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Study Number/Acronym	STEMS
Study Title	Neural Stem Cell Transplantation in Multiple Sclerosis Patients: a phase I study

Version number	1.2	**Date**	10 March 2017
Compound	human Neuronal Progenitor/stem Cells (hNPCs)		
EudraCT number	2016-002020-86		

Signature:

Herewith I confirm that I read the study protocol carefully and declare my consent with it. I will treat and examine the patients in accordance with the study protocol, the national applicable laws, the international guidelines on good clinical practice (ICH-GCP) and the Declaration of Helsinki.

Date: **10 March 2017**

Sponsor Representative: Prof. Giancarlo Comi

PI: Prof. Gianvito Martino

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Table of Content

1 Synopsis

1.1 General Information

Title (Acronym)	STEMS
Sponsor	Ospedale San Raffaele
Principal Investigator	Gianvito Martino
Sponsor Representative	Giancarlo Comi
Objectives **Primary Objective**	The primary objective of the study is to evaluate the feasibility, safety and tolerability of intrathecally administered hNPCs, at an escalating dose ranging from $0.7x10^6$±10% cells to $5.7x10^6$±10% cells/kg of body weight, in patients affected by progressive MS.
Endpoints **Primary Endpoints**	The following safety endpoints will be used to address the primary objectives: a) SHORT TERM (0-24 hours) - overall survival - overall safety and tolerability measured by AE recording - changes in neurological conditions not related to disease - proportion of successful intrathecal administration procedure (feasibility) Day 0 = hNPCs administration b) MID TERM (day 1-day 14) - overall survival - overall safety and tolerability measured by AE recording - changes in neurological conditions not related to disease c) LONG TERM (day 15 - week 96) - overall survival - overall safety and tolerability measured by AE recording - long term incidence of malignancies - percentage of subjects with treatment–related mortality defined as death due to procedure and not to the course of disease - evaluation of changes in quality of life measures - changes in neurological conditions
Research Endpoints	The research endpoints are evaluated to obtain preliminary data on the effect of hNPCs in patients affected by progressive MS.

Criteria of assessment for safety/efficacy/effectiveness	The safety profile will be monitored from the baseline visit to the last follow-up visit. The SHORT TERM safety profile will be evaluated trough: - Vital signs**:** During the injection of hNPCs vital signs including oxygen saturation will be monitored before and for 24 hours after hNPCs injection. - Physical and Neurological examination will be performed before the hNPCs injection and before the discharge of the patient. - Cerebrospinal fluid examination (CSF): a CSF examination will be performed in concomitance with hNPCs administration. - Routine Clinical Laboratory tests will be performed before the hNPCs injection and before the discharge of the patient. MID TERM (DAY 1 – DAY 14) safety profile will be evaluated trough: - Vital signs including oxygen saturation will be monitored during visit at week 1 and week 2. - Physical and Neurological examination will be performed during visit at week 1 and week 2. - examination: a neurological examination will be performed during visit at week 1 and week 2. - Haematological evaluation: will be performed during visit at week 1 and week 2. - Routine Clinical Laboratory tests will be performed during visit at week 1 and week 2 (see appendix for detailed tests). The LONG TERM safety profile will be evaluated trough: At every scheduled visit the following procedures will be performed: - Vital signs - Physical examination - Neurological examination - Clinical Laboratory tests - Haematological evaluations - Instrumental follow-up: • Week 48 and week 96 electrocardiogram, chest Xray; • Week 96 abdominal ultrasound. - Administration of MsQuol54 will be performed at 24, 48 and 96 weeks. - Brain and spinal cord MRI with gadolinium: will be performed at 12, 24, 48 and 96 weeks. - Cerebrospinal fluid examination (CSF): a CSF examination will be performed 12 weeks ± 2 weeks after hNPCs administration.

Protocol design	This is a prospective, monocentric, national, therapeutic exploratory, phase I, not randomized, open label, not controlled, single dose escalation clinical trial
Experimental drug description (dose/regimen/etc)	The Drug Product (DP) can be classified as an Advanced Therapy Investigastional Medicinal Product (ATIMP) belonging to the class of Cell Therapy Medicinal Products. The ATIMP consists of human Neural Precursor Cells (hNPCs) re-suspended in their final formulation medium as defined in IMPD. The product will be administered intrathecally through lumbar puncture. **Dosage indications** The protocol will consist of a total of four TC (from A to D), each of which receiving a single escalating dose of hNPCs injected intrathecally as it follows: - TC-A: $0.7x10^6$ ± 10% cells/kg of body weight; - TC-B: $1.4x10^6$ ± 10% cells/kg of body weight; - TC-C: $2.8x10^6$ ± 10% cells/kg of body weight; - TC-D: $5.7x10^6$ ± 10% cells/kg of body weight.
Main selection criteria:	**Inclusion criteria** 1. Signature of the Informed Consent Form (ICF) by the patient or patients' legal tutors 2. Age 18 to 55 years 3. Diagnosis of a. Progressive MS as per the revised MC Donald 2010 criteria with a progressive course according to 2013 Lublin phenotypes classification (PMS) with failure or intolerance to all approved therapies according to the disease course or without any alternative approved therapy 4. Evidence of progression of the disease defined by an increase of ≥ 0.5 EDSS points in the last 12 months 5. Disease duration 2 to 20 years (included) 6. EDSS ≥ 6.5 7. Presence of oligoclonal band in the cerebrospinal fluid (CSF) is required for Primary Progressive MS

	Exclusion criteria They will be excluded from the study patients: 1. with any active or chronic infection or diseases other than MS, including but not limited to infection with HIV1-2, Hepatitis B or Hepatitis C and tuberculosis or immune deficiency syndromes; 2. treated with any immunosuppressive therapy, including but not limited to natalizumab and fingolimod, within the 3 months prior to screening; 3. treated with interferon-beta or glatiramer acetate within the 30 days prior to screening; 4. treated with corticosteroids within the 30 days prior to screening; 5. if relapse occurred during the 30 days prior to screening; 6. with contraindications for or intolerance to any medication, treatments and procedures that will be used in the study; 7. pregnant or in lactation or of childbearing age who are not willing to use a contraceptive method effective* for the entire duration of the study; 8. who, in the opinion of the investigator, showing any condition that would preclude study participation. * refer to guideline http://www.hma.eu/fileadmin/dateien/Human_Medicines/01_About_HMA/Working_Groups/CTFG/2014_09_HMA_CTFG_Contraception.pdf
Sample size	Max 24 patients (max 6 patients for each cohort)
Accrual/duration timeline	Start: 2017 Recruitment period: For each subject the study phase will be completed after 96 weeks follow up after ATIMP administration.
Statistical considerations	Descriptive statistics (including means, standard deviations, and medians for continuous variables and proportions for discrete variables) will be used to summarize data as appropriate. A statistical analysis plan (SAP) with detailed statistical methods and techniques used for data analysis will be prepared before conducting the analysis.

