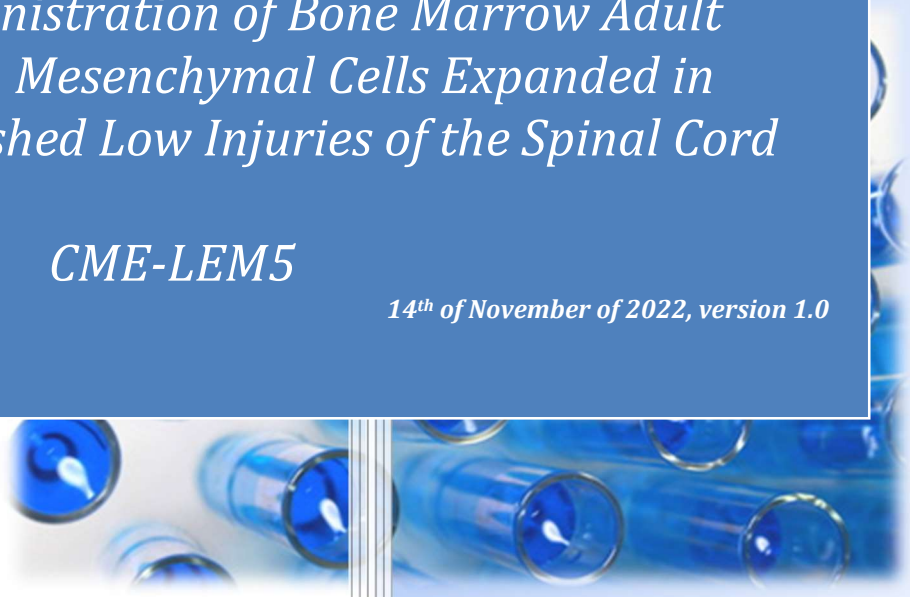
Summary of clinical trial results

Intrathecal Administration of Bone Marrow Adult Autologous Stem Mesenchymal Cells Expanded in Chronically Established Low Injuries of the Spinal Cord

CME-LEM5

14th of November of 2022, version 1.0



Foundation for Biomedical Research of the Hospital Universitario Puerta de Hierro-Majadahonda

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CONTENT.

1. Study Overview.

1.1. Study title.

Intrathecal Administration of Bone Marrow Adult Autologous Stem Mesenchymal Cells Expanded in Chronically Established Low Injuries of the Spinal Cord

1.2. Protocol code.

CME-LEC5.

1.3. Development phase.

Clinical Trial Phase II.

1.4. Description.

A phase II, single-center, non-randomized, uncontrolled, prospective open-label follow-up clinical trial has been conducted in a cohort of patients with chronically established, traumatic lower spinal cord injuries, who were administered autologous adult stem mesenchymal cells from the bone marrow. The expanded cells were administered into the subarachnoid space by lumbar puncture. The minimum duration of follow-up for each patient was 12 months after the first administration.

1.5. Research product.

Medicinal product NC1. Registered in the AEMPS (Spanish Agency of Medicines and Medical Devices) as IMP No. 12-141 and authorized as a Cell Therapy Medicinal Product of non-industrial manufacture with Authorization Number: 83796. Autologous bone marrow stromal cells expanded in vitro and suspended in autologous plasma for intrathecal administration (intramedullary and subarachnoid).

1.6. Therapeutic indication.

Spinal cord injury of traumatic or ischemic cause, chronically established and considered irreversible. For the purpose of this study, it was considered a chronically established lesion in those cases where there were no signs of functional recovery, after a minimum follow-up period of 6 months.

1.7. Sponsor.

Foundation for Biomedical Research of the Hospital Universitario Puerta de Hierro-Majadahonda. Joaquín Rodrigo St. 2. Majadahonda 28222 (Madrid, Spain).

1.8. Principal Investigator.

Principal Investigator: Dr. Jesús Vaquero Crespo.
Neurosurgery Service, Hospital Universitario Puerta de Hierro Majadahonda.
Joaquín Rodrigo St. 2, Majadahonda 28222-Madrid.

Co-Principal Investigator: Dr. Mercedes Zurita Castillo.
Cell Therapy Unit. Hospital Universitario Puerta de Hierro-Majadahonda.
Joaquín Rodrigo St. 2, Majadahonda 28222-Madrid.

