protocol. For this purpose, questionnaires will be sent directly from the trial centre to the patient's home by the investigator using a local courier service. Receipt of the shipment will be acknowledged by the patient to the trial site. The investigator must advise the patient to send back or bring back all questionnaires to the trial centre as soon as a personal patient visit is possible again. These changes do not apply for the screening and IMP administration visits (V1-8), due to patient hospitalization (inpatient period).

Central laboratory analyses:
If blood samples cannot be collected at patients´ home due to a pandemic, samples will not be collected. CSF samples will not be collected at patients` home.

Remote monitoring:
Should physical monitoring visits at the centres not be feasible or restricted for some time due to a pandemic situation, either a combined remote and on-site monitoring or full remote monitoring visit may be conducted during these periods. In line with local laws and regulations remote SDV may be part of the remote monitoring visits and if so, has to be agreed between the sponsor designee and the trial centre. If necessary, the relevant trial documents (e.g. monitoring plan) will be adjusted to reflect these activities. The ICH GCP requirements and applicable data protection and privacy regulations must be met in any case and for any selected monitoring approach. In case of a remote monitoring of data, the patients need to agree to it in the informed consent form.

1.4 **Relevant data summary**

1.4.1 Non Clinical Pharmacology

NG-101 (also known as ATI355) is a recombinant fully human monoclonal antibody of the IgG4/κ class, directed against the human Nogo-A protein. It is derived from Medarex mice which are genetically reconstituted with human immunoglobulin genes. The monoclonal antibody is produced in a recombinant Sp2/0 derived cell line under GMP conditions.

NG-101 has the following general properties:

- Recognizes human Nogo-A protein with high affinity with K_D = 410 pM.
- The epitope is highly conserved in rhesus and Cynomolgus monkey Nogo-A and shows high affinity to its epitope peptide with K_D = 750 pM.
- The epitope is not well conserved in rat and mouse Nogo-A and the binding to rodent Nogo-A is very weak.
- Potently neutralizes the inhibitory activity of cynomolgus brain extract for neurite outgrowth of rat cerebellar granule cells (0.1-10 µg/mL)

In vivo data showing therapeutic potential of anti-Nogo-A antibodies in SCI and brain injury models were obtained using the mouse anti-Nogo-A monoclonal antibodies IN-1, 11C7 and 7B12 in both rat and monkey models (2, 13) and also using the human monoclonal antibody NG-101 in a non-human primate SCI model (5, 12). These antibodies recognize different epitopes in the Nogo-A protein. Nonetheless, they all neutralize Nogo-A in vitro and in vivo and induce cellular internalization and downregulation of Nogo-A

protein in vivo, emphasizing that neutralization of Nogo-A activity is achievable using antibodies that recognize at least three different linear as well as non-linear epitopes of Nogo-A.

Preclinical safety

Malfunctions potentially caused by antibody treatment such as neuropathic pain, seizures or spasticity, have not been observed in either rats or monkeys treated subchronically with anti-Nogo-A antibodies (5, 6, 20). Moreover, Nogo-A KO mice develop normally and show no overt behavioral alterations (21).

Continuous intrathecal administration of NG-101 for 4 weeks to cynomolgus monkeys did not result in organpathological changes. There was no evidence for systemic toxicity or immunogenicity. All the animals were tested negatively for anti-NG-101 antibodies in serum samples during the study. The no observed adverse effect level (NOAEL) could therefore be established at 60 mg/day, the maximum daily dose which could technically be administered (due to solubility and infusion volume limitations).

A tissue distribution study revealed wide-spread staining of different organs with NG-101 suggesting expression of Nogo-A in many tissues. This assumption has been confirmed by testing Nogo-A gene expression in a variety of cynomolgus and human tissues and was also corroborated by literature. Although a high exposure to NG-101 could be demonstrated in preclinical studies, a detrimental effect on the function of these organs could not be detected. The comparable pattern of Nogo-A staining in monkey and human tissues therefore supports the assumption that treatment of patients with NG-101 will not lead to severe side effects. This conclusion is further corroborated by the fact that IgG4 isotype antibodies do not facilitate ADCC (antibody dependent cellular cytotoxicity) or CDC (complement dependent cytotoxicity) reactions.

Intrathecal bolus injection of NG-101 in male cynomolgus monkeys (three injections in one week) did not reveal any adverse, NG-101-mediated changes in CNS organ morphology nor safety pharmacology-relevant endpoints (respiratory, cardiovascular and neurological). The transient tremors/shivering observed shortly (5 to 10 minutes) after the first dose showed a declining tendency after further doses and was also evident in one control animal after the third dose, and as such, are considered related to the dosing procedure rather than to NG-101. The intrathecal bolus injection of NG-101 in male cynomolgus monkeys was well tolerated in doses of up to 25 mg/animal.

The in vivo pharmacokinetic and toxicokinetic investigations in cynomolgus monkeys characterized NG-101 as a typical IgG-type antibody with low serum clearance and a long terminal half-life. The half-life in monkeys following i.v. administration was 9.5 ±1.3 days and the pharmacokinetics in monkeys were dose proportional. After intrathecal administration, NG-101 cleared from cerebrospinal fluid (CSF) with a Tl/2 of approximately one to two days, probably to a large extent into the blood compartment. NG-101 distributed into spinal cord tissue at the cervical region when injected as a bolus dose at the lumbar site. The CSF concentrations in a 28 day toxicology study were similar or slightly lower than those in man after intrathecal infusion of the highest dose. When concentration profiles are extrapolated to the time of injection, bolus injection maximum would be predicted to be higher, although no direct comparison of Cmax values is available. Immunogenicity against NG-101 was not detected in a 4-week monkey study.

Application of NG-101 to humans is considered safe on the basis of the toxicology and safety pharmacology evaluations.

1.4.2 Previous clinical studies

NG-101 was tested in a phase 1, open-label, multicenter study (NCT00406016). A total of 52 acute ASIA-A spinal cord injury patients (27 paraplegics, 25 tetraplegics) were enrolled to assess the acute safety, tolerability, feasibility, and pharmacokinetics of NG-101 administered either by continuous i.t. infusion (highest exposure: 15 mg/day for a maximum of 28 days) or repeated i.t. bolus injection (up to 6 × 45 mg over four weeks).

PK of NG-101 was assessed by sparse sampling in CSF and intensive sampling in serum. Across all cohorts, a high degree of variability in CSF NG-101 concentration was observed after both i.t. infusion and bolus injection. Nevertheless, the NG-101 concentrations detected in CSF confirmed the suitability of the intrathecal administration regimen. NG-101 appeared to be rapidly transferred to the blood circulation after intrathecal administration with a half-life in serum of approximately 20-30 days. Despite the variability observed in the PK analysis, the NG-101 concentrations detected in both CSF and serum confirmed the suitability of the intrathecal administration regimens.

Overall, NG-101 was well tolerated, with no NG-101-related serious adverse events (SAEs) reported. Except for one moderate skin rash observed approximately 3 days after an initial i.t. bolus injection and investigator-rated as potentially related to study medication, no NG-101-related AEs led to premature discontinuation of study medication. The vast majority of the AEs was of mild severity and was in line with the findings in acute spinal cord injury patients receiving conventional treatments. No dose dependency was observed regarding frequency and nature of AEs. No anti-NG-101 antibodies were detected in blood samples from any patients, confirming absence of immunogenicity. Based upon the currently available data, repeated i.t. bolus injections appear to be safer and less prone to technical complications compared to the continuous infusion mode of administration.

A major study objective was to identify a viable route of administration for NG-101 and the 6 intrathecal bolus injections with 45 mg NG-101 each (given over a period of approximately 4 weeks) as investigated in Cohort V appear to meet tolerability and pharmacokinetics expectations. A dose of 45 mg/injection is currently the highest dose which can be dissolved in an injection volume of 3 ml. Such volume injected over one minute was found very well tolerated in acute sensorimotor complete SCI patients. The CSF concentrations of NG-101 produced by this dosing regimen appear to be sufficient to block its target (Nogo-A) in the CNS tissues although this would need confirmation by an adequately designed and sized proof of efficacy study.

1.5 Dose rationale

Clinical dose estimates for the preceding phase-I clinical trial were calculated from the results obtained in the monkey (macaque) pharmacodynamic studies, where internalization of the NG-101/Nogo-A complex is demonstrated after four weeks of continuous intrathecal infusion of 1.08 mg/day. Considering the inter-species difference in NG-101's affinity to human and monkey Nogo-A protein, the estimated effective dose in man is 5 mg/day.

Since a dose of 60 mg/day is well tolerated by monkeys in the 28-day i.t. infusion toxicity study it provided the basis for estimating the safe starting dose for the completed first-in-man study in acute paraplegic SCI patients. The human dose equivalent, 300 mg/day, is based on interspecies differences in compartmental volumes (CSF volume) and antibody affinity. Division by the default safety factor of 10 would result in a maximum safe starting dose of 30 mg/day. However, this dose level is already well above the estimated effective dose in humans of 5 mg/day, which is derived from the 1 mg/day dose level used in the above mentioned proof of efficacy study in monkeys. Therefore, a starting dose of 5 mg/day has been selected for the first into man clinical trial.

NG-101 in the first-in-man study (NCT00406016) was safe and well tolerated in spinal cord injured subjects at doses up to 15 mg/day for a maximum of 28 days using continuous i.t. infusion and at doses up to 6 × 45 mg over four weeks using repeated i.t. bolus injection. Repeated i.t. bolus injections appeared to be safer and less prone to technical complications compared to the continuous infusion mode of administration, and appear to meet tolerability and pharmacokinetics expectations. Based on the data from the first-in-man study (NCT00406016), the treatment regimen in the phase II study in spinal cord injury tetraplegic patients will be repeated i.t. bolus injection of 6 × 45 mg NG-101 over four weeks.

1.6 Data Safety Monitoring Board (DSMB)

A Data Safety Monitoring Board made up of independent experts will be set up.

The DSMB consists of 4 international clinical experts on spinal cord medicine and neurology. The DSMB will meet on a regular basis (approx. every year, meetings can also be held via phone conferences). After reviewing the data on the study conduct (recruitment, protocol adherence/ protocol deviations) and on safety issues, the DSMB will make recommendations to the Steering Committee (SC) on the further study conduct (modification, continuation, closure).

Detailed working procedures are described in the DSMB charter (separate document).

1.7 Scientific Advisory Board (SAB) and Steering committee (SC)

The Scientific Advisory Board comprises the coordinating investigator and his supporting co-investigators, clinical experts not directly involved in the clinical trial and the responsible biometrician.

The steering committee is responsible for the scientific integrity of the study protocol, the quality of the study conduct as well as for the quality of the final study report. The Steering committee will decide on the recommendations made by the DSMB.

2 TRIAL OBJECTIVES AND ENDPOINTS

2.1 Primary Objective and Primary Endpoint

The purpose of the study is to confirm in a network of leading international Spinal Cord Injury Centers that acute treatment (initiation of drug treatment within 4-28 days post-injury) of the anti-Nogo-A antibody NG-101 by repeated intrathecal bolus injections is **safe**, well tolerated and **efficacious** in patients with acute cervical SCI.

Primary objective:

To evaluate efficacy of acute treatment (initiation of drug treatment within 4 - 28 days post-injury) with NG-101 by repeated intrathecal (i.t.) bolus injections on day 168.

Primary efficacy endpoint:

Upper extremity motor scores (UEMS) according to the International Standards for the Neurological Classification of Spinal Cord Injury (ISNCSCI)

2.2 Secondary Objectives and Secondary Endpoints

Secondary objectives and endpoints:

- Effect on motor and sensory function according to the ISNCSCI protocol (ASIA impairment scale, ASIA lower extremities motor score (LEMS) and sensory scores (light touch (LT), pin prick (PP)) on day 168.
- Effect on autonomic dysfunction (i.e. bladder function as measured by bladder diary, Qualiveen questionnaire, bladder function assessment on day 168.
- Effect on functioning evaluated by the Spinal Cord Independence Measure (SCIM-III) on day 168.
- Effect on hand/upper limb function as assessed by the Graded and Redefined Assessment of Strength, Sensibility and Prehension (GRASSP) subscales on day 168.
- Effect on the Walking Index for Spinal Cord Injury (WISCI), 10-meter walk test (10mWT) and the 6-minute walking test (6MWT) on day 168.
- Effect on neurophysiological parameters (nerve conducting velocity, Somatosensory evoked potentials) on day 168.
- To evaluate the pharmacokinetics (PK) and immunogenicity of NG-101. All PK/ IG samples collected from day 0 until day 84 will be included in the respective PK and immunological response analyses.

Overview: secondary objectives, endpoints and assessment tools

For the exact time points of evaluation refer to assessment schedule.

Objective and *endpoint*	**assessment by**
effect on *motor and sensory function*	International Standards for Neurological Classification of Spinal Cord Injury by the American Spinal Injury Association (ASIA): • ASIA impairment scale (AIS) • Motor and sensory levels • ISNCSCI lower extremities motor scores (LEMS) • Sensory scores (light touch (LT), pin prick (PP))
effect on *autonomic (i.e. bladder) dysfunction*	• Bladder diary (items: fluid intake, urine by urination and/or catheter, urinary urgency, urine loss, pads, bladder pain) • Qualiveen questionnaire

Objective and *endpoint*	**assessment by**
	• Bladder function assessment (items: voiding, catheter, bladder sensation)
effect on *functioning*	Spinal Cord Independence Measure (SCIM-III), Total score - SCIM-III self-care - SCIM-III respiration/ sphincter - SCIM-III mobility subscores
effect on *hand/upper limb function*	Graded and Redefined Assessment of Strength, Sensibility and Prehension (GRASSP) subscales
effect on *walking function*	• Walking Index in Spinal Cord Injury (WISCI II) • 10-meter walk test (10mWT) • 6-minute walking test (6MWT)
effect on *neurophysiological parameters*	electrophysiological examinations: • Nerve Conduction Studies (nerve conduction velocity, compound action muscle potential) • Somatosensory evoked potentials (SSEP dermatomal and tibial nerves) (Amplitude and latencies)

2.3 Safety endpoints

Safety objectives and endpoints:

To evaluate the safety of acute treatment (initiation of drug treatment within 4 -28 days post-injury) with NG-101 by repeated intrathecal bolus injections (6 injections of 45 mg each over 4 weeks)

Safety endpoints:

- Adverse Events (Frequency, type, duration and intensity of AEs and SAEs)
- Relationship of AE/SAE frequency and time and duration of study medication administration
- Documented reasons for any unplanned study medication interruptions and/or withdrawal from the study
- Vital signs (blood pressure, heart frequency, body temperature)
- Muscle spasticity measured by the Modified Ashworth Scale
- Effect on pain (neuropathic pain and non-neuropathic pain) assessed by SCI pain data set, allodynia questionnaire & SCIPI

2.4 Substudies

Objective	**assessed by**
Proteomics and Future Research (WP3)** – to identify proteins in CSF and serum correlating with clinical prognosis and / or clinical outcome – to identify proteins in serum and CSF correlating with drug response or non-response and or indicating functional/neurological recovery – long term storage of bio samples for future research purposes (genotyping excluded)**	Proteomics analyses in CSF and serum samples by the “Medizinisches Proteom-Center” Bochum, Germany Samples will be provided by to research institutes worldwide after request and Sponsor approval.**

Objective	assessed by
Neuroimaging (WP4) to evaluate changes of grey and white matter of the CNS	Neuroimaging (**MRI**), performed by the „Max-Planck-Institut für Kognitions- und Neuro-wissenschaften, Abteilung Neurophysik" Leipzig, Germany

**Future research refers to analysis of serum and CSF not directly related to the IMP, which will be covered by a separate study protocol and a separate patient informed consent form.

3 TRIAL DESIGN AND DESCRIPTION

3.1 Trial design

This is a placebo controlled, randomized, double blind, multicenter, multinational study to assess the safety, tolerability, feasibility and preliminary efficacy of early (within 4-28 days post injury) initiation of treatment with repeated bolus injections of NG-101 in cervical acute SCI patients. The study has 3 phases: screening/baseline Phase, treatment phase, and a follow-up phase.

The actual study design provides a novel state of the-art trial design in human SCI. The study design will allow enrolling simultaneously patients with complete and incomplete SCI. The enrollment and stratification of the patients is based on their individual prediction of outcomes.

Originally, the trial was planned with balanced (1:1 NG-101-placebo-ratio) randomization. During the course of the trial, it was decided that the ratio be changed to 3:1 in order to aim for more subjects who received verum and therefore gain more information about the reaction of the human body to NG-101.

The proposed study protocol goes far beyond the so far state-of-the-art clinical SCI trial designs. It is very ambitious in providing novel prediction algorithms for stratification and enrollment of patients as well as applying well informed clinical outcome parameters. In addition, the development and application of surrogate markers is the first of its kind in the context of SCI clinical trials and holds promise to improve the sensitivity and responsiveness of these findings. The study has tremendous innovation potential and, if successful, will have a profound impact on the design of future SCI clinical trials. Positive findings of this study will likely influence clinical studies related to other CNS disorders, such as stroke, traumatic brain injury, and multiple sclerosis. These disease entities have shown extensive improvements after anti-Nogo-A administration in respective animal models (2).

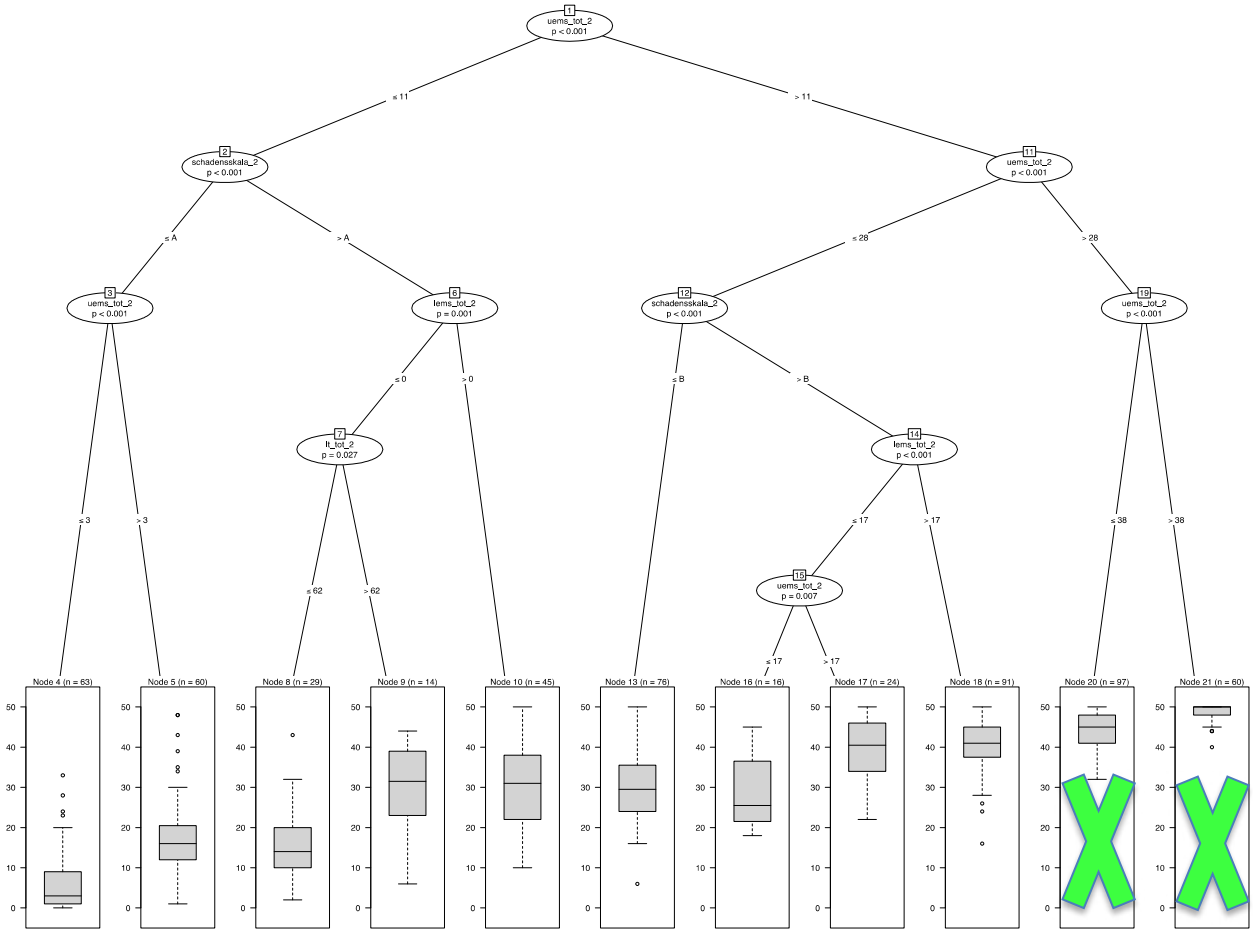


Figure 1 Unbiased Recursive Partitioning tree from EMSCI subset. Tree has been grown with respect to UEMS outcomes at 6 months (66, 67).

We will implement (Biostatistics (**substudy WP2**) a recently developed stratification algorithm that is based on the individual prediction of the patients with acute cervical SCI (see figure 1). Using Unbiased Recursive Partitioning (URP) (22) we will be able to reveal the distribution of UEMS outcomes at 6 months and to distinguish different cohorts of outcome of UEMS recovery (nodes in the interference tree). This will enable us to exclude those patients who are expected to recovery in mean UEMS > 41 regardless of treatment group (mean UEMS above 41/50). These cohorts (node 20 and 21) clearly will reach ceiling effects that will impact to reveal treatment effects as the outcome is already that good that further improvements can't be revealed by measuring the UEMS. On the contrary we can predict these outcome cohorts (node 4, 5, 8, 9, 10, 13, 16, 17, 18) that have a limited outcome and further improvements can be discerned in the treatment group compared to the control group.

This approach allows us to enroll about 73% of the patients with cervical AIS A - D which represents a reasonable high inclusiveness while applying this predictive stratification that will avoid enrolling patients that might likely less benefit from the intervention.

URP prediction of UEMS outcome at 6 months after acute cervical SCI AIS A - D.
The analysis is based on the assessment of 575 patients with acute cervical SCI scored as AIS A - D (assessment within the first 2 weeks following acute SCI). The inclusiveness (i.e. the percentage of patients that might be recruited) is about 73% when excluding node 20 and 21.

3.2 Need for placebo

Spontaneous partial recoveries of lower body functions are often observed within the first weeks after SCI. They are mostly interpreted as a reflection of the difficulty of a precise diagnosis. All functional improvements obtained must therefore be compared between the NG-101 and a placebo group. In addition, it is also well known in neurological rehabilitation that the personal motivation of each patient is an important determinant of the outcome of the rehabilitation process. The perception by the patients of the NISCI trial that they receive a regeneration-enhancing drug could stimulate their motivation to train hard and achieve a maximal possible functional improvement. A strictly controlled double blind procedure is therefore required.

3.3 Trial Duration and Schedule

The duration of the trial for each subject is expected to be 168±7 days including the follow ups after treatment (see assessment schedule).

The actual overall duration or recruitment may vary. The study end is defined as "last subject out" (LSO).

Total trial duration:	108 months		
Duration of clinical phase:	48 months		
Beginning of the preparation phase:	Q1 2016		
FSI (first subject in):	Q2 2019	48 months	108 months
LSI (last subject in):	Q2 2022		
LSO (last subject out):	Q4 2022		
DBL (database lock):	Q4 2022		
Statistical analyses completed:	Q2 2023		
Trial report completed:	Q4 2023		

4 SELECTION OF SUBJECTS AND TRIAL SITES

4.1 Number of Subjects

As calculated in section 9 Sample Size Calculation, 114 subjects will be expected to become enrolled in the clinical trial. The total number may vary, depending on the recruitment status at the timepoint of randomization switch to 3:1 ratio.

No interim analysis is planned.

4.2 Recruitment – Study Population

The study population will consist of tetraplegic patients ranging from 18 to 70 years of age, with an acute cervical SCI classified as AIS A-D at screening. The study will be conducted in Europe and Switzerland in conjunction with the European multinational spinal cord injury trial network (EMSCI) network (www.emsci.org). All participating sites provide comprehensive care for people with SCI, from acute treatment to long-term rehabilitation. Patients from any primary acute care hospitals can be referred to any of the specialized sites, which will be part of the trial for screening and for possible study participation. On a regular basis referring hospitals will be informed and updated about the NISCI-trial.

Furthermore, the EMSCI network may involve additional backup sites that are already actively participating in the EMSCI observational study if recruitment falls behind schedule.

4.3 Sites

The study will be conducted on a multinational and multicenter basis. It is intended that the study will take place at approximately 14 sites out of the following countries: Germany, Switzerland, Czech Republic, Italy and Spain.

Stopping rule: A site can be closed unless it has recruited at least 2 patients within 12 months after initiation as discussed with the steering committee.

4.4 General Criteria for Subjects' Selection

The gender ratio in traumatic SCI is about 4 males: 1 female that has been fairly consistent over the last several decades. While female SCI patients suffer from specific challenges regarding conception, pregnancy and giving birth as well as few aspects of bladder/ bowel management the issues of sensory and motor recovery as well as functional outcomes are considered to be less gender specific. Therefore, preclinical research and clinical trials so far have not been specifically designed or required specific adjustments for either male or female SCI. Overall traumatic SCI primarily affects male subjects due to work, traffic, violence, war (soldiers) and sports related injuries and interestingly the percentage of female SCI did not increase in the last 2-3 decades.

There will be no preferences on the selection of gender to be included, since there are no gender specific differences concerning efficacy and safety of the investigational diagnostic process expected. It is anticipated that the study results will give a representative gender distribution, which should reflect the natural gender distribution in the underlying disease.

4.5 Inclusion Criteria

Subjects meeting all of the following criteria will be considered for admission to the trial:

1. Male or female, 18 through 70 years of age
2. Acute cervical spinal cord injury (SCI) (Neurological Level of Injury C1 ≤ lesion ≤ C8) with confirmed classification of ASIA impairment scale (AIS) A-D at screening and predicted mean upper extremities motor score (UEMS) recovery less than 41/50 (according to the URP prediction model)
3. 4-28 days post-injury (i.e. initiation of bolus injection within 4-28 days post injury)

4. Tetraplegic patients who are allowed to start treatment are those who either do not require mechanical ventilation or who do not completely depend on mechanical ventilation but show some degree of spontaneous ventilation. Only those modes of ventilation where the patient show active initiation of breathing are allowed (e.g., continuous positive airway pressure [CPAP])
5. Hemodynamically and clinically stable according to the acute SCI condition at baseline
6. For patients of childbearing potential, use of reliable means of contraception as described below during the treatment period and for at least six months after the last dose of study drug:

   Males and Females of child bearing potential, who are willing to use a highly effective method of contraception [either combined hormonal contraception associated with inhibition of ovulation (oral, intravaginal, transdermal), progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable, implantable), intrauterine device, intrauterine hormone-releasing system, bilateral tubal occlusion, vasectomized partner or sexual abstinence)], or women not of child bearing potential, defined as women who have been surgically sterilized (total hysterectomy or bilateral oophorectomy, bilateral tubal ligation, staples, or another type of sterilization) or are postmenopausal for at least 2 years. Individuals who are convincingly sexually abstinent are also eligible.

