

A multi-centre, double-blind, randomized, placebo-controlled, delayed-start Phase II/III study to assess the efficacy and safety of Neuro-Cells in (sub)acute spinal cord injury patients

Introduction

This study was a delayed-start randomized placebo-controlled double blind multicentred Phase II trial (Sponsor ID number: A2017SCI03 / EudraCT: 2018-000805-22 / NTC trial number: NCT03935724) with autologous Neuro-Cells in patients with a (sub)acute traumatic spinal cord injury. The study started in March 2022 with the first visit of the first patient and formally ended with the last visit last patient in June 2024. The first visit first patient took place in March 2022.

The study started with the initial submission of Protocol version 1.0 date 31MAR2019 and the first patients were enrolled with Protocol version 4.0 date 14FEB2022. Approval for Denmark was given in July 2019 and the final approval for Spain was given in January 2022.

4 amendments were filled to enhance the enrolment period from 8 to 10 weeks, to add high cervical lesions to the inclusion criteria, to add an extra ISNCSCI assessment at 90 days after the start of the treatment and to adjust the study timelines due to recruitment delays.

The coordinating Principal Investigator was Prof.dr.Fin Biering-Soerensen, MD, PhD. In Denmark Prof.dr.Fin Biering-Soerensen, dr.Claus Andersen, MD, PhD and Christian Tersbøl Pinkowsky, MD, were responsible for the clinical trial site. In Spain dr.Antonio Oliviero, MD, PhD and Prof.dr.Jörg Mey, PhD was responsible for the clinical trial site. All the mentioned investigators were part of the blinded team performing the assessments of the patients. Unblinded and only responsible for the safe administration of the treatment/placebo were dr. Lars-Henrik Krarup, MD, PhD (Denmark) and dr. Angela Brocalero-Camacho, MD, PhD (Spain), both trained neurologists.

The CRO responsible for the data management and the trial monitoring was QbD Clinical Wilrijk Former (Trium Clinical Lier) Belgium.

A data safety monitoring board (DSMB) was installed. Prof.dr.Daniel Anthony, PhD, of Oxford was chairing the DSMB. In the DSMB a member of the European Spinal Cord Injury Association was added to assure the contribution of the patients with a spinal cord injury.

Summary study design

The intervention was administered at a single time point between 6 to 8 weeks postinjury to patients with C6-T11 AIS grade A, B or C SCI. Due to the COVID-19 quarantine rules of the hospitals, the inclusion criteria were later enhanced to C4-T11 and 6 to 10 weeks post injury. Participants were followed for 6 months and after 6 months the patients initially with the placebo treated were offered the treatment with Neuro-Cells® and followed again for 6 months. All patients were followed a year after the receiving of the initial intervention. To ensure

blinding for both the assessor and the patient during the first 6 months, a blinded and an unblinded team were installed. The unblinded team consists of a Neurologist and a research nurse to perform the lumbar puncture with the administration of the placebo or the cell treatment. The overview of the study scheme with the assessments are presented in Figure 1.

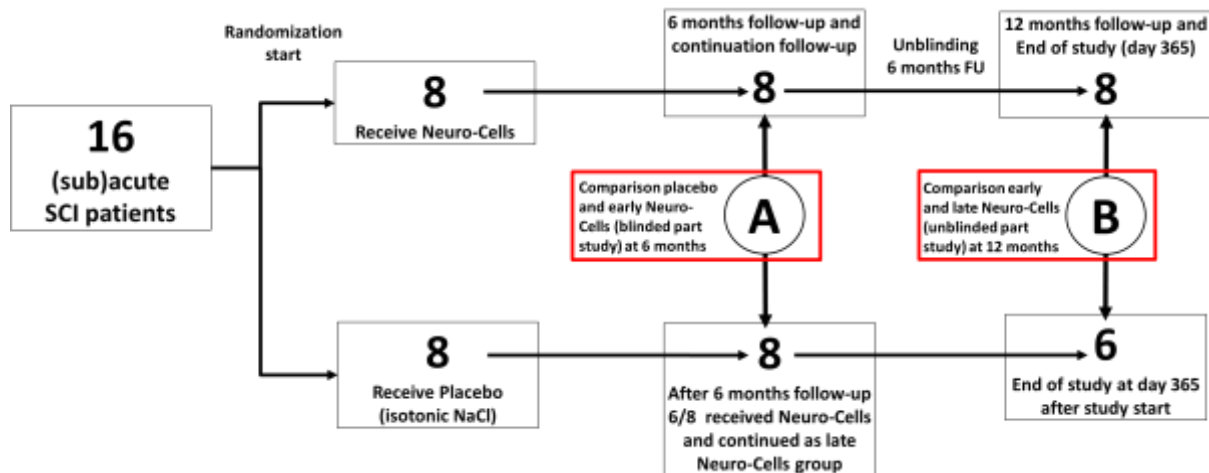


Figure 1. Randomization and delayed start design of the Phase II study with a placebo-controlled double-blind part (A) and an early and late administration of Neuro-Cells part (B).