1.9. Contact person(s).

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1.10. Relevant dates.

- Date of first authorization by the AEMPS: 25/09/2017
- Date of first authorization Ethics Committee of reference: 11/09/17
- Start date of the trial: 20/10/2017
- Date of inclusion of the first patient: 20/10/2017
- End date of study: 02/01/2021
- Date of the Final Report of the study: 07/04/2022

1.11. Compliance with Good Clinical Practices (GCP).

The principal investigator committed to carry out the trial in accordance with the study protocol, to follow Good Clinical Practices (GCP), WHO recommendations, the deontological code, Spanish legislation on clinical trials, and international ethical recommendations for research and clinical trials in humans contained in the international ethical declarations of Helsinki (World Medical Association Declaration of Helsinki, 59th General Assembly, Seoul, Korea, October 2008). For the execution of the study, it was evaluated and approved by the Ethical Committee for Clinical Research of Medicinal Products (IEC) and the Spanish Agency of Medicines and Medical Devices (AEMPS).

1.12. Confidentiality.

The patient's identity was kept confidential throughout the study.

The data obtained were treated in accordance with Organic Law 15/1999 on the Protection of Personal Data.

2. Study Summary.

2.1. Study title.

Intrathecal Administration of Bone Marrow Adult Autologous Stem Mesenchymal Cells Expanded in Chronically Established Low Injuries of the Spinal Cord

2.2. Name of the finished medicinal product.

Medicine NC1. Registered in the AEMPS as IMP No. 12-141 and authorized as a Cell Therapy Medication of non-industrial manufacture with Authorization Number: 83796.

2.3. Name of the active substance.

Autologous bone marrow stromal cells expanded in vitro and suspended in autologous plasma for intrathecal administration (intramedullary and subarachnoid).

2.4. Investigators.

Principal Investigator: Dr. Jesús Vaquero Crespo

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Joaquín Rodrigo St. 2, Majadahonda 28222-Madrid.

Co-Principal Investigator: Dr. Mercedes Zurita Castillo

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2.5. Study Site(s).

Hospital Universitario Puerta de Hierro Majadahonda (Madrid).

Joaquín Rodrigo St. 2, Majadahonda 28222-Madrid.

2.6. Publications.

N/A

2.7. Study period (years-months).

- Recruitment period. 1 year.
- Treatment period and estimated follow-up. 12 months.
- Maximum estimated duration per patient in the study. 12 months.
- Date of inclusion of the first patient. 20/10/2017
  End date of study. 02/01/2021.

2.8. Study development phase.

Clinical Trial Phase II.

3. Objectives.

3.1. Primary objectives.

The primary objective of the study was to evaluate the efficacy of subarachnoid administration of autologous adult spinal cord mesenchymal cells of expanded bone marrow in the sequelae of lower traumatic spinal cord injuries.

3.2. Secondary objectives.

The secondary objective of the study was to confirm the safety of the treatment, at the doses proposed in the present study.

4. Methodology.

A phase II, single-center, non-randomized, uncontrolled, prospective open-label follow-up clinical trial has been conducted in a cohort of patients with chronically established, traumatic lower spinal cord injuries, who were administered autologous adult stem mesenchymal cells from the bone marrow. The expanded cells were administered into the subarachnoid space by lumbar puncture. The minimum duration of follow-up for each patient was 12 months after the first administration.

5. Number of patients.

The number of patients planned to be included in the study was 15, a sufficient number to have a first approximation of the efficacy profile of the proposed regimen for the subarachnoid administration of bone marrow adult mesenchymal stem cells expanded as a treatment of chronically established lesions of the lower segment of the spinal cord. Although 17 patients were initially enrolled, during the course of the trial 2 of them withdrew.

Due to the exceptional situation produced by SAR-COV2 and the personal situation of the principal investigator of the trial, the treatment of patients was interrupted in March 2020 and at the end of the same year it was decided to end the trial. So far, a total of 13 patients had been treated with one dose of the medication, of whom 7 patients had received the full treatment (2 doses of the medication 6 months apart). Due to the situation caused by COVID19, of the total number of patients treated, only 4 patients were able to carry out the complete study, until the end of the final visit corresponding to month 12.