   Sexual inactivity by abstinence must be consistent with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g., calendar ovulation, symptothermal, or post-ovulation methods) and withdrawal are not acceptable methods of contraception
7. Written informed consent by patient before any study assessment is performed. If the patient is only able to consent orally a witness signs and confirms the patient's consent
8. Cooperation and willingness to complete all aspects of the study
9. Ability of subject to understand character and individual consequences of clinical trial

4.6 Exclusion Criteria

Subjects presenting with any of the following criteria will not be included in the trial:

1. Complete anatomical transection confirmed by magnetic resonance imaging (MRI)
2. Trauma caused by ballistic or other injury that directly penetrates the spinal cord including gunshot and knife wounds
3. Multiple levels of clinically relevant spinal cord lesions
4. Major brachial or lumbar plexus damage/trauma
5. Significant head trauma (e.g. cortical damage/lesion), or other injury that was, in the opinion of the investigator, sufficient to interfere with the assessment of the spinal cord function or otherwise compromise the validity of the patient's data
6. Other significant pre-existing or current severe systemic disease such as lung, liver (exception: history of uncomplicated Hepatitis A), gastrointestinal, cardiac, immunodeficiency (including anamnestic known HIV) or kidney disease; or active malignancy or any other condition as determined by history or laboratory investigation that could cause a neurological deficit including syphilis, myelopathy, clinically relevant polyneuropathy, etc.
7. History of or an acute episode of Guillain-Barre syndrome
8. History of recent (6 months) meningitis or meningoencephalitis
9. History of refractory epilepsy
10. Patients with uncontrolled bleeding diathesis and/or who require uninterrupted concomitant therapeutic anticoagulation (e.g. phenoprocoumon (Marcumar®), heparin/heparinoids and new oral anticoagulants at a higher dose than for the prophylaxis of venous thromboembolism)
11. Presence of any unstable medical or psychiatric condition (defined by the Diagnostic and Statistical Manual of Mental Disorders, Edition 4 [DSM-IV]) that could reasonably have been

expected to subject the patient to unwarranted risk from participation in the study or result in a significant deterioration of the patient's clinical course

12. Drug dependence (as defined by DSM-IV) any time during the 6 month's preceding study entry (Screening)
13. Pregnant or nursing (lactating) women, where pregnancy is defined as the state of a female after conception and until the termination of gestation, confirmed by a positive human chorionic gonadotropin (hCG) laboratory test (> 5 mIU/mL)
14. History of a life-threatening allergic or immune mediated reaction
15. Patients with the presence of infection around the location where the spinal needle insertions are planned for applying the intrathecal injections
16. Inability to communicate effectively with the neurological examiner such that the validity of the patient's data could be compromised
17. Participation in any clinical investigation within 4 weeks prior to dosing or longer if required by local regulations, and for any other limitation of participation based on local regulations
18. Patients who are unconscious, including those patients who are unconscious due to medication causing marked sedation
19. History of hypersensitivity to the investigational medicinal product or to any drug with similar chemical structure

No subject will be allowed to enroll in this trial more than once.

4.7 Randomization

After a patients' eligibility according to inclusion and exclusion criteria has been confirmed, the patient will be registered at the randomization server via https://randomizer.at.

The randomization server will provide the number of a package available at the site. Neither package number nor study medication, even if unopened, may be reassigned after an erroneous randomization. The allocation of treatment will use a balancing algorithm (Big stick allowing for an imbalance of up to 3 patients per cohort) stratified according to the cohorts obtained by the algorithm referred to in section 3 to the EMSCI data base. The cohorts are derived from the screening (not baseline) measurements because the model has been developed on data obtained about 2 weeks after the incident that led to SCI. They are defined as follows:

1. UEMS total score ≤ 3, AIS 2 = A
2. 3 < UEMS total score ≤ 11, AIS 2 = A
3. UEMS total score ≤ 11, AIS 2 > A, LEMS total score = 0, light touch total score ≤ 62
4. UEMS total score ≤ 11, AIS 2 > A, LEMS total score = 0, light touch total score > 62
5. UEMS total score ≤ 11, AIS 2 > A, LEMS total score > 0
6. 11 < UEMS total score ≤ 28, AIS 2 = A or B
7. 11 < UEMS total score ≤ 17, AIS 2 > B, LEMS total score ≤ 17
8. 17 < UEMS total score ≤ 28, AIS 2 > B, LEMS total score ≤ 17
9. 11 < UEMS total score ≤ 28, AIS 2 > B, LEMS total score > 17

The code list will be generated within the system of the randomization server. It will be kept in safe and confidential custody at KKS Heidelberg. A copy of the list will be sent to the distributor.

4.8 Criteria for Withdrawal

4.8.1 Withdrawal of Patients from Treatment and withdrawal from the whole study

Any patient can withdraw from the treatment at any time without personal disadvantages and without having to give a reason. Patients who discontinue participation in the clinical study on their own or patients who

are withdrawn by the investigator, for reasons other than disease progression (i.e. in case of AEs, protocol violation), will be defined as premature withdrawals.

Decision to replace a patient may be considered on a case-by-case basis by the sponsor representatives, the biometrician and the Principal Investigator (PI). These patients are considered withdrawn from the Treatment Phase of the study. Reason for premature discontinuation must be noted in the eCRF. These patients, however, are encouraged to complete the Follow-up Phase visit assessments and can remain as participants in the study. These patients will be included in the Full Analysis Set for the assessment of the primary endpoint.

Replacement patients will be randomized just as regular patients.

The study medication will be discontinued based on the discussion with PI and repeated injection stopped if one or more of the following pertained

- Infections (local to region of injection, signs/symptoms of encephalitis, meningitis, or other systemic infections)
- Pregnancy

Additionally, the i.t. injection of the study medication will be stopped prematurely (and the i.t. needle subsequently removed) when:

- The PI (or his deputy), based on his/her clinical judgment of the patient's mental physical status, considers that the number and/or severity of AE(s) justify the discontinuation of the study medication

The time of treatment discontinuation must be documented in the patient file and on the eCRF.

The investigator can also discontinue the study / study treatment after considering the risk-to-benefit ratio, if he/she no longer considers the further treatment of the patient according to study protocol justifiable. The date of and the primary reason for the withdrawal, as well as the observations available at the time of withdrawal are to be documented in the source data and subsequently in the eCRF. Reasons leading to the withdrawal of a patient can include the following (**one primary reason must be determined**):

- **Lack of efficacy** of the study medication, e.g.
  - Progress of study disease compared to baseline
  - Need for a prohibited concomitant medication by the perception of patient and the consideration of PI for the treatment of study disease
- **Intolerable adverse events** to be determined by PI
- **Lack of patient's cooperation**, e.g.
  - Patient's request to withdraw
  - Lack of compliance, patient fails to attend the interim visits as agreed
  - Existing or intended pregnancy, lactation
- **Other reasons** (noting reason), e.g.
  - Other diagnosis than study disease

In all patients who finish the study prematurely, a withdrawal examination at least with respect to the primary endpoint should be carried out (ASIA/ SCIM-III). The withdrawal examination will then be documented in the eCRF.

If a patient does not come to a visit, the reason should be clarified. Every effort should be made by the investigator to contact him/her or a knowledgeable informant by telephone or by sending appropriate correspondence (i.e. certified letter) that will become part of the investigator's file to record the efforts made to reach the patient. If the patient fails to return for or comply with these visits, the investigator must determine the primary reason for a patient's premature withdrawal from the study and record this information on the Study Completion eCRF page. If the patient wants to withdraw, the reason should be

documented in the patients file and in the eCRF. If the patient withdraws, the reason should be asked for in detail and documented in detail.

For documentation of AE and SAEs see chapter 8.

4.8.2 Premature Closure of the Clinical Trial or a Site

If new information on the risk-to-benefit ratio of the drug or on the treatment methods used in the study is obtained in the meantime and safety concerns arise, the sponsor representatives reserve the right to interrupt or terminate the project. Premature termination is also possible if the sponsor representatives notice and agree upon that patient recruitment is insufficient and that this cannot be expedited by appropriate measures.

Premature termination of a single site is also possible upon sponsor's decision, as a result of the clinical research associate (CRA/ monitor) or sponsor representatives noticing or reporting that the conduction of the trial is not compliant with ICH-GCP and / or is not according to the protocol, the patient recruitment and / or the quality of the data is insufficient.

The DSMB can recommend interruption or termination of the study or of treatment arms based on the results of the intermittent SAE evaluation or of accumulating information on the above-mentioned reasons.

The ethics committee (EC) and the competent authorities must be informed about the premature closure of the trial or one of the treatment arms. Furthermore, the ethics committee(s) and competent authorities themselves may decide to stop or suspend the trial.

All involved investigators have to be informed immediately about a cessation / suspension of the trial. The decision is binding to all trial sites and investigators.

5 INVESTIGATIONAL MEDICINAL PRODUCT (IMP)

5.1 Dose and Schedule

Patients will be randomly assigned to either NG-101 or placebo in a 3:1 ratio. For allocation to a treatment arm (randomization) see section 4.7.

Investigational medicinal product (IMP) dose and schedule details are as follows:

	Investigational product	
Product Name	**NG-101**	**Placebo**
Formulation description	NG-101 concentrate solution	Placebo (same composition as the active drug product without NG-101)
Route of administration	Intrathecal bolus injection	Intrathecal bolus injection
Dose strength	45 mg/ injection	NA
Regimen	6 bolus injections of 45 mg NG-101 within 4 weeks (on Day 0, 5, 10, 15, 20 and 25 ± 2 days) *Note: Bolus injection time intervals should not fall below 3 days and should not exceed 7 days (5 ± 2 days)*	6 bolus injections of placebo within 4 weeks (on Day 0, 5, 10, 15, 20 and 25 ± 2 days)

For further details on IMPs, please refer to the Investigator's Brochure.

5.2 Packaging, Labeling and Handling

Packaging of the study drug will be overseen by the sponsor (University of Zurich). The study medication labels will be designed in accordance with the local legal requirements. IMP will be received by designated personnel at the study site. Upon receipt of the IMP at the site, the site personnel must verify the shipment condition (including a check of the temperature logs provided by the courier) and content; and the site will acknowledge receipt of IMP delivery. Site personnel should report any deviations or product complaints to a designated person of the sponsor immediately upon discovery. If the deviation requires, the IMP will be replaced.

Adequate records of the receipt and disposition of IMP must be documented in the "*Site Study Drug Accountability Log*".

IMP must be dispensed or administered according to procedures described herein. Only subjects enrolled in the study protocol may receive IMP. Only authorized site staff may supply or administer IMP. All IMPs must be stored in a secured area with access limited to authorized site staff. Long-term storage temperature must not exceed -60°C.

For IMP reconstitution NG-101/placebo solutions must be thawed on the day of the first injection: Use a water bath (30-35°C) for approximately 10 minutes or until all ice particles have melted without shaking the vials. Once thawed, NG-101/placebo must be stored at 2-8°C, not be frozen, and be protected from light. Maintenance of a "*Temperature Log for Study Drug*" is required. **Prior to further use, the refrigerated NG-101/placebo solutions must be equilibrated to room temperature (below 25°C). Vials equilibrated to room temperature for less than 24 hours may be refrigerated again at 2-8°C. Re-equilibration at controlled room temperature and subsequent use is acceptable only once.**

The remaining components can be destroyed at site according to local practice. The site's destruction process has to be documented on the "IMP on-site destruction authorization form" and has to be authorized by the Sponsor. On-site destruction of IMP vials has to be documented on the "IMP inventory Log" or "IMP preparation and administration Log"

5.3 Preparation and Administration

Detailed instructions for the preparation and administration of the study medication are described in the corresponding manual "Instructions for IMP preparation and administration". Each vial of IMP and diluent is for single use and must not be used for the preparation of a second reconstitution of injection solution.

At the investigator's site, qualified site staff with experience in aseptic technique will dispense the study medication in individual patient-specific vials according to the treatment schedule defined in this protocol. Administration of IMP by intrathecal injection will be performed by the patient's treating Investigator.

IMPORTANT NOTE: By preparing the IMP the designated site staff may become unblinded. In no case any of the unblinding information may be disclosed to any other treating study personnel. To prevent unintentional unblinding, the Sponsor will provide working instructions and sheets.

5.4 Assessment of Compliance

Subjects are dosed at the study site and will receive IMPs directly from the Principal Investigator or designee. Information related to accountability and compliance such as number of vials, date and time of each dose administered in the clinic will be recorded in the "*IMP Inventory Log*" and the "*IMP Subject Accountability Log*", respectively. Relevant details will also be recorded in the dedicated eCRF forms. All drug vials should be kept for inspection and evaluation of compliance by the CRA.

5.5 Prior and Concomitant Treatment

The treatment of accompanying illnesses not subject to the exclusion criteria is permissible if this is not expected to have any effect on the outcome measures used in this study and to interfere with the trial medication.

In particular, the following drug groups are **not permitted** as concomitant medication:

- concomitant therapeutic anticoagulation (e.g. phenoprocoumon (Marcumar®), heparin/heparinoids and new oral anticoagulants) at a higher dose than for the prophylaxis of venous thromboembolism, which cannot be interrupted.
- Other investigational therapies are prohibited 4 weeks prior to enrollment and throughout the study period
- Subjects should not receive vaccinations with live viruses (e.g. Measles, Mumps and Rubella, Varicella) while on NG-101 therapy

The following drug groups are **permitted under restriction** as concomitant medication:

Metamizole (non-steroidal anti-inflammatory drug; frequent brandnames: Novalgin, Analgin, Berlosin, Metalgin, Metamizol-Puren, Nolotil, Novaminsulfon)

Attention should be paid to patients under treatment with Metamizole where adjusted control of leucocytes is recommended (agranulocytosis). In the phase I study, one case of agranulocytosis/leucopenia unrelated to NG-101 was seen, which most probably was related to the use of concomitant treatment of Metamizole.

Medication (or diagnostics) taken prior to first dosing: All prescription medications and over-the-counter drugs (including vitamins) taken during the screening phase and throughout the study must be recorded in the patient's file and on the Concomitant Medications / Non-Drug Therapies page of the eCRF. New medications administered to the patients (e.g. to treat an AE) must also be recorded accordingly. Medication entries should be specific to generic name, the start and end date, and the reason for therapy.

5.6 Dose Modifications

No dose modifications are foreseen in this study.

5.7 Blinding and Unblinding

This will be a double-blind study. All patients and study site staff (excluding staff receiving IMP shipments and preparing the injections) will remain blinded to the treatment assignment. By handling and preparing IMP, the designated site staff responsible for receipt of IMP shipments and preparation of injections may become unblinded to treatment assignment and must maintain the blind and not reveal any unblinding information to other study personnel.

Unblinding of a subject's treatment assignment should not occur except in the case of a medical emergency or in the event of a serious adverse event (SAE), when the identity of the IMP is essential for the clinical management of the subject. In such circumstances, unblinding will be performed by means of the web-based randomization tool. As a back-up option (just in case of system or power failure), the investigational site was provided with sealed envelopes. An identical set of sealed envelopes will be held at the pharmacovigilance of the KKS. These envelopes contain information on the subjects' trial medication / treatment assignment and are to be opened only under circumstances in which the unblinding option via the web-based randomization tool cannot be accessed (see also above). The emergency envelopes are not to be opened by the investigator at the end of the trial. The completeness and integrity of the envelopes will be checked during regular monitoring visits by the CRA. All envelopes will be collected by the CRA at the end of the trial. In association with the modification of the randomization ration to 3:1, the availability of envelopes for new produced IMP kits will end, thus offering the randomizer.at server as the single-point unblinding facility

The Investigator should make every effort to contact the representatives of the Sponsor (see cover page) before unblinding to discuss options. If the blind is broken for any reason and the Investigator is unable to contact the Sponsor representatives prior to unblinding, the Investigator must notify the Sponsor as soon as possible following the unblinding incident without revealing the subject's study treatment assignment. The Investigator must record the date and reason for breaking the blind in the subject's medical records and in the eCRF.

As per regulatory reporting requirements, the Sponsor will unblind the identity of the treatment assignment for all unexpected SAEs that are considered by the Investigator to be related to study drug (SUSAR, see chapter "Adverse Events") as per the relevant safety reference document(s) (e.g. IB) in order to initiate

expedited reporting. The unblinding by the safety officer will be carried out according to the applicable standard operating procedures (SOPs) of the KKS.

In order to facilitate analysis of the biological samples collected in this study, the treatment code can be released to the responsible analytical person if required for analysis. Analysis results must not be released with individual identification of the patient until the database is closed.

6 TRIAL VISITS

6.1 Time Sequence and Frames

The study period for an individual patient consists of a screening phase and a twenty-five-day treatment period. The follow-up phase ends on day 168.

Phase	**SCR**	**BL**	**Treatment** *bolus injection time intervals must not fall below 3 days and must not exceed 7 days (5 ± 2 days)*						**Follow-up**		
Hospital	in-patient								out-patient		
Visit	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*9*	*10*	*11*
Days	-28* to -2	-1	Days 0 to 25						Days 30 to 168		
			0	**5**	**10**	**15**	**20**	**25**			
			6 bolus injections**								

*SCR: Screening – The duration of the Screening Phase cannot exceed the protocol requirement that treatment must be initiated from 4–28 days post-injury;
BL: Baseline
**6 intrathecal (i.t.) bolus injections each administered over 60 seconds, over 4 weeks, each injection containing 45 mg NG-101 [in 3 mL] or Placebo

Patients are hospitalized during the whole bolus treatments with study medication. All bolus intrathecal injections of NG-101 should be administered in accordance to standard hospital settings and the patient should remain supine in that setting for 4-6 hours post intrathecal injection. Upon completion of treatment, the patient will enter a Follow-up Phase. Follow-up Phase assessments will occur on Days 30, 84 and 168.

Important note: The bolus injections should be administered as per the visit windows detailed in the assessment schedule. **The time interval between two bolus injections must be at least 3 days and should not exceed 7 days.**

6.2 Description of Trial Visits

6.2.1 Screening and Baseline (visit 1 & 2) in-patient

All patients will participate in a Screening/Baseline Phase (Day -28 to Day -2), which consists of a Screening visit and a Baseline visit. During the Screening visit (on or within Day-28 to Day -2), the patient will be assessed for study eligibility. The duration of the Screening/Baseline Phase should be as short as possible; this duration will be dependent on the time necessary to obtain all data from the Screening visit, as well as the continued stability of the patient.

Patients who remain eligible after the Screening visit will then participate in the Baseline visit on Day -1 for re-evaluation of eligibility (applicable criteria during baseline see below) in the study. Additionally, **the Baseline visit should occur no later than 27 days post SCI injury** (i.e. the study medication NG-101 or placebo should be administered no more than 28 days post injury).

Patients will be admitted to the study if they meet the inclusion/exclusion criteria at both the Screening and Baseline visits (reevaluation of a subset of inclusion/exclusion criteria) and after confirmation with the Investigator. All baseline evaluation results must be available prior to receiving the first intrathecal (i.t.) bolus injection of NG-101 or placebo. However, safety laboratory results from the Screening visit can be utilized at baseline to assess the patient's eligibility if screening and baseline visits are scheduled within 3 days.

During the Screening Phase, the following assessments will be conducted and recorded in the source documentation and in the eCRF:

1. Informed consent
2. Review of inclusion/exclusion criteria
3. Medical history (prior and concomitant diseases)
   Relevant additional diseases present at the time of informed consent and during the screening phase are regarded as concomitant diseases (medical history) and will be documented on the appropriate pages of the eCRF. Included are conditions that are seasonal, cyclic, or intermittent (e.g. seasonal allergies; intermittent headache).
4. Vital signs
   Systolic and diastolic blood pressure, respiratory rate and pulse rate will be assessed after the patient has rested in the supine position for at least 3 minutes. Blood pressure should be assessed at the same arm for each time of determination
5. Height/weight (according to assessment schedule)
   Body weight taken at screening will be utilized for all pharmacokinetic calculations.
6. Pregnancy test (in serum)
7. Concomitant medication
8. ISNCSCI protocol (ASIA)
9. Pain assessment (SCI pain data set, Allodynia questionnaire & SCIPI)
10. Bladder assessments:
    a. Qualiveen Questionnaire (self-reported by the subject or with support of the investigator or relative/ accompanying person). The situation before the accident will be recorded.
    b. Bladder function assessment
11. Neurophysiological assessments:
    a. dSSEPs
    b. SSEPs
    c. NCVs
12. MRI
13. Blood sample

During the Baseline Phase, the following assessments will be conducted and recorded:

1. Review of inclusion/exclusion criteria (inclusion criteria No 5; exclusion criteria No 11, 15, 18)
2. Physical examination/neurological examination (see appendix)
   This evaluation will include an examination of general appearance, skin, neck, HEENT (head, eyes, ears, nose, throat), lungs, heart, abdomen, back and reflexes
   Information about the physical and neurological examination must be present in the source documentation at the study site.
   Significant findings that are present prior to the start of study medication must be included in the relevant medical history/current medical conditions of the eCRF page.
3. Medical history (prior and concomitant diseases)
4. Vital signs
5. ECG
6. Concomitant medication
7. Adverse events during screening/baseline have to be reported in the medical history section of the eCRF).
8. ISNCSCI protocol (ASIA)
9. Modified Ashworth Scale
10. SCIM-III
11. GRASSP (partial)
12. WISCI II
13. 6mWT & 10MWT (mandatory if the patient is able to walk)

14. MART & activity counts: assessments will be done consecutively three to five days per week
15. Blood sample
    If screening and baseline visits are scheduled within 3 days, only the screening lab has to be done.

After reevaluation of inclusion/exclusion criteria and after the subject is found to be eligible randomization can take place.

Screening failure

If a patient who has signed consent but did not continue in the study to receive the study medication (i.e. screening failure), information regarding the screening number, screening date and reason for discontinuation must be recorded in a paper-based screening-log in the investigator site file.

6.2.2 Treatment Phase (Visit 3-8) in-patient

Patients should be hospitalized for the entire duration of treatment with study medication and at least 6 weeks (assessment at day 84) post study medication administration.

The day after the Baseline visit, patients will enter the Treatment Phase (Days 0 to 25). On the morning of day 0, patients will receive the initial i.t. needle insertion (lumbar puncture) for bolus study medication administration. Local blood results should be obtained and checked before lumbar puncture (LP).

Contraindications for a lumbar puncture according to the Consensus guidelines for lumbar puncture in patients with neurological diseases (68) will be ruled out:

- It is advised to perform brain imaging before LP, whenever an intracranial lesion with mass effect, abnormal intracranial pressure due to increased CSF pressure, or tonsillar herniation is suspected based on medical history or neurological examination, and in case of recent seizures, impaired consciousness, or papilledema.
- Coagulation status (Quick >50% or INR<1.5 and aPTT within normal limits) and platelet count (should be higher than $40/10^9$ /L) should be checked by blood analysis within 24h before LP.
- Concomitant medication should be checked before LP. In case of concomitant therapeutic anticoagulation (e.g. phenoprocoumon (Marcumar®), heparin/heparinoids and new oral anticoagulants) at a higher dose than for the prophylaxis of venous thromboembolism), which cannot be interrupted, an LP is contraindicated. An LP can be performed without substantial risk when patients take one type of antiplatelet drug.
- Infections at the LP site are relative contraindications.

During the Treatment Phase, the following assessments will be conducted and recorded on days 0, 5, 10, 15, 20 and 25:

1. Adverse events
2. Physical/neurological exam
   Significant findings made after the start of study medication which meet the definition of an AE must be recorded in the Adverse Event eCRF page.
3. Vital signs
4. ISNCSCI protocol (ASIA) (between injection 3 (visit 5) and 4 (visit 6).
5. Concomitant medication
6. Blood samples and CSF samples according to Assessment Schedule
7. MART & activity counts: assessments will be done three days per week

6.2.3 Follow up Phase (visits 9-11) in-/out-patient

Upon completion of treatment, the patient will enter a 20-week (day 26 to 168) follow-up phase. Follow-up phase assessments will occur on days 30 (± 2 days), 84 and 168 (± 7 days). The follow up visits itself can be timed according to the condition of the patient lasting up to 48 hrs.

During the Follow-up Phase, the following assessments will be conducted and recorded on days 30, 84 and 168:

1. Adverse events
2. Physical/neurological exam
3. Vital signs
4. Concomitant medication
5. ISNCSCI protocol (ASIA)
6. GRASSP (complete)
7. SCIM-III
8. WISCI II
9. 6MWT (mandatory if the patient is able to walk)
10. 10mWT (mandatory if the patient is able to walk)
11. MART & activity counts for in-patients only: assessments will be done consecutively over the study period during three to five days per week
12. Pain assessment (SCI pain data set, Allodynia Questionnaire & SCIPI)
13. Modified Ashworth Scale
14. Bladder assessments (only day 84-168):
    a) Bladder diary
    b) Qualiveen Questionnaire
    c) Bladder function assessment
15. Neurophysiological assessments:
    a) dSSEPs
    b) SSEPs
    c) NCVs
16. MRI (only on day 30 and 168)
17. Blood samples and CSF samples according to Assessment Schedule. CSF collection on day 84 should be done the day prior to bladder assessments

For all patients, study completion evaluation will be performed at the last visit or at early discontinuation.

6.2.4 Early termination visit

In the event of an early trial termination the following basic clinical requirement will be conducted and recorded:

1. Adverse events
2. Physical/neurological exam
3. Vital signs
4. Concomitant medication
5. ISNCSCI protocol (ASIA)
6. GRASSP (complete)
7. SCIM-III
8. WISCI II
9. 6MWT
10. 10mWT
11. Pain assessment (SCI pain data set, Allodynia Questionnaire & SCIPI)
12. MRI

6.3 **Planned treatment after study end**

Patients included in the study will have the best medical therapy and will be observed and followed up at the investigator's discretion. The investigator will continue to observe all patients (also withdrawals) because of intolerable AEs/ SAEs until the findings have been clarified or became stable. The patients will be seen on a regular basis in each participating clinic (site) during their routine check-ups. If any unexpected condition occurs, that might be related to the study medication, they could contact the investigator/study nurse at any time. When the subject's participation in the clinical investigation has been completed, the subject shall return to the medical care as per physician's recommendation. After end of the study, patient will remain in the EMSCI network.

7 METHODS OF DATA COLLECTION

7.1 Safety Parameters

Adverse events (for definition see 8.1.1.) will be interrogated for at each contact between the responsible investigator and the study subject. All pathological and clinically relevant findings in physical and neurological examinations, vital signs, 12-lead ECGs, clinical chemistry, hematology, and clotting will be documented as adverse events.

7.1.1 Vital signs

Body height, body weight and body temperature according to sites clinical practice will be obtained at specified times during the study. Body weight taken at screening will be utilized for all pharmacokinetic calculations.

Systolic and diastolic blood pressure, respiratory rate and pulse rate will be assessed after the patient has rested in the supine position for at least 3 minutes. Blood pressure should be assessed at the same arm for each time of determination.

Vital signs (pulse rate, systolic and diastolic blood pressure and body temperature) determined on predefined study days will be documented as numerical values in the appropriate eCRF (see also appendix form for vital signs). Furthermore, vital signs may be recorded at any time, if medically imperative for clarification of clinical signs and symptoms. Pathological and clinically relevant findings will be documented as adverse events/ serious adverse events.

7.1.2 Physical/neurological Examination

Following parameters will be examined on the predefined study days and documented in the corresponding form: general appearance, skin, HEENT, neck, respiratory, cardiovascular, gastrointestinal, back, reflex left and right for biceps, knee & ankle, Babinski left and right for Plantar response. Pathological and clinically relevant findings will be documented as adverse events/serious adverse events.

7.1.3 12-lead Electrocardiogram (ECG)

Paper copies of the ECGs will be obtained at the site as a back-up and for review by the investigator (or deputy) at their discretion. Paper tracings will be dated and signed by the individual who reviews the ECGs at the clinical site. The patient's number and initials, the date and actual time of the tracing, and the study code (site number/name) must appear on each page of the tracing. These tracings will be archived with the source documents together with the ECG analysis report. If the ECG was not performed, the reason should be noted on the appropriate eCRF page. 12-lead ECG may be recorded at any time at discretion of the responsible investigator, if medically imperative for clarification of clinical signs and symptoms. Pathological and clinically relevant findings will be documented as adverse events/ serious adverse events.