1.1.1 FLOW-CHART STUDIO CLINICO

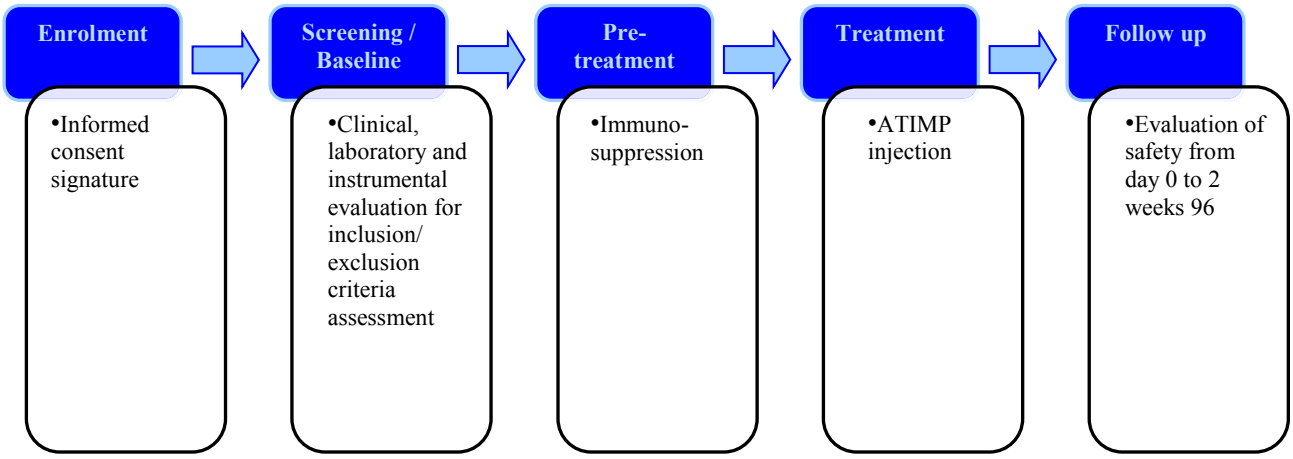


Figure 1

1.2 Treatment and Assessment Schedule

Tests and assessment	Screening/ Baseline (from -28th to -7th day)	Pretreatment (from end of screening phase to day -1)		Day 0 hNPCs Injection	V1 Week 1	V2 Week 2	V3 Week 3	V4 Week 4	V5 Week 8	V6 Week 12	V7 W 16	V8 W 20	V9 Week 24	V10 W 30	V11 Week 36	V12 W 42	V13 Week 48	V14 W 54	V15 Week 60	V16 W 66	V17 Week 72	V18 W 78	V19 Week 84	V20 W 90	V21 Week 96	V22 Week 104 FU
		Day 1 (Friday)	Day-1																							
Informed Consent	X																									
Inclusion / exclusion criteria	X	X																								
Medical History	X																									
General Physical examination with Height and Weight	X	X		X[1]	X	X	X	X	X	X			X		X		X		X		X		X		X	X
Vital Signs[2]	X	X	X	X[3]	X	X	X	X	X	X			X		X		X		X		X		X		X	X
Routine Clinical Laboratory tests	X[4]	X[5]	X[6]			X[5]		X[4]	X[5]	X[4]	Emo 1	Emo 1	X[4]	Emo 1	X[5]	Emo 1	X[4]	Emo 1	X[5]	Emo 1	X[5]	Emo 1	X[5]	Emo 1	X[4]	X[5]
VitB12/folates/TSH	X																X								X	X
Infectivological screening	X[7]							X[8]		X[8]			X[8]				X[8]								X[8]	X[8]
Neurological examination with EDSS	X		X					X		X			X		X		X		X		X		X		X	
Haematological evaluation	X	X						X		X			X		X		X		X		X		X		X	X
Research Samples/ Immunological analysis[9]	X			X				X		X					X										X	
Pregnancy Test	X[15]		X					X		X			X		X		X		X		X		X		X	
ECG	X												X				X								X	
Chest X ray	X																X								X	
Abdominal Ultrasound	X																								X	
Treatment – Tacrolimus and blood levels monitoring[10]		X----------patient continues treatment----------X																								
Prophilactic Treatment[11]		X----------patient continues treatment----------X																								
Treatment – Prednisone		X----------decalage[12]----------X																								
CSF examination				X[13]						X																
Treatment – hNPCs				X																						
Brain MRI	X									X[14]			X				X								X	
Spinal Cord MRI	X									X[14]			X				X								X	

T25FW, SDMT, 9-HPT	X												X				X				X				X	
OCT, VEP, SEP, MEP	X												X				X								X	
MSQol		X											X				X				X				X	
Concomitant Medication revision	X	X	X	X	X	X	X	X	X	X			X		X		X		X		X		X		X	X
AEs, SAEs record		X	X	X	X	X	X	X	X	X			X		X		X		X		X		X		X	X
Relapse Assesment		X	X	X	X	X	X	X	X	X			X		X		X		X		X		X		X	X

Table 1

1: General Physical examination on Day 0 will be performed before hNPCs Injection and the day after before discharge. 2: Pulse rate, systolic and diastolic blood pressure, respiration rate and axillary temperature. 3: Vital signs (pulse rate, systolic and diastolic blood pressure, respiration rate and axillary temperature) will be obtained before hNPCs injection and then after 15', 60' and 3 hours after the end of injection. 4: Complete blood cell count; Extensive clinical laboratory evaluations: PCR, LDH, Total Protein, albumin, IgG, IgA, IgM, AST, ALT, GGT, ALP, Na+, K+, Ca++, urea, uric acid, alkaline phosphatase, Total Bilirubin, Direct Bilirubin, Glucose, creatinine, creatinine clearance, ferritin, Serum Iron, transferrin, transferring saturation, PT, PTT, urin analyisis. 5: complete blood cell count, blood chemistry and urin analysis: PCR, AST, ALT, GGT, Na+, K+, Ca++, Total Bilirubin, Direct Bilirubin, Glucose, creatinine, creatinine clearance, LDH, urin analyisis; 6: Blood chemistry (note 5) will be performed at day -1 only if not performed within seven days. 7: infectivological panel at screening: HBs-Ag, HBs-Ab, HBe-Ag, HBc-IgG, HBc-IgM, HBV-DNA, HCV-Ab, HCV-RNA, HIV-Ab, VZV-IgG, VZV-IgM, HSV1-IgG, HSV1-IgM, EBV-IgG, EBV-IgM, EBV-DNA, CMV-IgG, CMV-IgM, CMV-DNA, Toxo-IgG, Toxo-IgM, HSV2-IgG, HSV2-IgM, Galactomannan detection, TPHA, Quantiferon. 8: infectivological panel during follow-up: CMVDNA, EBV-DNA Galactomannan detection. 9: explained in detail in the main test, overall 25 mLs heparinized blood+ 5 mLS serum. 10: tacrolimus: will be administered as explained in the text and monitored as explained in the specified chapter. 11: prophilactic treatment are explained in the specified chapter. 12: Oral prednisone (deltacortene) will be administered only from day -1 till week 4 as explained in the text. 13: CSF examination will be performed at the same time of hNPCs administration. 14: At week 12 we will perform a brain and total spinal cord MRI with gadolinium for safety reason heparinized blood+ 5 mLS serum. 15: The pregnancy test will be performed always by urine, except the control before inclusion that will be done by serum. Emo1 = Complete blood cell count

2 Lists

2.1 Abbreviations and definitions

A	
ADR	Adverse Drug Reaction
AE	Adverse Event
Anti-CD 20	Rituximab
ATIMP	Advanced Therapy Investigational Medicinal Product
B	
BMSCs	Bone Marrow Stem Cells
C	
CBMP	Cell-Based Medicinal Product
CMV	Cytomegalovirus
CNS	Central Nervous System
CRF	Case Report Form
CRP	C-reactive protein
CSF	Cerebrospinal fluid
CTCAE	Common Terminology Criteria for Adverse Events
D	
ddPCR	droplet digital Polymerase Chain Reaction
DMDs	Disease-Modifying Drugs
DMSO	Dimethyl Sulfoxide
E	
EAE	Experimental Autoimmune Encephalomyelitis
EBV	Epstein Barr Virus
EDSS	Expanded Disability Status Scale
rh-EGF	recombinant human Epidermal Growth Factor
EKG	Electrocardiography
ESV	Erythrocyte Sedimentation Rate/Velocity
F	
rh-bFGF	recombinant human basic Fibroblast Growth Factor
FK506	Tacrolimus
FSS	Functional System Scores
G	
GA	Glatiramer Acetate
Gd	Gadolinium
GMP	Good Manufacturing Practice
H	
hNPCs	human Neural stem/Precursor Cells
HSA	Human Serum Albumin
HSCT	Human Stem Cell Transplantation
I	
IMP	Investigational Medicinal Product
InSPE	Institute of Experimental Neurology
i.t.	intrathecally
i.v.	intravenously