6. Diagnosis and main inclusion criteria.

6.1. Inclusion criteria.

The study subjects had to be between 18 and 70 years old, with chronically established lower spinal cord injury, in good general condition and without systemic pathologies that could condition an important risk factor to the treatment. For the purpose of this study, it was considered a chronically established lesion in those cases where there were no signs of functional recovery, after a minimum follow-up period of 6 months.

The inclusion criteria required of patients to participate in this study are listed below:

1. Spinal cord injury at low bone marrow level (below the dorsal region), clinically stable at least in the 6 months prior to recruitment.
2. Studies of clinical scales, as well as studies of neurophysiology, urodynamics, defecatory function and progress that allow to have useful baseline values, so that they could be compared with the same examinations after treatment, and obtain objective data of possible efficacy.
3. Age between 18 and 70 years old.
4. Women and men of childbearing age, for safety, should commit to using contraception measures from the time they had cells removed from their bone marrow until 6 months after the last administration of mesenchymal stromal cells (MSCs) by lumbar puncture.
5. Possibility of evolutionary follow-up and commitment to perform outpatient physiotherapy, throughout the treatment period.
6. Written informed consent, in accordance with current legislation.
7. Haematological and creatinine parameters, SGOT and SGPT, in normal range, according to laboratory standards, accepting, however, modifications that are considered non-

significant in the context of the treatment to be performed, according to the clinical criteria of the research team.

6.2. Exclusion criteria.

The exclusion criteria for this study were as follows:

1. Age under 18 or over 70 years old.
2. Pregnancy or lactation.
3. Patients with systemic disease that were considered by the research team to represent an added risk to treatment.
4. Alterations in the genetic study carried out to rule out the risk of cell transformation in the expansion process.
5. Patients with doubts about their possible cooperation in the maintenance of physiotherapy or controls during the study.
6. Added neurodegenerative disease.
7. Drug addiction or psychiatric illness, current or past, as well as current or past neoplastic disease, which in the opinion of the researchers could interfere with the study.
8. HIV and/or syphilis positive serology or allergy to protein products used in the cell expansion process.
9. Active Hepatitis B or Hepatitis C, according to the serology analysis.
10. If in the opinion of the researcher there had been any other cause for which the patient was not considered a candidate for the study.

7. Investigational medicinal product, dosage and mode of administration, batch number.

The investigational medication is NC1, registered in the AEMPS as IMP No. 12-141 and authorized as a Cell Therapy medication of non-industrial manufacture with Authorization No.: 83796. It consists of autologous bone marrow stromal cells expanded in vitro and suspended in autologous plasma at a concentration of 100,000 cells/microliter.

The administration was carried out intrathecally, in the subarachnoid space, by lumbar puncture. The total dose received by patients was 300 x 10^6 Mesenchymal Stromal Cells (MSCs), in 2 injections of 150 x 10^6 MSCs, with an interval of 6 months between each administration.

The batch numbers of the NC1 medicinal product used are specified below:

CME-MO1-066-1D, CME-MO1-066-2D, CME-MO1-067-1D, CME-MO1-067-2D, CME-MO2-068-1D, CME-MO2-068-2D, CME-MO1-069-1D, CME-MO1-069-2D, CME-MO1-071-1D, CME-MO1-071-2D, CME-MO2-072-1D, CME-MO2-072-2D, CME-MO2-075, CME-MO2-076, CME-MO1-077-1D, CME-MO1-077-2D, CME-MO1-089, CME-MO1-090, CME-MO1-092, CME-MO2-093.

Regarding the times of the study, it was considered for each patient as day 1 of the treatment that of the first cell administration. The second dose was administered 6 months after the first administration.

8. Assessment criteria.

8.1. Efficacy.

The main endpoints were those related to the possible efficacy of the treatment and were measured by:

- Modifications in the ASIA scales and their subsections, as well as in the IANR-SCIFRS, PENN, ASHWORTH, EVA, GEFFNER and BDS scales.
- Modifications in neurophysiological records (somatosensory evoked potentials, motor evoked potentials and EMG).
- Modifications in urodynamic records, defecatory function and gait.