7.1.4 Mechanical ventilation status

Details on patient's status of mechanical ventilation: start / end date / time of ventilation, invasive or non-invasive type of ventilation, pressure and volume mode, start of weaning off, potential complications and other relevant detail.

7.1.5 Imaging Assessment - MRI

Conventional diagnostic magnetic resonance imaging (MRI) primarily focuses on the cord macrostructure (e.g. lesion length, compression ratio, haemorrhage or edema) (Miyanji et al., 2007) and do not provide information on microstructural changes secondary to the injury (cell apoptosis, de- and remyelination, axonal degeneration vs repair). Within the NISCI trial MRIs will be reviewed by local site staff or radiologists for safety purposes and confirmation of Exclusion criteria 1. The scientific aim of the MRI study is to implement a refined spinal cord and brain quantitative MRI protocol next to the conventional MRI, to tract microstructural changes (e.g. iron and myelin) induced by therapeutic treatments. The advanced MRI readouts (Stroman et al., 2013; Wheeler-Kingshott et al., 2014) include cross-sectional cervical cord area, brain volume changes, myelin-sensitive magnetization transfer (MT) and longitudinal relaxation rate (R1, R2*) maps which are sensitive to iron and myelin level (Callaghan et al., 2014a, 2014b; Dick et al., 2012;

Freund et al., 2013; Weiskopf et al., 2013). We believe this information will help us to improve stratification of the patient groups and identify even subtle treatment effects in the clinical trial.

Therefore, the main aim of the neuroimaging work-package in NISCI trial is to develop and implement quantitative MRI biomarkers sensitive to the potential adverse effects of the anti-Nogo-A antibody treatment in SCI patients.

Patients with acute spinal cord injury will undergo a qMRI (quantitative MRI) protocol within three time points prior and after drug administration. The MR images will be coded (pseudonymized) and sent electronically to the **Swiss Center For Musculoskeletal Imaging (SCMI) in Switzerland** for quality assurance (QA) and will be stored securely there. Subsequently, if patients have signed the corresponding Informed Consent Form (ICF) the MRI data will be evaluated by experienced researchers qualified for the same purpose, as described in the study synopsis, at Max Planck Institute in Leipzig and Balgrist University Hospital Zurich.

7.1.6 Standard/routine clinical laboratory evaluations

Local laboratories at all sites will be used for all routine laboratory analyses.

Following parameters will be determined on the predefined study days:

Blood chemistry:

Albumin, alkaline phosphatase (AP), total bilirubin, calcium, cholesterol, chlorine, creatinine, creatine kinase (CK), glucose, gamma glutamyl transferase (y-GT), lactate dehydrogenase (LDH), lipase, amylase, potassium, total protein, aspartate transaminase (AST), alanine transaminase (ALT), sodium, triglycerides, uric acid and c-reactive protein (CRP).
If the total bilirubin concentration is increased above 1.5 times the upper limit of normal, direct and indirect bilirubin should be differentiated.

Hematology, complete Blood Count (CBC):

Hemoglobin, hematocrit, WBC (count with differential if medical indicated), RBC (absolute value), platelet count (absolute value)

Clotting:

Quick/INR, aPTT

Urine analysis:

Specific gravity, pH, glucose, protein, bilirubin, ketones, leucocytes, blood

After collection the samples will immediately be delivered to the local and/or central laboratory for respective determinations. All parameters will be documented in the appropriate eCRF.

Further laboratory parameters may be determined at any time during the study at the discretion of the responsible investigator. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms, are considered clinically significant, or require therapy or intervention.

7.1.7 CSF evaluations

CSF sampling (routine) during study drug application

CSF will be taken before each bolus injection (Day 0, 5, 10, 15, 20 and 25) and during follow up on day 84. It will be analyzed at the laboratory of each clinical site according to local standards. Routine CSF laboratory parameters (including cell count, both RBC and WBC, glucose, lactate and protein) to assess infections

will be performed. All data from this analysis must be noted directly into the corresponding eCRF. The volume of CSF sample for safety analysis is estimated to be 1 to 2 ml (24).

7.1.8 Muscle spasticity

Measured with the ***Modified Ashworth Scale (MAS).***

Therapies that aim to improve the growth of injured connections in the spinal cord could possibly also stimulate the growth of any fiber type in the spinal cord, resulting in an unknown spectrum of side effects including worsened spasticity. A velocity-dependent, abnormal increase in muscle tone with exaggerated tendon jerks is one definition of spasticity, which is a common complication of SCI. Spasticity, can lead to incoordination of muscle action, reduced functional limb movement, and in its more severe forms may result in chronic pain, muscle contracture, and permanent muscle shortening. The level of spasticity is known to vary over time, thus a single clinical assessment will not necessarily reflect accurately an individual's overall level of spasticity. The principal clinical outcome measure for spasticity is the Ashworth Scale. This scale may have less than ideal inter-rater reliability and poor correlation with self-rated assessments of spasticity (25). The scale determines the amount of resistance felt during the passive displacement of a limb, but it does not accurately account for the dependence of the resistance to the velocity of the stretch, which can be highly variable from examiner to examiner. (The intake of caffeine, nicotine, alcohol and prescribed spasticity medications will be assessed).

7.1.9 Pain Assessment

Effect on pain (neuropathic pain and non-neuropathic pain) assessed by **pain data set, incl. Allodynia & SCIPI**.

Pain in SCI tends to be chronic, interferes with functioning, and is resistant to medical treatment. Major SCI pain classification systems (26) (27) agree that neuropathic pain is typically perceived at or below the level of injury in areas without normal sensation, and that nociceptive pain (primarily musculoskeletal pain) most often occurs in areas of normal sensation and high activity such as the shoulder. If occurring in areas of impaired sensation, differentiation of nociceptive and neuropathic pain can be challenging which again underlines the need of appropriate and standardized pain assessments (28).

Pain is commonly assessed on uni-dimensional self-reported measures (e.g., perception of magnitude or severity of pain on 11-point Likert scale with anchors at ‘0’ [no pain] and ‘10’ [worst pain imaginable, pain as bad as it could be] and with relation to the impact of pain on functioning e.g., pain interference). Numerical pain ratings scales have been shown to have good test-retest reliability and adequate validity in terms of associations with other pain measures and treatments (29). Pain interference can be measured on the degree to which pain interfered with daily activities during the past week. For example, scores on a scale range from 0 to 10, with higher scores indicating greater pain interference with activities of daily life.

Therapies that aim to improve the growth of injured connections in the spinal cord could possibly also stimulate the growth of damaged sensory/nociceptive fibers or sprouting from undamaged pathways, resulting in increased pain levels that may be permanent or poorly responsive to therapy. Causing pain as a result of an experimental treatment is a major concern, especially as NG-101 has the potential to stimulate axonal fiber outgrowth or functional plasticity within central pain pathways. Thus, the ICCP Clinical Guidelines Panel recommended inclusion of specific pain measures as an important component of SCI therapeutics' outcome testing (24).

As a standardized way to evaluate and report pain in SCI patients (30) the latest version of the **International Spinal Cord Injury Pain Data Set (ISCIPDS)** will be included. It represents a structured interview and includes 2 assessment components (assessment of overall pain symptoms and specific assessment of up to the three worst pain sites). It is designated as clinical tool for specific characterization of both SCI-associated neuropathic and non-neuropathic pain in terms of localization, severity, time course, duration, response to pharmacological and non-pharmacological treatment and also its clinical classification. For that purpose, data is recorded by means of Likert-, and Rating-scales, as well as by “Yes-No” questions (polar questions). Furthermore, the **Spinal Cord Injury Pain Instrument (SCIPI)** which is a short questionnaire containing 7 items with “Yes-No” questions (polar questions) to specifically screen for the presence of SCI-associated neuropathic pain is added (31). Accordingly, a cutoff of ≥ 4 decisions with “Yes” indicates probable neuropathic pain for the 7 item SCIPI (33).

Aspects of pain measurement

- Pain description according to type, location and intensity.
- Pain perception in terms of intensity and sensory description, time course of pain.
- Pain interference (alleviating and enhancing factors) and side effects.
- Time for completion: 10 minutes

7.2 **Efficacy Parameters**

*7.2.*1 Efficacy outcome measures (providing anatomical or neurological assessments for the connectivity of the spinal cord)

All assessments will be done according to international standards.

a) ISNCSCI by the **A**merican **S**pinal **I**njury **A**ssociation (**ASIA** protocol)

International **s**tandards for the **n**eurological **c**lassification of **s**pinal **c**ord **i**njury (**ISNCSCI**)

Neurological condition is assessed using the International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI also called the ASIA protocol) (34, 35). The ASIA Impairment Scale (American Spinal Injury Association) has become a standardized and routinely adopted classification for most patients suspected of suffering a SCI (36). During the acute stages of SCI, there have been concerns about how soon after injury the ASIA examination can provide useful prognostic information about the eventual degree of impairment. It has been argued that an ASIA assessment within the first 24 hours may not provide an accurate prognosis and that a later 72-hour examination is a more reliable indicator, as the patient is medically more stable (24).
Perceptions of light touch (LT) and pinprick (PP) stimuli are scored as 0 for absent, 1 for impaired and 2 for normal. Each sensory dermatome is tested for light touch and pinprick, and a summary score that ranges from 0 to 112 is calculated by adding up the dermatome scores. Motor function is scored on the Medical Research Council Scale of 0 for total paralysis to 5 for normal strength. Ten muscles are tested bilaterally and individual muscle scores are added together, yielding an ASIA motor score that ranges from 0 to 100. The level of injury (LOI) is assigned by determining the lowest spinal level with normal neurological function. In addition, an ASIA Impairment Scale (AIS) grade (A, B, C, D) is assigned using ASIA Motor and Sensory Scores. Briefly, AIS grade A denotes no motor function beneath the LOI and no sensory function at the lowest sacral level (i.e., on rectal examination), AIS grade B denotes presence of rectal sensation but no motor function below the LOI, AIS grade C denotes sensory function and some motor function below the LOI, and AIS grade D denotes sensory function and substantial motor function beneath the LOI (American Spinal Injury Association 2002).

In many respects, the ASIA motor score is considered more reliable than the ASIA sensory score in predicting functional outcome after SCI (24, 37). It is recommended that upper and lower limb motor scores should be compiled separately as the upper-extremity motor score (UEMS) and lower-extremity motor score (LEMS) (24). This enables a change in motor function to be more clearly tracked and recorded as specific to either the cervical or lumbar levels. Separation of the motor scores into UEMS and LEMS also reduces the influence that a large change in the functional strength in one or a few muscles might have on the interpretation of therapeutic benefit.

Key muscles used for ASIA motor score assessment, with muscle grades categorizing functional assessment of each muscle's contraction

Left side (max. grade)	Key muscles for ASIA motor score assessment and primary level of spinal innervation	Right side (max. grade)
5	Elbow flexors (biceps brachialis) – C5	5
5	Wrist extensors (extensor carpi radialis longus and brevis) – C6	5
5	Elbow extensor (triceps) – C7	5
5	Finger flexors (flexor digitorum profundus, middle finger) – C8	5
5	Finger abductors (abductor digiti minimi, little finger) – T1	5
25	**Upper Extremity Motor Score (UEMS)**	**25**
5	Hip flexors (iliopsoas) – L2	5
5	Knee extensors (quadriceps) – L3	5
5	Ankle dorsiflexors (tibialis anterior) – L4	5
5	Long toe extensors (extensor hallucis longus) – L5	5
5	Ankle plantar flexors (gastrocnemius, soleus) – S1	5
25	Lower Extremity Motor Score (LEMS)	25
50	Total ASIA motor score (=100 for both sides)	50

ASIA muscle grades: 0=total paralysis; 1=palpable or visible contraction; 2=active movement, gravity eliminated; 3=active movement, against gravity; 4=active movement, against some resistance; 5=active movement

b) Graded **R**edefined **A**ssessment of **S**trength **S**ensibility and **P**rehension (GRASSP) (according to GRASSP version NISCI, Copyright 2008 International GRASSP Research and Design Team)

Cervical SCI now accounts for approximately 50% of all people living with a SCI. Validating a functional outcome tool to assess arm and hand capacity after a cervical spinal injury was identified as a top priority (24, 38).
The GRASSP has been designed to capture information regarding hand function from the whole cervical SCI (C0-T1) population, capture integrated sensory and motor impairment data, and to discriminate the population according to level of lesion (39). This measure also belongs in the section on functional assessments. The GRASSP is clinician administered and scored, and has an estimated time for completion of 45 minutes. The three modules - strength (motor), sensibility (sensory) and prehension - complement the ISNCSCI neurological assessment and the testing of functioning on the SCIM-III (40).
The GRASSP is designed to capture information of hand function from the whole cervical SCI population. Its neurological assessment can capture integrated sensory and motor impairment data and can discriminate the population according to the level of the lesion (41). The separate modules of the GRASSP allow for a comprehensive assessment at multiple time points post-injury. Unlike the SCIM-III or walking tests, the prehension subscale of the GRASSP can be administered at baseline in treatment studies of acute SCI. Using a functional outcome tool to assess arm and hand capacity is recommended; the GRASSP has demonstrated sensitivity to track small, but potentially meaningful functional gains in hand function (42).

It does not only evaluate changes within the motor and sensory systems, but also has a prehension component to relate impairment level changes to complex hand function tasks.

c) Electrophysiological Assessments (dSSEP, SSEP and NCV)

Somato-Sensory Evoked Potentials (SSEP)

Conventional *somatosensory evoked potentials* (SSEP) provide information regarding conduction in large diameter fibers that ascend in the dorsal column. Conventional SSEPs are examined by peripheral electrical stimulation of the median (arm) and tibial (leg) nerves with electrical pulses and recording electroencephalography (EEG) from scalp electrodes. Common readout parameters are the signal amplitude, latency, and velocity. SSEP are a useful adjunct to assess patients with various spinal cord pathology including trauma (44). SSEP has been correlated with prognosis, wherein acute patients without signal transmission from the posterior tibial nerve have poor ambulatory recovery and patients with some signal will tend to recover some ambulatory capacity at one year after injury. Strict correlation with outcome and SSEP results is not always present as patients can recover clinical function without changes in SSEP and likewise patients with recovery in SSEP may not have correlating improvement in motor function (45,46). Therefore, SSEP are being utilized in this study as an adjunct to the clinical examination and to determine if a signal change following injection may be reflective of a biological effect of the intervention that is below the clinical threshold.

However, it is difficult to precisely determine the level of SCI derived from findings of conventional SSEPs. For this reason, electrical stimulation of distinct individual dermatomes using similar EEG methodology as employed for conventional SSEPs allows a segmental neurophysiological assessment of posterior spinal cord innervation (dorsal root entry and ascending dorsal column conduction). The dermatomal SSEP's (dSSEP) are particularly applicable to injuries involving the cervical cord and are considered reproducible and able to detect changes in sensory level. This test is feasible in the study population and may represent a potential surrogate marker for a biological effect of application of NG-101 in patient with complete motor injury involving the cervical spinal cord. The dSSEP will be performed over the ASIA sensory key points above and below the segmental spinal cord injury (47). The test involves a controlled and standard cutaneous electrical stimulus at the ASIA sensory key points and recording of the cortical response by surface scalp electrodes. The latencies and amplitudes of the waveforms will be analyzed for determining changes following spinal cord intrathecal application of NG-101. Intercostal SSEP have been studied in patients with thoracic paravertebral blockades with predictive and consistent results (48).

Ulnar Nerve Conduction Velocity (NCV)

Nerve conduction velocity testing may be used to determine the adequacy of nerve impulse transmission of the ulnar nerves in the upper extremities. The NCV of the ulnar nerves may be used by the Investigator to assess centro-medullar function as part of the screening evaluation (49, 50).

The test is performed by placing electrodes over the skin along various sites of the nerve anatomy. One electrode stimulates the nerve and the transmission of the electrical impulse is recorded by the other skin surface electrodes. The distance between the electrodes and the time required results in the determination of the speed of transmission (nerve conduction velocity) (47, 51-56).

d) Bladder Function

The outcome of bladder function has a tremendous effect on the quality of life in patients with SCI. To address the burden of neurogenic bladder dysfunction on patient`s daily life and to understand the specific pathological conditions of bladder function a bladder diary, Qualiveen questionnaire as well as specific questions about bladder voiding and sensation will be applied according to the European Association of Urology (EAU) Guidelines on Neuro-Urology (57, 58). Furthermore, a very basic bladder function assessment which has shown to be feasible in the daily clinical routine will be applied to patients during screening and at 3 and 6 months visits. This assessment is tailored according to the patient's current neuro-urological condition, i.e. the patient empties the bladder spontaneously/ voluntarily or the patient relies on an indwelling catheter or performs intermittent self-catheterization. It is recommended to conduct the assessment with qualified medical staff. The patient's bladder will be slowly filled with 0.9% NaCl at a temperature of 4°C and a filling speed of 80-100mL/min until any sensation will be noticed by the patient or upon a maximum bladder filling volume of 500 mL. In the case of autonomic

dysreflexia, the bladder filling is stopped and the bladder is emptied immediately. The bladder-function assessment allows us to roughly assess if there are changes in lower urinary tract function as most patients will develop detrusor overactivity needing neuro-urological treatment.

For details see bladder-function assessment under Appendices and neuro-urology manual.

Bladder diary

By completing the three-day bladder diary the following information will be obtained:

Daytime, frequency, nighttime frequency, voiding e.g. spontaneous, catheter (transurethral, suprapubic, self-catheterization), voided volume, post void residual, urinary urgency, incontinence episodes, pad use, fluid intake per 24 hours, amount of urine per 24hours, pain (visual analogue scale 0-10).

The bladder diary must be completed during three days before the follow up visit of Day 84 or 168

In case of indwelling catheter and no application of bladder retraining or any oral fluid intake, the bladder diary must not be completed.

Bladder function / Qualiveen Questionnaire

Bladder function is assessed by Qualiveen questionnaire and specific questions about bladder emptying (spontaneous voiding, catheter) and bladder sensation (yes/no).

The 30-item Qualiveen is a specific health related quality of life questionnaire for urinary disorders in patients with neurological conditions, such as multiple sclerosis and spinal cord injury. Previous studies have demonstrated the reliability, validity and responsiveness of Qualiveen (59).

e) MART and activity counts

The Mapping of Rehab Training (MART) consists of a form which allows reporting of physio- and occupational therapy content and duration based on the SCI-Intervention Classification System (SCI-ICS) (43). This form is filled out by the therapist and offers the opportunity to track therapy in more detail.

Activity counts will be measured to record patients` overall activity level during their in-patient stay and follow ups. They will be applied to in-patients during three to five consecutively days per week except on IMP administration days. Data are transferred via SSL-encrypted links (https) established between sites and Swiss Federal Institute of Technology Zurich (ETH). Each measurement file contains a header part that includes the encrypted study subject ID. No further patient-related information will be stored in the files. Encrypted data will then be evaluated by Balgrist research workers.

7.2.2 Efficacy outcome measures (categorizing a subject's functional ability to engage in activities of daily living)

In general, assessments of recovery after CNS damage have been developed to be useful for planning clinical care rather than for clinical trials. For clinical trials, objective electrophysiological, functional imaging and behavioral outcome measures are required. The second International Campaign for Cures of spinal cord injury Paralysis (ICCP) Clinical Guidelines Panel has provided recommendations for the valid conduct of clinical trials in SCI patients (24). The panel focused on outcomes measures that are relevant to clinical trials of experimental cell-based and pharmaceutical drug treatments. They have categorized outcomes measures into three main classes:

1. Those that provide an anatomical or neurological assessment for the connectivity of the spinal cord

2. Those that categorize a subject's functional ability to engage in activities of daily living

3. Those that measure an individual's quality of life.

It is essential that steps be taken to standardize and optimize the accuracy of the ASIA assessment. It is recommended (24, 60) that ASIA assessors undergo standardized training in the EMSCI network

(central training courses) with an intra-and inter-rater reliability test being completed at the end of the training session. This is especially important in the NISCI study as this is a multicenter, multinational clinical trial. Although the ASIA assessment paradigm seems simple in its description, experience has indicated that rigorous adherence to the definitions, based on training, is necessary to obtain consistent data that can be meaningfully compared both within and across clinical studies or centers.

a) Spinal Cord Independence Measure (SCIM-III)

The International Campaign for Cures of spinal cord injury Paralysis (ICCP) Clinical Guidelines Panel recommends that an improvement in the measurable performance of meaningful function is necessary for any therapeutic intervention to be universally accepted as beneficial (24). In addition, functional measures may become standard tools for decisions on reimbursement with payors looking for reductions in the 'burden' of care.

The Spinal Cord Independence Measure (SCIM-III) is a comprehensive functional outcome measure, and it appears to be sensitive and accurate functional assessment for ability to perform activities of daily living after SCI. SCIM has gone through a few iterations and SCIM-III is the latest version (61, 62). The SCIM-III is a 100-point disability scale developed specifically for SCI with emphasis on 18 activities associated with:

1. Self-care (feeding, bathing, dressing, grooming), maximum (max)=20 points
2. Respiration and sphincter management (ventilation, bladder, bowel, use of toilet), max=40 points (clinically weighted)
3. Mobility (in bed, transfers, indoors and outdoors, wheelchair, walking), max=40 points.

b) Walking Index for Spinal Cord Injury II (WISCI-II)

For clinical trials involving people with motor-incomplete SCI, several validated tests of ambulatory performance have been developed including the Walking Index for Spinal Cord Injury (WISCI-II) and a number of timed walking tests (63, 64). WISCI-II is a 21-level hierarchical scale of walking based on physical assistance, need of braces and devices, with an ordinal range from 0 (unable to walk) to 20 (walking without assistance for at least 10m). It is an example of a more sensitive and precise scale for rating a specific functional activity in people with incomplete SCI. WISCI-II is currently a valid outcome measure for strategies directed to improve ambulation by subjects with incomplete SCI (63).

Although the WISCI-II has been validated as a qualitative outcome measure for the assessment of standing and walking after incomplete SCI, the opinion of the ICCP Clinical Guidelines Panel (24) is that a more accurate assessment may be provided by a combination of WISCI and some of the more quantitative timed walking tests. Such quantitative walking tests include the distance traversed during a 6-minute walk test (6mWT) (63). The WISCI-II evaluates the patients' ability to ambulate 10m with assistive devices. In contrast the SCIM-III evaluates mobility for moderate distances 10-100m, and outdoors at more than 100m. There are no actual items included in the 6mWT. The 6mWT is a simple test that requires a 100-feet, quiet, indoor, flat, straight rectangular hallway. The walking course must be 30m in length. The length of the 30m corridor must be marked by colored tape at every 3m. The turnaround must be marked with a cone.

c) 10-Meter Walk Test (10MWT)

The timed 10-Meter Walk Test is a quantitative measurement of lower extremity function. The patient is directed to one end of a course with 10 meters clearly marked. They are instructed to walk 10 meters at preferred speed. The task is immediately administered again by having the patient walk back the same distance. Patients may use assistive devices when doing this task. In consultation with the patient, the investigator selects the appropriate assistive device for each patient.

The individual is instructed to walk a set distance (10 meters) at preferred walking speed. Time in minutes is measured while the individual walks the set distance (often the individual is given space to accelerate to his/her preferred walking speed (this distance is not included when determining speed). The distance covered is divided by the time it took the individual to walk that distance.

d) 6-Minute Walk Test (6mWT)

This assessment is a submaximal test that will be used as a global and easy indicator of the locomotor performance. It is valid and reliable for many healthy or frail populations such as elderly, stroke, chronic lung disease, heart disease, Parkinson's disease, and Spinal Cord Injury. It allows therapists to assess the recovery of the patient's walking ability throughout their rehabilitation with a fast and simple measurement. Additionally, it allows therapists to adjust the training targets between 2 neuro rehabilitation sessions. From the patient's perspective, this test is a source of motivation, because it allows the objective monitoring of one's own progress.

Patients will be instructed to walk as far as possible during 6 minutes, taking rests whenever required. The distance covered (in meters (m)) and the number/ time of rests required will be recorded.

7.3 Pharmacokinetics and Immunogenicity Assessments

One of the secondary outcome measures for this clinical trial is the assessment of the pharmacokinetic (PK) and immunogenicity (IG) of NG-101 based on serial samples. The concentrations of NG-101 in serum and CSF will be analyzed by validated enzyme-linked immunosorbent assay (ELISA) methods. Samples for analysis of anti-NG-101 immunogenicity (antibody detection) will be screened using an immunoassay and possibly go through other characterization assays. See section 7.5 for sampling procedures, storage conditions, and shipment instructions.

7.4 Proteomics and Future Research

This substudy includes a proteomics analysis (see synopsis: Proteomics (WP3)), which as a first step aims to identify biomarkers measured in CSF and serum by proteomic analysis only in subjects, who gave written informed consent to the storage for proteomics analysis and future research (see below). Biomarker candidates will be correlated with the treatment, clinical prognosis and clinical outcome as well as neurological and functional parameters of patients with acute SCI. Proteomics analyses will be performed at the Medical Proteome Center at the Ruhr University Bochum (RUB). In the context of a first screening phase, whole CSF and corresponding exosome fractions will be analyzed in a label-free quantitative study to compare the proteome profiles in order to identify differential proteins. Afterwards, a systematic categorization of the proteomes will be performed with comprehensive bioinformatic tools. Differentially expressed proteins of CSF and exosome will be mapped among: 1) proteomes of different time points and 2) proteomes of different treatment groups. The most promising protein candidates will be validated for their suitability as biomarkers. Therefore, an independent targeted mass spectrometric method based on multiple reactions monitoring (MRM) will be used to analyse CSF and serum samples. In parallel, Western blot and enzyme linked immunosorbent assay (ELISA) analysis will be performed for further validation experiments. Suitable biomarkers will be used to develop a sensitive, multiplexed MRM assay for CSF und serum diagnostics.

Candidate protein biomarkers planned to be analyzed in CSF or blood serum is: NgR, myocilin, secreted frizzled-related protein 2 and 4 (sFRP2, sFRP4) and Matrix metalloprotease-9 (MMP9). The levels of candidate biomarkers will be determined by ELISA methods to be developed.

Depending on the CSF and serum volume used for the proteomic analysis as described remaining samples will be stored beyond the termination of the clinical trial to be available for future research within the NISCI consortium as well as national and international non-profit institutions dedicated to SCI research. Future research refers to analysis of serum and CSF not directly related to the IMP, which will be covered by a separate study protocol and a separate patient informed consent form. Future research analyses may include protein, lipid or metabolite related investigations aiming to detect molecular signatures of central nervous system injury and regeneration, prognostic markers and biological signals indicating treatment response and elucidating related mechanisms. Only for non-commercial purposes, respective analyses can be transferred to companies, which are capable to carry out such investigations. In any case, transfer of samples to partners outside of the consortium will be secured with respective material transfer agreements, which guarantee the adherence to applicable laws. There will be no commercial use of samples, meaning sharing CSF or serum samples for profit. The Biobank will be entrusted to further store the samples beyond

the project duration or transfer the samples to another high-quality storage unit for up to 20 years after the end of the main study lifetime.

7.5 Collection and preparation of biological samples

Pharmacokinetics/ Immunogenicity (main study), Proteomics (substudy)

CSF and blood samples will be collected from SCI patients according to the informed consents given to enable local safety, **pharmacokinetics and immunogenicity analyses** as well as a **substudy (proteomics)**. For details see also the Assessment Schedule and table of sampling details pages 83, 84.

In the following, the general process of sample collection (i.e. CSF and blood or serum derived from blood samples) and pre-processing is described. Specific details about sampling procedures, local (clinical SCI centers) and central (**central Biobank in Heidelberg/Germany**) storage conditions and shipment instructions are described in corresponding SOPs of an extra manual.