J	
JCV	John Cunningham Virus
K	
L	
M	
MCB	Master Cell Bank
MEP	Motor Evoked Potentials
MHC-I	Major Histocompatibility Complex-I
MHC-II	Major Histocompatibility Complex-II
MRI	Magnetic Resonance Imaging
MS	Multiple sclerosis
MSFC	Multiple Sclerosis Functional Composite
MTR	Magnetization Transfer Ratio
N	
sNPCs	somatic Neural stem/Precursor cells
O	
P	
PB	Peripheral Blood
PCR	Polymerase Chain Reaction
PCS	Primary Cell Stock
PML	Progressive Multifocal Leukoencephalopathy
PP-MS	Primary Progressive Multiple Sclerosis
PTLD	Post Transplant Lymphoproliferative Disease
Q	
R	
RRMS	Relapsing-Remitting Multiple Sclerosis
S	
S1P	Sphingosine-1-phosphate
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SCI	Spinal Cord Injury
SEP	Sensory Evoked Potentials
SP-MS	Secondary Progressive Multiple Sclerosis
SUSAR	Suspected Unexpected Serious Adverse Reaction
SVZ	SubVentricular Zone
T	
TC	Treatment Cohorts
U	
UAR	Unexpected Adverse Reaction
V	
VEP	Visual Evoked Potentials
W	
wpc	weeks post-conception
Y	
Z	

2.2 Tables

2.3 Figures/Flow-charts

3 Introduction: background information and scientific rationale

3.1 Background information

Multiple sclerosis (MS) is an acquired inflammatory and neurodegenerative immuno-mediated disorder of the central nervous system (CNS), characterized by inflammation, demyelination and primary or secondary axonal degeneration (Confavreux & Vukusic, 2006). It is a complex multi-factorial disorder, in which environmental factors are hypothesized to interact with genetically susceptible individuals. Genetic susceptibility plays a role in disease initiation and currently unidentified environmental factors are also involved.

The prevalence rate of MS varies between races and geographical latitude, ranging from more than 100 cases out of 100,000 in Northern and Central Europe to 50 cases out of 100,000 in Southern Europe.

Three major clinical forms of MS are recognized: primary progressive multiple sclerosis (PP-MS), secondary progressive multiple sclerosis (SP-MS), and relapsing-remitting multiple sclerosis (RRMS) (Confavreux, Vukusic et al., 2000). RR-MS and SP-MS are probably different stages of the same disease while PP-MS may imply different processes (Lublin, Reingold et al., 2014). RR-MS represents the more frequent form of the disease (80-85%). It is characterized by the occurrence of acute episodes of neurological dysfunction named relapses, followed by variable recovery and periods of clinical stability. The majority of patients who start with RR-MS eventually develop SP-MS, 50% at 10 years and 90% at 25 years (Kragt, van der Linden et al., 2006). During the progressive phase subjects develop sustained and progressive clinical deterioration with or without superimposed relapses(Lassmann, 2007a). PP-MS is mainly characterized by a progressive accumulation of disability since the onset of the disease (Bramow, Frischer et al., 2010, Lassmann, 2007b).

The disease shows heterogeneity with respect to its pathogenesis, clinical manifestations, prognosis and, most interestingly, with respect to its pathology. The essential structural features of MS are focal demyelination associated with inflammation and axonal damage (Frischer, Bramow et al., 2009). Acute inflammation, present in the early phases of the disease, produces demyelination and axonal loss.

The etiology of MS is unknown, although an autoimmune process has been implicated. It is hypothesized that CNS auto-reactive T cells are stimulated in the peripheral circulation to become active and proliferate. The expression of adhesion molecules on the surface of T cells allows adhesion to activated endothelial cells with subsequent migration of T cells into the CNS compartment. These cells upon interacting with CNS myelin antigens again proliferate, and initiate a pro-inflammatory cascade within the brain that results in either target-directed immune damage or bystander damage. Important cellular and humoral elements are T-lymphocytes, macrophages, microglial cells, metalloproteinases and cytokines, including interferon-gamma, and tumor necrosis factor (TNF)-alpha.

3.2 The treatment of Progressive Multiple Sclerosis

There are currently no approved therapies to slow down or prevent the accumulation of disability that occurs independently of relapses (Kurtzke, 1983). The current standard of care for patients with SP-MS is to use immunomodulatory drugs to reduce the frequency and severity of relapses, to provide mental health care for treatment of depression and anxiety, physical therapy to help preserve ambulatory function, and occupational therapy to help adapt to the loss of upper extremity and other functions. Short courses of corticosteroids are occasionally given but their efficacy is not proven, while the side effects associated with their long-term use are well established. Chemotherapeutic agents, such as mitoxantrone, are occasionally used in cases of rapidly pro-

gressing SP-MS (Martinelli, Cocco et al., 2011) but without conclusive evidence that they slow down the progression of disability independently of their effects on relapses (Fox, 2006).
In marked contrast to relapsing forms of MS, the majority of therapeutic clinical studies for progressive MS have failed to show a benefit compared to placebo. Only the European study of interferon beta-1b for treatment of relapsing SP-MS (Kappos, 1998, Kappos, Polman et al., 2001) and the MIMS study of mitoxantrone for treatment of relapsing or rapidly worsening MS (including SP-MS) (Hartung, Gonsette et al., 2002) have demonstrated positive results, but they enrolled subjects that looked a lot more like RRMS than SP-MS.
Recently, Ocrelizumab has been demonstrated to induce beneficial effects on PP-MS patients, but without conclusive evidence that they slow down the progression of disability independently of its effects on the inflammatory processes related to the disease. The poor results of available treatment obtained in SP and PP-MS are related to the fact that they are effective in the early inflammatory phase of MS, whereas they have a very poor impact on disease course during the progressive phase when inflammation fades and neurodegeneration leads to disability. A possible explanation is that other mechanisms, in part independent from early inflammation mainly mediated by cells of the acquired immunity (Magliozzi, Howell et al., 2007, Serafini, Rosicarelli et al., 2004), may lead to neurodegeneration (Lassmann, 2007a); this could arise from an exhaustion of the properties of self-repair by local precursors cells harmed by chronic inflammation and consequent degeneration of axons and neural cells.
The lack of efficacious treatments for these patients is indicative of the high unmet need for effective alternative therapeutically strategies with different mechanisms of action to manage the irreversible progression of disability.

3.3 Somatic Neural stem precursor cells

Somatic neural stem/precursor cells (NPCs) represent a heterogeneous population of mitotically-active, self-renewing and multipotent cells of both the developing and the adult CNS showing complex patterns of gene expression that may vary both in space and time (Temple, 2001). Neural stem/precursor cells can be isolated and established as growth factor-dependent 'virtually perpetual' cell lines from both the developing and adult human CNS, the subventricular zone (SVZ) and hippocampal regions of the adult mammalian brain being found to harbor undifferentiated neural cells which proliferate *in vitro* in response to rh-bFGF and rh-EGF and in many ways appear similar to their embryonic counterparts.
In defined-medium growth condition, these cells can be grown *in vitro* for long periods of time, while retaining the potential to differentiate into post-mitotic daughter neural cells. There is still much debate over how to classify these germinal cells – whether they are stem cells, progenitors or precursors – particularly when they are removed from the developing or adult CNS and grown *in vitro*. A number of papers confirm that cells derived from the embryonic (fetal) human CNS can be isolated and cloned in culture and grown for extended periods of time *in vitro*. These cells display extensive self-renewal capacity and functional stability, ability to maintain a steady growth profile over time, multipotency, and a constant potential for neuronal and glial differentiation over years of continuous *in vitro* culturing. Human NPCs are also cryopreservable and incorporate into the developing and adult rodent CNS. Tumor formation is not observed, even when human NPCs are transplanted into immuno-deficient hosts (Garitaonandia, Gonzalez et al., 2016, Mazzini, Gelati et al., 2015, Pluchino, Gritti et al., 2009).
As a consequence of their inherent biology, human NPCs from fetal human CNS can establish stable, transplantable non-immortalized cell lines *in vitro* by epigenetic stimulation (Vescovi, Parati et al., 1999). In particular, due to their capability to generate *in vitro* large numbers of cells in standardized and quality-controlled preparations, these lines enable the development of clinical strategy of neural transplantation (Martino, Franklin et al., 2010).