Efficacy was assessed taking into account the variation in the score of the different scales and records throughout the study, comparing the final values with those obtained before starting treatment.

8.2. Safety.

As a secondary endpoint, we assessed possible adverse effects during the administration of MSCs together with the occurrence of complications and other adverse effects after the administration and during the follow-up period.

Foreseeable adverse events were those related to the lumbar puncture procedure and administration of autologous bone marrow cells in the intrathecal compartment of the spinal cord (subarachnoid space): slight pain at the puncture site, in patients with tenderness in the area, transient headache, and transient meningeal or radicular irritation. Likewise, foreseeable adverse events in the course of the study are those inherent to spinal cord injury, such as urine infections, appearance of pressure ulcers or transient intensifications of spasms or neuropathic pain. The research team monitored adverse events related to the treatment, including the time of onset, duration, intensity, course and outcome.

Clinical assessment of possible adverse effects was performed at the injection of the MSCs and throughout the study, during established assessment visits, or at any time when the possibility of

their existence was communicated to the research team. At each of the follow-up visits, patients or their families were questioned about the occurrence of new adverse experiences since the last visit and about the evolution of adverse events reported at previous visits.

9. Statistical methods.

9.1. Analysis criteria.

The statistical method originally proposed in the protocol is described below:

The data would be presented as absolute frequencies and percentages in the case of qualitative variables. For quantitative variables, central tendency statistics, such as mean and median, and dispersion statistics, such as standard deviation (SD) and interquartile range, would be used. In the case of ordinal variables, depending on the number of categories, one form or another of description would be used.

To evaluate the efficacy of the treatment through the evaluation of clinical and neurophysiological parameters, a descriptive analysis of the scales and subscales of ASIA, IANR-SCIFRS, PENN, ASHWORTH, EVA, BDS and GEFFNER would be performed at each of the visits. The comparison of intensity between visits would be carried out by the chi-square test or Mc Nemar's test, in case of qualitative scales, or by Student's t test for scales with quantitative score. The parameters of possible modifications of evoked, somatosensory and motor potentials, and EMG study, throughout the study, as well as the modifications, where appropriate, of the values collected in the tests of urodynamic function, defecatory function, or gait analysis, would be subject to quantitative and qualitative evaluation and would be compared with respect to baseline values, according to the same statistical treatment.

For the analysis of adverse events, a descriptive analysis of them would be carried out throughout the study, presenting a list of them, grouped according to severity, intensity and relationship with the treatment of the study. The computer program to be used would be the SPSS (Chicago, IL).

9.2. Sample size.

It was proposed to include 15 patients, a sufficient number to have a first approximation of the efficacy profile of the proposed regimen for the subarachnoid administration of expanded adult bone marrow mesenchymal stem cells as a treatment of chronically established lesions of the lower segment of the spinal cord and.

9.3. Statistical analysis.

Finally, a descriptive analysis of categorical variables was performed using absolute and relative frequencies; and in numerical variables, using the median and 25 and 75 percentiles. To evaluate the evolution of numerical variables over time, in those patients with at least two determinations, generalized estimating (GEE) equation models have been performed, setting the corresponding variable as dependent variable and time as independent variable. For each of the variables, the estimate of the effect at each time, the linear predictor at each time, and a graphical representation of that evolution in time predicted by the model were shown. The significance level was set at 0.05 for all contrasts.

The statistical package used was Stata/IC v.16 (StataCorp. 2019. Stata Statistical Software: Release 16. College Station, TX: StataCorp LLC.).

9.4. Randomization.

N/A.

10. Summary of results.

15 patients eventually remained in the study, of whom 10 were men and 5 women. The ages of the participants were as follows: 2 were between 18-25 years old, 3 between 26-35, 6 between 36-45 and 4 were 46 years old or older. The distribution of patients by Autonomous Communities was as follows: 4 patients came from Andalucia, 1 from Cantabria, 1 from Catalonia, 2 from Extremadura, 1 from La Rioja, 5 from the Community of Madrid and the rest from the Basque Country.

Due to the drawbacks produced throughout the study, only 7 patients in the study received the administration of the two doses: 2 patients from the Community of Madrid, 1 from Cantabria, 1 from Catalonia and 3 from Andalucia. Of these 7, only 4 completed follow-ups until the final visit and therefore conclusive efficacy results could not be obtained.