In general, CSF and blood samples will be collected. Blood will be centrifuged to gain serum. CSF will be centrifuged to remove blood cells. Both, serum and CSF centrifugation will be performed at the local trial sites (clinical SCI centers). In accordance with the appropriate informed consent signed by the patients`, CSF and serum samples will then be stored at the local trial sites at ≤-70°C until shipment to the central Biobank in Heidelberg. At the central Biobank in Heidelberg, CSF and serum samples will be included in the Laboratory Information Management System (LIMS, micronic and CentraXX) and after gentle thawing, samples will be aliquoted to standard secondary tubes and stored in the fully automated and sealed cryostore at ≤-70°C.
The samples will be shipped to the respective central laboratories for the pharmacokinetics and immunogenicity analyses **(USZ, Zurich/Switzerland)** and proteomics analysis within the substudy (Medical Proteome Center at **Ruhr University Bochum (RUB)/ Germany**).

7.6.1 CSF sample collection

CSF samples (details see Appendix “Blood and CSF assessment schedule”) will be collected after i.t. needle insertion. The first sample taken will be collected in tubes, which are routinely used at the local trial sites for this purpose, for routine clinical laboratory analyses at the local trial site. If the routine analyses reveal more than 500 red blood cells/µl of CSF this information must be documented, as such high cell counts may impact the proteomics analysis. Subsequent CSF samples will be collected for PK analysis and the substudy (proteomics) in tubes provided centrally to all clinical SCI centers prior to clinical study start. The CSF collected for the for PK-analysis and the substudy will be centrifuged and stored in securely sealed tubes, which will be provided centrally to all clinical SCI centers prior to clinical study start. Respective CSF tubes will immediately be transferred to a local freezer (≤-70°C) until shipment to the **central Biobank in Heidelberg**.

In subjects not consenting to the substudy (proteomics) the same volume of CSF as in consenting subjects will be taken to avoid CSF withdrawal as confounding factor for potential effects and/or side effects. However, in non-consenting subjects CSF samples beyond the volume required for routine and PK analysis will not be used for the substudy.

At the local trial sites, a connection to alarm in case of failure and a basic temperature log of the freezer for quality control is recommended. The date and exact time of sample collection must be documented. Sampling problems will also be noted. Shipment of samples from the trial site to the Biobank will be organized by the central Biobank in Heidelberg.

7.6.2 Blood (serum) sample collection

All blood samples will be taken by either direct venipuncture or an indwelling cannula inserted in a forearm vein. Blood samples (details see Appendix “Blood and CSF assessment schedule”) will be collected into serum blood tubes, which are routinely used at the local trial sites. The freshly collected blood samples will be allowed to clot during 45 minutes at room temperature. For most serum tubes on the market, clotting is recommended in an upright (i.e. the tip upright) position of the serum tube, please consider the respective manufacturer`s requirements. The serum will then be centrifuged for

10 minutes at approximately 2500 x g. After centrifugation is completed, the serum will be transferred into tubes provided centrally prior to clinical study start to all local trial sites. Serum tubes will be stored within a total time of 60 minutes after venipuncture at ≤-70°C until shipment to the central Biobank in Heidelberg. The exact clock time of the current sample collection (date and time) will be documented. Sampling problems will also be noted.

In respect to serum in subjects not consenting to the substudy, only 1 container of blood instead of 2 containers will be collected. Serum obtained after centrifugation will be used for PK/IG analysis. The remaining serum will be stored at the Heidelberg Biobank for additional PK/IG analysis, if needed. However, remaining serum will not enter the substudy.

8 ADVERSE EVENTS

8.1 Definitions

Information about all adverse events, whether volunteered by the patient, discovered by investigator questioning, or detected through physical examination, laboratory test or other means, will be collected and recorded on the Adverse Events page of the eCRF and followed as appropriate.

8.1.1 Adverse Event

According to the International Conference of Harmonization (ICH), an adverse event (AE) is any untoward medical occurrence in a patient administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign, including an abnormal laboratory finding, symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

An AE may be:

- New symptoms/ medical conditions
- New diagnosis
- Changes of laboratory parameters

  Following criteria have to be considered when deciding whether to report an abnormal test finding as adverse event:

  - test result is associated with accompanying clinical signs or symptoms, and/or
  - test result requires diagnostic testing or medical/surgical intervention, and/or
  - test result lead to a change in trial dosing, or discontinuation from the trial, significant additional concomitant drug treatment, or other therapy, and/or
  - test result is considered by the investigator or sponsor to be an adverse event

- Intercurrent diseases and accidents
- Worsening of medical conditions/ diseases existing before clinical trial start
- Recurrence of disease
- Increase of frequency or intensity of episodical diseases.

A pre-existing disease or symptom will not be considered an adverse event unless there will be an untoward change in its intensity, frequency or quality. This change will be documented by an investigator. Pre-existing conditions will be reported in the medical history.

Surgical procedures themselves are not AEs; they are therapeutic measures for conditions that require surgery. The condition for which the surgery is required may be an AE. Planned surgical measures permitted by the clinical trial protocol and the condition(s) leading to these measures are not AEs, if the condition leading to the measure was present prior to inclusion into the trial. In the latter case the condition should be reported as medical history.

AEs are classified as "non-serious" or "serious".

8.1.2 Serious Adverse Event

A serious adverse event (SAE) is any experience that suggests a relevant hazard, contraindication, side effect or precaution. It is any AE that, at any dose, fulfills at least one of the following criteria:

- Results in death (please note: death is an outcome, not an event)
- Is life-threatening (the term life-threatening refers to an event in which the subject was at risk of death at the time of event and not to an event which hypothetically might have caused death if it was more severe)
- Requires hospitalization or prolongation of existing hospitalization*
- Results in persistent or significant disability/ incapacity**
- Is a congenital anomaly/ birth defect or
- Is otherwise medically relevant (i.e. an event that jeopardizes the subject or may require medical or surgical intervention to prevent one of the outcomes listed above) ***

* Hospitalization for performing protocol-required procedures or administration of study treatment is not classified as an SAE. Hospitalizations for disease-related procedures (surgery, imaging, laboratory tests) or any procedures planned before entry into the study (elective or pre-planned) are not considered SAEs. Hospitalizations for treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of a SAE given below and not resulting in hospital admission are not classified as SAE. Hospitalizations for social reasons in the absence of an adverse event are not classified as SAEs either.

** Persistent or significant disability or incapacity means that there is a substantial disruption of a person's ability to carry out the life functions he/she performed before. The irreversible injury of an organ function (e.g. paresis, diabetes, cardiac arrhythmia) fulfills this criterion.

*** Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations - such as important medical events that may not be immediately life threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed above. These should also usually be considered serious (examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasia or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse).

8.1.3 Serious Adverse Reaction

SAEs that potentially may be attributed to the investigational medicinal product (IMP) are to be classified as Serious Adverse Reactions (SARs).

8.1.4 Expectedness

An “unexpected” adverse reaction is one the nature or severity of which is not consistent with the applicable product information, in this case the Investigator’s Brochure (IB). Furthermore, reports which add significant information on specificity or severity of a known adverse reaction constitute ‘unexpected’ events.

8.1.5 Suspected Unexpected Serious Adverse Reaction (SUSAR)

SAEs that are both ‘suspected’, i.e., possibly related to the study medication (investigational medicinal product (IMP)) and ‘unexpected’, i.e., the nature and/ or severity of which is not consistent with the applicable product information are to be classified as Suspected Unexpected Serious Adverse Reactions (SUSARs).

In case, either the investigator who primary reported the SAE or the second assessor, classifies the SAE as ‘**suspected**’ and the SAE is **unexpected** it will be categorized as a SUSAR.

All SUSARs are subject to an expedited reporting to the responsible ethics committee(s), the competent authorities in all the member states concerned and to all participating investigators.

8.1.6 Grading of AEs

Difference in meaning between "serious" and "severe":

The terms “serious” and “severe” are not synonymous but are often used interchangeably. The term ‘severe’ is often used to describe the intensity (severity) of a specific event; the event itself, however, may be of relatively minor significance (such as severe headache). This is not the same as “serious”, which is based on the existence of one of the seriousness criteria (chapter 8.1.2).

The classification of AE-intensity in this trial will be carried out on the basis of a 3-grade scale as follows:

Mild:	signs and symptoms which can be easily tolerated. Symptoms can be ignored or disappear when the subject is distracted.
Moderate:	symptoms cause discomfort but are tolerable, they cannot be ignored and affect normal activity.
Severe:	symptoms strongly affect normal activity.

If there is a change in the intensity of an adverse event, different approaches for documentation may be applied:

- If the change in the intensity changes the medical relevance of the event, an additional AE should be documented.
- If the changes follow an expected pattern in the course of the event, the most representative or medically relevant grade may be documented and if appropriate an addition ‘intermittent’ may be recorded in the diagnose/description field.

The responsible investigator will choose the most appropriate way of documentation.

8.1.7 Relationship and Outcome of AEs

The investigator will evaluate each AE that occurred after administration of the IMP regarding the **relationship** with the administration of the IMP:

Not related:	The temporal relationship of the clinical event to the route of administration (i.t. injection) or to the IMP makes a **causal relationship unlikely**, or other drugs, therapeutic interventions or underlying conditions provide a sufficient explanation for the observed event.
Related:	The temporal relationship of the clinical event to the route of administration or to the IMP makes a **causal relationship possible**, and other drugs, therapeutic interventions or underlying conditions do not provide a sufficient explanation for the observed event.

“Related” should be chosen if there is a reasonable possibility for a causal relationship between the investigation product and the AE.

All subjects who have reportable AEs, whether considered associated with the use of the trial medication or not, must be monitored to determine the **outcome**. The clinical course of the AE will be followed up until resolution or normalization of changed laboratory parameters or until it has changed to a stable condition. This also holds for ongoing AEs/SAEs of withdrawn subjects.

The outcome of an AE at the time of the last observation will be classified as:

Recovered / resolved:	All signs and symptoms of an AE disappeared without any sequels at the time of the last interrogation.
Recovering / resolving:	The intensity of signs and symptoms has been diminishing and / or their clinical pattern has been changing up to the time of the last interrogation in a way typical for its resolution.

Not recovered / not resolved:	Signs and symptoms of an AE are mostly unchanged or worsened at the time of the last interrogation.
Recovered / resolved with sequel:	Actual signs and symptoms of an AE disappeared but there are sequels related to the AE.
Fatal:	Resulting in death. If there are more than one adverse event only the adverse event leading to death (possibly related) will be characterized as 'fatal'.
Unknown	The outcome is unknown or implausible and the information cannot be supplemented or verified.

Clarification of the "onset" and "end date" of AEs and SAEs:

Onset date:
- of AE is defined as the onset of signs and symptoms or a change from baseline.
- of SAE is defined as the date the signs and symptoms/diagnosis became serious, i.e., met at least one of the criteria for seriousness.

End date:
- of AE is defined as the date when the symptoms resolve, or the event is considered stable.
- of SAE: same as AE. The end date of the SAE must not be later than the end date of the corresponding AE.

AEs and SAEs that are ongoing at the time of death and that are not the reason for the outcome "death" are considered "not resolved" or "resolving".

The action taken with the IMP will be assigned to one of the following categories:

Dose not changed:	No change in the dose of the IMP.
Drug interrupted:	Temporarily interruption of the IMP.
Drug withdrawn:	Discontinuation of the IMP.
Unknown:	The information is unknown or implausible and it cannot be supplemented or verified.
Not applicable:	The question is implausible (e.g. the subject is dead).

The term "countermeasures" refers to the specific actions taken to treat or alleviate adverse events or to avoid their sequels. Following categories will be used to categorize the countermeasures to adverse events:

None:	No action taken.
Drug treatment:	Newly-prescribed medication or change in dose of a medication.
Others:	Other countermeasures, e.g. an operative procedure.

Recommended treatment for expected AEs

Due to the route of administration, local adverse events at the access site of the intrathecal injection are expected to occur. Standard treatment of care should include treatment with an antiseptic solution and an appropriate course of antibiotic treatment (after identification and culture).

Information about possible side effects related to the investigational drug will be detailed in the Investigator Brochure or will be communicated between IB updates in the form of Investigator Notifications, if applicable. This information will be included in the patient informed consent and should be discussed with the subject during the study as needed.

8.2 Period of Observation and Documentation

Adverse events (AEs) will be ascertained by the investigators using non-leading questions, noted as spontaneously reported by the patients to the medical staff or observed during any measurements on all study days. The observation period begins with the first administration of the IMP. The period ends with the last study visit, i.e. 5 months after the last intake of study medication. AEs occurring before starting IMP but after signing the informed consent form are recorded on the Medical History page of the eCRF.

AEs will be documented in the patient file and in the eCRF. The investigator should always be notified of the abnormality of test results in a timely fashion. All subjects who present with AEs, whether considered associated with the use of the trial medication or not, will be monitored by the responsible investigator to determine their outcome; this applies to withdrawals, too (see also 8.1.7). Once an adverse event is detected, it should be followed until its resolution, (or until it is judged to be permanent), and assessment should be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the study medication, the interventions required to treat it, and the outcome.

The patients should report any AEs occurring during the outpatient part to the study site by phone.

The end date of the SAE is defined the same as for AEs. The end date of the SAE must not be later than the end date of the corresponding AE.

AEs and SAEs that are ongoing at the time of death are considered not resolved or resolving (if they are not the reason for death).

All SAEs and their relevance for the benefit/risk assessment of the study will be evaluated continuously during the study and for the final report. All SAEs will be documented in the "Serious Adverse Event" form (see 8.3). The period of observation and documentation is the same as for AEs.

Based on the phase-I trial, the follow-up after end of treatment could be reduced to five months. Five months follow up can be considered reasonable as during this time period patients will show most of their neurological and functional recovery.

Based on the results of the phase-I study the timelines for follow ups were set up accordingly.
The half-life of NG-101 in CSF and brain is about 4-7 days and in the blood about 3-4 weeks. NG-101 levels are low after 3 months' time. No generation of anti-idiotypic antibodies are expected. No Adverse Reactions are anticipated within the treatment and follow-up period.

8.3 Reporting of Serious Adverse Events by Investigator

All SAEs, regardless of suspected causality and occurring from the first administration of the IMP until the end of the follow-up period (last study visit) (also until 4 weeks after the patient has stopped study participation), must be reported by the investigator to the responsible Safety Officer at the KKS Heidelberg within 24 hours after the SAE becomes known using the "Serious Adverse Event" form. The initial report will be completed in English and must be as complete as possible including details of the current illness and (serious) adverse event and an assessment of the causal relationship between the event and the trial medication. The SAE Report Form and the fax confirmation sheet must be kept with the Source Documentation at the study site.

The reporting will be performed by faxing a completed 'SAE Form' to KKS Heidelberg

within 24 hours,

fax number:

Pharmacovigilance: +49 (0) 6221 56 33725

or via email:

pharmakovigilanz.KKS@med.uni-heidelberg.de

Pharmacovigilance: Providing professional staff (safety officer and safety data manager) and infrastructure to collect initial SAE reports, to keep an eye on data consistency and follow up reports, and to ensure in time reporting to ethic committees, investigators and competent authorities, as

requested. Development Safety Update Reports (DSURs) will be issued in close cooperation with the coordinating investigator.

Follow-up reports

Recurrent episodes, complications, or progression of the initial SAE must be reported as follow-up to the original episode within 24 hours of the investigator receiving the follow-up information. An SAE occurring at a different time interval or otherwise considered completely unrelated to a previously reported one should be reported separately as a new event.

Follow-up information is sent to the responsible Safety Officer, using a new SAE Report Form stating that this is a follow-up to the previously reported SAE and giving the SAE number of the respective SAE. Each re-occurrence, complication, or progression of the original event should be reported as a follow-up to that event even if it occurs at a different time interval. The follow-up information should describe whether the event has resolved or continues, if and how it was treated and whether the patient continued or withdrew the study medication.

8.4 Expedited Reporting

SUSARs are to be reported to the responsible ethics committee(s), the competent authorities in all the member states concerned, to all participating investigators and if applicable to further bodies according to international law within defined timelines, i.e. if they are subject to an expedited reporting.

All SAEs will be forwarded to the EU coordinator Professor Curt and the coordinating investigator Prof. Weidner. All SAEs are subject to a second assessment by a designated person. The designated person for the present trial, referred to as the second assessor is the EU-coordinator Professor Curt, deputy in case of absence is the coordinating investigator Professor Weidner.

The second assessor will fill out a ‘Second Assessment Form’ for each SAE and send it back per fax to the responsible person at KKS Heidelberg within 48 hours, SAE-fax-number:

+49 (0) 6221 56 33725

The ‘Second Assessment Form’ will contain the following information:

I) assessment of relationship between SAE and IMP (causality)
II) assessment of relationship between SAE and underlying disease
III) assessment of expectedness of SAE (derived from IB or SmPC)
IV) statement if the benefit/ risk assessment for the trial did change as a result of SAE.

Fatal and life-threatening SUSARs must be reported to EC/CA without delay, but not later than 7 calendar days after becoming aware of the minimum reporting criteria. SUSARs that are not fatal or life threatening must be reported without delay, at least within 15 calendar days after becoming aware of the minimum reporting criteria. Reporting will be carried out centrally by the staff responsible for pharmacovigilance.

The Safety Officer has to break the blind for expedited reporting. Only SUSARs occurring after administration of IMP (verum) will undergo expedited reporting.

8.5 Pregnancies

To ensure patient safety, each pregnancy in a patient on study medication or each pregnancy in a female partner of a male patient on study medication must be reported on the comment field on the SAE form by the investigator to the Safety officer within 24 hours of learning of its occurrence. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence of any birth defects, congenital abnormalities, or maternal and/or newborn complications.

Pregnancy follow-up should be recorded on the same form and should include an assessment of the possible relationship to the study medication of any pregnancy outcome.

Any SAE experienced during pregnancy must be reported on the SAE Report Form.

The related SAEs (SARs) will be handled as SUSARs with expedited reporting (in case unblinding revealed application of verum).

9 STATISTICAL PROCEDURES

9.1 Sample Size Calculation

The primary efficacy endpoint (UEMS recovery) will be analysed by the comparison of the mean of the control and treatment groups. Also, the secondary efficacy endpoints will be analysed by comparing the means of outcomes between the control and treatment groups.

The power calculation is based on the mean delta changes in the EMSCI data of the control group (nodes 4, 5, 8, 9, 10, 13, 16, 17, and 18 have a mean delta UEMS of 14.3 +/-SD of 10.8 motor scores) and a 42% treatment effect (mean delta change of 20.3 motor scores). Using t-tests means with an allocation 3:1, an estimated α error probability of 0.05 and a power (1-β err prob) of 0.8 a total of about 106 patients would be required. For an adequate powering of the study, we assume that 20 per cent of patients will drop out of follow-up, which means that at approximately 114 patients will be needed to compensate.

The protocol is being amended in order to get more subjects exposed to NG-101. After approval of the amendment the randomization ratio will be changed from 1:1 to 3:1 (NG-101:placebo). If the randomization ratio is altered after 30 subjects have been included at 15:15 and the remaining subjects will be recruited at 63:21, the resulting number of subjects is expected to be 78:36. The power of the test is expected to go down to 66 per cent. It is planned to compensate this loss of power by filling up the pool of control subjects by historical controls from the EMSCI database. The EMSCI database has repeatedly been used for publications guiding treatment development strategies. The demographic and prognostic variables have been documented consistently, and do not display overt trends over the years. These will be drawn randomly from the database matched by the cohorts which have been used for stratification of randomization (see section 3.1). This will permit sensitivity analyses with a tradeoff aiming for a higher precision (power going up to 86 per cent) in return for an unknown bias stemming from selecting historical controls.

The test of the primary hypothesis is outlined in section 9.5. The power calculation based on a t-test is a reasonable (and conservative given that the variance of the estimate should be lower with explanatory variables explaining some of the variance) approximation to parameter tests from the model outlined there.

The analysis of delta UEMS changes between 2 weeks-6 months compared to 2 weeks-12 months (see table below) reveals that the UEMS scores have reached about 90% of their recovery within the first 6 months after injury and late changes are rather minor. These findings allow for a total study duration of 5.5-6 months for each patient to reveal the effectiveness of NG-101. As patients become seamlessly embedded in the EMSCI long-term observation (i.e. 1-year observation) later changes can be retrieved.

9.2 Analysis Variables

The primary criterion is the UEMS recovery score at day 168.

Secondary variables are the following:

- Effect on changes in motor and sensory function according to the ISNCSCI protocol (ASIA impairment scale, ASIA lower extremities motor score (LEMS) and sensory scores (light touch (LT), pin prick (PP)) at day 168.
- Effect on autonomic dysfunction (i.e. bladder function as measured by bladder diary, Qualiveen questionnaire and bladder function assessment during screening and days 84 and 168.
- Effect on pain (neuropathic pain and non-neuropathic pain) assessed by short pain assessment questionnaire at day 168.
- Effect on functioning evaluated by the Spinal Cord Independence Measure (SCIM-III) at day 168.

- Effect on hand/upper limb function as assessed by the Graded and Redefined Assessment of Strength, Sensibility and Prehension (GRASSP) subscales at day 168.
- Effect on the Walking Index for Spinal Cord Injury (WISCI), 10-meter walk test (10mWT) and the 6-minute walking test (6MWT) at day 168.
- Effect on neurophysiological parameters (nerve conducting velocity, Somatosensory evoked potentials)
- To evaluate the pharmacokinetics (PK) and immunogenicity of NG-101.

Safety endpoints are:

- Adverse Events (Frequency, type, duration and intensity of AEs and SAEs)
- Relationship of AE/SAE frequency and time and duration of study medication administration
- Documented reasons for and unplanned study medication interruptions and/or withdrawal from the study.
- Vital signs (blood pressure, heart frequency, body temperature)
- Muscle spasticity measured by the Modified Ashworth Scale
- Effect on pain (neuropathic pain and non-neuropathic pain) assessed by SCI pain data set, allodynia questionnaire and SCIPI.

9.3 Definition of Trial Population to be analyzed

The primary analysis will be performed for the full analysis set which comprises all patients randomized into the trial. In this set, every patient is analyzed according to the group randomized into. Exceptions to this rule can be made for patients for whom non-eligibility became apparent after randomization, provided all three of the following conditions hold:

1. The patient has not been administered study medication yet
2. The actual measurement of the violation of the criterion was made prior to randomization
3. All patients hold up to the same scrutiny with respect to this criterion, regardless of treatment group.

The per-protocol set will comprise all patients who were treated according to the randomized treatment as outlined in the protocol. Specifically, patients have to be eligible according to in- and exclusion criteria. Before the trial team is unblinded, rules for selecting the per-protocol set will be selected by a steering board with at least the Coordinating Investigator (“LKP”) and study statistician as members.

The safety set will comprise all patients who have received study medication at least once, and will allocate the patients to the treatment they actually received, regardless of randomization.

The augmented set will contain the full analysis set and a sample from the EMSCI database from the recent 8 years containing subjects matched by stratification cohorts. Sensitivity analyses of the primary endpoint and select secondary endpoints will be performed on the augmented set.

9.4 Missing values

Missing values pertaining to exploratory variables will be replaced using simple imputation if their frequency is not more than, or multiple imputation if more than five percent. Missing values in response variables will not be replaced as GLMM will allow unbiased estimation of the treatment effect under the Missing At Random assumption.

9.5 Statistical Methods

The primary criterion is the UEMS recovery score at day 168. The impact of randomized treatment on this score is estimated using a linear mixed model with the 1 month, 12 weeks and 24 weeks measurements as response variable, randomization cohort, baseline value, time of measurement, treatment group and interaction of treatment group with time as fixed and subject and subject-time interaction as random explanatory variables. The actual effect will be estimated as a contrast between treatment arms at day 168. The null hypothesis of no treatment effect on UEMS recovery score can be rejected at the .05 level if the 95 per cent confidence interval does not cover 0.

In a sensitivity analysis, the primary analysis will be repeated for the per protocol set only. In another sensitivity analysis, the primary analysis will be repeated for the augmented data set.

In a secondary analysis and as part of project package 2, the UEMS constituents will be interpreted as a vector of ordinal variables and analyzed using an autoregressive transitional ordinal model as described in Tanadini et al..

For safety analysis, all adverse events will be tabulated by grading and treatment group, by relatedness and treatment group, and by SOC/PT and treatment group using number of events and number of subjects with events. This tabulation will be repeated for adverse events starting within 30 days and adverse events starting more than 30 days after randomization.

Other safety relevant variables are vital signs (blood pressure, heart frequency, body temperature) and muscle spasticity measured by the Modified Ashworth Scale. These will be tabulated by treatment arm and visit using minimum, maximum, the quartiles, mean and standard deviation.

Scale variables obtained as secondary and safety (Modified Ashworth Scale) endpoints will be analysed in the same fashion as the primary endpoint.

A more detailed description of the analysis will be available in a statistical analysis plan which will be finalized and approved before unblinding of sponsor and biometrician.

Unblinded analysis of all endpoints will not begin before the study data base is closed.

For Pharmacokinetic analyses see section 7.3.

9.6 Interim Analysis

Interim analyses are not planned for the trial.

10 DATA MANAGEMENT

10.1 Data Collection and Source Data

The timing of assessments required during the study is delineated in the Study Synopsis and the Assessment Schedule. All data obtained from these assessments must be supported in the patient's source documentation. Source documentation must be available for all data entered in the eCRFs. eCRFs are not substitute for source documents. In advance exceptions to this rule can be defined. Regardless, there must be a minimum documentation, which provides information on study participation and includes all medical information necessary for appropriate medical care outside of the clinical trial in the patient record.

If an assessment is unable to be performed, the reason must be noted on the appropriate eCRF.

All findings including clinical and laboratory data will be documented in the subject's medical record. As a general rule, medical information that is not specifically required by the study (e.g. patient's sex, prior medical history, prior medication, type of surgical procedure, etc.) but necessary for an adequate medical treatment during routine clinical care must be found in source documents (and on the eCRF). Information specifically required by the protocol and not required by routine clinical care may be recorded into the local site source documents.

In addition, source documents must mention that the patient has been included in an investigational study. Finally, there must be no data that are inconsistent between eCRF and source documents.

The investigator is responsible for ensuring that all sections of the eCRF are completed correctly and that each entry can be verified against source data (this must also be ensured for **study specific data like intensity or causality of (S)AEs** that are normally not documented in detail in the subject records). Any errors should have a single line drawn through them so that the original entry remains legible and the correct data should be entered at the site (eCRF) with the investigator's signature, date and reason for change on the Source Data. Self-explanatory corrections need not to be justified.

All protocol-required information collected during the trial must be entered by the investigator, or a designated representative, in the eCRF. Patient data will be documented pseudonymously. The investigator, or a designated representative, should complete the eCRF pages as soon as possible after the information is collected, preferably on the same day when a trial subject is seen for an examination,

treatment, or any other trial procedure. Any pending entry must be completed immediately after the final examination. Explanation should be given for all missing data.

The correctness of entries in eCRF will be confirmed by dated signature of the responsible investigator.

10.2 Data Handling

Data entries will undergo an automatical online check for plausibility and consistency. In case of implausibilities, 'warnings' will be produced during data entry (edit checks). A responsible investigator or a designated representative will be obliged either to correct the implausible data or to confirm its authenticity and to give appropriate explanation. The responsible data manager will check all explanations and resolves the warnings if the explanation is appropriate. The responsible CRA/monitor can generate special questions (CRA/monitor query), that will be sent back to the responsible investigator. The investigator or a designated representative will have to answer them all. The responsible CRA/monitor will check all answers and resolves the CRA/monitor query if the answer is appropriate. Analogue queries can be used by the data manager (dm query).

The investigator has to confirm the accuracy of all data by signing sections online in the eCRF.