3.4 Rationale of NPCs for the treatment of MS

Inflammation and degeneration are the prototypical pathological processes promoting irreversible tissue damage and clinical disability in CNS disorders. To prevent irreversible tissue damage, the CNS has evolutionarily developed a series of molecular and cellular mechanisms of repair that are activated in response to chronic inflammation and neurodegeneration. Endogenous somatic neural stem/precursor cells (NPCs) – the self-renewing and multipotent cells of the CNS capable of driving neurogenesis and gliogenesis in adult life – migrate into damaged areas to promote functional and structural tissue repair.

However, reactive innate 'protective' CNS mechanisms are not robust enough to promote the full recovery of the cytoarchitecture of the damaged CNS. In order to foster and reset such mechanisms, NPC transplantation strategies have been developed and may represent one of the possible and most promising approaches to generate new therapeutic options for still incurable chronic neurological disorders.

Several preclinical and clinical studies using cells obtained either from embryonic, fetal or adult tissues are already available (Chen, Sakowski et al., 2016, Chu, Kim et al., 2003, Feldman, Boulis et al., 2014, Jeong, Chu et al., 2003, Park, Teng et al., 2002). Altogether, these studies indicate that transplantation of NPCs is nowadays promising based on the ability of transplanted NPCs to adapt their behavior and fate to the CNS microenvironment and to promote neuroprotection *via* different and articulated strategies (Pluchino, Zanotti et al., 2005a). Despite the initial perception that transplantation of NPCs could only serve to replace the lost cells, recent experimental studies have shown that transplanted NPCs could also exert additional neuroprotective, as well as immunomodulatory, "bystander" effects capable of promoting tissue repair by preventing tissue damage, interfering with the pathogenic process, or rescuing endogenous neural cells (Muller, Snyder et al., 2006). Thus, NPCs transplantation might represent an ideal therapeutic strategy also in neurological disorders characterized by neurodegenerative processes secondary to inflammation as MS. The "bystander" effect is a multistep process depending on the timing of cell injection and the route of cell transplantation. Once injected intraparenchymally or systemically [intravenously (i.v.) or intrathecally (i.t.)], NPCs show a certain degree of pathotropism (i.e. migration and homing to pathological sites) (Aboody, Brown et al., 2000). Upon migration into inflamed CNS areas, transplanted NPCs survive in close proximity to blood vessels where they interact with CNS-infiltrating blood borne inflammatory cells, endothelial cells and CNS-resident astrocytes and microglia. Within perivascular areas, these latter cell populations secrete a series of molecules that are capable, on one hand, to recapitulate the microenvironment of the prototypical SVZ germinal niche, and, on the other hand, to inhibit the NPC cell cycle by upregulating the expression of cell cycle-dependent kinase inhibitors. As a consequence of these cellular interactions, transplanted NPCs retain an undifferentiated phenotype forming an atypical ectopic perivascular niche. There, undifferentiated NPCs continue to express and produce a wide array of constitutive transmembranal and secreted molecules that, in turn, promote tissue repair through immunomodulation and stimulation of endogenous reparative mechanisms (e.g. trophic support, cellular plasticity) (Lu, Jones et al., 2003).

In experimental autoimmune encephalomyelitis (EAE), the animal model of MS, intrathecal (i.t.) or intravenous (i.v.) transplantation of NPCs inhibits T-cell proliferation and promotes the apoptosis of encephalitogenic CNS-infiltrating T cells (Einstein, Karussis et al., 2003, Pluchino, Quattrini et al., 2003, Pluchino, Zanotti et al., 2005b). Similarly to other stem cell types (i.e. mesenchymal stem cells), NPCs can exert a bystander immunomodulatory effects also outside the CNS upon systemic transplantation. Indeed, in EAE mice, i.v. injected NPCs may also inhibit the initiation and maintenance of the inflammatory events occurring within the secondary lymphoid organs. The immunomodulatory properties of NPCs seem to be a constitutive and evolu-

tionarily conserved signature. Indeed, fetal human NPCs constitutively express a considerable amount of immune related genes (around 18% of the total number of expressed genes) and inhibit T-lymphocyte proliferation as well as dendritic cell maturation in vitro. Indeed, in nonhuman primates with EAE, systemically transplanted fetal human NPCs ameliorate disease severity, while persisting long-term within the host CNS and in the draining lymph nodes. NPCs also have the ability to stimulate endogenous reparative mechanisms. This effect is mainly attributed to the capacity of undifferentiated NPCs to secrete a plethora of trophic factors able to protect and prevent neural cell damage and to re-establish the functional interactions between neural and glial cells. In animal models of both MS and spinal cord injury (SCI) (Fujiwara, Tanaka et al., 2004), enhanced endogenous remyelination following NPC transplantation has been consistently reported (Pluchino, Furlan et al., 2004).
In diseases in which the stem cell-mediated bystander effect is desirable – for example, diseases characterized by primary inflammation (MS, SCI and ischemic stroke) – we still need to understand how to optimize the treatment in order to avoid unwanted side-effects. This is particularly true in diseases such as MS, in which repeated inflammatory insults determine the coexistence of variable proportions of both neurodegenerative and regenerative processes. As recently suggested, treatment optimization might be achieved by tightly controlling the timing and route of cell administration. An early time-window for NPC transplantation seems to be the most appropriate approach, as the expression of genes coding for molecules supporting tissue growth predominates over genes coding for molecules exerting an antiplasticity effect occurring soon after CNS damage.
Concerning the administration route, orthotopic transplantation should be preferred as recent data indicate that NPCs – but also other sources of somatic stem cells (i.e. BMSCs) – might form tumors, in response to microenvironment mediated signals, when implanted in heterotopic tissues (Melzi, Antonioli et al., 2010).

3.5 Rationale of first dose of NPCs for the treatment of MS

The number of cells to be injected into patients via lumbar puncture has been chosen according to the data obtained in the preclinical trial we conducted in non human primates (Pluchino et al., 2009). The maximal dose of cells intracisternally injected in non human primates was $5x10^6$/kg cells per animal. This is the very same maximal dose we will reach in humans, as we will start our dose escalating protocol from $0.7x10^6$/kg cells per patient and, if no side effects will be recorded, we will proceed up to $5.7x10^6$/kg cells per patients.

3.6 Risk benefit assessment

3.6.1 Risk benefit definitions

Risk is defined as the probability of physical, psychological, social, or economic harm occurring as a result of participation in a research study. Both the probability and magnitude of possible harm in human research may vary from minimal to considerable.
Minimal risk exists where the probability and magnitude of harm or discomfort anticipated in the proposed research are not greater, in and of themselves, than those ordinarily encountered in daily life or during the performance of routine physical or psychological examinations or tests.
Risk above this standard is more than minimal (moderate, maximal) and that imposes limitations on the conduct of the research and increases the requirements for monitoring. It also requires more stringent approval processes when studying children or otherwise vulnerable populations. Increased risk should be accompanied by the probability of appropriately increased benefits.

