Summary of clinical trial results

Intrathecal administration of autologous adult bone marrow mesenchymal stem cells expanded in the diffuse axonal injury
CME-LEC1

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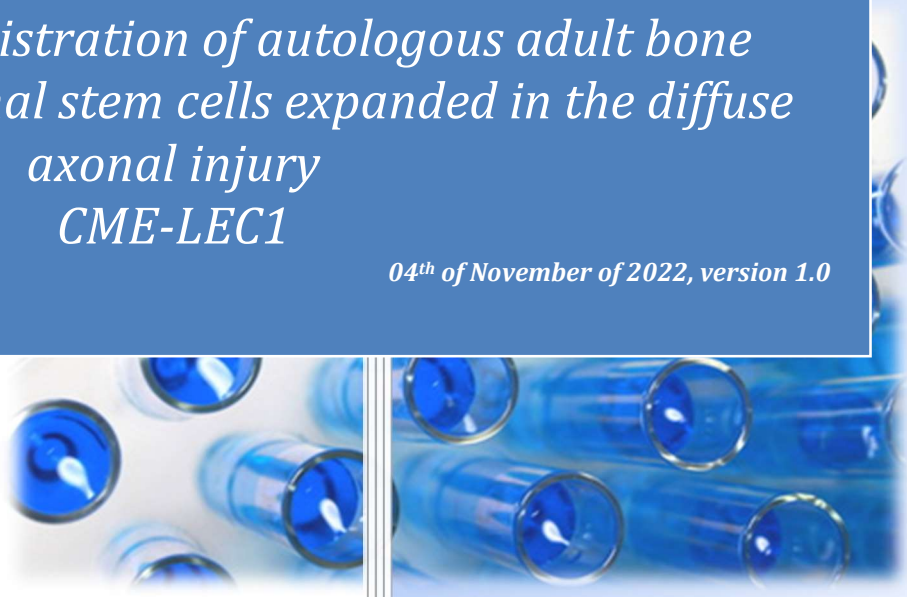

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CONTENT.

1. General study information.

1.1. Study title.

Intrathecal administration of autologous adult bone marrow mesenchymal stem cells expanded in the diffuse axonal injury.

1.2. Protocol code.

CME-LEC1.

1.3. Development phase.

Clinical trial phase II.

1.4. Description.

A Phase II, single-center, non-randomized, non-controlled, open-label, prospective follow-up clinical trial was conducted in a cohort of patients with post-traumatic brain injury and a diagnosis of chronically established diffuse axonal damage who were administered autologous adult bone marrow mesenchymal stem cells. The expanded cells were administered into the subarachnoid space by lumbar puncture. The minimum follow-up duration for each patient was 12 months after the first administration.

1.5. Research product.

NC1 (IMP No. 12-141). Autologous bone marrow stromal cells expanded *in vitro* and suspended in autologous plasma for intrathecal administration.

1.6. Therapeutic indication.

Chronically established traumatic brain injury attributed to diffuse axonal damage.

For the purpose of this study, a lesion was considered to be chronically established in those cases in which 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 University Hospital Puerta de Hierro-Majadahonda.

Joaquín Rodrigo St., 2. Majadahonda 28222 (Madrid, Spain).

1.8. Principal investigator.

Dr. Jesús Vaquero.

Neurosurgery Service, University Hospital 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: 29/11/2017
- Date of the first authorization by the ethics committee of reference: 23/10/2017
- Trial start date: 29/12/2017
- Date of inclusion of the first patient: 03/01/2018
- End of study date: 15/12/2020
- Date of the End of study Report: 17/02/2022

1.11. Compliance with Good Clinical Practices (GCP).

The principal investigator undertook to conduct the trial in accordance with the study protocol, to follow Good Clinical Practice (GCP), WHO recommendations, the code of ethics, Spanish legislation on clinical trials and the international ethical recommendations for research and clinical trials in humans contained in the international ethical declarations of Helsinki (Declaration of Helsinki of the World Medical Association, 59th General Assembly, Seoul, Korea, October 2008). For the execution of the study, it was assessed and approved by the Clinical Research Ethics Committee (REC) and the Spanish Agency of Medicines and Medical Devices (AEMPS).

1.12. Confidentiality.

The patient's identity was kept confidential throughout the entire 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 autologous adult bone marrow mesenchymal stem cells expanded in the diffuse axonal injury.

2.2. Name of the finished medicinal product.

NC1 (IMP No. 12-141).

2.3. Name of the active substance.

Autologous bone marrow stromal cells expanded *in vitro* and suspended in autologous plasma for intrathecal administration.

2.4. Investigators.

Dr. Jesús Vaquero.

Neurosurgery Service, University Hospital Puerta de Hierro Majadahonda. Joaquín Rodrigo St., 2, Majadahonda 28222-Madrid.