After signing the informed consent and the confirmation of the inclusion and not the exclusion criteria, all patients were scheduled for a bone marrow procurement (visit 2) and within 48 hours (visit 3) for a lumbar puncture to administer the assigned intervention (placebo or Neuro-Cells). The intervention was prepared and packed for direct application in the GMP facility of Neuroplast BV located in the South of the Netherlands. The list of codes to unblind the individual status of the patients was locked and the only person able to retrieve this information was the qualified person of Neuroplast BV.

All participants underwent the comprehensive baseline assessments and a bone marrow procurement collecting between 75 and 100 ml of bone marrow out of the iliac crests. The bone marrow was transported under ambient conditions to the GMP facility of Neuroplast BV. If the viability of the cells and the total number of nucleated cells fulfilled the acceptance criteria for manufacturing, the patients were randomly assigned to the placebo or the treatment group by the qualified person of Neuroplast BV. Only the bone marrow from participants assigned to the treatment group was processed to Neuro-Cells®. After release at the end of the manufacturing, the Neuro-Cells® batch or a placebo batch were packed in a locked and sealed passive cooled box (between 4-8 degrees Celsius) for transport to the hospital. To ensure that the patient receives Neuro-Cells® processed from his/her own bone marrow, a unique code and the date of birth with the expiration date/time were put on the ready-to-use syringe with the Neuro-Cells® or the placebo solution. The stem cells of interest needed to fulfil the minimum dose for release (at least 4 million hematopoietic stem cells (HSCs) and 4 million mesenchymal stem cells (MSCs) in 8 ml buffer solution). After arrival at the hospital, the locked transport box was put in quarantine until the final release was performed based on the outcome of the anti-inflammatory potency assay.

After the final release, the box was opened by the unblinded neurologist and applied by lumbar puncture to the patient. A research nurse monitored that the lumbar puncture was performed in the trained way to ensure a complete blinded procedure for the patient.

This design was chosen in discussion with the ethical committees of Spain and Denmark, the European Patients Organisation and the coordinating Investigator Prof.dr.Fin Biering-Soerensen. A follow-up of 6 months and then offering a treatment to the initially placebo assigned patients was mandated by the ethic committees as the product Neuro-Cells influences the inflammation and assigning the treatment to the controls after 12 months provides no benefit at all for the patients.

Safety and outcome assessments

The primary outcome of the trial was confirming the safety of the intrathecal administration of Neuro-Cells and the first efficacy assessed by the International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI) examinations. Safety is assessed by physical examinations, vital signs, adverse events (AEs) reporting, concomitant medications and comprehensive laboratory tests including blood chemistry and urine chemistry. The adverse events were reported to the medical monitor of the sponsor (Neuroplast bv) and to an independent data and safety monitoring board (DSMB). Regarding the frequency and the severity of the adverse events, the sponsor advised by the DSMB could prematurely terminate the clinical trial. The ISNCSCI is an internationally accepted assessment to establish the neurologic impairment of the spinal cord injured patient. In this trial all the ISNCSCI assessors were trained physicians or trained physical therapists with more than 5 years' experience with this assessment. During the study follow-up, ISNCSCI assessments were performed and documented at the start before the application of the treatment/placebo (visit 2), at day 90 (visit 6) at day 180 (visit 7) and at day 365 (visit 12) after the administration of the intervention treatment/placebo. Based on the input of the European Spinal Cord Injury Patient Organisation, Neuroplast added a comprehensive package of assessments to the study as secondary outcomes. This package of standardized assessments was performed to describe the general and specific functioning of the patient at different time points during the follow-up (Figure 2):

- SCIM III
- Pain Basic Data Set version 2.0
- Modified Ashcroft Scale (MAS)
- Quality of Life basic data set
- Neurogenic bowel dysfunction score
- Lower Urinary Tract Function Basic Data Set version 2.0

During the study follow-up, the first 4 assessments were performed and documented at the start before the application of the treatment/placebo (visit 2), at day 90 (visit 6) at day 180 (visit 7) and at day 365 (visit 12) after the administration of the intervention treatment/placebo.