Benefit applies to the potential of the research treatment to ameliorate a condition or treat a disease. This can apply to an individual participant or to a population. In research as in clinical medicine, results cannot be guaranteed but, as a consequence of prior work, a benefit may appear to be a reasonable expectation. Since this is research, an advantage for the treatment groups cannot be presupposed. Since the risks have not been fully evaluated, a statement of individual benefit should be made most cautiously if at all. The investigator should always distinguish between research and treatment and never lure the patient into participating in hopes of remission or cure.

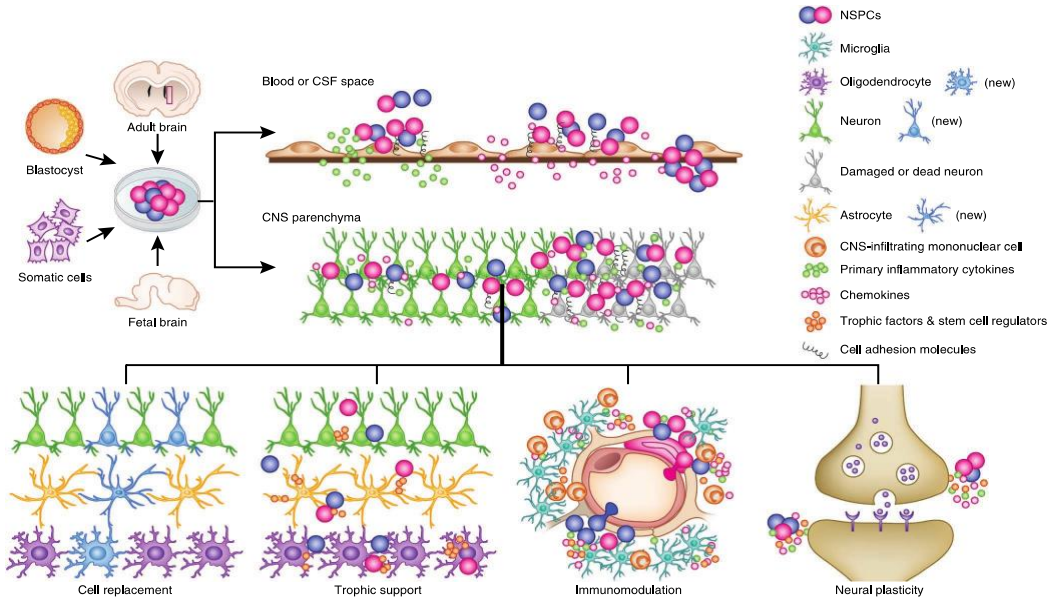
3.6.2 Risk benefit ratio

The final goal of this study and future studies is to develop a curative treatment which can be offered to any patient affected by Progressive MS. The proposed clinical trial is designed to appropriately assess the safety of the ATIMP and applied procedures. The potential risks associated to the use of a novel technology and a reduced toxicity of immunosuppresion regimen are well balanced by the potential benefit of a potentially curative therapy for patients to whom cannot be offered therapeutic options.

3.6.2.1 Benefit

This approach might represent one of the first cell-based therapy promoting neuroprotection in diseases in which neurodegeneration is the final outcome of the tissue pathology. This is because, despite the initial perception that transplantation of NPCs could only serve to replace the lost cells, recent experimental studies from our own and other groups have shown that transplanted NPCs could also exert additional neuroprotective, as well as immunomodulatory, bystander effects capable of promoting tissue repair by preventing tissue damage, interfering with the pathogenic process, or rescuing endogenous neural cells (Kokaia et al., 2012). As a matter of fact, such results do support the concept that somatic stem cells might promote CNS repair via several and articulated strategies in inflammatory and degenerative disorders such as MS (see Figure 2).

Figure 2 – Benefit assessment



Transplanted NPCs adapt their fate and function(s) to specific environmental needs occurring as a result of different pathological conditions and therefore are capable of supporting neuroprotection not only by promoting cell replacement but also via the so-called bystander effect that is mainly aimed at rescuing endogenous neural cells. The bystander effect is made possible by the fact that NPCs sense the inflammatory environment (pathotropism) and, within such an environment, promote tissue homeostasis and repair by releasing, at the site of tissue damage (ectopic atypical perivascular niche), a milieu of constitutively expressed molecules capable of immunomodulation and trophic support. The net and final effect is neuroprotection because transplanted NPCs not only rescue damaged cells but also promote cellular and molecular plasticity. Thus, NPC transplantation might represent an ideal therapeutic strategy also in neurological disorders characterized by multifocal and diffuse inflammatory and degenerative processes caused by the maladaptive interactions among different cell types

3.6.2.2 Risk

The risks the patients enrolled in the trial can be subjected to might be due to:
(1) the procedure of cell injection per se (discussed in details other sections of this project);
(2) allergic reactions to the medicinal product (discussed in details other sections of this project);
(3) infections due the non sterility of the cell product: this risk has been very much minimized by the extensive in vitro examinations of the medicinal product we performed (see IMPD);
(4) tumor formation: this risk has been minimized by the in vivo extensive assessment we have performed using supra maximal doses of cells infused in immunosuppressed mice (see preclinical in vivo toxicy data).

3.6.2.3 Ratio

In conclusions, while the risk patients enrolled in the trial can be subjected to the risks that we usually take when we explore a new potential therapy, the benefits might overtly overcome the risks since the therapeutic approach we are proposing might represent one of the very first – if not the first - neuroprotetective therapies for conditions in which neurodegeneration is due to neuroinflammation, i.e. progressive MS patients.
Moreover, if efficacious such therapy might also represent an approach to treat several other neurodegenerative/neuroinflammatory CNS disorders such as stroke and spinal cord injury.

4 Objectives

4.1 Primary Objective

The primary objective of the study is to evaluate the feasibility, safety and tolerability of intrathecally administered hNPCs, at an escalating dose ranging from $0.7x10^6$±10% cells to $5.7x10^6$±10% cells/kg of body weight, in patients affected by progressive MS.

5 Study design and Endpoints

5.1 Description of Study Design

This is a prospective, monocentric, national, therapeutic exploratory, phase I, not randomized, open label, not controlled, single dose escalation clinical trial.

5.2 Duration of study

Each subject will participate in the study for approximately 96 weeks. Participation will include a screening evaluation between -28 and -7 days before the ATIMP administration. A follow-up visits will be performed from 1 to 96 weeks.

5.3 Primary End-point

The following safety endpoints will be used to address the primary objectives:

a) SHORT TERM (0-24 hours)
- overall survival
- overall safety and tolerability measured by AE recording
- changes in neurological conditions not related to disease
- proportion of successful intrathecal administration procedure (feasibility)

Day 0 = hNPCs administration

b) MID TERM (day 1-day 14)
- overall survival
- overall safety and tolerability measured by AE recording
- changes in neurological conditions not related to disease

c) LONG TERM (day 15 - week 96)
- overall survival
- overall safety and tolerability measured by AE recording
- long term incidence of malignancies
- percentage of subjects with treatment–related mortality defined as death due to procedure and not to the course of disease
- evaluation of changes in quality of life measures
- changes in neurological conditions

5.4 Research endpoints

The research endpoints are evaluated to obtain preliminary data on the effect of hNPCs in patients affected by progressive MS.

