SUPPLEMENTARY MATERIAL: ***Data about the NC1 medicament***

INTRATHECAL CELL THERAPY WITH AUTOLOGOUS BONE MARROW STROMAL CELLS AS A NEW TOOL FOR NEUROLOGIC SEQUELS AFTER SPINAL CORD SURGERY: A REPORT OF TWO CASES.

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NC1 medicament

We used the NC1 medicament. It is a cell therapy medicament developed after preclinical studies by our group and currently approved by the Spanish Agency of Medicament and Health Products (AEMPS). The medicament consists of autologous MSCs and autologous plasma as its excipient. Previous to NC1 preparation, a sample of peripheral blood was retrieved from each patient for genomic studies in order to rule out chromosomal abnormalities that could discourage cell expansion, and to obtain a genetic fingerprint (KaryoNIM Stem Cells and KaryoNIM STR test, respectively, NIMGenetics, Madrid, Spain).

The genetic studies included: 1) Analysis of the cellular genome by techniques Array CGH to ensure that the starting cells are genetically stable and the expansion process produces no genetic modification. 2) Analysis of genetic fingerprinting (STR, Short Tandem Repeat) with the aim of having no cross contamination with other cells in the manufacturing process. The genetic fingerprint analysis was done by the external laboratory NIMGenetics, with a panel of 20 markers + amelogenin. The panel was amplified by multiplex PCR using the PCR kits PlusTM AmpFlSTR® Identifiler Amplification Kit and / or PCR ™ NGM AmpFlSTR® Amplification Kit. The analysis by capillary electrophoresis of the amplified products was performed on an ABI automated sequencer 3100 - Avant.

For the analysis of the profiles obtained, Gene Mapper software v 3.2.1 was used. The analysis of possible genetic abnormality was performed by the external laboratory NIMGenetics using

a platform CGH Array (KaryoNIM STEM) designed by the same laboratory (NIMGenetics) and optimized for use in cell therapy projects. This platform consists of 60,000 probes distributed throughout the genome with a probe designed each 60 Kb and enables efficient detection of amplifications and deletions over 200Kb and analyze in detail 407 genes related to genomic instability and abnormal proliferation according with the Cancer Gene Census list. It has an enrichment probes designed specifically for the detection of 395 cancer-related genes, included in the Cancer Gene Census list (genes for which have been described causal mutations involved in cancer) with an average of 5 probes per gene. The platform includes 15 specific probes for detecting each of the 23 oncogenes commonly used in clinical diagnosis (see table at the end of the text). In addition, there are probes designed specifically for the detection of six genes associated with stem cells. The minimum degree of mosaicism detected by array CGH is 20% -30%. The scanning process allows the use of arrays 8x60 k with a resolution of 2 microns.
For obtaining the excipient, as a first step in the preparation of the NC1, we start with the removal of 500 mL of peripheral blood from each patient. In our cleanroom, blood was centrifuged at 900g for 8 minutes to obtain the plasma fraction, which is aliquoted in 15 ml tubes and stored at -80 ° C until the medicament formulation.
Approximately two weeks later, 50 mL of bone marrow was aspirated under aseptic conditions from the iliac bones of each patient, immediately anticoagulated by a 5 mL solution composed by 100 IU/mL sodium heparin Chiesi (ChiesiEspaña, L'Hospitalet de Llobregat, Spain) and 10^4 IU / 10^4 µg penicillin-streptomycin (BioWhittaker-Lonza, Madrid, Spain) and sent to our cleanroom for culture and expansion under good manufacturing practice (GMP).
Mononuclear cells (MNC) were separated by density gradient, using an automated cell-processing system (SEPAX, BioSafe, Madrid, Spain). Then, they were plated at a density of 16 x 104 to 20 x 104 cells / cm^2, in 175 cm^2 flasks on Alpha-Minimum Essential Medium (MEM) with Earle's Balanced Salt Solution (BSS), and supplemented with 20% prion-free LGC standard serum (SLU ATCC.SCRR-3020, lot 63753841 (Salvador Spriu, Barcelona, Spain), and 10^4 IU / 10^4 µg penicillin-streptomycin (BioWhittaker-Lonza). The cultures were maintained at 37ºC in a humedified 5% CO_2 atmosphera for 3 days, after which nonadherent cells were removed by replacing the medium. When the cultures approached confluence (90-100%), adherent cells were detached by treatment with trypsin/ethylenediaminetetraacetic acid (EDTA) solution (BioWhittaker-Lonza). Neutralization of trypsin and subsequent washing was performed with Alpha-MEM medium supplemented with 10% FBS and 2 mM L-glutamine, centrifuging at 1,250 rpm for 10 minutes. After study of viability, cells were cultured to obtain the required number according to the plan previously made for each patient. Cells were replated at a density of 3,000-5,000 cells / cm^2 in factory farming of 4 floors with free-antibiotic Alpha-

MEM medium supplemented with 10% FBS and 2 mM L-glutamine, and the culture was maintained renewing the medium every 3-4 days until a confluence of 90-100%. Once the culture reached confluency, it was prepared to obtain the bulk of MSCs. At this time, MSCs were detached with trypsin/EDTA and washed with Hank's BSS medium (BioWhittaker-Lonza) supplemented with 5% albumin (20% albumin Grifols, Barcelona, Spain). After it, MSCs were resuspended with the previously obtained autologous plasma in order to remove traces of the washing medium. After cell counting, MSCs for the successive doses were separated and then cryopreserved, in 1.8 mL cryotubes at a concentration of 2.2 x 10^6 cells / mL, in a FBS solution with 10 % dimethylsulphoxide (DMSO) (Miltenyi Biotec, Madrid, Spain). For it, we used the standard technique of cryopreservation in isopropanol chamber. Finally, the MSCs for surgical administration were formulated, according to the number scheduled for each patient, after a new centrifugation at 1,250 rpm for 10 minutes.