All missing data or inconsistencies will be reported back to the site(s) and have to be clarified by the responsible investigator prior to database lock. If no further corrections are to be made in the database it will be declared locked and used for statistical analysis.

All data management activities will be done according to the current SOPs of the KKS.

10.3 Archiving of Essential Documents

The investigator(s) will archive all trial data (source data and Investigator Site File (ISF) including subject identification list and relevant correspondence) according to the section 4.9 of the ICH Consolidated Guideline on GCP (E6) and to local law or regulations, in any case at least 10 years after the end of the trial. These procedures shall include:

- the protocol including the rationale, objectives and statistical design and methodology of the trial, with conditions under which it is performed and managed, and details of the investigational product used.
- standard operating procedures
- all written opinions on the protocol and procedures,
- final report,
- case report forms,
- audit certificate(s), if available.
- all other relevant documents of the trial master file, according to the ICH-GCP guideline

Any change of data ownership shall be documented. All data shall be made available if requested by relevant authorities.

11 ETHICAL AND LEGAL ASPECTS

11.1 Good Clinical Practice

The procedures set out in this trial protocol, pertaining to the conduct, evaluation, and documentation of this trial, are designed to ensure that all persons involved in the trial abide by ICH harmonized tripartite guideline on Good Clinical Practice (ICH-GCP) and the ethical principles described in the applicable version of the Declaration of Helsinki.

11.2 Legal basis

The trial will be carried out in keeping with local legal and regulatory requirements. The trial has to be conducted in compliance with the protocol, ICH-GCP and the applicable regulatory requirements in all participating countries.

11.2.1 Declaration of Helsinki

The study will be carried out in conformity with the "Ethical principles for medical research involving human subjects" version 1964 including all amendments.

11.2.2 Other Legal Bases

The other legal bases of this clinical trial are as follows (including their amendments/ up-dates, if applicable):

- Guideline for Good Clinical Practice E6(R2), EMA/CHMP/ICH/135/1995
- Directive 2001/20/EC (April 4, 2001)
- Commission Directive 2005/28/EC (April 8, 2005)
- National regulatory requirements/guidelines of the participating countries concerning Clinical Trials
- General national regulatory requirements
- local data protection regulations
- EU-data protection regulation, where applicable

The Coordinating Investigator and all investigators will be given an up-to-date investigator's brochure containing full details of the status of the pre-clinical and clinical knowledge of the study medication. As soon as new information is obtained, an updated version will be supplied or an amendment added to the existing investigator's brochure.

11.3 Approval of Trial Protocol and Amendments

Before the start of the trial, the trial protocol, informed consent document, and any other appropriate documents will be submitted to the independent Ethics Committees (EC) as well as to the competent authorities of each participating country.

A written favorable opinion/vote of the EC and an approval by the competent authority are a prerequisite for initiation of this clinical trial. The statement of EC should contain the title of the trial, the trial code, the trial site, and a list of reviewed documents. It must mention the date on which the decision was made and must be officially signed by a committee member. This documentation must also include a list of members of the EC present on the applicable EC meeting and a GCP compliance statement.

The investigator and the Sponsor (trial master file) will keep a record of all communication with the EC and the regulatory authorities.

Before the first subject is enrolled in the trial, all ethical and legal requirements must be fulfilled.

All planned substantial amendments (e.g. trial protocol amendments) have to be signed by the sponsor and biometrician and will be submitted to EC and the competent authority for approval.

11.4 Notification to other Regulatory Authorities or boards according to local law/ regulations

In addition to the approval of the competent authority (see 11.3) further notifications must be done or approvals received prior, during and at the end of the trial as required by local law of each participating country.

Each investigator is obliged to inform the sponsor about his/her legal obligations to notify/inform his/her local authorities or applicable boards and must fulfill this duty in all cases where this responsibility is not delegated by contract to the Sponsor or another institution (e.g. contract research organization (CRO)).

11.5 Subject Information and Informed Consent

Before being admitted to the clinical trial, the patient must consent to participate after being fully informed by the investigator or a designated member of the investigating team about the nature, significance and implications (risks) and individual consequences of the clinical trial and his/her right, to terminate the participation at any time.

The patient should also have the opportunity to consult the investigator, or a physician who is member of the investigating team about the details of the clinical trial. The informed consent to participate in the clinical trial may be withdrawn by the patient verbally in the presence of, or in written form directed to, the investigator or a physician member of the investigating team at any time during the trial. The patient must not entail any disadvantage therefor or be coerced or unduly influenced to continue to participate. Furthermore, the patient is not obligated to disclose reasons for the withdrawal of the consent.

If the patient has a primary physician, the investigator should inform him or her about the patient's participation in the trial, provided the patient agrees hereto.

The informed consent should be presented in a private setting, apart from noise and other distractions that may put pressure on the subject to sign the consent. Sufficient/ adequate ample time must be provided to allow the patient to consider the study (e.g. at least 24 hours; allow the patient to consider his decision over night and to sign at a subsequent visit).

After reading the informed consent document, the patient must give consent in writing. The patient's consent must be confirmed by a personally dated signature of the patient and by a personally dated signature of the physician conducting the informed consent discussion.

A copy of the signed informed consent document must be given to the subject; the original will be filed at the investigator's site. The documents must be in a language understandable to the subject and must specify who informed the subject (according to local legal requirements).

The subjects will be informed as soon as possible if new information may influence his/her decision to participate in the trial. The communication of this information should be documented in the patient's file.

Subjects unable to write:

If the patient is unable to write, oral presentation and explanation of the content of the informed consent form and of the data protection information must take place in the presence of an impartial witness. The witness and the physician conducting the informed consent discussions must also sign and personally date the consent document. The witness must not be in any way dependent on the sponsor of the trial, the trial site or any member of the investigating team (e. g. an employee at the trial site.).

This clinical trial includes optional substudies which require **a separate informed consent and signature** if the patient agrees to participate. It is required as part of this protocol that the Investigator presents these options to the patient.

11.6 Insurance

Prior to the start of the trial the sponsor has to subscribe to an insurance policy covering, in its terms and provisions, its legal liability for injuries caused to participating persons and arising out of this research performed strictly in accordance with the scientific protocol as well as with applicable laws and regulations in each country where the trial is conducted and according to professional standards.

Any impairment of health which might occur in consequence of trial participation must be notified to the insurance company. The subject is responsible for notification. The insured person will agree with all appropriate measures serving for clarification of the cause and the extent of damage as well as the reduction of damage.

During the clinical trial, the subject must not undergo any other additional interventional trial treatment. The subject is bound to inform the investigator immediately about any adverse events and drugs additionally taken. The terms and conditions of the insurance should be delivered to the subject.

The insurance company has to be informed about all amendments that could affect subjects' safety.

11.7 Continuous Information to the Ethics Committee and the Competent Authority

The responsible EC, the competent authority and all participating investigators will be informed of all suspected unexpected serious adverse reactions (SUSARs) occurring during the trial. Both institutions will be informed in case the risk/ benefit assessment did change or any others new and significant hazards for subjects' safety or welfare did occur. Furthermore, a report on the subject's safety will be submitted once a year – Development Safety Update Report (DSUR).

The EC and the regulatory authorities must be informed of the end of the trial. They will be provided with a summary of trial results within one year after the end of clinical phase (LSO) or within the time frame required according to local law of participating countries.

12 QUALITY CONTROL AND QUALITY ASSURANCE

The sponsor, the investigators, and all involved study personnel agree to conduct this clinical trial in accordance with the ICH Guideline for Good Clinical Practice.

12.1 Direct Access to Source Documents According to ICH GCP

According to ICH-GCP the investigator(s)/institution(s) must provide direct access to source data/documents for trial related monitoring, audits and regulatory inspection. Each subject has consented - via written informed consent - to direct access to his/her original medical records for trial-related monitoring, audit and regulatory inspection. Content of the protocol must be the identification of any data to be recorded directly on the eCRFs (i.e., no prior written or electronic record of data), and to be considered to be source data (see 10.1).

In the absence of either an audit-trail or limited access for the CRA (access only to source data of trial participants) the electronic record of data must be printed out.

12.2 Data Protection

During the clinical trial, subjects will be identified solely by means of their individual identification code (randomization number). Trial findings stored on a computer will be stored in accordance with local data protection law and will be handled in strictest confidence. For protection of these data, organizational procedures are implemented to prevent distribution of data to unauthorized persons. The appropriate regulations of local data legislation will be fulfilled in its entirety.

The subject consents in writing to release the investigator from his/her professional discretion in so far as to allow inspection of original data for monitoring purposes by health authorities and authorized persons (inspectors, CRAs/monitors, auditors). Authorized persons (clinical monitors/CRAs, auditors, and inspectors) may inspect the subject-related data ensuring the appropriate effective data protection law.

The investigator will maintain a subject identification list (subject numbers with the corresponding subject names) to enable records to be identified. **Subjects who did not consent to circulate their pseudonymized data will not be included into the trial.**

This protocol, the eCRFs, other results forms, laboratory data must be handled with strict confidentiality and not be disclosed to third parties except with the express of prior consent of the Sponsor. In particular, it must be ensured that study medication is kept out of reach of third parties. Staffs of the investigators involved in this study are also bound by this agreement.

12.3 On-site Monitoring / Central Monitoring / Risk based quality management

Monitoring will be done by personal visits from a clinical research associate (CRA) according to SOPs of the KKS and centrally by all applicable functions (e.g. data manager, biometrician) according to a risk-based quality management approach (remote evaluation of the ongoing trial in a timely manner). During on-site visits the CRA will review the entries into the eCRFs on the basis of source documents and spot checks the trial conduct and protocol compliance. The investigator must allow the CRA to verify all essential documents and must provide support at all times to the CRA.

Frequency and details of monitoring as well as central procedures in context of the risk based quality management and the rationale for the chosen monitoring strategy will be defined by all participating functions such as data manager, biometrician, CRA/clinical monitor, project manager, pharmacovigilance and the sponsor and described in applicable manuals (e.g. monitoring manual, data management plan and further manuals (if necessary)).

12.4 Inspections and Audits

Regulatory authorities and/ or auditors authorized by the sponsor may request access to all source documents, eCRF, and other trial documentation. Direct access to these documents must be guaranteed by the investigator who must provide support at all times for these activities.

The investigator will inform the sponsor immediately about a planned inspection.

12.5 Responsibilities of the Investigator

The investigator ensures that all team members are informed adequately about the protocol, all amendments to the protocol, the current investigator's brochure, the study procedures und study specific duties and tasks.

The investigator nominates adequately qualified members of the investigating team and must instruct and supervise them in order to ensure that they are adequately informed about relevant information regarding the trial, especially the trial protocol and Investigators Brochure. Furthermore, he has to nominate a **deputy** with qualification comparable to his/her own. The Sponsor has to be informed in case of replacements of the investigator or his deputy, as well as substantial changes of the trial site qualification (where applicable according to local law this also needs to be approved by the local ethics committee).

The investigator will maintain a **list of the members** of the investigating team and other persons to whom he/she has delegated significant trial-related duties.

The investigator must inform the Sponsor in case of a planned involvement of third parties to which particular tasks will be delegated by contract.

He/she also is responsible for supervising any individual or party to whom the investigator delegates study tasks at the trial site ensuring that this party/person is qualified to perform those study tasks.

13 ADMINISTRATIVE AGREEMENTS

13.1 Financing of the Trial

The trial will be supported by the **European Commission (EC) HORIZON 2020**, by the Swiss State Secretary for Education, Research and Innovation (SERI), by the Swiss Paraplegic Foundation and by the Wings for Life Spinal Cord Research Foundation.

13.2 Financial Disclosure

Before the start of the trial, the investigator will disclose any proprietary or financial interests he or she might hold in a funding organization, in the investigational product(s) or any commercial organization being involved in the clinical trial. The investigator has also to confirm that he/she has not entered into any financial arrangement, whereby the value of compensation paid could affect the outcome of the clinical trial.

The investigator agrees to update this information in case of significant changes.

13.3 Reports

After conclusion of the trial, a report (or alternatively the publication) shall be written by the sponsor's delegate, the coordinating investigator and / or principal investigators. The report will include a statistical analysis and an appraisal of the results from a medical viewpoint. It will be based on the items listed in this trial protocol. The KKS Heidelberg will prepare the biometrical part of this report.

Within the defined timeframe (e.g. for Germany within one year after completion of the trial (trial end is defined as last patient out)) the competent authorities and the ethics committees will be supplied with this final report or a summary of the final report containing the principle results.

Dependent on national regulations the trial report will be published in a clinical trial register via the competent authority. By signing this protocol the investigators agree to disclose their names/ clinic address in the trial report.

13.4 **Registration of the Trial**

Prior to the beginning of the clinical phase (FPI) the Sponsor will register the trial in an official online register (e.g. Current Controlled Trials (http://www.controlled-trials.com/), (http://www.clinicaltrials.gov) or other accepted registers). Thus, the trial will be given a unique registration number, which is a prerequisite for a publication in a peer-review paper. If further registrations are necessary according to local requirements each trial site will be responsible thereof.

13.5 **Publication**

All information concerning the trial is confidential before publication.

Publication(s) and/or presentation(s) of the study results is encouraged after appropriate time for review and written agreement by the sponsor/sponsor representatives. The sponsor/sponsor representatives have to be provided with a draft of the abstract and/or manuscript for review and editorial comments at least 30 days prior to submission and/or presentation. Neither the sponsor nor the Coordinating Investigator (respectively the representatives of the sponsor) has the right to prevent publication, except for patent or copyright reasons.

KKS staff members who gave relevant scientific support to the study design, conductance and/or analysis of results will be included as coauthors, if applicable. A copy of all publications will be sent to the KKS.

Any publication of the results, either in part or in total (articles in journals or newspapers, oral presentation, etc.) by the investigators or their representatives shall require the approval of the sponsor representatives.

It is planned to publish the results of the trial as an original article in an appropriate medical journal as well as presentation at congresses. The CONSORT guidelines for publishing study results will be observed. The sponsor representatives have the right to name the first and last author of the article and select the presenter of the data at major congresses. The choice of the journal for the publication will be made by the sponsor representatives in agreement with the co-authors. Besides the Principal Investigator, further authors of this article have to meet the following criteria:

- Contribution in the design of the trial and/ or
- Substantial contribution to the trial in form of recruitment of patients and/ or
- Substantial contribution to the interpretation of the data and/ or
- Substantial contribution to drafting the article or revising it critically for important intellectual content.

<u>Declaration regarding Data-Sharing</u>

Data-sharing according to the requirements of the International Committee of Medical Journal Editors:

After publication of the primary objective, the data might be provided to interested scientists on request (e.g. for meta-analyses, health related registers or other scientific questions) in an anonymized way.

14 SIGNATURES

The present trial protocol was subject to critical review and has been approved in the present version by the persons undersigned. The containing information is consistent with:

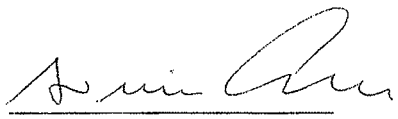
- the current risk-benefit assessment of the investigational medicinal product.
- the moral, ethical, and scientific principles governing clinical research as set out in the latest relevant version of Declaration of Helsinki, the principles of the guidelines of ICH Good Clinical Practices and the applicable legal and regulatory requirements.

The investigator will be supplied with details of any significant or new finding including AEs relating to treatment with the investigational medicinal product.

It will be ensured that the first subject is enrolled only after all ethical and regulatory requirements are fulfilled. Written consent from all subjects or witness if subject can only consent orally is received after detailed oral and written information and according to the requirements of local law. All study participants will be informed on the type of encoding their personal data (pseudo-anonymization) and who receives or has access to such data. Subjects who do not agree to this data encoding and transfer will not be enrolled into the trial. In this context it will be assured that all investigational sites comply with the local regulatory requirements for data protection.

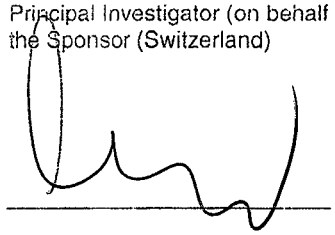
No subjects in a relationship of any dependence to the investigator or sponsor will be included.

Via current versions of the clinical trial protocol and the investigator's brochure (IB) it will be ensured that all principal investigators are informed about the pharmacological-toxicological assessments and results regarding the benefits and risks of the clinical trial.

Date: 27.10.2020 Signature: 

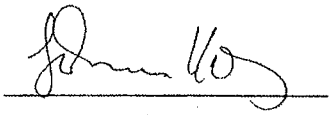
Name (block letters): Professor Armin Curt

Function: Principal Investigator (on behalf of the Sponsor (Switzerland)

Date: 30/10/20 Signature: 

Name (block letters): Professor Norbert Weidner

Function: Coordinating investigator (on behalf of the legal representative of the Sponsor (European union))

Date: 2020-10-27 Signature: 

Name (block letters): Dr. Johannes Huesing

Function: Biometrician

15 DECLARATION OF INVESTIGATOR

I have read the above trial protocol and confirm that it contains all information to properly conduct the clinical trial. I pledge to conduct the clinical trial according to the protocol.

I will enroll the first subject only after all ethical and regulatory requirements are fulfilled. I will obtain written consent for trial participation from all subjects or witness if subject can only consent orally after detailed oral and written information and according to the requirements of local law. All study participants will be informed on the type of encoding their personal data (pseudo-anonymization) and who receives or has access to such data. Subjects who do not agree to this data encoding and transfer will not be enrolled into the trial. In this context I confirm that my investigational site complies with all local regulatory requirements for data protection.

Furthermore, I declare that to the best of my knowledge no subjects in a relationship of any dependence to the investigator or sponsor will be included.

I know the requirements for accurate notification of serious adverse events and I will document and notify such events as described in the protocol.

I declare that I am informed about the pharmacological-toxicological assessments and results regarding the benefits and risks of the clinical trial by reading the description in the clinical trial protocol and in the current version of the investigator's brochure (IB). I ensure that all investigators/ relevant staff at my site will be informed of this results and possibly new risks that are forwarded by the sponsor later on (e.g. via new version of the investigator's brochure).

I confirm that every staff will be adequately trained to guarantee compliance to the trial protocol incl. subsequent amendments.

I will retain all trial-related documents and source data as described. I will provide a current Curriculum Vitae (CV) before the start of the trial. I agree that the CV and Financial Disclosure (FD) may be submitted to the responsible EC.

As the clinical trial and the results have to be published in a clinical trial register and forwarded to the competent authorities I agree that my name and clinic address will be part of this final trial (summary) report / public register and are disclosed for that purpose.

Date: _____ Signature: _____

Name (block letters): _____

Function: Principal Investigator (PI)

Investigational Site (address): _____

_____

_____

Date: _____ Signature: _____

Name (block letters): _____

Function: Deputy of the PI

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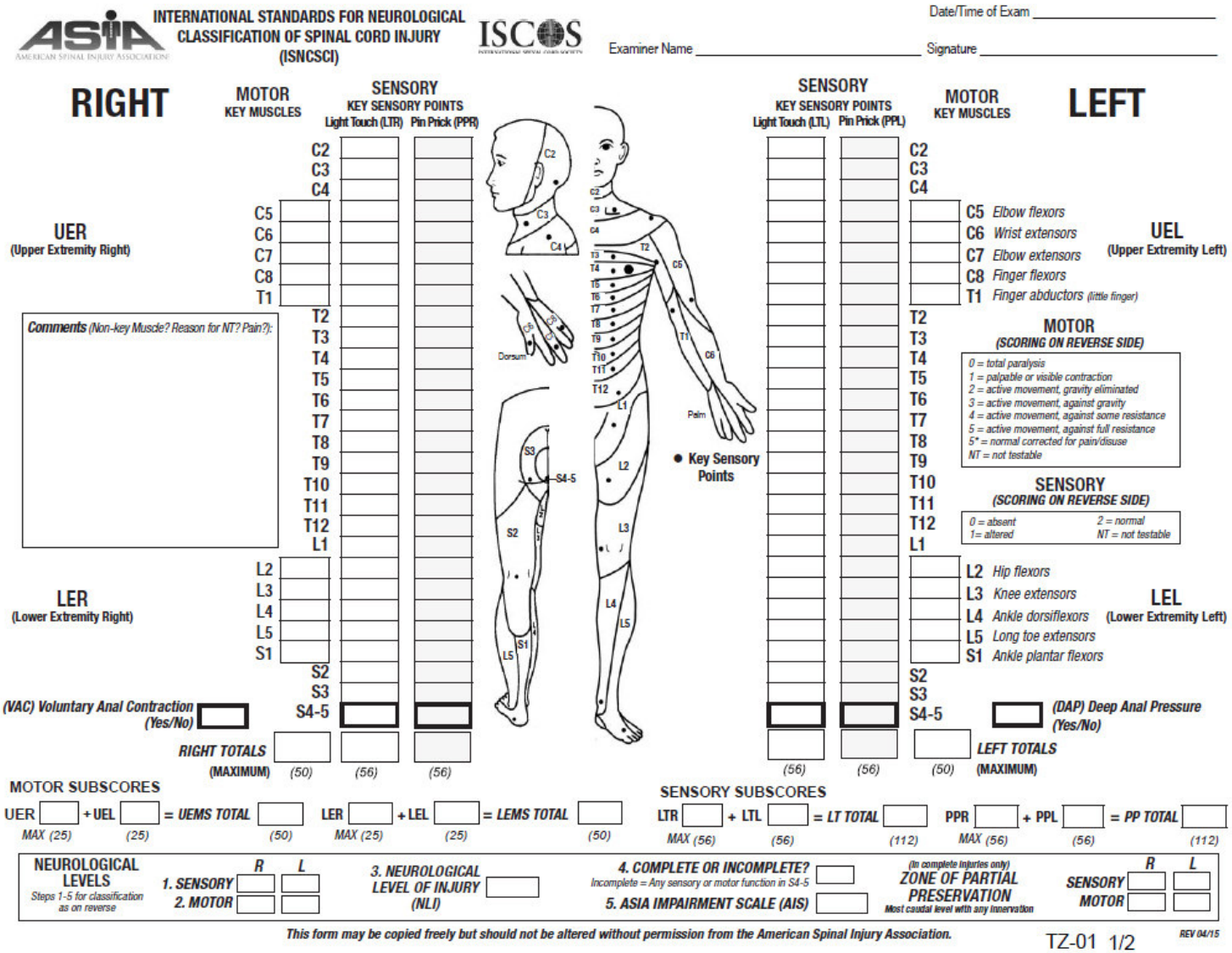
17 APPENDICES

17.1 Appendix: ASIA protocol (Standard Neurological Classification of SCI)

according to revised Version TZ-01 Rev 04/15

(William P. Waring III, MS, MD et al.2009 Review an Revision of the International Standars for the Neurological Classification of Spinal Cord Injury). J Spinal Cord Med. October 2010;33(4):346-352)



INTERNATIONAL STANDARDS FOR NEUROLOGICAL CLASSIFICATION OF SPINAL CORD INJURY (ISNCSCI)

Date/Time of Exam _____

Examiner Name _____ Signature _____

RIGHT | **LEFT**

MOTOR KEY MUSCLES | SENSORY KEY SENSORY POINTS Light Touch (LTR) Pin Prick (PPR)

SENSORY KEY SENSORY POINTS Light Touch (LTL) Pin Prick (PPL) | MOTOR KEY MUSCLES

C2 C3 C4 C5 C6 C7 C8 T1 T2 T3 T4 T5 T6 T7 T8 T9 T10 T11 T12 L1 L2 L3 L4 L5 S1 S2 S3 S4-5

C5 Elbow flexors
C6 Wrist extensors
C7 Elbow extensors
C8 Finger flexors
T1 Finger abductors (little finger)

UER (Upper Extremity Right)

UEL (Upper Extremity Left)

LER (Lower Extremity Right)

LEL (Lower Extremity Left)

L2 Hip flexors
L3 Knee extensors
L4 Ankle dorsiflexors
L5 Long toe extensors
S1 Ankle plantar flexors

Comments (Non-key Muscle? Reason for NT? Pain?):

MOTOR (SCORING ON REVERSE SIDE)

0 = total paralysis
1 = palpable or visible contraction
2 = active movement, gravity eliminated
3 = active movement, against gravity
4 = active movement, against some resistance
5 = active movement, against full resistance
5* = normal corrected for pain/disuse
NT = not testable

SENSORY (SCORING ON REVERSE SIDE)

0 = absent 2 = normal
1= altered NT = not testable

● Key Sensory Points

(VAC) Voluntary Anal Contraction (Yes/No)

(DAP) Deep Anal Pressure (Yes/No)

RIGHT TOTALS (MAXIMUM) (50) (56) (56)

(56) (56) (50) LEFT TOTALS (MAXIMUM)

MOTOR SUBSCORES

UER ____ + UEL ____ = UEMS TOTAL ____ (MAX (25) (25) (50))

LER ____ + LEL ____ = LEMS TOTAL ____ (MAX (25) (25) (50))

SENSORY SUBSCORES

LTR ____ + LTL ____ = LT TOTAL ____ (MAX (56) (56) (112))

PPR ____ + PPL ____ = PP TOTAL ____ (MAX (56) (56) (112))

NEUROLOGICAL LEVELS (Steps 1-5 for classification as on reverse)

1. SENSORY R ____ L ____
2. MOTOR R ____ L ____

3. NEUROLOGICAL LEVEL OF INJURY (NLI) ____

4. COMPLETE OR INCOMPLETE? ____ (Incomplete = Any sensory or motor function in S4-5)

5. ASIA IMPAIRMENT SCALE (AIS) ____

ZONE OF PARTIAL PRESERVATION (In complete injuries only) (Most caudal level with any innervation)
SENSORY R ____ L ____
MOTOR R ____ L ____

This form may be copied freely but should not be altered without permission from the American Spinal Injury Association.

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Muscle Function Grading

0 = total paralysis

1 = palpable or visible contraction

2 = active movement, full range of motion (ROM) with gravity eliminated

3 = active movement, full ROM against gravity

4 = active movement, full ROM against gravity and moderate resistance in a muscle specific position

5 = (normal) active movement, full ROM against gravity and full resistance in a functional muscle position expected from an otherwise unimpaired person

5* = (normal) active movement, full ROM against gravity and sufficient resistance to be considered normal if identified inhibiting factors (i.e. pain, disuse) were not present

NT = not testable (i.e. due to immobilization, severe pain such that the patient cannot be graded, amputation of limb, or contracture of > 50% of the normal ROM)

Sensory Grading

0 = Absent

1 = Altered, either decreased/impaired sensation or hypersensitivity

2 = Normal

NT = Not testable

When to Test Non-Key Muscles:

In a patient with an apparent AIS B classification, non-key muscle functions more than 3 levels below the motor level on each side should be tested to most accurately classify the injury (differentiate between AIS B and C).

Movement	Root level
Shoulder: Flexion, extension, abduction, adduction, internal and external rotation **Elbow:** Supination	C5
Elbow: Pronation **Wrist:** Flexion	C6
Finger: Flexion at proximal joint, extension. **Thumb:** Flexion, extension and abduction in plane of thumb	C7
Finger: Flexion at MCP joint **Thumb:** Opposition, adduction and abduction perpendicular to palm	C8
Finger: Abduction of the index finger	T1
Hip: Adduction	L2
Hip: External rotation	L3
Hip: Extension, abduction, internal rotation **Knee:** Flexion **Ankle:** Inversion and eversion **Toe:** MP and IP extension	L4
Hallux and Toe: DIP and PIP flexion and abduction	L5
Hallux: Adduction	S1

ASIA Impairment Scale (AIS)

A = Complete. No sensory or motor function is preserved in the sacral segments S4-5.

B = Sensory Incomplete. Sensory but not motor function is preserved below the neurological level and includes the sacral segments S4-5 (light touch or pin prick at S4-5 or deep anal pressure) AND no motor function is preserved more than three levels below the motor level on either side of the body.