The last 2 assessments were performed at the start (visit 2) and at the end of the study on day 365 (visit 12). It was permitted to perform the assessments for 2 days when the health situation of the patient did not allow to perform all the assessments in one day.

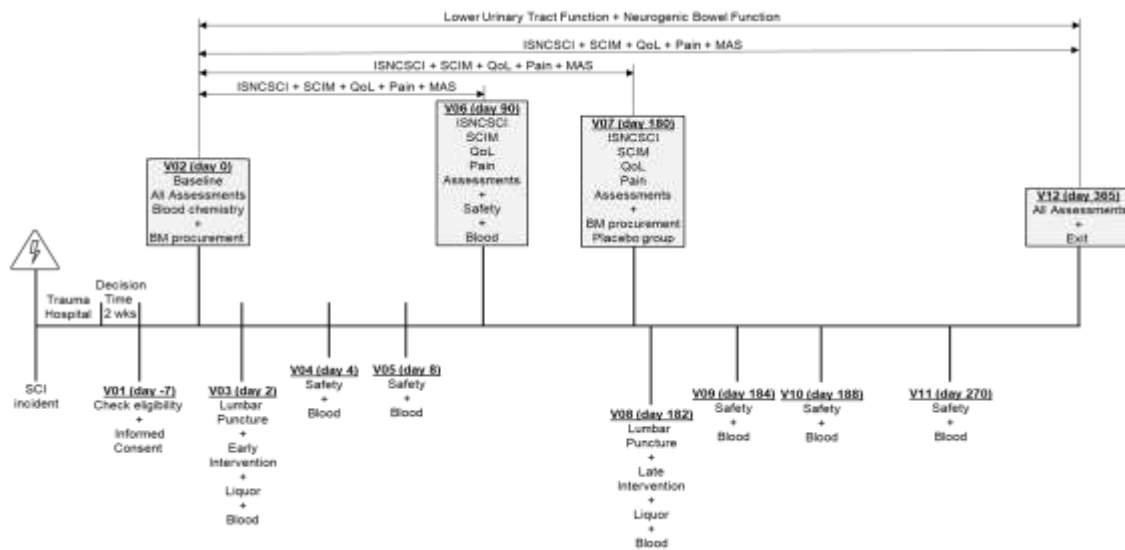


Figure 2. Overview of the individual assessments with the time points/visits.

Product composition

The uniqueness of Neuro-Cells® is that it contains a natural mixture of fresh not-cryopreserved naïve stem cells. The cell composition is minimally manipulated, and this is a key element of the manufacturing. Neuro-Cells® is an autologous fresh product and the quality, and the number of specific cells depend completely on what is harvested during the bone marrow procurement process. Table 1 gives an overview of the total number of cells of the 2 mentioned cell-types per patient (HSCs and MSCs).

Randomization in early and late Neuro-Cells group	Patient batch number	Volume (ml) applied intrathecally	Viable number Total Nucleated Cells (TNC)	Total number of Hematopoietic Stem cells (HSCs)	Total number of Mesenchymal stem cells (MSCs)
Early	A22NC001	8,31	2,64E+08	1,12E+07	1,98E+07
Early	A22NC002	8,57	3,37E+08	2,31E+06	1,08E+07
Early	A22NC006	8,00	9,67E+07	6,30E+06	8,29E+06
Early	A22NC009	7,98	2,64E+08	4,82E+06	1,58E+07
Early	A22NC012	8,32	2,77E+08	9,52E+06	2,52E+07
Late	A22NC013	8,75	2,01E+08	1,01E+07	1,59E+08
Late	A23NC002	7,84	2,56E+08	9,35E+06	1,75E+07
Late	A23NC003	8,12	7,49E+08	1,71E+07	2,66E+07
Late	A23NC004	8,05	1,96E+08	1,06E+07	1,60E+07
Early	A23NC005	9,03	4,53E+08	1,56E+07	2,53E+07
Early	A23NC008	7,99	6,05E+08	1,52E+07	3,36E+07
Late	A23NC010	7,99	1,42E+08	5,95E+06	1,85E+07
Early	A23NC011	7,96	1,84E+08	8,86E+06	1,05E+07
Late	A23NC013	8,11	9,36E+07	5,79E+06	8,99E+06

Clinical experience of Neuro-Cells® in Spinal Cord Injury

From March 2022 until June 2024, Neuro-Cells® was tested in a randomized placebo-controlled double-blind multicentre Phase II study (NCT03935724) in (sub)acute spinal cord injury patients (between 6 to 10 weeks after the SCI onset) to confirm safety and efficacy.