5.5 Safety criteria

5.5.1 Safety profile (hNPC cells*)*

The safety profile of hNPCs cells are unknown. Other heterogenic clinical studies on Parkinson disease, ataxia-teleangectasia, Huntington disease, spinal cord injury and inherited cerebellar atrophy were conducted and two cases of late onset graft-derived tumours were observed. To

date hNPCs cells have never been used in Multiple Sclerosis. However, two phase I trials and one phase II trial have been performed in adult patients affected by Amyotrophic Lateral Sclerosis and other two phase I trials have been performed in children affected by neuronal ceroid lipofuscinosis and and Pelizaeus-Merzbacher disease. The total number of patients exposed to hNPCs are 42. In all these studies a surgical injection of stem cell directly in the brain or the spinal cord was performed. Overall no side effects or adverse events related to hNPCs were reported. Also anatomo-pathological studies conducted in subjects who died during the follow-up of the previous studies for the underlying disease confirmed the feasibility and the therapeutic potential of this treatment.
Preclinical studies in non-human primates with hNPCs did not shown any safety issue.

The safety profile will be monitored from the baseline visit to the last follow-up visit.

The SHORT TERM safety profile will be evaluated trough:

- Vital signs**:** During the injection of hNPCs vital signs including oxygen saturation will be monitored before and then after 15 minutes, 60' and 3 hours after the end of injection. Vital signs will be then monitored before discharge.
Particularly, during the treatment visit acute injection toxicity and allergic reactions will be monitored, and it will be defined as any of the following events within three hours post injection:
  - Systolic blood pressure (mmHg) <90 or >180, or >33% decrease or increase from baseline
  - Diastolic blood pressure (mmHg) <50 or >100, or >33% decrease or increase from baseline
  - Heart rate: <60 or >33% decrease; >110 or >33% increase
  - Oxygen saturation: hypoxia (grade ≥2 according to CTCAE)
  - Body temperature (°C) >37.5°C or >1°C increase
  - Any other toxic side effect (according to CTCAE)

- Physical examination will be performed before the hNPCs injection and before the discharge of the patient.
- Neurological examination will be performed before the hNPCs injection and before the discharge of the patient.
All new or worsening neurological signs and symptoms will be monitored, evaluated and recorded. In the presence of newly identified or worsening of neurological sign or symptom the investigator should assess whether the patient is experience a relapse of MS or another neurological (non-MS related) disorder.

- Cerebrospinal fluid examination (CSF): a CSF examination will be performed in concomitance with hNPCs administration.
- Routine Clinical Laboratory tests will be performed before the hNPCs injection and before the discharge of the patient (see appendix for detailed tests).

The following procedures might be performed, at investigator discretion, in the presence of a new or worsening neurological or systemic condition:
- Haematology and biochemistry examination complete of Erythrocyte Sedimentation Rate/Velocity (ESV) and CRP
- Chest X ray
- Brain and spinal cord Magnetic Resonance Imaging (MRI) with gadolinium
- Lumbar puncture

- Electrocardiography (EKG)

MID TERM (DAY 1 – DAY 14) safety profile will be evaluated trough:

- Vital signs including oxygen saturation will be monitored during visit at week 1 and week 2.
- Physical examination will be performed during visit at week 1 and week 2.
- Neurological examination: a neurological examination will be performed during visit at week 1 and week 2.

All new or worsening neurological signs and symptoms will be monitored, evaluated and recorded. In the presence of newly identified or worsening of neurological sign or symptom the investigator should assess whether the patient is experience a relapse of MS or another neurological (non-MS related) disorder.

- Haematological evaluation: will be performed during visit at week 1 and week 2.
- Routine Clinical Laboratory tests will be performed during visit at week 1 and week 2 (see appendix for detailed tests).

The following procedure might be performed, at investigator discretion, in the presence of a new or worsening neurological or systemic condition:
- Haematology and biochemistry examination complete of ESV e CRP
- Chest X ray
- Brain and spinal cord MRI with gadolinium
- Lumbar puncture
- EKG

The LONG TERM safety profile will be evaluated trough:
At every scheduled visit the following procedures will be performed:
- Vital signs
- Physical examination
- Neurological examination
- Clinical Laboratory tests
- Haematological evaluations
- Instrumental follow-up:
  - Week 48 and week 96 electrocardiogram, chest Xray;
  - Week 96 abdominal ultrasound.

- Administration of MsQuol54 will be performed at 24, 48 and 96 weeks.
- Brain and spinal cord MRI with gadolinium: will be performed at 12, 24, 48 and 96 weeks.
- Cerebrospinal fluid examination (CSF): a CSF examination will be performed 12 weeks ± 2 weeks after hNPCs administration.

All new or worsening neurological signs and symptoms will be monitored, evaluated and recorded.
In the presence of newly identified or worsening of neurological sign or symptom the investigator should assess whether the patient is experience a relapse of MS, any neurological disorder related to the recent injection procedure or any other neurological (non-MS related) disorder.

The following procedure might be performed, at investigator discretion, in the presence of a new or worsening neurological or systemic condition:
- Haematology and biochemistry examination complete of ESV e CRP
- Chest X ray
- Brain and spinal cord MRI with gadolinium
- Lumbar puncture
- EKG

5.5.2 Adverse Event (AE)

An adverse event (AE): any untoward medical occurrence in a patient or clinical trial subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavourable 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.
In this protocol the follow AEs (not related to the IMP) are expected:

AEs related to the use of tacrolimus:
The most common adverse events reported with use of tacrolimus are listed below.
Nervous System: very common **(frequency >10)**: headache and tremors. Common (> 1:100 < 1/10): paresthesias or dysesthesias mental status changes, seizures, encephalopathy, dizziness, impaired writing, and peripheral neuropathy. Late neurotoxicity was highly associated with severe infections and multi-organ failure.
Respiratory System: common: pleural effusion, dyspnea, lung disorders, cough, pharyngitis rhinitis and sinusitis.
Renal System: very common: renal function impairment; common: renal insufficiency acute renal failure oliguria, nephrotoxicity, kidney tubular necrosis, urinary disturbances.
Psychiatric Symptoms: very common: insomnia; common: nightmares, depression, irritability, agitation, anxiety, hallucinations, abnormal dreams.
Immunologic System: Immunologic side effects have included infectious complications as a result of immunosuppression. Cytomegalovirus is the most frequently encountered viral pathogen in patients treated with tacrolimus. Bacterial infections, primarily gram-positive and gram-negative aerobes, and fungal infections are also encountered. Progressive multifocal leukoencephalopathy (PML) related to a JC virus infections have been reported.
Hematologic System: common: anemia, leukopenia, leukocytosis, thrombocytopenia hematocrit alterations.
Cardiovascular system: very common: hypertension; common: tachycardia hemorrhage, myocardial infarction, thrombosis, hypotension, phlebitis.
Gastrointestinal system: very common: diarrhea, nausea; common: elevations in liver function tests cholangitis cholestatic jaundice hepatitis gastrointestinal hemorrhage stomatitis, gastrointestinal perforation.
Metabolic adverse events: very common: hyperkalemia, hyperglycemia, diabetes mellitus; common: hypomagnesemia, hypokalemia, hyperlipidemia, acidosis, hypocalcemia, hypophosphatemia, hyponatremia, anorexia.
Oncologic adverse events: in one study of 936 transplant patients receiving tacrolimus as primary immunosuppressive therapy, post transplant lymphoproliferative disease (PTLD) occurred in 15 (1.6%) patients. Serologic evidence of Epstein-Barr virus infections was found in all 15 patients. Oncologic side effects, or the development of new malignancies, are of particular concern

in post transplant patients. Lymphoproliferative disorders are most commonly encountered. Lymphadenopathy and monoclonal and polyclonal B cell hyperplasia as well as malignant, often fatal, B cell lymphomas have been reported.
Hypersensitivity: Hypersensitivity side effects including allergic reaction have been reported infrequently.

AEs related to chronic steroids treatment methylprednisolone and prednisone:

Expected AEs include: systemic hypertension; glaucoma; fluid retention; hyperglycemia and diabetes; mood and emotional disorders, central obesity; osteoporosis; cataracts; increased risk of infections; suppressed adrenal gland hormone production; thin skin, easy bruising and slower wound healing.