2.5. Study site(s).

University Hospital 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. 12 months.
- Estimated treatment and follow-up period. 12 months.
- Estimated end of study date. 15/12/2020.
- Estimated treatment duration per patient in the study. 12 months.
- Date of inclusion of the first patient. 03/01/2018.

2.8. Study development phase.

Clinical trial phase II.

3. Objectives.

3.1. Primary objectives.

The primary objective of the study was to analyze the possible clinical efficacy of intrathecal administration, in the subarachnoid space, of autologous adult bone marrow mesenchymal stem cells expanded *in vitro* in the treatment of a homogeneous group of patients with chronically established brain injury (BI) and previous diagnosis of diffuse axonal damage.

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, non-controlled, open-label, prospective follow-up clinical trial was conducted in a cohort of patients with post-traumatic brain injury and a diagnosis of chronically established diffuse axonal damage who were administered autologous adult bone marrow mesenchymal stem cells. The expanded cells were administered into the subarachnoid space by lumbar puncture. The minimum follow-up duration 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 10, as this is the minimum number of patients calculated by the Biostatistics Unit of the Institute of Biomedical Research of the Puerta de Hierro-Majadahonda Hospital, necessary to achieve a power of 90% to detect differences in related samples. Although eleven patients were initially recruited, one of them was a screening failure. However, due to the pandemic context and the personal situation of the principal investigator, at the time of the trial discontinuation in March of 2020 only a total of 6 patients had been treated.

6. Diagnosis and main inclusion criteria.

6.1. Inclusion criteria.

The study subjects should be between 18 and 70 years of age, with a chronically established brain lesion, with a diagnosis of diffuse axonal injury and without systemic pathologies that could condition an important risk factor for treatment. For the purpose of the present study, a lesion was considered to be chronically established when there were no signs of functional recovery after a minimum follow-up period of 3 months.

Patients to be included in the study had to meet the following criteria:

1. History of traumatic brain injury with cognitive sequelae and clinical diagnosis of diffuse axonal damage.
2. Clinical scale studies (GCS, FIL, ASHWORTH, GOAT, DRS) as well as neurophysiology and PET-CT studies that would provide useful baseline values, so that they could be compared

with the same examinations after treatment, and obtain objective data on possible efficacy.

3. Age between 18 and 70 years old.
4. Possibility of evolution follow-up and neuro-rehabilitation support during the follow-up period.
5. Written informed consent, in accordance with current legislation.
6. Hematological and creatinine parameters, SGOT and SGPT, in normal range, in accordance with laboratory standards, accepting, however, modifications that are not considered 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 are listed below:

1. Age under 18 or over 70 years old.
2. Pregnancy or breastfeeding.
3. Patients with systemic disease that was considered by the research team to represent an added risk to the treatment.
4. Alterations in the genetic study performed to rule out the risk of cell transformation in the expansion process.
5. Patients with doubts about their possible follow-up during the study.
6. Added neurodegenerative disease.
7. Drug addiction or psychiatric disease, current or past, as well as current or past neoplastic disease, which in the opinion of the investigators could interfere with the study.
8. Positive serology for HIV and/or syphilis or allergy to the protein products used in the cell expansion process.
9. Active Hepatitis B or Hepatitis C, according to serology analysis.
10. If in the opinion of the investigator there is any other reason why the patient is not considered a candidate for the study.

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

The investigational medicinal product is NC1, registered with the AEMPS as IMP No. 12-141. It is composed of autologous bone marrow stromal cells expanded *in vitro* and suspended in autologous plasma at a concentration of 100,000 cells/microliter.

Administration was performed intrathecally, in the subarachnoid space, by lumbar puncture. The total dose received by the patients was 300 x 10^6 Mesenchymal Stromal Cells (MSC), administered in 2 injections of 150 x 10^6 MSC, with an interval of 3 months between each administration.
The batch numbers of the NC1 medicinal product used in this clinical trial are specified below: CME-MO1-073-1D, CME-MO1-073-2D, CME-MO1-078-1D, CME-MO1-078-2D, CME-MO1-082-1D, CME-MO1-082-2D, CME-MO1-085-1D, CME-MO1-085-2D, CME-MO1-086-1D, CME-MO1-086-2D, CME-MO2-087-1D, CME-MO2-087-2D.
Regarding the timing of the study, month 1 of treatment for each patient was considered to be that of the first cell administration. The second dose was administered 3 months after the first (month 3). At months 3, 6, 9 of follow-up and at the end of the study (month 12), a complete assessment of the clinical variables collected throughout the study was performed.

8. Assessment criteria.

8.1. Efficacy.

The main efficacy variable was based on the modification of the initial neurological deficit at the end of the follow-up period, assessed by means of clinical scales: Disability Rating Scale (DRS), Glasgow Coma Scale in adults (GCS), Galveston test (GOAT), ASHWORTH and Functional Independence Level Scale (FIL), as well as neurophysiological and brain metabolism studies (TAC-PET). An efficacy assessment was planned to be performed by means of the variation in the scores of the different scales and registers throughout the study, comparing the final values with those obtained before starting treatment.