The patients of the Phase II study were followed for 1 year. All patients received a 1-time injection by lumbar puncture of Neuro-Cells®. There were no product-related adverse events reported, and the treatment was well tolerated by the patients (table 2). The Phase II confirmed the safety outcome of the Phase I study with intrathecally applied Neuro-Cells®. So far, Neuro-Cells® was given to 27 patients, and no product related adverse events were reported during a follow up of respectively 2 years (Phase I /NCT04205019) and 1 year (Phase II /NCT03935724).

Table 2. Overview of the reported adverse events during the study follow-up of 1 year (following the MEDRA coding).					
Patient	Event	Reported Term for the Adverse Event	Outcome of Adverse Event	Relationship to Neuro-cells	Relationship to procedure
ES-001-003	AE	headache	Recovered/resolved	Not related	Not related
ES-001-003	AE	constipation	Recovered/resolved	Not related	Not related
ES-001-003	AE	urinary infection	Recovered/resolved	Not related	Not related
ES-001-003	AE	pressure ulcer	Recovered/resolved	Not related	Not related
ES-001-005	AE	headache	Recovered/resolved	Unlikely to be related	Possibly related
ES-001-005	AE	vomiting	Recovered/resolved	Not related	Possibly related
ES-001-007	AE	headache	Recovered/resolved	Unlikely to be related	Possibly related
ES-001-013	AE	urinary infection	Recovered/resolved	Not related	Not related

Primary outcomes ISNCSCI

The ISNCSCI assessment consists of a motor scoring and a sensory scoring part (ASIA Motor score and ASIA Sensory score). The motor scoring measures the strength and resistance in the upper limbs (Fingers, hand, elbow and arm) and the lower limbs (Toes, ankle, knee and leg) and the sensory scoring measures the soft and sharp sensations of the skin of the patient.

Figure 3 and figure 4 report the changes in scores to baseline for both motor and sensory scores. Part A is the double-blind comparison between the Neuro-Cells and the placebo group, and part B is the comparison between the early and the late administered Neuro-Cells groups. There is a positive effect in the ISNCSCI scores for both the motor and the sensory scores between the Neuro-Cells treated groups compared to the placebo treated groups (only significant for the sensory scores at day 90 after the treatment with Neuro-Cells with a $p < 0.05$).

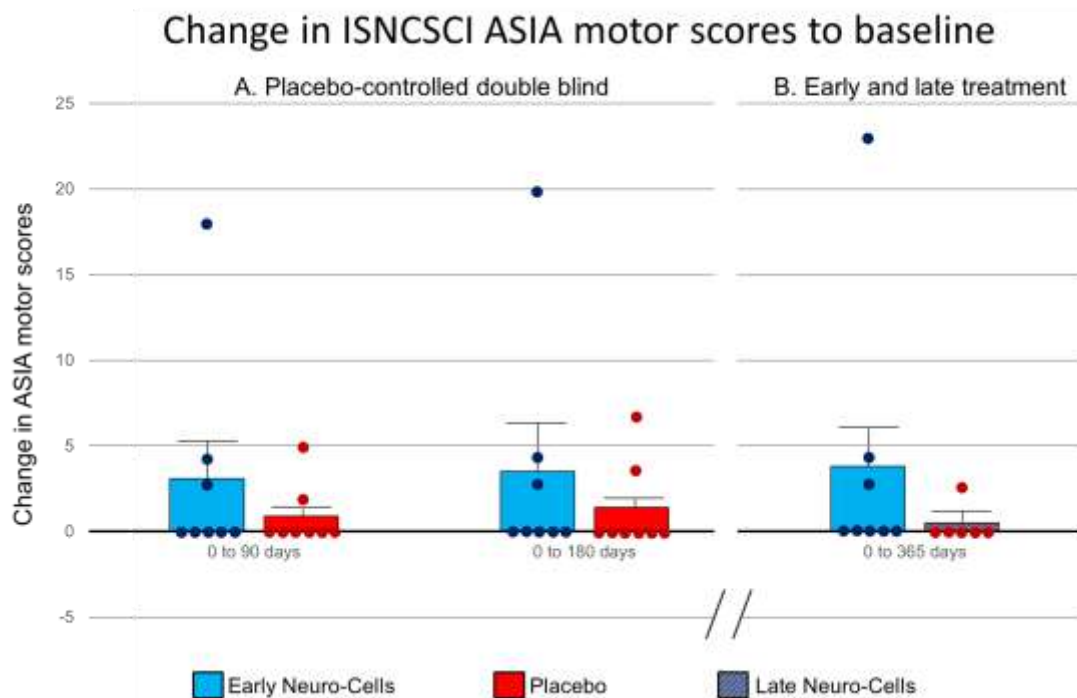


Figure 4. Change to baseline in ISNCSCI ASIA motor scores in the Neuro-Cells® and placebo treated groups (Part A) and in the early and late treatment groups (Part B).