AEs related to lumbar puncture and injection procedure:
Lumbar puncture: is a minimally invasive, diagnostic or therapeutic procedure that involves the removal of a small amount of cerebrospinal fluid (CSF) - the fluid that surrounds the brain and spinal cord - followed by the injection of neural cells into the lumbar region of the spinal column. In this study, the quantity of collected CSF will be replaced by the same amount of cell culture medium.
The most frequent adverse events related to this procedure is the orthostatic headache and/or back pain. The frequency of these events is about 15-30%. Normally they resolve in few days and they can be treated with bed rest, anti-inflammatory drugs and hydration. The injection of any substance in the spinal canal could induce an inflammatory/irritative reaction with highly variable severity and duration of the AE. This kind of reaction is unpredictable.
Overall, rare adverse events (<1%) are related to the simple Lumbar Puncture Procedure and consist in: risk of infection; risk of bleeding (epidural or subdural hematoma); risk of cranial nerve palsy and cauda equina syndrome; hypotension, bradycardia with or without syncope.

Infective Complication due to Immunosuppressive treatments
To address the risk of opportunistic infections and organ toxicity due to combined steroid and tacrolimus treatment, periodic clinical, biochemical (full blood count, renal and liver function) drug level (FK506), immunological and virological monitoring (PCR for cytomegalovirus-CMV and Epstein virus-EBV) will be instituted at fixed intervals (see details in the specific chapter) and depending on patient's clinical and biochemical condition and previous findings, for the entire duration of the immunosuppressive treatment.
Pre-emptive CMV reactivation will be treated by standard treatment for immunosuppressed patients (i.e. ganciclovir or foscarnet). Immunosuppression reduction or discontinuation can be considered depending on severity.
EBV reactivation will be treated with immunosuppressive drug reduction or discontinuation and/or anti-CD20 (Rituximab) depending on the copy level and patient's clinical condition.
Recurrent episodes of CMV reactivation will be addressed with reduction of the immunosuppressive drug tacrolimus. Immunosuppression discontinuation will be considered depending on severity.
Bacterial infection will be treated according to standards. Repeated infections will be addressed by reduction or discontinuation of immunosuppression depending on site of infection, severity and patient's condition.

The safety profile related to concomitant medication will be assessed trough general physical examination, neurological visit and trough blood examination specifically hematology and chemistry parameters. At every visit signs and symptoms of possible infective events will be recorded.

For all the concomitant medication and procedure used in the protocol only AEs exceeding the grade 3 of severity according of the international definition of AEs will be reported (see also CTCAE).

5.5.2.1 Documentation of (serious) AE

Between screening and last follow–up visit the patient will be asked and examined by the investigator for the occurrence of AEs. All AEs will be documented using CTCAE terms (see Appendix), including

- Severity (listed under)
- Onset date
- Stop date
- Causality assessment with study drug
- Action taken
- Outcome

The severity of AEs is defined as (see also CTCAE):

- Grade 1 mild
- Grade 2 moderate
- Grade 3 severe
- Grade 4 life-threatening
- Grade 5 death

All clearly related signs, symptoms and abnormal diagnostic procedures should be grouped together and recorded as a single term in the CRF.

5.5.2.2 Forwarding

All the Serious AE (SAE) and the ADR will be notified, as specified in Table 2.

	Documentation	**Report**	**Time**
Serious (AE, ADR)	Patient's chart CRF SAE-Form	Investigator forwards SAE-form to Local E.C.	Within 24 h
Non-serious (AE, ADR)	Patient's chart	-	-

Table 2 – SAE/ADR timeline

5.5.3 Adverse Drug Reaction (ADR)

An Adverse reaction (ADR): all untoward and unintended responses to an investigational medicinal product related to any dose administered. The phrase responses to an investigational medicinal product means that a causal relationship between the investigational medicinal product and an adverse event is at least a reasonable possibility, i.e. the relationship cannot be ruled out.

5.5.3.1 Attribution Definitions

Not related: An adverse event that is not related to the use of the investigational product.
Doubtful: An AE for which an alternative explanation is more likely, e.g., concomitant drug(s), concomitant disease(s), or the relationship in time suggests that a causal relationship is unlikely.
Possible: An AE that might be due to the use of the investigational product. An alternative explanation, e.g., concomitant drug(s), concomitant disease(s), is inconclusive. The relationship in time is reasonable; therefore, the causal relationship cannot be excluded.
Probable: An AE that might be due to the use of the investigational product. The relationship in time is suggestive (e.g., confirmed by de-challenge). An alternative explanation is less likely, e.g., concomitant drug(s), concomitant disease(s).
Very likely: An AE that is listed as a possible adverse event reaction, and cannot be reasonably explained by an alternative explanation, e.g., concomitant investigational drug(s), concomitant disease(s). The relationship in time is very suggestive (e.g., it is confirmed by de-challenge and re-challenge).

5.5.4 Serious adverse event (SAE) or Serious Adverse Reaction (Serious ADR)

SAE or SAR: any untoward expected medical occurrence or effect that at any dose results in death, is life-threatening, requires hospitalisation or prolongation of existing hospitalisation, results in persistent or significant disability or incapacity, or is a congenital anomaly or birth defect.

5.5.5 Unexpected adverse reaction (UAR)

UAR: an adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g. investigator's brochure for an unauthorised investigational product or summary of product characteristics for an authorised product).

5.5.6 Suspected unexpected serious adverse reaction (SUSAR)

SUSAR: an adverse event assessed as serious and unexpected and for which there is a reasonable suspected causal relationship with an investigational medical product.

5.5.7 Pharmacovigilance Procedure

Serious Adverse Event: The Investigator shall report all SAE immediately to the Sponsor and Ethics Committee. The Investigator must fill the SAE report no later than 15 days. For reported deaths of a subject, the Investigator shall supply the Sponsor and Ethics Committee with any additional information requested.
Suspected Unexpected Serious Adverse Reaction (SUSAR): In case of a Serious ADR that is unexpected, the Investigator/Sponsor must report the reaction as soon as possible (and in any case no later than 7 days for reactions that are fatal or life-threatening, and no later than 15 days for all other SUSARs) to the competent Regulatory Authorities and to the competent Ethics Committee (and that relevant follow-up information is subsequently communicated within an additional eight days).
Periodic reporting: Once a year throughout the trial, the Investigator should provide to Competent Regulatory Authorities and the Ethics Committee an Annual Safety Report, that is, a listing of all suspected Serious ADRs, which have occurred over this period and a report of the subjects' safety.
Adverse event recording: All AEs/ADRs regardless of seriousness or relationship to Medicinal Product or expectedness are to be recorded on the corresponding page(s) included in the Case Report Form. Whenever possible, symptoms should be group as a single syndrome or diagnosis. The Investigator should specify the date of onset, maximal intensity, action taken with respect to

Medicinal Product, corrective therapy given, outcome and his/her opinion as to whether there is a reasonable possibility that the event was caused by the study Medicinal Product.
The investigator shall keep detailed records of all adverse events, which are reported. These reports must be provided to the competent Authorities if they so request.

5.5.8 Follow-up of AE / SAE / ADR

The Investigator should take all appropriate measures to ensure the safety of the patients, notably he/she should follow up the outcome of any event (clinical signs, laboratory values or other, etc.) until the return to normal or until consolidation of patient conditions.
In the case of any **Serious Adverse Event or Serious Adverse Drug Reaction**, the patient must be followed up until clinical recovery is complete and laboratory results have returned to normal, or until progression has been stabilized. This may imply that follow-up will continue after the patient left the study and if the Ethical Committee requests additional investigations.