C = Motor Incomplete. Motor function is preserved at the most caudal sacral segments for voluntary anal contraction (VAC) OR the patient meets the criteria for sensory incomplete status (sensory function preserved at the most caudal sacral segments (S4-S5) by LT, PP or DAP), and has some sparing of motor function more than three levels below the ipsilateral motor level on either side of the body.
(This includes key or non-key muscle functions to determine motor incomplete status.) For AIS C – less than half of key muscle functions below the single NLI have a muscle grade ≥ 3.

D = Motor Incomplete. Motor incomplete status as defined above, with at least half (half or more) of key muscle functions below the single NLI having a muscle grade ≥ 3.

E = Normal. If sensation and motor function as tested with the ISNCSCI are graded as normal in all segments, and the patient had prior deficits, then the AIS grade is E. Someone without an initial SCI does not receive an AIS grade.

Using ND: To document the sensory, motor and NLI levels, the ASIA Impairment Scale grade, and/or the zone of partial preservation (ZPP) when they are unable to be determined based on the examination results.

ASIA
AMERICAN SPINAL INJURY ASSOCIATION

INTERNATIONAL STANDARDS FOR NEUROLOGICAL CLASSIFICATION OF SPINAL CORD INJURY

ISCOS
INTERNATIONAL SPINAL CORD SOCIETY

Steps in Classification

The following order is recommended for determining the classification of individuals with SCI.

1. Determine sensory levels for right and left sides.
The sensory level is the most caudal, intact dermatome for both pin prick and light touch sensation.

2. Determine motor levels for right and left sides.
Defined by the lowest key muscle function that has a grade of at least 3 (on supine testing), providing the key muscle functions represented by segments above that level are judged to be intact (graded as a 5).
Note: in regions where there is no myotome to test, the motor level is presumed to be the same as the sensory level, if testable motor function above that level is also normal.

3. Determine the neurological level of injury (NLI)
This refers to the most caudal segment of the cord with intact sensation and antigravity (3 or more) muscle function strength, provided that there is normal (intact) sensory and motor function rostrally respectively.
The NLI is the most cephalad of the sensory and motor levels determined in steps 1 and 2.

4. Determine whether the injury is Complete or Incomplete.
(i.e. absence or presence of sacral sparing)
*If voluntary anal contraction = **No** AND all S4-5 sensory scores = **0** AND deep anal pressure = **No**, then injury is **Complete**.*
*Otherwise, injury is **Incomplete**.*

5. Determine ASIA Impairment Scale (AIS) Grade:

Is injury Complete? **If YES, AIS=A** and can record ZPP (lowest dermatome or myotome on each side with some preservation)

NO ↓

Is injury Motor Complete? **If YES, AIS=B**

NO ↓ (No=voluntary anal contraction OR motor function more than three levels below the motor level on a given side, if the patient has sensory incomplete classification)

Are at least half (half or more) of the key muscles below the neurological level of injury graded 3 or better?

NO ↓ **AIS=C** **YES** ↓ **AIS=D**

If sensation and motor function is normal in all segments, AIS=E
Note: AIS E is used in follow-up testing when an individual with a documented SCI has recovered normal function. If at initial testing no deficits are found, the individual is neurologically intact; the ASIA Impairment Scale does not apply.

TZ-01 2/2

17.2 Appendix: SCI Pain data set, Allodynia questionnaire and SCIPI

SCI Pain data set and SCIPI: Version EPAF in its current version

Name / EMSCI ID: _____ **Exam. Date:** __ __ / __ __ / __ __ __ __ **Exam. stage:** ☐ acute I - ☐ acute II - ☐ acute III - ☐ chronic **Examiner:** _____	**Signature:** _____	EPAF 1 04/2016 TZ-19 i

1. Assessment of Overall Pain Symptoms

1.1. Basic Dataset

a.) Have you had any pain during the last seven days including today?
☐ No ☐ Yes

b.) In general, how much has pain interfered with your day-to-day activities in the last week?
No interference ☐ 0 - ☐ 1 - ☐ 2 - ☐ 3 - ☐ 4 - ☐ 5 - ☐ 6 - ☐ 7 - ☐ 8 - ☐ 9 - ☐ 10 Extreme interference

c.) In general, how much has pain interfered with your overall mood in the last week?
No interference ☐ 0 - ☐ 1 - ☐ 2 - ☐ 3 - ☐ 4 - ☐ 5 - ☐ 6 - ☐ 7 - ☐ 8 - ☐ 9 - ☐ 10 Extreme interference

d.) In general, how much has pain interfered with your ability to get a good night's sleep?
No interference ☐ 0 - ☐ 1 - ☐ 2 - ☐ 3 - ☐ 4 - ☐ 5 - ☐ 6 - ☐ 7 - ☐ 8 - ☐ 9 - ☐ 10 Extreme interference

e.) How many different pain problems do you have?
☐ 1; ☐ 2; ☐ 3; ☐ 4; ☐ ≥5

1.2. Extended Dataset

a.) Number of days with pain in the last 7 days including today:
☐ none - ☐ 1 - ☐ 2 - ☐ 3 - ☐ 4 - ☐ 5 - ☐ 6 - ☐ 7 - ☐ unknown

b.) Average pain unpleasantness in the last week:
0 = not at all unpleasant; 10=the most unpleasant pain imagine
☐ 0 - ☐ 1 - ☐ 2 - ☐ 3 - ☐ 4 - ☐ 5 - ☐ 6 - ☐ 7 - ☐ 8 - ☐ 9 - ☐ 10

c.) Number of days with manageable/tolerable pain in the last 7 days including today:
☐ none - ☐ 1 - ☐ 2 - ☐ 3 - ☐ 4 - ☐ 5 - ☐ 6 - ☐ 7 - ☐ unknown

1.3. Are you taking any oral and/or topical medication?

☐ Antidepressants	☐ Benzodiazepines	☐ Paracetamol
☐ NSAID/aspirin	☐ Tramadol	☐ topical anaesthetics
☐ Antiepileptics	☐ Antispasticity drugs	☐ topical capsaicin
☐ Pregabalin	☐ Opioids	☐ others: _____
☐ Gabapentin	☐ Cannabinoids	
☐ Other		

SCI Pain data set and SCIPI (continuation)

2. Assessment of the *two* worst pain sites No.: ☐ 1 / 2; ☐ 2 / 2

2.1. Date of onset: _ _ / _ _ / _ _ _ _

2.2. Are you using or receiving any treatment for your pain problem?

☐ Yes	☐ No	→*If yes – are/were the treatments helpful or not?* (over the last 12 months):
☐ Yes;	☐ No; ☐ Uncertain	Physiotherapy
☐ Yes;	☐ No; ☐ Uncertain	Passive and stimulation therapy
☐ Yes;	☐ No; ☐ Uncertain	Relaxation and Psychotherapy
☐ Yes;	☐ No; ☐ Uncertain	Oral and topical medication
☐ Yes;	☐ No; ☐ Uncertain	Procedural interventions
☐ Yes;	☐ No; ☐ Uncertain	Surgical interventions
☐ Yes;	☐ No; ☐ Uncertain	Other treatment: _____

2.3. Factors that trigger or intensify your pain:

☐ Yes;	☐ No; ☐ Uncertain	Change in temperature
☐ Yes;	☐ No; ☐ Uncertain	Mental stress
☐ Yes;	☐ No; ☐ Uncertain	Relaxation
☐ Yes;	☐ No; ☐ Uncertain	Being touched
☐ Yes;	☐ No; ☐ Uncertain	Spasticity
☐ Yes;	☐ No; ☐ Uncertain	Constipation
☐ Yes;	☐ No; ☐ Uncertain	Bladder complications
☐ Yes;	☐ No; ☐ Uncertain	Other: _____

2.4. Time course of Pain

How long does your pain usually last?
☐ ≤ 1 min; ☐ > 1 min but < 1 hr; ☐ ≥ 1 hr but < 24 hrs; ☐ ≥ 24 hrs;
☐ constant or continuous; ☐ unknown

When during the day is the pain most intense?

☐ morning	(06:00 – 12:00)
☐ afternoon	(12:01 – 18:00)
☐ evening	(18:01 – 24:00)
☐ night	(00:01 – 06:00)
☐ unpredictable	(pain is not consistently more intense at any one time of day)

2.5. Intensity and duration of pain

Average pain intensity in the last week: 0 = no pain; 10 = pain as bad as imaginable
☐ 0 - ☐ 1 - ☐ 2 - ☐ 3 - ☐ 4 - ☐ 5 - ☐ 6 - ☐ 7 - ☐ 8 - ☐ 9 - ☐ 10

Pain intensity in present moment: 0 = no pain; 10 = pain as bad as imaginable
☐ 0 - ☐ 1 - ☐ 2 - ☐ 3 - ☐ 4 - ☐ 5 - ☐ 6 - ☐ 7 - ☐ 8 - ☐ 9 - ☐ 10

SCI Pain data set and SCIPI (continuation)

2.6. Localization of pain 1/2:				2.7. Type of pain	
	R	M	L	According to the International Spinal Cord Injury Pain (ISCIP) Classification (check one):	
Head				**Pain (sub-)Type**	**Examples / etiology**
Neck/shoulders				**Nociceptive**	
throat					
neck				☐ Musculoskeletal	e.g. glenohumeral arthritis, muscle spasms, fractures
shoulder					
Arms/hands					
upper arm				☐ Visceral	e.g. myocardial infarction, pain due to bowel impaction
elbow					
forearm					
wrist				☐ Other	e.g. autonomic dysreflexia Headache, migraine, surgical skin incision
hand/fingers					
Frontal torso/genitals					
chest				**Neuropathic**	
abdomen					
pelvis/genitalia				☐ At-level	e.g. spinal cord compression, Nerve root compression, Cauda-equina compression
Back					
upper back					
lower back					
Buttocks/hips				☐ Below-level	e.g. spinal cord compression, Spinal ischemia
buttocks					
hip					
anus				☐ Other	e.g. carpal tunnel syndrome, trigeminus neuralgia, diabetic polyneuropathy
Upper legs/thighs					
Lower legs/feet					
knee					
shin				☐ **Other**	e.g. fibromyalgia, complex regional pain syndrome type I,
calf					
ankle				☐ **Unknown**	
foot/toes					

SCI Pain data set and SCIPI (continuation)

2.8. Description of painful and non-painful sensations within the painful area

Grading: 0 = not at all; 10 = strongest intensity

Quality of pain

Burning:

☐ 0 - ☐ 1 - ☐ 2 - ☐ 3 - ☐ 4 - ☐ 5 - ☐ 6 - ☐ 7 - ☐ 8 - ☐ 9 - ☐ 10

Shooting, electrifying pain attacks:

☐ 0 - ☐ 1 - ☐ 2 - ☐ 3 - ☐ 4 - ☐ 5 - ☐ 6 - ☐ 7 - ☐ 8 - ☐ 9 - ☐ 10

Movement-related pain:

☐ 0 - ☐ 1 - ☐ 2 - ☐ 3 - ☐ 4 - ☐ 5 - ☐ 6 - ☐ 7 - ☐ 8 - ☐ 9 - ☐ 10

Cramp-like pain:

☐ 0 - ☐ 1 - ☐ 2 - ☐ 3 - ☐ 4 - ☐ 5 - ☐ 6 - ☐ 7 - ☐ 8 - ☐ 9 - ☐ 10

In the abdomen:

☐ 0 - ☐ 1 - ☐ 2 - ☐ 3 - ☐ 4 - ☐ 5 - ☐ 6 - ☐ 7 - ☐ 8 - ☐ 9 - ☐ 10

Paraesthesia

Do you have non-painful sensations, such as tingling, numbness or subjective sensations of cold or warmth?

☐ 0 - ☐ 1 - ☐ 2 - ☐ 3 - ☐ 4 - ☐ 5 - ☐ 6 - ☐ 7 - ☐ 8 - ☐ 9 - ☐ 10

Allodynia

Do you have pain due to a stimulus which does not normally provoke pain?

Please estimate allodynia for the following stimuli:

Light touch (for example by a blanket):

☐ 0 - ☐ 1 - ☐ 2 - ☐ 3 - ☐ 4 - ☐ 5 - ☐ 6 - ☐ 7 - ☐ 8 - ☐ 9 - ☐ 10

Warmth or cold (cold air/warm shower):

☐ 0 - ☐ 1 - ☐ 2 - ☐ 3 - ☐ 4 - ☐ 5 - ☐ 6 - ☐ 7 - ☐ 8 - ☐ 9 - ☐ 10

Low pressure (for example with a finger):

☐ 0 - ☐ 1 - ☐ 2 - ☐ 3 - ☐ 4 - ☐ 5 - ☐ 6 - ☐ 7 - ☐ 8 - ☐ 9 - ☐ 10

2.9. Spinal Cord Injury Pain Instrument (SCIPI)

A	Is the quality of pain electrical or electric shock like?	☐ No	☐ Yes
B	Is the quality of pain like pins and needles, or tingling?	☐ No	☐ Yes
C	Does the skin over the area of pain or inside your body where the pain is located feel hot or burning or cold or freezing?	☐ No	☐ Yes
D	Is the skin over the area of pain abnormally sensitive to touch and without any surgical scars, ulcers or breaks in the skin?	☐ No	☐ Yes
E	Is the pain usually unchanged with movement of the painful area?	☐ No	☐ Yes
F	Do you experience pain all the time without any breaks when you are awake (although it may vary in intensity during different times)?	☐ No	☐ Yes
G	Does the pain only occur in an area of the body in which you have no feeling on the skin overlying that area?	☐ No	☐ Yes

17.3 Appendix: MART

Mart (Mapping of Rehab Training v1, according to (43))

MART v1 - MApping of Rehab Training v1 Date (dd/mm/yy): __ / __ / 20__

Surname* _____ First name* _____ EMSCI-ID _____

* Mask name before sending

Current therapy session

❑ Individual ❑ Group Therapist _____ ❑ PT ❑ OT Duration scheduled _____

cancelled, due to : (max. one cross!)
❑ Bladder/Bowel ❑ Fatigue/infection
❑ Orthostatic problem ❑ requested by patient
❑ Pain ❑ other: _____
❑ Conflicting dates

NISCI ❑ affected by CSF Injection ❑ other NISCI activities

Interventions on body function and structure level

	LE	UE	T	Passive	Assistive	Active
Strength training	❑	❑	❑		_____	_____
Tone regulation	❑	❑	❑	_____	_____	_____
Joint mobility	❑	❑	❑	_____	_____	_____
Sensory function	❑	❑	❑	_____	_____	_____
Pain reduction	❑	❑	❑	_____	_____	_____
Stretching	❑	❑	❑	_____	_____	_____
Skin/lymphatic system/scars	❑	❑	❑	_____		
Cardiovascular endurance				_____	_____	_____
Respiratory training				_____	_____	_____

Interventions on activity level

Transition in bed / bed mobility	_____	_____	_____
Transfer (bed – wheelchair)	_____	_____	_____
Sitting (balance training / endurance)	_____	_____	_____
Wheelchair skills	_____	_____	_____
Stand up (sit-to-stand transition)	_____	_____	_____
Standing (balance-training / endurance)	_____	_____	_____
Walking	_____	_____	_____
Stairs	_____	_____	_____
Dressing	_____	_____	_____
Grooming	_____	_____	_____
Eating / Drinking	_____	_____	_____
Arm / hand use (e.g. reaching, grasping and dexterity)	_____	_____	_____
Domestic activities	_____	_____	_____
Recreation and leisure activities	_____	_____	_____
Sports	_____water	_____ sitting	_____ standing	_____ walking

Other inventions	Duration
Assessment	_____
Education / Conversation	_____
Evaluation / adaptation of assistive devices	_____

Assistive devices

❑ Orthoses
❑ E-Wheelchair
❑ Manual wheelchair
❑ Add-on drive for wheelchair
❑ Wheeled forearm walker
❑ Walker (non wheeled)
❑ Wheeled walker
❑ 2 walking poles / crutches
❑ 1 walking poles / crutches
❑ Walking stick (1 or 2)
❑ Shower wheelchair / WC
❑ Shower seat
❑ _____

Training aids

❑ Strength training device
❑ Stander / tilt table (e.g. Erigo)
❑ Driven gait orthosis (e.g. Lokomat)
❑ Parallel bars
❑ Treadmill
❑ Body weight support system
❑ Vibratory training devices
❑ Electrical stimulation
Ergometer: ❑ Arms ❑ Legs
❑ Bed
❑ Car
❑ Bath tube
❑ Training device UE
❑ Therapy in water
❑ _____

Legend: CSF Injection, Cerebral Spinal Fluid Injection; LE, Lower Extremities; UE, Upper Extremities; T, Trunk

17.4 Appendix: Bladder function assessment

NISCI	Protocol: NISCI-Study EudraCT: 2016-001227-31	Subject ID: _____	Visit 10: Follow Up day 84

Bladder-function Assessment – Visit 10

Preparations *for* Visit 10

1. distribute bladder diary and Qualiveen Questionnaire *(4 days before visit 10)*
2. 1 day before visit 10 put 500ml or 1 liter of NaCl 0,9% (depending on bottle size available at site) in fridge (4°C)
3. arrange together with nursing team that patient has a comfortably full bladder for visit 10

Visit 10

1. Collect the bladder diary and Qualiveen Questionnaire *at* visit 10 and carefully check completeness. If data are missing immediately inform study coordinator or study nurse
2. if feasible assess the patient with comfortable full bladder
3. measure blood pressure before the procedure and after instillation (full bladder)
4. patient position should be supine with 30° inclination and patient should lie on surgical drape

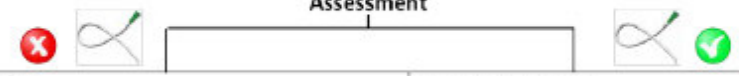
Timing and procedure *for* Visit 10

➔ ***Proceed according to the actual patient conditions:***

→ If the patient can void the bladder spontaneously/voluntarily (left pathway), or

→ If the patient has a permanent catheter (right pathway)

Assessment



Timing

No indwelling catheter	***Indwelling*** catheter
1) Prepare the material ✓ Bladder syringe 80-100ml (depending on syringe size that is available) ✓ Set for catheterization ✓ Sterile gloves ✓ Measuring cup (accuracy: 10ml) ✓ *Catheter* a. Immediately before testing, ask patient to voluntarily empty the bladder (toilette) b. Measure blood pressure (baseline) c. Insert catheter, completely empty bladder and measure post void residual (PVR) d. Allow 3-4 minutes to let the sensation settle (irritation due to catheter) e. In the meantime collect the cold NaCl 0.9% (4°C) for instillation from fridge	1) Prepare the material ✓ Bladder syringe 80-100ml (depending on syringe size that is available) ✓ Set for catheterization ✓ Sterile gloves ✓ Measuring cup (accuracy: 10ml) a. Measure blood pressure (baseline) b. Collect the cold NaCl 0.9% (4°C) for instillation from fridge

2) Instill 80-100ml/min NaCl 0.9% (4°C) and make sure patient does not know the level of filling!

3) Tell patient: "*we now slowly will fill your bladder. Please tell us as soon as you become aware of any sensation in/from your bladder*" (irrespective of temperature and apart from catheter sensation) and mark the answer (see table below)

4) Mark answer and proceed as following if patient has:
   - *Any* sensation → stop instillation, measure blood pressure, immediately empty bladder and report instillation volume (see table below)
   - No sensation → continue instillation up to 500ml, measure blood pressure, empty bladder

Date of examination __ __ / __ __ / 2 0 __ __ D D M M Y Y Y Y	Blood pressure [mm Hg]	Baseline ___ / ___ systolic / diastolic	Stop of instillation or up to 500ml ___ / ___ systolic / diastolic

Catheter (patient normally relying on catheterization to empty bladder)	☐ No	
	☐ Yes	☐ Transurethral ☐ Suprapubic ☐ Intermittent catheterization
Voiding (spontaneously/voluntarily)	☐ No	
	☐ Yes Mean voided volume:_____ ml/per void (according to 3-day bladder diary) Post void residual (PVR):_____ ml (via catheter)	
Any sensation during instillation of NaCl 0.9% (4°C)?	☐ No	
	☐ Yes, after instillation of:_____ ml	Desire to void: ☐ No / ☐ Yes

Completed by: _____ (Initials / Date)

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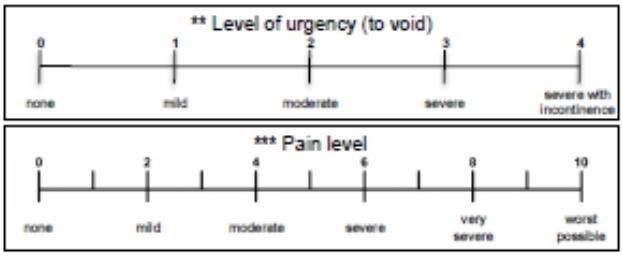
17.5 Appendix: Bladder Diary

Wake-up time:_____:_____ Bedtime:_____:_____ ID: _____

Date: ____/____/20____ (Day 1)

Time (hh:mm) *until next morning/ wake-up time*	Oral fluid intake* in mL	Voided volume through urethra in mL	Cathe-terized volume in mL	Level of urgency** 0	1	2	3	4	Pain level*** (Score:0-10)	Urine leakage none	slight	moderate	heavy	Pad replaced (X)	Wetness of pad dry	moist	soaked

Comments / special events:
Example: forgot medication, diarrhoea, traveling



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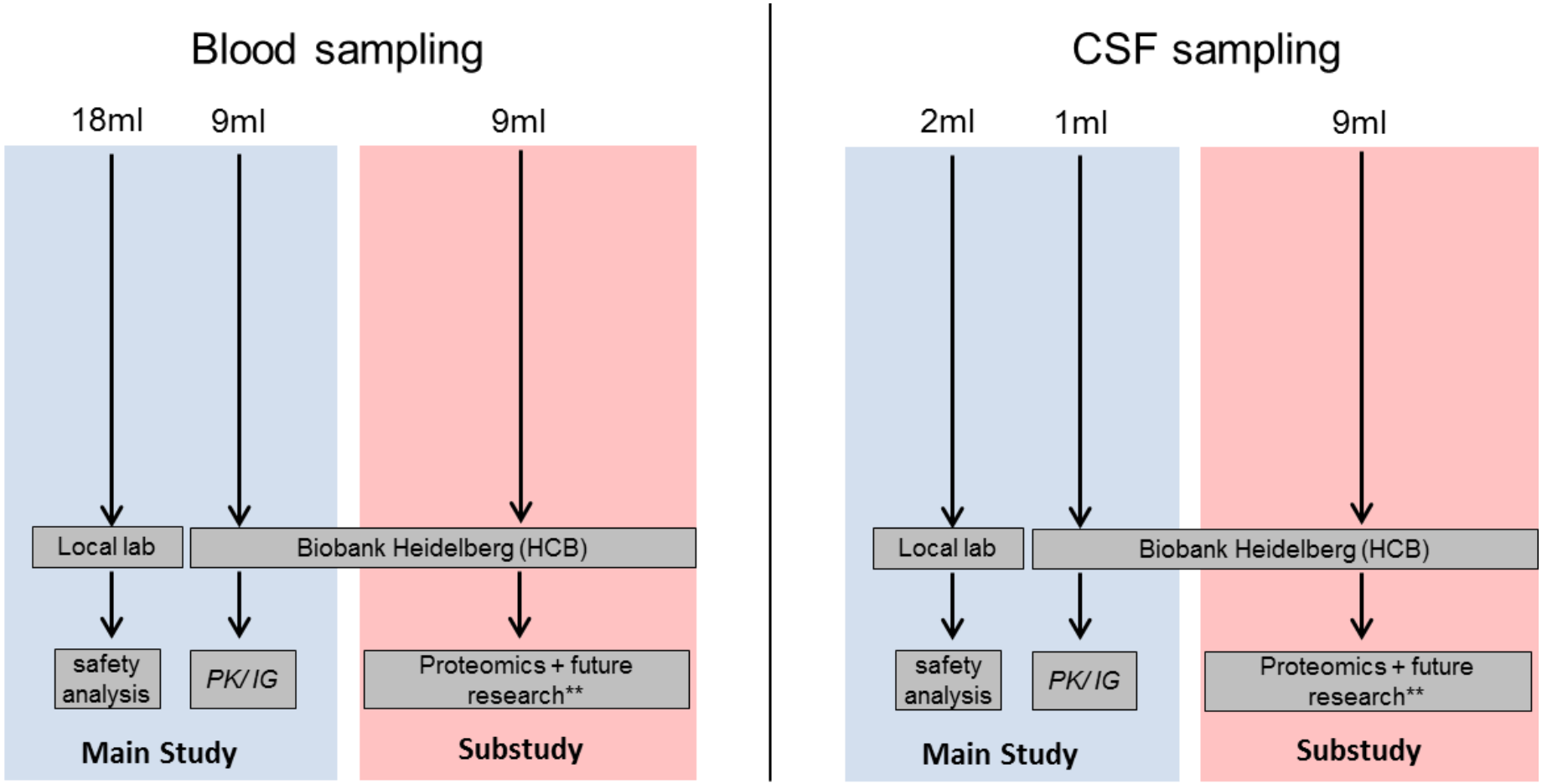
17.6 Appendix: Blood and CSF assessment schedule

		Main Study				Substudy	
Sample Type		**Blood samples**		**CSF samples**		**Blood samples**	**CSF samples**
Analysis (Lab)		**Safety** (local Lab)	**PK/IG** (central Lab)	**Safety** (local Lab)	**PK** (central Lab)	**Proteomics/ Future Research****	**Proteomics/ Future Research****
Time point		ml*	ml*	ml	ml	ml*	
Screen. (V1)	**Day-28 to-2**	**30.0**					
Screen. (V1)	**Day-28 to-2**	**2,5 Preg. test**					
Baseline (V2)	**Day-1**	**(30.0)**					
Day 0 (V3)	before IMP admin	**18**	**9**	**2**	**10**	9	**aliquot from PK sample**
Day 5 (V4)	before IMP admin	**18**	**9**	**2**	**10**	9	**aliquot from PK sample**
Day 10 (V5)	before IMP admin	**18**	**9**	**2**	**10**	9	**aliquot from PK sample**
Day 15 (V6)	before IMP admin	**18**	**9**	**2**	**10**	9	**aliquot from PK sample**
Day 20 (V7)	before IMP admin	**18**	**9**	**2**	**10**	9	**aliquot from PK sample**
Day 25 (V8)	before IMP admin	**18**	**9**	**2**	**10**	9	**aliquot from PK sample**
Day 30 (V9)		**30**	**9**			9	
Day 84 (V10)		**30**	**9**	**2**	**10**	9	**aliquot from PK sample**
Total, ml		***228***	***72***	***14***	***70***	*72*	

Blood Total (main study)	300 ml
Blood Total (main study & substudy)	372 ml
CSF Total	84 ml/ patient

*Listed are maximum blood sample volumes. Actual blood sample volumes may vary due to different local standards of blood collection equipment at study sites.
**in subjects not consenting to the substudy only 1 container of blood (9ml) instead of 2 containers (18ml) will be collected. 1ml of serum obtained after centrifugation will be used for PK/IG analysis. The remaining serum will be stored at the Heidelberg Biobank for additional PK/IG analysis, if needed. However, remaining serum will not enter the substudy.
In respect to CSF collection in non-consenting subjects, the same volume of CSF (12ml) will be collected in order to exclude CSF withdrawal volume as a potential confounding factor for therapeutic effects and/or side effects. In non-consenting subjects remaining CSF samples beyond routine analysis will be stored at the Heidelberg Biobank for additional PK/ analysis, if needed. However, remaining CSF will not enter the sub study.
Future research refers to analysis of serum and CSF not directly related to the IMP, which will be covered by a separate study protocol and a separate patient informed consent form.