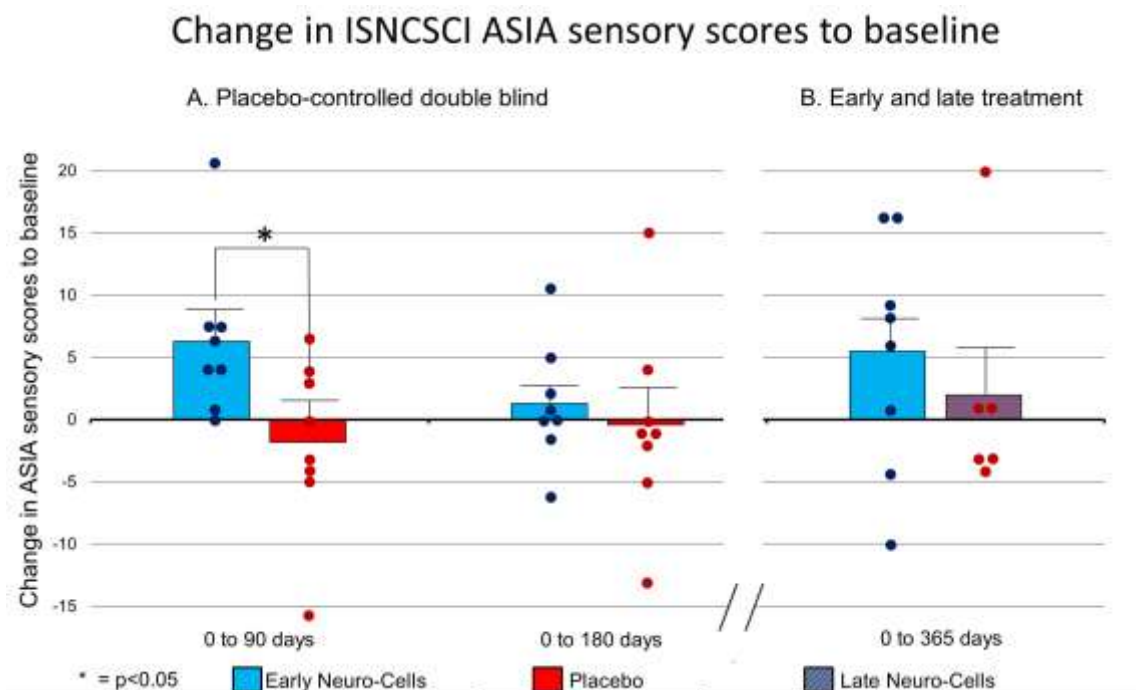


Figure 3. Change to baseline in ISNCSCI ASIA sensory scores in the Neuro-Cells® and placebo treated groups (Part A) and in the early and late treatment groups (Part B).

The difference between the late and the early Neuro-Cells treatment confirms the beneficial effect of the early application interfering with the secondary inflammation following the initial insult of the Spinal Cord Injury. Another aspect is that the positive effect following one injection of Neuro-Cells® in the beginning still gives an effect one year later.

Secondary outcomes

Important parameters impacting the quality of life of patients with a spinal cord injury are bowel and bladder functioning, spasm and neuropathic pain. The SCIM and the Quality-of-Life basic data set judge as a kind of overall scoring all the mentioned aspects resulting in dependency to caregivers and effect on mood and acceptance of the status of life of patients. Spinal Cord Injury patient group confirmed that not controlling bowel and/or bladder and taking all kind of heavy medication suppressing neuropathic pain have a bigger impact on the quality of life than being dependent on a wheelchair. Especially when patients want to participate in their community or start to work again, not controlling bowel/bladder or taking heavy pain medication limit substantially their opportunities.

Bowel functioning (SCIM and Neurogenic Bowel Dysfunction Score):

Figure 5 provides an overview of the change to baseline on bowel management and figure 6 independently provides an overview of the change to baseline of the neurogenic bowel dysfunction score in relation to early and late treatment with Neuro-Cells®. An increase in the SCIM score expresses an improvement of the condition. In contrast to the SCIM, the opposite is the case regarding the Neurogenic Bowel Dysfunction Score (the higher the score, the worse is the bowel condition).