11. Efficacy results.

Within the statistical analysis carried out, significant differences have been detected at 6 and 12 months of evolution in the following scales and subscales:

- ASIA Total
- IANR-SCIFRS Global
- IANR-SCIFRS control of sphincters
- EVA

- Movement of lower limbs
- Rectal sphincter control

As for the ASIA LIGHT TOUCH SCORE and GEFFNER, statistical significance was achieved at 6 months, but not at 12 months. As for BDS, statistical significance was obtained only at 6 months, at 12 months the p value obtained was 0.055. Finally, in the ASIA PRICK SCORE, no statistical significance was reached, obtaining a p value of 0.058 at 12 months.

Since the statistical analysis has been performed on a very small number of patients (only 3 of the 4 patients who completed the study), it is not possible to speak of efficacy in those patients in whom statistical significance has been obtained with respect to baseline. But we could speak of a possible trend of improvement in some cases, especially when analyzing that the statistical significance is very clear in the Total ASIA scale, as in other scales that involve the assessment of pain (VAS) or sensitivity.

12. Safety Results.

During the development of this clinical trial, and until its completion, the following serious adverse events (SAEs) have been detected:

- SAE1: the patient (09/CME-LEM5) suffered a rectorrhagia and was treated with a diet rich in fiber.
- SAE2: the patient (09/CME-LEM5) suffered orchitis and the treatment administered consisted of Cefixime, Metamizole, Paracetamol and Omeprazole.
- SAE3: the patient (09/CME-LEM5) suffered a urinary tract infection and was treated with Cefixime.
- SAE4: the patient (08/CME-LEM5) required ulcer surgery and was treated with rest, analgesics and the patient's usual medication.

During the development of the present clinical trial, and until its completion, no deaths have been described.

Therefore, with the data collected from the CME-LEM5 clinical trial, with the treated patients and the completed unfinished trial, the data seem to indicate that the NC1 cell therapy medication is safe.

13. Conclusions.

The results obtained in the present clinical trial are consistent with those derived from previous studies (clinical trials CME-LEM1, CME-LEM2, CME-LEM3 and CME-LEM4) (Vaquero J et al. 2016;

Vaquero J et al, 2017; Vaquero J et al, 2018a; Vaquero J et al, 2018b; Vaquero J et al, 2018c). The doses used in patients in these trials have reached 300 million MSCs intrathecally, with no signs of drug-related adverse events. This proves once again the safety of the medication NC1 for the treatment of spinal cord injuries. On the other hand, although the efficacy results obtained from the CME-LEM5 clinical trial were not conclusive, they do give an idea of how this therapy can influence the quality of life of patients, as derived from the results obtained in terms of pain reduction, increased sensitivity or sphincter control, among others.

Finally, due to the exceptional situation produced by SAR-COV2 and the personal situation of the principal investigator of the trial, the treatment of the patients was interrupted in March 2020 and at the end of the same year it was decided to end the trial. Up to that time, a total of 13 patients had been treated with one dose of the medication, of whom 7 patients had received the full treatment (2 doses of the medication 5 months apart). Due to the situation caused by COVID19, of the total number of patients treated, only 4 patients were able to carry out the complete study, until the end of the final visit corresponding to month 12. All these alterations produced numerous deviations in the development of the clinical trial protocol so that no conclusive results can be drawn, beyond the efficacy trend derived from the statistical study carried out and the demonstrated safety of the NC1 medication.

14. Bibliography.

Vaquero J, Zurita M, Rico MA, Bonilla C, Aguayo C, Montilla J, et al. An approach to personalized cell therapy in chronic complete paraplegia: The Puerta de Hierro phase I/II clinical trial. Cytotherapy 18: 1024-1035, 2016.

Vaquero J, Zurita M, Rico MA, Bonilla C, Aguayo C, Fernández C, et al. Repeated subarachnoid administrations of autologous mesenchymal stromal cells supported in autologous plasma improve quality of life in patients suffering incomplete spinal cord injury. Cytotherapy 19: 349-359, 2017.

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15. Annexes.

N/A