8.2. Safety.

As a secondary variable, we took into account the possible adverse effects during the administration of MSCs, the appearance of complications and other adverse effects after administration and during the follow-up period. Adverse events related to the lumbar puncture procedure and administration of autologous bone marrow cells in the intrathecal compartment of the spinal cord (subarachnoid space) were considered foreseeable adverse events: slight pain in the puncture area, in patients with sensitivity in the area, transient headache, and transient meningeal or radicular irritation. The research team followed up on treatment-related adverse events, recording the time of onset, duration, intensity, course and outcome.

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

possibility of their existence was reported to the research team. At each of the follow-up visits, patients or their relatives 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: In general, 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 or another form of description would be used.

To assess treatment efficacy by assessing clinical and neurophysiological parameters, a descriptive analysis of the GCS, DRS, FIL and Ashworth scales and subscales would be performed in each of the visits described in the section, and the intensity between visits would be compared using the chi-square or Mc Nemar test, in the case of qualitative scales, or Student's t-test for scales with quantitative scores.

The parameters of possible changes in evoked, somatosensory and motor potentials would be assessed quantitatively or qualitatively and compared with baseline values, according to the same statistical treatment.

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

9.2. Sample size.

The number of patients planned to be included in the study was 10, as this is the minimum number of patients calculated by the Biostatistics Unit of the Institute of Biomedical Research of the Puerta de Hierro-Majadahonda Hospital, necessary to achieve a power of 90% to detect differences in related samples.

9.3. Statistical analysis.

A descriptive analysis was made of the 6 patients who received the intervention. Categorical variables were described by absolute and relative frequencies and numerical variables by median and 25th and 75th percentiles. The evolution over time of each patient was described using graphical methods for each scale assessed. Given the small sample size, no statistical inference analysis was performed. The software used was Stata v.17.

9.4. Randomization.

N/A.

10. Summary of results.

All 6 patients who finally completed the study were male. The ages of the participants were as follows: two of them between 18-25 years, 3 between 26-35 and the last one 46 years or older. As for the distribution by autonomous community, 4 of the 6 patients belonged to the Community of Madrid, one came from Aragon and the remaining one from Catalonia.

With the data obtained to date from these 6 patients treated with the medicinal product NC1, no conclusive results about the therapy have been obtained.

11. Efficacy results.

With the data analyzed from the study, given that it was not possible to complete the number of patients initially recruited due to complications generated by the COVID 19 pandemic and the illness and death of the study's principal investigator, it is not possible to draw conclusions about efficacy with the data obtained from the CME-LEC1 clinical trial.

12. Safety results.

During the development of the present clinical trial, and until its completion, no Adverse Events have been described by patients or their relatives. There have also been no deaths, other serious adverse events and/or other significant adverse events. This indicates that, with 6 patients treated and the trial not fully completed, the cell therapy medicinal product NC1 is safe for treatment of diffuse axonal damage.

13. Conclusions.

The exceptional situation caused by SARS-CoV2, together with the personal situation of the trial's principal investigator, meant that the treatment of the CME-LEC1 patients was interrupted in March 2020, and at the end of that year it was decided to terminate the trial. Up to that moment, a total of 6 patients had been treated, of which 4 could not receive the face-to-face follow-up visits required by the trial protocol, due to the health safety measures established at that time. All these alterations produced numerous deviations in the development of the clinical trial protocol. From the results obtained to date, after the closure of the CME-LEC1 clinical trial, no conclusive results can be obtained due to the disruption produced by SAR-COV2, as mentioned above.

The few conclusions that can be drawn from the treatment of NC1 for Diffuse Axonal Damage (DAD) are related to the follow-up and safety of the treatment. Patients have been followed up on some occasions for more than 12 months after the administration of the first dose, with no serious adverse events that could indicate any contraindication of the treatment either in the short or long term. This indicates that, with 6 patients treated and the trial not fully completed, the cell therapy medicinal product NC1 is safe for the treatment of DAD. These results have already been corroborated in the pharmacovigilance studies carried out since the beginning of the first clinical experiences developed by the Unit and collected by the AEMPS, which show the biosafety of the NC1 medicinal product. More than 200 intrathecal administrations have been performed and signs of therapeutic efficacy have been obtained on both complete and incomplete spinal cord injuries (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 the trials have reached 300 million MSC intrathecally, with no signs of adverse events related to the NC1 medicinal product. Although no significant results could be obtained from the CME-LEC1 clinical trial, with 6 patients treated with the NC1 medicinal product, one of the few conclusions that can be drawn, together with the Unit's experience, is that therapy with the NC1 medicinal product to treat the sequelae of DAD is a safe treatment.

14. Bibliography.

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improve quality of life in patients suffering incomplete spinal cord injury. Cytotherapy 19: 349-359, 2017.

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15. Annexes.

N/A