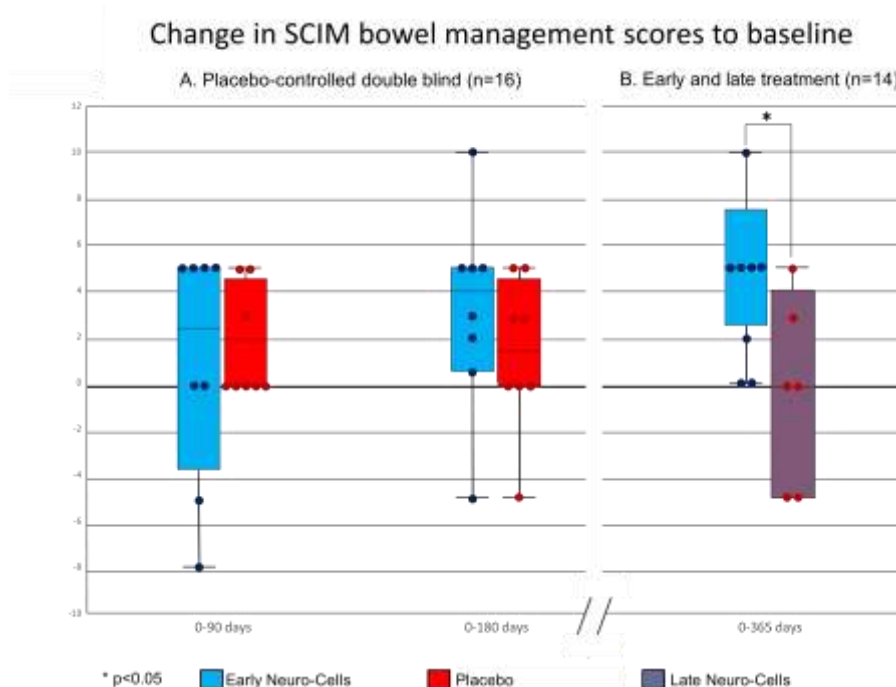


Figure 5. Change to baseline of the SCIM in the Neuro-Cells® and placebo treated groups (Part A) and in the early and late treatment groups (Part B).

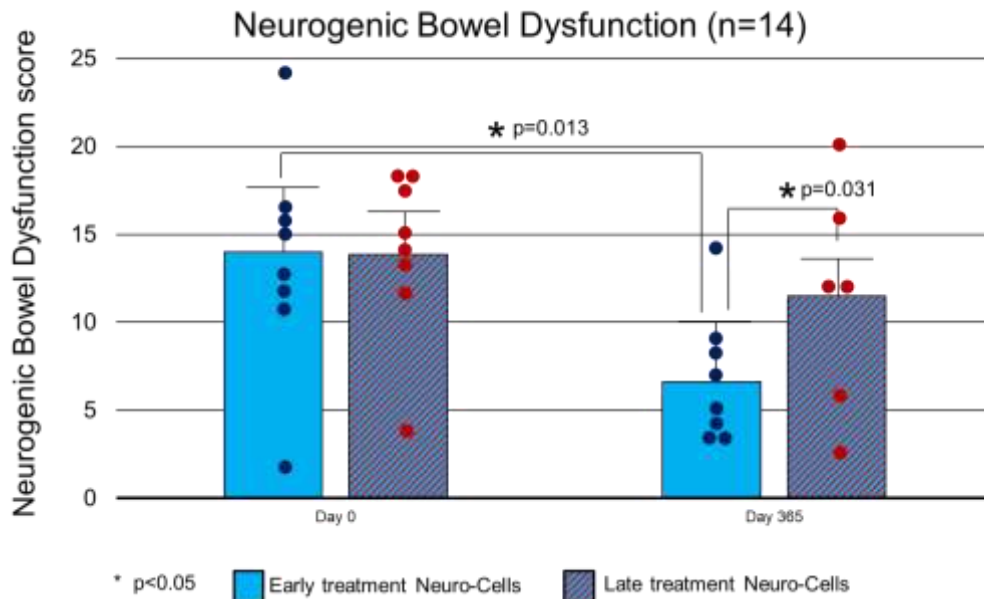


Figure 6. The Neurogenic Bowel Dysfunction score at time point 0 and at time point 365 days for the early Neuro-Cells® and the late Neuro-Cells® treatment groups. There is a significant difference between the 2 groups ($p=0.031$) and a significant change in score in the early treatment group ($p=0.013$).

Neuropathic Pain:

Figure 7 gives an overview of the change in pain sensation to baseline of the several patients groups (Neuro-Cells® compared to Placebo groups and Early Neuro-Cells® compared to Late Neuro-Cells® groups). The pain intensity score is increasing in the placebo and in the late Neuro-Cells® treatment group and is decreasing or stable in the early Neuro-Cells® treatment group. This difference is significant in favour of the early Neuro-Cells® treatment group.