17.7 Appendix: Sampling flow chart



**Future research refers to analysis of serum and CSF not directly related to the IMP, which will be covered by a separate study protocol and a separate patient informed consent form

Antibodies against Nogo-A to enhance plasticity, regeneration and functional recovery after acute spinal cord injury (NISCI)

EudraCT No. 2016-001227-31

Statistical Analysis Plan

Version number: V02

Version date: 28.04.2023

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* According to § 40 German Drug Law (AMG)

CONTENT

1 Study background

1.1 Study objective

The main objective of the NISCI trial is to evaluate the efficacy of acute treatment (initiation of drug treatment within 4-28 days post-injury) with NG-101 by repeated intrathecal bolus injections (6 injections of 45 mg each over 4 weeks) on day 168.

1.2 Study design

Multi-center, international, placebo controlled, double-blind and randomized phase II (2 parallel treatment groups) clinical proof of concept trial.

1.3 Substudies

The substudies: Biostatistics (WP2), proteomics (WP3) and neuroimaging (WP4) and the exploratory objectives and endpoints: Effect on outcome of the Spinal Cord Ability Ruler (SCAR), activity counts (sensors) and mapping of rehab training (MART) will not be analyzed by the KKS and therefore, are not part of this SAP.

2 Analysis sets

2.1 Definitions

The full analysis set (FAS) comprises all patients, with a valid informed consent, who were randomized into the trial and received the study medication at least once. In this set, every patient is analyzed according to the group they are randomized into.

The per-protocol set (PPS) comprises all patients of the FAS. Specifically, no major protocol violation should have occurred. See Section 2.3.

The safety set (SAF) is identical to the FAS. Patients will be analyzed according to the treatment they actually received, regardless of randomization. Subjects, who received at least twice a verum vial, will be assigned to the verum arm.

The augmented set (AMS) comprises the full analysis set and a sample from the EMSCI database containing subjects fulfilling the following criteria:

- Age (18-70),
- Traumatic cause,
- DAI (less than 28 days),
- not a NISCI patient, country (Germany, Switzerland, Spain or Czechia),
- NLI (C1-C8),
- AIS (A-D),
- Randomization node (strictly less than 20),
- DOI (between 01.01.2013 and 09.07.2022).

2.2 Scope

The full analysis set will be used for all efficacy analyses, especially the primary analysis. In a sensitivity analysis, the primary analysis will be repeated for the per-protocol set only. In another sensitivity analysis, the primary analysis will be repeated for the augmented data set. The safety set will be used for all safety analyses.

2.3 Major protocol violations

The following protocol violations will lead to exclusion from the per protocol set:

- receiving only one dose of the treatment as randomized
- are not eligible according to in- and exclusion criteria

2.4 Deviations from study protocol

The primary analysis will be performed as described in study protocol version 3.2 not the current version (4.0).

Contrary to the original plan, a package number or study medication, if unopened, were reassigned after an erroneous randomization due to the low number of verum kits. Furthermore, patients are excluded from the FAS if treatment was not started.

Pharmacokinetic and immunology serum analyses will not be part of this SAP.

The spasticity measured by the Modified Ashworth Scale results will not be analyzed in the same fashion as the primary endpoint and will be analyzed descriptively.

The effect on pain assessed by short pain assessment questionnaire will not be analyzed and only be listed. The analysis of the type of pain will be evaluated as described in Section 6.7.6.

3 Study time frame, proceedings and patient flow

3.1 Study time frame

The clinical study time frame is defined by the following events:

- Accrual of first subject (FSI)
- Accrual of last subject for final analysis (LSI)
- Last time point of documented findings and activities for final analysis (LSO)

Patient related data recorded into the study data base occur at the following visits:

- Screening
  - Visit 1: Day -28 to day -2, within day 4 to day 28 post-injury
- Baseline/Randomization
  - Visit 2: Day -1
- Treatment visits
  - Visit 3: Day 0 ± 0 of treatment
  - Visit 4: Day 5 ± 2 of treatment
  - Visit 5: Day 10 ± 2 of treatment
  - Visit 6: Day 15 ± 2 of treatment
  - Visit 7: Day 20 ± 2 of treatment
  - Visit 8: Day 25 ± 2 of treatment
- Follow-up visits
  - Visit 9: 30 ± 2 days after randomization
  - Visit 10: 84 ± 7 days after randomization
  - Visit 11: 168 ± 7 days after randomization

3.2 Randomization

After patient eligibility according to inclusion and exclusion criteria has been confirmed, the patient will be registered at the randomization server via https://randomizer.at.
The randomization server will provide the number of a package available at the site. The allocation of treatment will use a balancing algorithm (Big stick allowing for an imbalance of up to 3 patients per cohort) stratified according to the cohorts obtained by the algorithm referred to in section 3 in the study protocol to the EMSCI data base. The cohorts are derived from the screening (not

baseline) measurements because the model has been developed on data obtained about 2 weeks after the incident that led to SCI. They are defined as follows:

1. UEMS total score ≤ 3, AIS 2 = A
2. 3 < UEMS total score ≤ 11, AIS 2 = A
3. UEMS total score ≤ 11, AIS 2 > A, LEMS total score = 0, light touch total score ≤ 62
4. UEMS total score ≤ 11, AIS 2 > A, LEMS total score = 0, light touch total score > 62
5. UEMS total score ≤ 11, AIS 2 > A, LEMS total score > 0
6. 11 < UEMS total score ≤ 28, AIS 2 = A or B
7. 11 < UEMS total score ≤ 17, AIS 2 > B, LEMS total score ≤ 17
8. 17 < UEMS total score ≤ 28, AIS 2 > B, LEMS total score ≤ 17
9. 11 < UEMS total score ≤ 28, AIS 2 > B, LEMS total score > 17

The randomization was originally 1:1. With protocol version 4.0 it was changed to 3:1 to get a ratio of 2:1 because recruitment was low.

3.3 Sample size

The power calculation is based on the mean delta changes in the EMSCI data of the control group (nodes 4, 5, 8, 9, 10, 13, 16, 17, and 18 have a mean delta UEMS of 14.3 +/-SD of 10.8 motor scores) and a 42% treatment effect (mean delta change of 20.3 motor scores). Using t-tests means with an allocation 1:1, an estimated α error probability of 0.05 and a power (1-β err prob) of 0.8 a total of about 106 patients would be required. For an adequate powering of the study, we assume that 20 per cent of patients will drop out of follow-up, which means that 132 patients will be needed to compensate.

The randomization was originally 1:1. With protocol version 4.0 it was changed to 3:1 to get a ratio of 2:1 because recruitment was low. The power of the test was expected to go down to 66 per cent. It was planned to compensate this loss of power by filling up the pool of control subjects by historical controls from the EMSCI database. These will be drawn randomly from the database matched by the cohorts which have been used for stratification of randomization. This will permit sensitivity analyses with a tradeoff aiming for a higher precision (power going up to 86 per cent) in return for an unknown bias due to the introduction of historical controls.

4 Analysis variables

Whether the assessment was performed/available/collected or not, the reason why it was not performed/available/collected and each date of assessment will be provided.

4.1 Disposition

- Informed consent
- Inclusion/exclusion criteria
- Premature termination of the study and treatment and the relevant reasons are collected as well.
- Unblinding
- Protocol deviations are collected in addition: Timepoint of deviation
    - Date of initial entry
    - Type (derived, monitor detected, site detected)
    - Category of deviation (patient related, site related)
    - Subcategory of description as defined in eCRF
    - Description of deviation (incl. reason)
    - Timepoint of deviation
    - Date of deviation

- Date of awareness
- Deviation confirmed by investigator (according to monitor follow-up list)
- Reason if deviation not confirmed

4.2 Baseline characteristics

Baseline characteristics

The following variables are collected:

- Age (years)
- Gender (male, female, intersexual/diverse, unknown)
- Year of birth
- Nodes

Age in years will be derived by subtracting year of birth from year of randomization. For analyses, age will be categorized as follows: 18-64 and ≥ 65 years old, with respect to meeting the inclusion criterion "Age 18-70".

Medical history (Acute (spinal cord) injury related disorders, other prior diseases)

- Start/end date
- Ongoing (other prior diseases)
- General medical history (other prior diseases as free text)
- Spinal cord injury (body parts affected) (yes/no)
  - Spine
  - Head and face
  - Chest
  - Abdomen, pelvic contents
  - Extremity pelvic girdle
  - Other (as free text)

Procedure

- Start-/end date
- Patient is on invasive ventilation (at baseline) (yes/no)
- Ventilation ongoing during drug application (yes/no)

4.3 Concomitant medication/therapy

- Active component
- Indication for use
- Start/end date

4.4 Exposure

- Date of injection and start time of injection
- Treatment code
- Injection volume (3 ml (45mg), other volume)
- Duration of injection (sec)
- Total dose injected (calculated mg)
- Injection performed
- Reason if injection not performed

4.5 Primary variable

The primary efficacy variable is the upper extremity motor scores (UEMS) according to the international standards for the neurological classification of spinal cord injury.

4.6 Secondary variables

4.6.1 Efficacy

The efficacy is measured by the following variables. Each item will be listed and all (sub)scores will be analyzed.

ISNCSCI

- Motor Single Scores – Upper Extremity (Assessed region, right/left side)
- Motor Subscores – Upper Extremity (Right/left/both side(s))
- Motor Single Scores - Lower Extremity (Assessed region, right/left side)
- Motor Subscores – Lower Extremity (Right/left/both side(s))
- Motor Subscores – Right and Left (Right/left/both side(s))
- Other Motor Data
    - Voluntary Anal Contraction (yes/no)
    - Deep Anal Pressure (yes/no)
    - Comments to ISNCSCI
- Sensory Scores - Light Touch Single Scores (Assessed region, right/left side)
- Sensory Scores - Light Touch Subscores (Right/left/both side(s))
- Sensory Scores - Pin Prick Single Scores (Assessed region, right/left side)
- Sensory Scores - Pin Prick Subscores (Right/left/both side(s))
- Neurological levels
    - Sensory level (right/left)
    - Motor level (right/left)
    - Neurological level of injury (NLI)
    - Complete or incomplete
    - If complete: Zone of partial prevention (ZPP) – Sensory/motor (right/left)
- ASIA impairment scale (AIS)

SCIM-III

- SCIM III - Self Care
- SCIM III - Self Care – Subtotal score (0-20)
- SCIM III - Respiration and Sphincter Management
- SCIM III - Respiration and Sphincter Management - Subtotal score (0-40)
- SCIM III - Mobility
- SCIM III - Mobility - Subtotal score (0-40)
- SCIM III - Total score (0-100)

GRASSP

- Position of GRASSP testing
- GRASSP Single Scores Strength (assessed region, right/left side)
- GRASSP Subscore Strength (0-50, subscore, right/left side)
- GRASSP Single Scores Sensation SWM Threshold (assessed region, right/left side)
- GRASSP Subscore Sensation (Palmar Total) (0-12, assessed region, right/left side)
- GRASSP Single Scores Prehension Ability (assessed region, right/left side)
- GRASSP Subscore Prehension Ability (0-20, subscore, right/left side)
- GRASSP Single Scores Prehension Performance for the complete GRASSP
    - Assessed region
    - Duration on right side (sec)
    - Score on right side

  - Drops on right side
  - Duration on left side (sec)
  - Score on left side
  - Drops on left side
- GRASSP Single Subscore Prehension Performance (subscore, right/left side) for the complete GRASSP
- GRASSP - Total Score (0-94, subscore, right/left side) for the complete GRASSP
- GRASSP - Partial Score (subscore, right/left side) for the partial GRASSP (Strength, sensation and prehension ability subscores)
- Comments to GRASSP

<u>6-Minute Walk Test (6mWT)</u>

- 6mWT aborted (yes/no)
- Distance (m) walked in 6 min
- Distance (m) walked until abort
- Duration (sec) of walk until abort

<u>10-Meter Walk Test (10MWT)</u>

- 10mWT aborted (yes/no)
- Duration (sec) for 10 meters with preferred speed
- Distance (m) walked until abort
- Duration (sec) of walk until abort
- Assistance needed (yes)
  - Parallel bars (yes)
  - 2 persons (yes)
  - 1 person (yes)
  - Walker (yes)
  - Walking frame (yes)
  - Quad canes (yes)
  - Crutches (yes)
  - 1 crutch (yes)
  - Cane (yes)
  - Braces (yes)

To analyze the type of assistance needed, all combinations will be deduced.

<u>Walking Index for Spinal Cord Injury II (WISCI II)</u>

- WISCI II level (0-20)

<u>Neurophysiological and electrophysiological exams</u>

- Nerve conduction velocity (NCV) of ulnaris nerve - Response (Exam, result right/left (cat))
- Nerve conduction velocity (NCV) of ulnaris nerve (Exam, result right/left, unit right/left)
- Somatosensory evoked potentials (SSEP) of tibial nerve - Response (Exam, result right/left (cat))
- Somatosensory evoked potentials (SSEP) of tibial nerve (Exam, response right/left yes/no, result right/left, unit right/left)
- Dermatomal SSEP (dSSEP) C6 – Response (Exam, result right/left (cat))
- Dermatomal SSEP (dSSEP) C6 (Exam, result right/left, unit right/left)
- Dermatomal SSEP (dSSEP) C8 – Response (Exam, result right/left (cat))
- Dermatomal SSEP (dSSEP) C8 (Exam, result right/left, unit right/left)

Bladder diaries

The following variables will be calculated:

- Median volume per void (through urethra) of all non-zero and non-missing values over 72 hours (ml)
- Median volume per catheterization of all non-zero and non-missing values over 72 hours (ml)
- At least once a level of urgency larger than 2 within 3 days
- Average over three days of the mean of pain level per day
- At least once a moderate to heavy urine leakage within 3 days
- Average over three days of the sum of replaced pad per day
- At least once a soaked pad within 3 days

Bladder function assessment

- 'Baseline' BP: Systolic (mmHg)
- 'Baseline' BP: Diastolic (mmHg)
- Change in BP during bladder filling (between baseline and stop of instillation): Systolic (mmHg)
- Change in BP during bladder filling (between baseline and stop of instillation): Diastolic (mmHg)
- Catheter
    - No
    - Yes, transurethral
    - Yes, suprapubic
    - Yes, intermittent catheterization
- Voiding usually spontaneously/voluntarily (yes/no)
- Any sensation during instillation of NaCl 0.9% (4°C) (yes/no)
- Sensation after instillation (ml)
- Desire to void (yes/no)
- Reason for stop of instillation
    - Any sensation
    - Bladder filled with 500 ml
    - Leakage
    - Symptoms of anatomic dysfunction (e.g. headache, sweating etc.)

Qualiveen questionnaire

- Every item with result
- Did you fill in this questionnaire on your own? (yes/no)
- Subscores
    - Inconvenience (0-4)
    - Restrictions (0-4)
    - Fears (0-4)
    - Impact on daily life (0-4)
- Total score (0-4)

Subscores and score will be calculated according to Qualiveen - SCIRE Professional (scireproject.com)

4.6.2 Safety

The safety is measured by the following variables.

Adverse events (frequency, type, duration and intensity of AEs and SAEs)

- AE No.

- Description of AE (only one finding per row)
- Start date of AE
- AE ongoing at end of study
- End date of AE
- Serious AE
- If serious, SAE Report No.
- Please specify seriousness criteria (multiple choice):
    - Results in death
    - Is life-threatening
    - Requires or prolongs hospitalization
    - Persistent or significant disability/incapacity
    - Congenital anomaly or birth defect
    - Other medically important serious event
- Severity/Intensity
- Pattern of adverse event
- Relatedness to IMP
- Action taken with study medication (Dose not changed, Dose reduced, Dose interrupted, Drug withdrawn, Not applicable, Unknown)
- Other action taken (None, Drug treatment, Others)
- Please specify other action
- Outcome (Recovered/resolved, recovering/resolving, not recovered/not resolved, recovered/resolved with sequel, fatal, unknown)

The adverse events will be coded using MedDRA.

Vital signs

For each vital sign parameter, a 'not done' entry (if not performed), a value, a unit, clinically relevant entry (yes/no) and a link between VS and AE in case of an adverse event are provided

- Time of vital signs
    - Body temperature (°C)
    - Systolic/diastolic blood pressure (mmHg)
    - Pulse rate (bpm)
    - Respiratory rate (breaths per min)
    - Height (cm)
    - Weight (kg)
    - BMI (kg/m²)

Physical examination

- Body (-system):
    - General appearance
    - Skin
    - Head, eyes, ears, nose, throat
    - Neck
    - Respiratory
    - Cardiovascular
    - Gastrointestinal
    - Back
    - Other

Result (Normal, not clinically relevant abnormal, clinically relevant abnormal)

Neurological examination

- Reflexes:
  - Biceps (right/left)
  - Knee (right/left)
  - Ankle (right/left)
  - Babinski Plantar Response (right/left)
  - Other
- Result (unable to test, not done, normal (reflex), normal, hypoactive, hyperactive with clonus, hyperactive without clonus, extension, absent)

Electrocardiogram

- Result of ECG (Normal, not clinically relevant abnormal, clinically relevant abnormal)

MRI

- Result of MRI (Normal, pathological)

Muscle spasticity measured by the Modified Ashworth Scale

- MAS – Joints tested (result right/left side)
  - Elbow flexors
  - Elbow extensors
  - Knee flexors
  - Knee extensors
- Result (0,1, 1+,2,3,4)

Pain assessments

- Pain site assessment performed
- Date of pain site assessment performed
- Assessment of overall pain symptoms
  - Basic dataset
    - Have you had any pain during the last seven days including today? (yes/no)
    - In general, how much has pain interfered with your day-to-day activities in the last week? (from 0 to 10)
    - In general, how much has pain interfered with your overall mood in the last week? (from 0 to 10)
    - In general, how much has pain interfered with your ability to get a good night's sleep? (from 0 to 10)
    - How many different pain problems do you have? (1/2/3/4/≥ 5)
  - Extended Dataset
    - Number of days with pain in the last 7 days including today (from 0 to 7 days or unknown)
    - Average pain unpleasantness in the last week (from 0 to 10)
    - Number of days with manageable/tolerable pain in the last 7 days including today (from 0 to 7 days or unknown)
  - Are you taking any oral and/or topical medication?
    - Each medication listed with result (yes) if taken
- Assessment of the two worst pain sites
  - Pain treatment
    - Are you using or receiving any treatment for your pain problem? (yes/no)
    - Is/was one of these treatments helpful (over the last 12 months)?
      Each treatment listed with result (yes/no/uncertain)
  - Factors that trigger or intensify your pain

- Each item with result (yes/no/uncertain)
- Time course of pain
  - How long does your pain usually last? (≤ 1 min, › 1 min but ‹ 1 hr, ≥ 1 hr but ‹ 24 hrs, ≥ 24 hrs, constant or continuous, unknown)
  - When during the day is the pain most intense? (morning, afternoon, evening, night, unpredictable)
- Intensity and duration of pain
  - Average pain intensity in the last week (from 0 to 10)
  - Pain intensity in present moment (from 0 to 10)
- Localization of pain
  - Localization and site (right/midline/left)
- Type of pain
- Description of painful and non-painful sensations within the painful area
  - Each item with result (from 0 to 10)
- Spinal Cord Injury Pain Instrument (SCIPI)
  - Each item with result (no/yes)

4.6.3 Laboratory parameters

Local laboratory:

All laboratory values are classified as clinically significant outside normal range or not (except pregnancy test).

Pregnancy test

- Result of pregnancy test (negative, positive)
- β-HCG (mIU/ml)
- Reason for negative assessment if β-HCG › 5 mIU/ml but result is assessed 'negative'

Blood

- Clinical chemistry
  - Sodium (mmol/l)
  - Chloride (mmol/l)
  - Potassium (mmol/l)
  - Calcium (mmol/l)
  - Glucose (mmol/l)
  - Aspartate Aminotransferase (ASAT, SGOT) (U/l)
  - Alanine Aminotransferase (ALAT, SGPT) (U/l)
  - Gamma Glutamyl Transferase (U/l)
  - Alkaline Phosphatase (U/l)
  - Total Bilirubin (mg/dl)
  - Direct Bilirubin (mg/dl)
  - Indirect Bilirubin (mg/dl)
  - Lactate Dehydrogenase (U/l)
  - Triglycerides (mmol/l)
  - Total Cholesterol (mmol/l)
  - Creatinine (mg/dl)
  - Uric Acid (mg/dl)
  - Creatine Kinase (U/l)
  - Lipase (U/l)
  - Pancreatic Amylase (U/l)

- Total Protein (g/l)
- Albumin (g/l)
- C Reactive Protein (mg/dl)

- Hematology
  - Hemoglobin (g/dl)
  - Hematocrit (%)
  - Erythrocytes, Red Blood Cells (/µl)
  - Platelets (/nl)
  - Leukocytes, White Blood Cells (/nl)
  - Neutrophils (/nl)
  - Monocytes (/nl)
  - Eosinophils (/nl)
  - Basophils (/nl)
  - Lymphocytes (/nl)
  - INR (no unit)
  - Activated Partial Thromboplastin Time (aPTT) (sec)

Urine
- pH (no unit)
- Specific gravity (g/ml)

The parameters below are given either qualitatively (negative, positive, normal, abnormal) or quantitatively with their respective units
- Ketones (mg/dl)
- Glucose (mmol/l)
- Leukocytes, White Blood Cells (/nl)
- Total Protein (g/l)
- Total Bilirubin (mg/dl)
- Blood (/µl)

CSF Safety
- Erythrocytes, Red Blood Cells (/µl)
- Leukocytes, White Blood Cells (/nl)
- Glucose (mmol/l)
- Lactate (mmol/l)
- Total protein (g/l)

4.7 Data known to the biostatistician

At the time of writing the SAP V01, the responsible biostatistician is blinded with respect to randomization. All subjects finished the study when the SAP was finished.

The KKS and WP2 were unblinded at the time when SAP V02 came into effect but the sponsor is still blinded.

5 Treating missing values and outliers

5.1 Missing values

Missing values in response variables will not be replaced as GLMM will allow unbiased estimation of the treatment effect under the Missing at Random assumption.

No further imputation of missing values is planned.

The SCIM subscores and total scores will not be computed if a single value is missing.

For GRASSP (partial/complete), the right (resp. left) subscores and right (resp. left) total scores will not be computed if a single value is missing on the right (resp. left) side. If one item value (or more) of the right (resp. left) GRASSP prehension performance score is missing but no right (resp. left) partial subscores (strength, sensation and prehension ability) were incomplete then the right (resp. left) total partial score will be computed whereas the right (resp. left) total complete score will not.

Likewise, the right (resp. left) ISNCSCI subscores and right (resp. left) total scores will not be computed if a single value is missing on the right (resp. left) side. Consequently, the ISNCSCI subscores on both sides and total scores will not be computed as well.

The Qualiveen subscores and total scores will not be computed if a single value is missing.

5.2 Incomplete values

Dates known up to month or year only will be set to a specific, arbitrary date whenever time spans or location measures are derived from them. In case of adverse events the least favorable option will be selected, i.e. start will be set to the earliest and end to the latest possible date. In every other case the middle date will be chosen (16th if the day is missing and July 1st if month and day are missing.

5.3 Time windows

The baseline value is the last value prior to treatment. All assessments on the day of treatment are regarded as prior to treatment. Day 0 is defined as the day of the start of medication. The observation period ends on the last follow-up visit (i.e. visit 11 on day 168).

In case of an early termination, the early termination visit will be allocated to a planned visit as follows (if applicable):

- Day 0 until 30: +/- 2 days
- Day 84: +/- 20 days
- Day 168: +/- 30 days

5.4 Outliers

Outliers will be evaluated as documented. Quartiles will be reported in addition to the mean, minimum and maximum. The latter have to be interpreted with caution.

6 Statistical analyses

All patient data entered in the CRF will be listed.

6.1 Subject disposition

A table following the CONSORT statement will be created depicting the following variables (including the reason for their exclusion): the number of patients in the full analysis set, the per-protocol set, in the safety analysis set.

In a table the actual treatment will be compared to the planned treatment. The absolute number of patients in the different groups will be shown.

A further table will be created displaying the number of patients who

- screened,
- randomized,
- started treatment,
- terminated the treatment earlier,
- completed treatment,
- terminated the study earlier,
- completed study,

(including the reasons).

Protocol deviations (subcategories) will be tabulated in total and against treatment arm (if applicable) by category of deviation.

In addition, subjects will be tabulated against treatment group and in total using absolute frequencies by study visits they attended.

6.2 Baseline characteristics

Sociodemographic and baseline characteristics mentioned above will be displayed against treatment group and in total for the FAS, PPS and SAF using number of non-missing values, mean, standard deviation, extrema and quartiles for continuous variables and absolute and relative frequencies for categorial variables.

The following variables will be analyzed:

<u>Sociodemographic</u>

- Sex, age (continuous and categorial)

<u>Spinal cord injury</u>

- Category

<u>Procedure</u>

- Patient is on invasive ventilation

All other data will only be listed.

The variables sex, age (continuous and categorial) at baseline, cause of injury, date of injury, time between date of injury and enrollment, node, neurological level of injury, ASIA impairment scale at baseline will be descriptively displayed for the AMS as well.

6.3 Concomitant medication

Concomitant medications will be listed by patient and start date.

6.4 Exposure to treatment, compliance

All tables will be displayed for the full analysis and safety set for the treated group.

Total dose of NG-101/placebo administrated after randomization and the duration of injection will be tabulated by visit using number of non-missing values, mean, standard deviation, extrema and quartiles.

Injection performed and volume will be displayed per visit using absolute and relative frequencies.

All other data will only be listed.

6.5 Primary analysis

All tables will be displayed for the full analysis set.

The primary criterion is the UEMS recovery score at day 168 and will be estimated using a linear mixed model with the baseline, 30-, 84- and 168-days measurements as response variable, and strata, time of measurement, treatment group and interaction of treatment group with time as fixed and subject and subject-time interaction as random explanatory variables. The actual effect will be estimated as a contrast between treatment arms and interaction between treatments arms and time at day 168 using the SAS function GLIMMIX. The null hypothesis of no treatment effect on UEMS recovery score can be rejected at the .05 level if the 95% confidence interval does not cover 0.

For the handling of missing values, see Section 5.1.

If we cannot estimate the effect between both groups at day 168 a simpler model will be required. The difference between 168-days and baseline measurements will be considered as the response variable and strata, treatment group and the baseline value as exploratory variable. In this case multiple imputation will be used to replace missing values taking the strata, the baseline, age and sex into account. If the model is still not estimable, the strata will be omitted.

Additionally, the UEMS recovery scores will be tabulated against treatment group by visit using non-missing values, mean, standard deviation, extrema and quartiles without replacing missing values.

6.6 Secondary efficacy analyses

All tables will be displayed for the full analysis set.

6.6.1 Neurophysiological and electrophysiological examinations

The following examinations on day 168 will be analyzed in the same fashion as the primary endpoint for**:**

- ISNCSCI
  - Upper and lower extremity motor scores and motor total scores (only both sides)
  - All sensory total scores (only both sides)

- SCIM III
  - All subscores
  - Total score

- GRASSP
  - All subscores except prehension performance
  - Partial score

The 6mWT,10MWT and WISCI II results, the ASIA impairment scale as well as the neurological level of injury will be tabulated against treatment group and in total by visit using non-missing values, mean, standard deviation, extrema and quartiles for continuous variables and absolute and relative frequencies for categorial variables.

The dSSEP, SSEP, NCV, GRASSP prehension performance results (except the duration and number of drops) and the total score will be tabulated against treatment group and in total by laterality and by visit using non-missing values, mean, standard deviation, extrema and quartiles for continuous variables and absolute and relative frequencies for categorial variables.

All remaining variables will be listed.

6.6.2 Effect on autonomic dysfunction

The Qualiveen questionnaire subscores and total score will be analyzed in a similar fashion as the primary endpoint but without the 30-days measurement as response. The question *Did you fill in this questionnaire on your own?* and single questions will only be listed.