To prepare the successive doses, cryopreserved MSCs were thawed in a thermostatic bath at 37 ° C, washed with antibiotic-free Alpha-MEM medium supplemented with 10% FBS and 2 mM L-glutamine, and centrifuged at 1,250 rpm for 10 minutes. After it, a cell count was performed and MSCs were plated at a concentration of 10,000-15,000 MSCs / cm^2 in 175 cm^2 culture flasks with antibiotic-free Alpha-MEM medium supplemented with 10% FBS and 2 mM L-glutamine, in order to reach a confluence of 90-100% over a period of 4-5 days, and then we proceeded in the same way as with the first MSCs dose.

Formulation and packaging

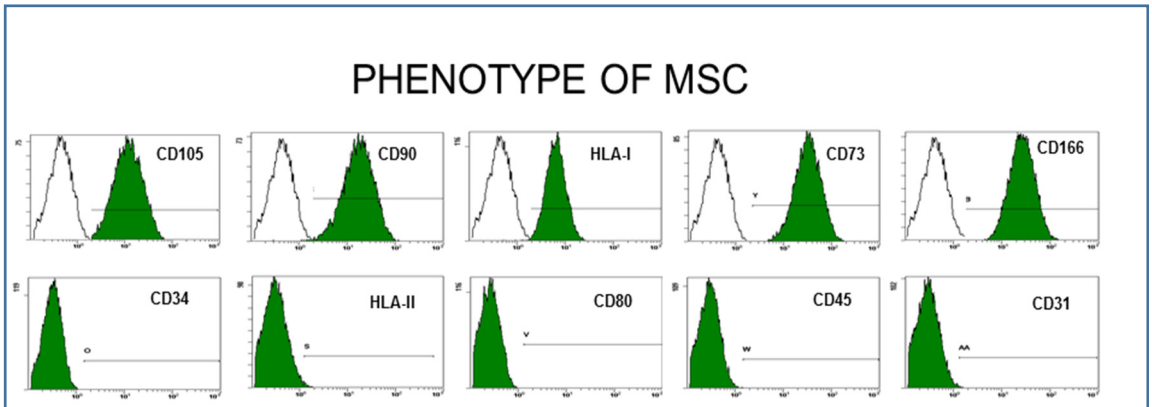
After obtaining the MSCs for first or successive dose administration, they were resuspended in the autologous plasma at a cell concentration of 10^4 cells / microliter. After formulation, the cell therapy medicament was packaged in sterile and endotoxins-free 1 mL-Hamilton microsyringes, with a 20G needle. Subsequently the needle is removed and a sterile luer plug nut was placed on the end of each preloaded syringes. Microsyringes with the medicament were placed inside a sterile metal box, which is also double bagged before being transported for cell administration.

Phenotypic characterization of MSCs

For phenotypic characterization of MSCs, monoclonal antibodies conjugated with different fluorochromes (Fluorescein (FITC) / Phycoerythrin (PE) / Alexa-647 (AL-647) which combine a number of both positive and negative MSCs membrane markers, were used. Positive markers used were CD105 FITC (R&D Systems,Minneapolis, USA); CD90 AL-647

(AbDSerotec,Oxford, OX5 1GE, UK); HLA Class I FITC (Cytognos, Salamanca, Spain); CD73 PE (BD Bioscience, New Jersey, USA) and CD166 PE (R&D Systems). Negative markers used were CD34 PE (BD Bioscience); HLA class IIPE (Cytognos); CD80 AL-647 (AbDSerotec); CD45 FITC (Cytognos) and CD31 FITC (Cytognos). Furthermore, suitable isotopic controls for FITC, PE (Cytognos) and AL-647 (AbDSerotec) respectively, were used as controls for specificity of the monoclonal antibodies.

The labeled cells were acquired with a flow cytometer FC500 MPL Cytomics (Beckman Coulter, California, USA) using the MXP software (Beckman Coulter). Nonviable cells were discarded using the labeling reagent LIVE&DEAD (Invitrogen, California, USA), and he collected data analyzed with the CXP analysis software, version 2.1 (Beckman Coulter). Criteria for the administration of MSCs in our present clinical trial included a viability greater than 95%, absence of microbial contamination (bacteria, fungus, virus, or mycoplasma), expression of CD105, CD90, HLA I, CD73 and CD166 for more than 90% of cells, and absence of CD34, CD80, HLA II, CD45 and CD31 (expression of each less than 5%), as assessed by flow cytometry (Supplementary Figure).



MARKER	CD105	CD90	HLA-I	CD73	CD166	CD34	CD80	HLA-II	CD45	CD31
REFERENCE PHENOTYPE	POSITIVE	POSITIVE	POSITIVE	POSITIVE	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
PHENOTYPE FOUND	POSITIVE	POSITIVE	POSITIVE	POSITIVE	POSITIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE	NEGATIVE
CRITERION	95% ± 5%	95% ± 5%	95% ± 5%	95% ± 5%	95% ± 5%	2.5% ± 2.5%	2.5% ± 2.5%	2.5% ± 2.5%	2.5% ± 2.5%	2.5% ± 2.5%

Supplementary Figure S1. Phenotypic characterization of the MSCs in our cell therapy medicament. MSCs showed expression of CD105, CD90, HLA I, CD73 and CD166 for more than 90%, and showed absence of CD34, CD80, HLA II, CD45 and CD31 expression (less than 5% for each).