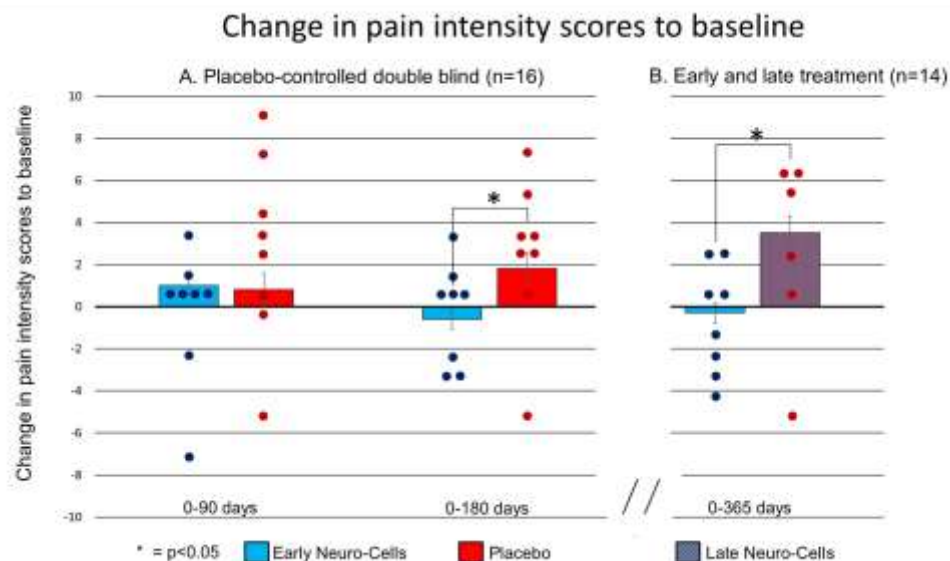


Figure 7. Change to baseline of the Pain intensity scores (Basic Pain dataset) in the Neuro-Cells® and placebo treated groups (Part A) and in the early and late treatment groups (Part B).

Modified Ashworth Scale (MAS):

Spasm is measured using the Modified Ashworth Scale (MAS) of the ankle and knee joints. The higher the score, the more rigidity caused by spasm. There is a positive effect of the Neuro-Cells® treatment compared to the placebo treated group (Figure 8 part A) and between the early Neuro-Cells and the late Neuro-Cells group (Figure 8 part B) but not significant.

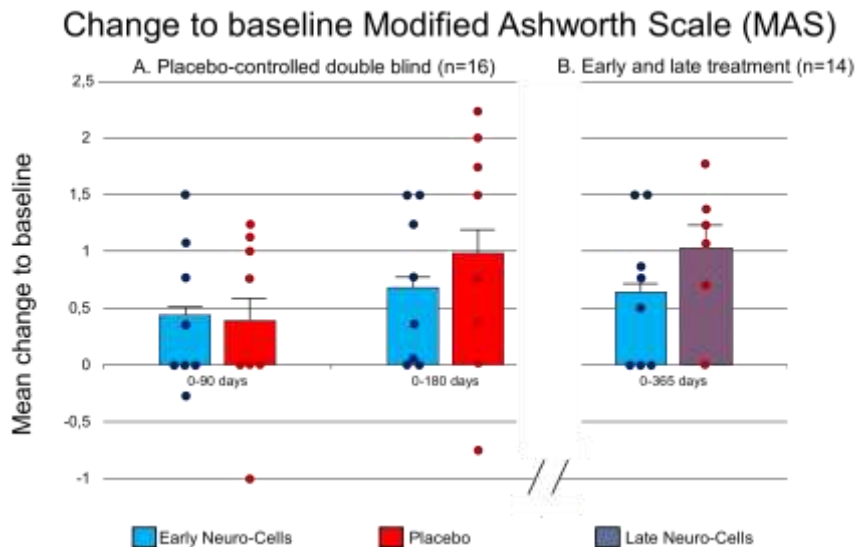


Figure 8. Change in Modified Ashworth Scale to baseline in ankle and knee joints in the Neuro-Cells® and placebo treated groups (Part A) and in the early and late treatment groups (Part B).

Quality of Life basic dataset (QoL):

Figure 9 presents the outcome of the Quality-of-Life basic dataset for Spinal Cord Injury. The differences between the groups were not significant. However, at day 365 100% of the patients treated with the early applied Neuro-Cells® reported an improved in Quality-of-Life perception to baseline compared to 50% of the patients treated with the late applied Neuro-Cells®.