The bladder function assessments and the calculated bladder diary variables will be tabulated against treatment group by visit using non-missing values, mean, standard deviation, extrema and quartiles for quantitative variables and will be displayed using absolute and relative frequencies for qualitative variables.

6.7 Safety/tolerability

All tables will be displayed for the safety set.

6.7.1 Adverse events

All tables will be displayed showing the number of events, number of subjects with at least one event and percentage of patients with events.

An overview of all AEs and SAEs will be tabulated against treatment group for the seriousness criteria, severity/intensity criteria, patterns of adverse event, relatedness to IMP, actions taken with study medication, other actions taken and outcomes.

The following tables will be displayed by system organ class (SOC) and preferred term (PT) sorted by frequency within each table:

- AEs and SAEs
- Non-serious AEs (excluding SAEs),
- AEs and SAEs resulting in death,
- (Possibly) related AEs and SAEs by mortality (resulting in death, not resulting in death and total).

In addition, a table showing the patients with AEs against treatment group and displayed by SOC and PT by severity criteria as well as relatedness will be created as well as a table showing deaths.

This tabulation will be repeated for adverse events starting within 30 days and adverse events starting more than 30 days after randomization.

The relationship between the AE/SAE frequency per patient and the duration of study medication taken will be also be shown using Spearman correlation by treatment group.

All fatalities will be listed. All serious adverse events will be listed in addition. A glossary of all adverse event terms will be displayed.

6.7.2 Laboratory parameters

The current values (only with additional total arm) and changes from baseline will be tabulated against treatment arm using non-missing values, mean, standard deviation, extrema and quartiles for the following laboratory values by visit:

Clinical chemistry

- Sodium, Chloride, Potassium, Calcium, Glucose, Aspartate Aminotransferase (ASAT, SGOT), Alanine Aminotransferase (ALAT, SGPT), Gamma Glutamyl Transferase, Alkaline Phosphatase, Total Bilirubin, Direct Bilirubin, Indirect Bilirubin, Lactate Dehydrogenase, Triglycerides, Total Cholesterol, Creatinine, Uric Acid, Creatine Kinase, Lipase, Pancreatic Amylase, Total Protein, Albumin, C Reactive Protein

Hematology:

- Hemoglobin, Hematocrit, Erythrocytes, Red Blood Cells, Platelets, Leukocytes, White Blood Cells, Neutrophils, Monocytes, Eosinophils, Basophils, Lymphocytes, INR, Activated Partial Thromboplastin Time (aPTT)

CSF Safety

- Erythrocytes, Red Blood Cells, Leukocytes, White Blood Cells, Glucose, Lactate, Total protein

In addition, shift tables (including urine) will be created based on abnormalities or clinically relevance showing the shift between the baseline results and the result on the relevant visit.

Pregnancy test results will only be listed.

6.7.3 Vital signs (and height, weight)

Vital signs (blood pressure, pulse rate, body temperature, respiratory rate, height, weight) will be tabulated by parameter and visit using number of non-missing values, mean, standard deviation, extrema and quartiles. The change from baseline will be tabulated as well.

6.7.4 Physical/neurological examination, electrocardiogram and MRI

The physical/neurological examination and electrocardiogram/MRI results will be displayed by visit using absolute and relative frequencies as well as by laterality for the physical examination. In addition, shift tables will be created based on abnormalities or clinically relevance showing the shift between the baseline results and the result on the relevant visit.

6.7.5 Modified Ashworth Scale

The muscle spasticity measured by the Modified Ashworth Scale results will be displayed by laterality and by visit using absolute and relative frequencies.

6.7.6 Pain assessments

The evaluation of the type of pain will be summarized once by visit (days 84 and 168) and once combined for the two most recent visits (days 84 and 168) for at least once-occurred neuropathic pain (at level/below level) versus never-occurred neuropathic pain (including neuropathic pain (other)).

The odds ratio is the ratio of the odds of sensing neuropathic pain, at least once at level/below level, in the verum arm to the placebo arm and will be displayed with a confidence interval.

All analyses will be displayed for the safety set as well as for the full analysis set. All other assessments regarding pain will be listed.

6.8 Subgroup analyses

The following variables will be used to identify relevant subgroups and will be analyzed as part of work package 2.

- Age of the subject at baseline
- Sex
- Nodes used for randomization
- ASIA impairment scale at baseline
- Neurological level of injury at baseline
- Bilateral pin prick score at baseline
- Bilateral light touch score at baseline
- Level of completeness of SCI at baseline
- Level of intact motor function at baseline (right and left) at baseline
- Level of sensory function at baseline (right and left) at baseline
- Level (right and left) of dermatomal SSEP (dSSEP) C6 – Response at baseline
- Level (right and left) of dermatomal SSEP (dSSEP) C8 – Response at baseline
- Level (right and left) of somatosensory evoked potentials (SSEP) of tibial nerve – Response at baseline
- Level (right and left) of nerve conduction velocity (NCV) of ulnaris nerve - Compound motor action potential at baseline

6.9 Interim analyses

No interim analyses are planned.

6.10 Sensitivity analyses

In one analysis, the primary analysis will be repeated for the per protocol set only.

In one analysis, the primary analysis will be repeated for the augmented set. Multiple times, each node will be filled with samples drawn from the EMSCI database, according to the criteria mentioned in Section 2.1 added to the full analysis set to reach a 1:1 ratio yielding multiple augmented sets. The primary analysis will be performed for every set. All results will be combined using multiple imputation technics.

Furthermore, the treatment effect and the confidence interval limits will be plotted against the augmented sets ordered by treatment effect size.

In further sensitivity analyses, the primary analysis will be repeated for the full analysis set using the software R with the functions glmmTMB (package glmmTMB) and lmer (package lme4). The

primary analysis will be repeated in SAS with a transformation which maps the UEMS to an unbounded scale using the quantile function of the standard normal distribution.

In addition, as part of work package 2, analyses will be performed to try to better model the UEMS.

7 Software

SAS Version 9.4 or higher will be used to analyze the data.

8 Appendix

8.1 References

Tanadini, L.G., Steeves, J.D., Curt, A. et al. Autoregressive transitional ordinal model to test for treatment effect in neurological trials with complex endpoints. *BMC Med Res Methodol* **16**, 149 (2016). https://doi.org/10.1186/s12874-016-0251-y

Steeves, J., Lammertse, D., Curt, A. et al. Guidelines for the conduct of clinical trials for spinal cord injury (SCI) as developed by the ICCP panel: clinical trial outcome measures. *Spinal Cord* **45**, 206–221 (2007). https://doi.org/10.1038/sj.sc.3102008

8.2 Tables, listings and figures

Tables and figures:

Section 14

14.1.1.1	Analysis sets
14.1.1.2	Subjects enrolled by site
14.1.1.3	Treatment assignment
14.1.1.4	Subject disposition
14.1.1.5	Visit attended
14.1.1.6	Protocol deviations
14.1.2.1	Baseline characteristics – part I (continuous)
14.1.2.2	Baseline characteristics – part II (categorial)
14.1.2.3	Baseline characteristics – part III (spinal cord injury)
14.1.2.4	Baseline characteristics – part IV (procedure)
14.2.1.1	Exposure - part I (continuous)
14.2.1.2	Exposure - part II (categorial)
14.2.2.1	ISNCSCI – part I (descriptive)
14.2.2.2	ISNCSCI – part II (regression model)
14.2.2.3	ISNCSCI - ASIA impairment scale
14.2.2.4	ISNCSCI - Neurological level of injury
14.2.3.1	SCIM III - part I (descriptive)
14.2.3.2	SCIM III - part II (regression model)
14.2.4.1	GRASSP - part I (descriptive)
14.2.4.2	GRASSP - part II (regression model)

14.2.5	6mWT / 10MTW / WISCI
14.2.6.1	Sensitivity analyses
14.2.6.2	Sensitivity analyses - plot
14.2.7.1	Neurophysiological – part I (continuous)
14.2.7.2	Neurophysiological – part II (categorial)
14.2.8.1	Electrophysiological – part I (continuous)
14.2.8.2	Electrophysiological – part II (categorial)
14.2.9.1	Qualiveen questionnaire – part I (descriptive)
14.2.9.2	Qualiveen questionnaire – part II (regression model)
14.2.10.1	Quantitative bladder function assessments – part I (continuous)
14.2.10.2	Quantitative bladder function assessments – part II (categorial)
14.2.11.1	Bladder diaries – part I (continuous)
14.2.11.2	Bladder diaries – part II (categorial)
14.3.1.1	Adverse Events - Overview
14.3.1.2	Adverse Events
14.3.1.3	Non-serious Adverse Events
14.3.1.4	Serious Adverse Events
14.3.1.5	Adverse Events resulting in death
14.3.1.6	Serious Adverse Events resulting in death
14.3.1.7	(Possibly) related Adverse Events by mortality
14.3.1.8	(Possibly) related Serious Adverse Events by mortality
14.3.1.9	Adverse Events by Severity Criteria
14.3.1.10	Adverse Events by Relatedness
14.3.1.11	Adverse Events/Serious Adverse Events frequency and duration of study medication relationship
14.3.1.12	Deaths
14.3.1.13.1	Hematology
14.3.1.13.2	Hematology - Change
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14.3.1.14.1	Clinical chemistry
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14.3.1.17.2	Vital signs - Change
14.3.1.18.1	Physical examination
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14.3.1.19.1	Neurological examination
14.3.1.19.2	Neurological examination – Shift
14.3.1.20.1	Electrocardiogram
14.3.1.20.2	Electrocardiogram - Shift
14.3.1.21.1	MRI
14.3.1.21.2	MRI - Shift
14.3.1.22	Modified Ashworth Scale
14.3.1.23	Pain assessments
14.3.2	Listing of Deaths
14.3.3	Listing of Serious Adverse Events
14.3.4	Listing of Abnormal Laboratory Values

Listings:

Section 16

16.2.1.1	Discontinued patients
16.2.1.2	Visits
16.2.2	Protocol deviations
16.2.3.1	Analysis sets
16.2.3.2	In-/Exclusion Criteria
16.2.4.1	Sociodemographic characteristics
16.2.4.2	Disease Characteristics
16.2.4.3	Medical History
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16.2.7.1	Adverse Events
16.2.7.2	Glossary
16.2.8.1	Laboratory
16.2.9	Vital Signs

8.3 Mockup tables

8.3.1 Disposition

Analysis sets:

	Treatment arm		
	Arm 1 (N=)	Arm 2 (N=)	Total (N=)
Subjects excluded from the full analysis set	y	y	y
Reason			
Reason 1	y	y	y
…			
Full analysis set	y	y	y
Subjects excluded from the per-protocol set	y	y	y
Reason			
Reason 1	y	y	y

…			
Per-protocol set	y	y	y
Subjects excluded from the safety set	y	y	y
Reason			
Reason 1	y	y	y
…			
Safety set	y	y	y

y = number of patients with events

Subjects enrolled by site:

	Treatment arm		
Site-ID	Arm 1 (N=)	Arm 2 (N=)	Total (N=)
ABC	y	y	y

y = number of patients with events

Treatment assignment:

		Planned treatment arm		
		Arm 1 (N=)	Arm 2 (N=)	Total (N=)
Actual treatment arm	Arm 1 (N=)	y	y	y
	Arm 2 (N=)	y	y	y
	Total (N=)	y	y	y

Subject disposition:

		Treatment arm		
		Arm 1 (N=)	Arm 2 (N=)	Total (N=)
Screened	Failed			y
	Not failed			y
Randomization	Randomized	y	y	y
	Not randomized			y
Treatment start	Started	y	y	y
	Not started	y	y	y

Treatment completion	Completed	y	y	y
	Discontinued: Intolerable adverse events	y	y	y
	….			
Study participation	Completed	y	y	y
	Discontinued:	y	y	y
	…			

y = number of patients with events

Visit attended:

	Treatment arm		
Visit	Arm 1 (N=)	Arm 2 (N=)	Total (N=)
Visit 1:	y	y	y
…			

y = number of patients with events

Protocol deviations:

	Treatment arm		
	Arm 1 (N=)	Arm 2 (N=)	Total (N=)
Category 1	y	y	y
…			

y = number of patients with events

8.3.2 Baseline characteristics

Continuous:

Parameter		n	Mean	SD	Min	Q1	Median	Q3	Max
Age (years)									
	Arm 1 (N=)								
	Arm 2 (N=)								
	Total (N=)								
…									

n = number of patients in the PPS, SAF and FAS sets with non-missing values on the relevant visit, SD = Standard deviation, Q1 = 25%-Quantile, Q3 = 75%-Quantile

Categories:

	Treatment arm	

		Arm 1 (N=)		Arm 2 (N=)		Total (N=)	
Parameter		#	%	#	%	#	%
Sex	n	y	100	y	100	y	100
	Female	y	z.z	y	z.z	y	z.z
	Male	y	z.z	y	z.z	y	z.z
	…						
…							

n = number of patients in the PPS, SAF and FAS sets with non-missing values at the relevant visit, y = number of patients with events, z.z = Percentage of patients with events

Spinal cord injury

		Treatment arm					
		Arm 1 (N=)		Arm 2 (N=)		Total (N=)	
Category		#	%	#	%	#	%
Category 1	n	y	z.z	y	z.z	y	z.z
	Yes	y	z.z	y	z.z	y	z.z
	No	y	z.z	y	z.z	y	z.z
…							

n = number of patients in the PPS, SAF and FAS sets with non-missing values at the relevant visit, y = Number of patients with events, z.z = Percentage of patients with events

8.3.3 Exposure

Total dose administrated (mg)/Duration of injection (sec)

‹Parameter (Unit)›

	Visit	n	Mean	SD	Min	Q1	Median	Q3	Max
Arm 1 (N=)	Baseline								
	V1								
	….								

n = number of patients in the FAS and SAF sets with non-missing values on the relevant visit, SD = Standard deviation, Q1 = 25%-Quantile, Q3 = 75%-Quantile

Injection volume/performed:

‹Parameter›

		Treatment arm					
		Arm 1 (N=)		Arm 2 (N=)		Total (N=)	
Visit		#	%	#	%	#	%
Baseline	n	y	z.z	y	z.z	y	z.z
	3 ml (45 mg)	y	z.z	y	z.z	y	z.z

	Other volume	y	z.z	y	z.z	y	z.z
…							

n = number of patients in the FAS and SAF sets with non-missing values at the relevant visit, y = Number of patients with events, z.z = Percentage of patients with events

8.3.4 Primary analysis

UEMS recovery scores:

‹Parameter›

Visit		n	Mean	SD	Min	Q1	Median	Q3	Max
Baseline	Arm 1 (N=)								
	Arm 2 (N=)								
	Total (N=)								
V1	Arm 1 (N=)								
	Arm 2 (N=)								
	Total (N=)								
…									

n = number of patients in the FAS set with non-missing values on the relevant visit, SD = Standard deviation, Q1 = 25%-Quantile, Q3 = 75%-Quantile

Regression model:
‹Parameter›

Effect	Estimate	Standard error	95%-CI	p-value
Effect of NG-101 against Placebo at day 168*				
Additional information:				
Intercept				
Node 4				
…				
Days after start of medication				
…				
NG-101 against Placebo				
..				
Interaction between days after start of medication and medication				
..				

CI = confidence interval

*Contrast between treatment arms and interaction between treatments arms and time at day 168

8.3.5 Neurophysiological and electrophysiological examinations/Effect on autonomic dysfunction

Quantitative assessments: See the descriptive mock-up table for the primary analysis.

Qualitative assessments:

‹Parameter›

		Treatment arm					
		Arm 1 (N=)		Arm 2 (N=)		Total (N=)	
Visit		#	%	#	%	#	%
Baseline	n	y	z.z	y	z.z	y	z.z
		y	z.z	y	z.z	y	z.z
		y	z.z	y	z.z	y	z.z
….							

n = number of patients in the FAS set with non-missing values at the relevant visit, y = number of patients with events, z.z = Percentage of patients with events

ISNCSCI (all subscores and total motor scores (only both sides), all total sensory scores (only both sides)), SCIM III (all subscores, total score), GRASSP (all subscores except prehension performance, partial score) and Qualiveen questionnaire (total score) will be analyzed in a similar fashion as the primary endpoint (8.3.4), but without the 30-days measurement as response for the Qualiveen questionnaire.

8.3.6 Adverse events

Overview:

	Treatment arm		
	Arm 1 (N=)	Arm 2 (N=)	Total (N=)
Total	x / y (z.z%)	x / y (z.z%)	x / y (z.z%)
Seriousness criteria			
Results in death	x / y (z.z%)	x / y (z.z%)	x / y (z.z%)
….			
Severity/ Intensity			
Mild	x / y (z.z%)	x / y (z.z%)	x / y (z.z%)
….			
Pattern of adverse event			
Intermittent	x / y (z.z%)	x / y (z.z%)	x / y (z.z%)
…			
Relatedness to IMP			
Related	x / y (z.z%)	x / y (z.z%)	x / y (z.z%)
Action taken with study medication			

Dose not changed	x / y (z.z%)	x / y (z.z%)	x / y (z.z%)
…			
Other action taken			
…	x / y (z.z%)	x / y (z.z%)	x / y (z.z%)
Outcome			
Recovered/ resolved			
…			

x/ y (z.z%): x = Number of events, y = Number of patients with events, z.z = Percentage of patients with events. Percentages are based on the number of patients in the SAF set.

AEs, Non-serious AEs (excluding SAEs), SAEs, AEs resulting in death, SAEs resulting in death, (Possibly) related AEs by mortality (AEs resulting in death, not resulting in death and total), (Possibly) related SAEs by mortality (AEs resulting in death, not resulting in death and total):

	Treatment arm		
	Arm 1 (N=)	Arm 2 (N=)	Total (N=)
SOC Preferred term			
Total	x / y (z.z%)	x / y (z.z%)	x / y (z.z%)
SOC 1	x / y (z.z%)	x / y (z.z%)	x / y (z.z%)
Preferred term 1	x / y (z.z%)	x / y (z.z%)	x / y (z.z%)
Preferred term 2	x / y (z.z%)	x / y (z.z%)	x / y (z.z%)
….			

x/ y (z.z%): x = Number of events, y = Number of patients with events, z.z = Percentage of patients with events. Percentages are based on the number of patients in the SAF set. Percentages within system organ classes might add up to more than the total of the system organ class if patients had more than one event. Coding according to MedDRAVersion xx.x.

Adverse Events by Severity Criteria, Adverse Events by Relatedness:

		Treatment arm		
		Arm 1 (N=)	Arm 2 (N=)	Total (N=)
SOC Preferred term	Characteristic			
Total	Total	x / y (z.z%)	x / y (z.z%)	x / y (z.z%)
	‹Severe›	x / y (z.z%)	x / y (z.z%)	x / y (z.z%)
	…			
SOC 1	Total	x / y (z.z%)	x / y (z.z%)	x / y (z.z%)
	‹Severe›	x / y (z.z%)	x / y (z.z%)	x / y (z.z%)
	…			
Preferred term 1	Total	x / y (z.z%)	x / y (z.z%)	x / y (z.z%)

	‹Severe›	x / y (z.z%)	x / y (z.z%)	x / y (z.z%)
	…			

x/ y (z.z%): x = Number of events, y = Number of patients with events, z.z = Percentage of patients with events. Percentages are based on the number of patients in the SAF set. Percentages within system organ classes might add up to more than the total of the system organ class if patients had more than one event. Coding according to MedDRAVersion xx.x.

Adverse Events/Serious Adverse Events frequency and duration of study medication relationship:

‹Parameter›

	Treatment arm	
	Arm 1 (N=)	Arm 2 (N=)
Spearman correlation coefficient	x	x

Deaths:

	Treatment arm			
	Arm 1 (N=)		Arm 2 (N=)	
	#	%	#	%
Reason				
Deaths	y	z.z	y	z.z
Reason1	y	z.z	y	z.z
….	y	z.z	y	z.z

= number of patients, % = percentages are based on the number of patients in the full analysis set (N).

8.3.7 Laboratory parameters and vital signs / Physical/neurological examination and electrocardiogram/MRI/ Modified Ashworth Scale

Absolute value per visit:

Clinical chemistry, hematology, CSF safety and vital signs:

‹Parameter›

	Visit		n	Mean	SD	Min	Q1	Median	Q3	Max
Arm 1 (N=)	Baseline									
	V1									
	….									
Arm 2 (N=)	Baseline									
	….									
Total (N=)	Baseline									
	….									

n = number of patients in the SAF set with non-missing values on the relevant visit, SD = Standard deviation, Q1 = 25%-Quantile, Q3 = 75%-Quantile

Absolute change from baseline per visit

Clinical chemistry, hematology, CSF safety and vital signs:

‹Parameter›

	Visit	Parameter	n	Mean	SD	Min	Q1	Median	Q3	Max
Arm 1 (N=)	Day 0	‹Parameter (unit)›								
	….									
Arm 2 (N=)	Day 0									
	….									
Total (N=)	Day 0									
	….									

n = number of patients in the SAF set with non-missing values on the relevant visit, SD = Standard deviation, Q1 = 25%-Quantile, Q3 = 75%-Quantile

Shift tables:

Clinical chemistry, hematology, CSF safety and urinalysis:
‹Parameter - visit›

		Baseline				
Arm		Missing	Normal	Abnormal, NCR	Abnormal, CR	Total
Arm 1 (N=)	Missing					
	Normal					
	Abnormal, NCR					
	Abnormal, CR					
	Total					
	…					
Arm 2 (N=)	Missing					
	…					

CR = clinically relevant, NCR = not clinically relevant. Percentages are based on the number of patients in the SAF set. Columns represent the baseline results, rows represent the results to show the shift.

8.3.8 Pain assessments

Type of pain:

			Treatment arm				Odds ratio	
Visit			Arm 1 (N=)		Arm 2 (N=)			
			#	%	#	%	Estimate	95%-CI
Day 84	Type of pain	n	y	100	y	100		

		Neuropathic - At level/Below level at least once	y	z.z	y	z.z	x	[a,b]
		Not neuropathic or neuropathic - other	y	z.z	y	z.z		
Day 168	Type of pain	n	y	100	y	100		
		Neuropathic - At level/Below level at least once	y	z.z	y	z.z	x	[a,b]
		Not neuropathic or neuropathic - other	y	z.z	y	z.z		
Day 84/ Day 168	Type of pain	n	y	100	y	100		
		Neuropathic - At level/Below level at least once	y	z.z	y	z.z	x	[a,b]
		Not neuropathic or neuropathic - other	y	z.z	y	z.z		

n = number of patients in the SAF and FAS sets with non-missing values on the relevant visit, CI = confidence interval, a = lower bound of the confidence interval, b = upper bound of the confidence interval, y = number of patients with events, z.z = Percentage of patients with events, x = odds ratio (ratio of the odds of sensing neuropathic pain, at least once at level/below level, in the verum arm to the placebo arm)

8.3.9 Sensitivity analyses

All sensitivity analyses will be displayed as in 8.3.4 except the analysis on the augmented set for which only the contrast part of the table will be displayed.

8.4 Mockup listings

For all listings, (study) day is defined as day after randomization. Therefore, day 0 is the day of the start of medication and negative days indicate days before randomization.

One row per patient:

Subject-ID	Parameter 1	Parameter 2	Parameter 3	...	...	...	...	...	...
01-001	....								

Many visits per patient – parameter horizontal:

Subject-ID	Visit	Date	Day	Parameter 1	Parameter 2	Parameter 3	...	...	...
‹01-001 characteristics›									
01-001	Screening								
01-001	Baseline								

	…								

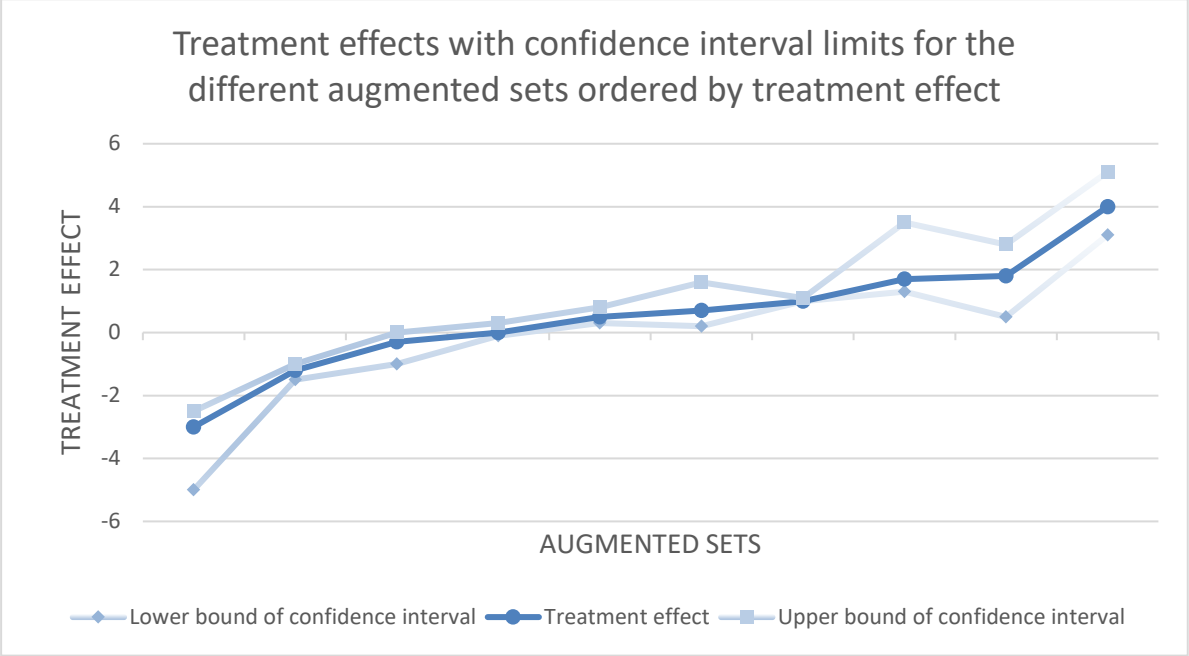
Many visits per patient – Parameter vertical:

Subject-ID	Visit	Date	Day	Parameter	Characteristic 1	Characteristic 2	…	…
‹01-001 characteristics›								
01-001	Screening			Parameter 1				
01-001				Parameter 2				
01-001				…				
01-001	Baseline							
	…							

Many events/measures/methods per patient - properties horizontal:

Subject-ID	Date	Day	Event	Characteristic 1	Characteristic 2	Characteristic 3	…	…	…
‹01-001 characteristics›									
01-001									
01-001									
01-001									

8.5 Mockup figures



9 Changes

Changes between SAP Version V02 and V01

- Nodes were added to the baseline characteristics.
- Not the nodes at baseline but the nodes used for randomization will be considered for the subgroup analyses and the primary analysis.
- Some changes in the analysis of the bladder diary and bladder function test were made.
- The Qualiveen questionnaire subscore names were corrected according to the names in the literature.
- The p-value and confidence interval were removed from the mockup table of the descriptive analysis of the primary endpoint.
- Reformulation of the primary analysis in the case where the effect between both groups at day 168 is not estimable.

By signing I declare to be the author of this document and that analysis of the study will be carried out according to this plan.

Date, signature: _____

Bérénice Robert, Biometrician, Coordination Centre for Clinical trials (KKS)

By signing I declare on behalf of the sponsor (Switzerland) that analysis of the study will be carried out according to this plan.

Date, signature: _____

Prof. Dr. Armin Curt, Principal Investigator, Balgrist University Hospital

By signing I declare on behalf of the legal representative of the sponsor (European union) that analysis of the study will be carried out according to this plan.

Date, signature: _____

Prof. Dr. med. Norbert Weidner, Coordinating Investigator, Heidelberg University Hospital