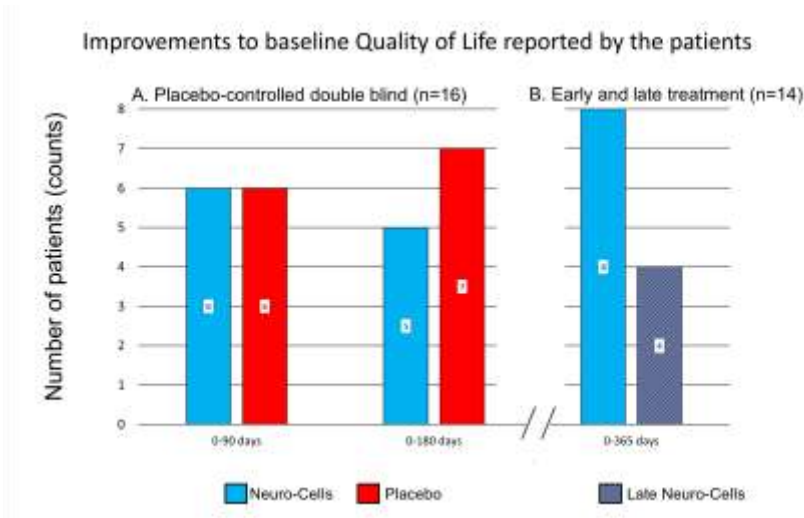


Figure 9. Change to baseline of the Quality-of-Life scores (Basic QoL dataset) in the Neuro-Cells® and placebo treated groups (Part A) and in the early and late treatment groups (Part B).

Reduced cardiovascular risk:

Finally, there was a normalisation of the heartrate and the blood pressure in the early Neuro-Cells® treated group compared to the late Neuro-Cells® treated group. In Spinal cord injured patients, there is an increased cardiovascular risk which is associated with bradycardia and hypotension. A positive effect, which was even significant for the normalisation of the heart rate was witnessed in the early treatment group at day 365 compared to the late treatment group (Figure 10).

Normalization vital functioning

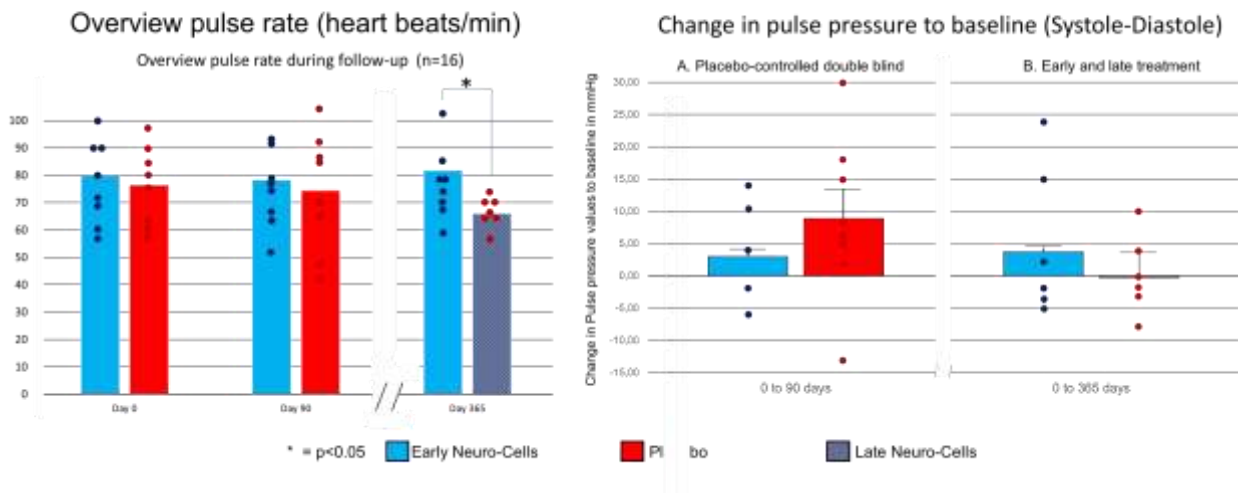


Figure 10. Tendency to normalization of the heart rate and the pulse pressure (Difference between systole and diastole in mmHg).

Summary and Conclusions

Regarding the primary endpoint there was only a significant improvement in the Asia sensory scores of more than 5 points at day 90 between the Neuro-Cells and placebo treated groups.

There was a significant improvement of the SCIM bowel management and the bowel dysfunction score between the early and late treatment group and a significant difference between the 2 groups at day 365 regarding neuropathic pain. Finally, a tendency to normalisation regarding the vital functions blood pressure and heartrate was reported in the early treatment group at day 365 compared to the late treatment group.