5.5.9 Pregnancy

Pregnant and lactating women will not be included in the study. Females must have a negative pregnancy test prior to be included in the study and will be required to use a reliable and effective form of birth control during the study. Male participants will also be required to prevent pregnancy in their partners. The birth control methods which may considered effective are: abstinence, oral contraceptives either combined hormonal or progestogen alone, injectable progestogen, subcutaneous contraceptive implants of etonogestrel or levonorgestrel, estrogenic vaginal ring, percutaneous contraceptive patches, intrauterine device or system (IUD) that has a certified failure rate less than 1% per year, male partner sterilization only with the declaration that he is the sole partner, male condoms combined with a female diaphragm either with or without a vaginal spermicide.
The birth control methods which are considered unacceptable in clinical trials are: periodic abstinence (calendar, symptothermal, post-ovulation methods), withdrawal (coitus interruptus), spermicides only, and lactational amenorrhoea method (LAM).For information refer to guidelines http://www.hma.eu/fileadmin/dateien/Human_Medicines/01About_HMA/Working_Groups/CTFG/2014_09_HMA_CTFG_Contraception.pdf

At every study visit the sexual activity of participants of reproductive age will be re-assessed. If a subject who was previously sexually inactive becomes sexually active, s/he will be counseled about the need to use a reliable and effective form of birth control. Female subjects of childbearing potential will also be required to undergo pregnancy tests at regular intervals, including and especially prior to hNPCs administration. Women who had a positive pregnancy test will not treated with hNPCs.

All pregnancies that are identified during the study must be followed to conclusion and the outcome of each must be reported. The investigator should be informed immediately of any pregnancy whether occurring in a female participant or the female partner of a male participant. The investigator should report all pregnancies to Sponsor within the same timeframe (24 hours) as SAEs, using the SAE report form. Monitoring of the participant should continue until the conclusion of the pregnancy, and a follow-up SAE report form detailing the outcome of the pregnancy should be submitted to the Sponsor.

5.6 Research criteria

In order to gather preliminary information on the efficacy of the experimental treatment clinical and MRI parameters will be recorded throughout the study.

We will evaluate:
- the proportion of disability progression-free patients at week 96;
- the longitudinal changes from baseline to visit week 96 in T25FW, 9-HPT, SDMT

Disability in MS is commonly measured by the Expanded Disability Status Scale (EDSS). The EDSS is based on a standard neurological examination, incorporating seven functional systems (visual, pyramidal, cerebellar, brainstem, sensory, bowel and bladder and cerebral) rated and stored as functional system scores (FSS). Each FSS is and ordinal clinical rating scale ranging from 0 to 5 or 6. These ratings are then used in combination with ambulation capacity to determine the EDSS score that ranges from 0 (normal) to 10 (death due to MS).
Sustained disability progression is defined as an increase of $\geq$ 0.5 EDSS points in the last 12 months. This change has not to be attributable to another etiology (i.e. fever, concurrent illness, MS relapse or concomitant medication) and has to be confirmed at a regularly scheduled visit after at least 12 weeks after the initial disease progression.

Brain MRI parameters: MRI is a useful tool for monitoring MS lesions. Different MRI derived parameters are used to assess paraclinical evidence of drug efficacy.
The following MRI parameters will be evaluated to address the research objective:
- brain and spinal cord atrophy as defined by percentage in brain and spinal cord volume change from baseline to week 96;
- number of new T2 brain and number of spinal cord lesions and changes in T2 lesion volume at week 96;
- number of new T1 hypointense lesions and changes in T1 hypointense lesion volume between baseline and week 96;
- total Gd-enhancing lesions in the brain and spinal cord MRI between baseline and week 96;
- other MR parameters including thalamic, cortical, white matter and normal appearing brain tissue average magnetization transfer ratio (MTR) – ancillary sub-study-.

- Multimodal Evoked Potentials are useful for the evaluation of sensory, motor and visual pathways; Motor Evoked Potentials (MEP), Visual Evoked Potentials (VEP) and Sensory Evoked Potentials (SEP) will be performed at screening, at six and 12 months follow-up and then at two years follow-up (week 24, 48 and 96). We will evaluate changes in amplitude and velocity conduction of MEP, SEP and VEP between the baseline and week 96.

- Optical coherence tomography (OCT) is a useful tool, especially in the field of research, to measure retinal nerve fiber layer (RNFL) thickness as a marker of axonal loss and to detect neurodegeneration in vivo and in a non-invasive way. OCT will be performed at screening, at six and 12 months follow-up and then at two years follow-up (week 24, 48 and 96). We will evaluate RNFL thickness changes between the baseline and week 96.

- to determine immune response in patients treated during immunosuppressive therapy with FK506, cellular and humoral immunological assays will be performed. Immune reconstitution by PB immunophenotyping (lymphocyte subpopulations) will be obtained on blood at screening, at weeks 4^{th}, 12^{th}, 24^{th}, 48^{th} and 96^{th}.

- We will collect data on donor-recipient chimerism on blood samples and CSF (approximately at screening, at weeks 4^{th} and 12^{th}; further evaluations only if clinically useful) with ddPCR assays. It will be performed using the Bio-Rad QX100 system.
- A dosage of tacrolimus on CSF will be performed at week 12^{th} to collect data of CNS drug delivery.

6 Study Enrolment and Withdrawal

6.1 Inclusion criteria

1. Signature of the informed consent by the patient or patients' legal tutors
2. Age 18 to 55 years
3. Diagnosis of
   a. Progressive MS as per the revised MC Donald 2010 criteria with a progressive course according to 2013 Lublin phenotypes classification (PMS) with failure or intolerance to all approved therapies according to the disease course or without any alternative approved therapy
4. Evidence of progression of disease defined by an increase of ≥ 0.5 EDSS points in the last 12 months
5. Disease duration 2 to 20 years (included)
6. EDSS ≥ 6.5
7. Presence of oligoclonal band in the CSF is required for Primary Progressive MS

6.2 Exclusion criteria

They will be excluded from the study patients:

1. with any active or chronic infection or diseases other than MS including but not limited to infection with HIV1-2, Hepatitis B or Hepatitis C and tuberculosis or immune deficiency syndromes;
2. treated with any immunosuppressive therapy, including but not limited to natalizumab and fingolimod, within the 3 months prior to screening;
3. treated with interferon-beta or glatiramer acetate within the 30 days prior to screening;
4. treated with corticosteroids within the 30 days prior to screening;
5. if relapse occurred during the 30 days prior to screening;
6. with contraindications for or intolerance to any medication, treatments and procedures that will be used in the study;
7. pregnant or in lactation or of childbearing age who are not willing to use a contraceptive method effective* for the entire duration of the study;
8. who, in the opinion of the investigator, showing any condition that would preclude study participation.

   * refer to guideline http://www.hma.eu/fileadmin/dateien/Human_Medicines/01_About_HMA/Working_Groups/CTFG/2014_09_HMA_CTFG_Contraception.pdf

6.3 End of Study Phase

For single subject: regular termination.

The participation of the patient in the study is regularly terminated:

- End of the follow-up.
- In case of death

For single subject: premature termination.

The patient may drop out of the clinical study at any time without stating reasons. This may not have any negative consequences for the patient's further treatment.

Possible reasons for withdrawal of the patient are:

- Withdrawal of the consent.
- Any clinical condition that in the Investigator's opinion could become dangerous for the patient and prevent the good conduction of the clinical trial.
- Protocol violation that could compromise the quality of study data.

- Lack of co-operation/compliance of the patient.
- Occurrence of new diseases that could influence the treatment efficacy, for which the study medication is contraindicated or that are treated with a medication that is not permitted as a concomitant medication.
- Lack of experimental treatment administration due to failure to release the Medicinal Product lot.
- Lost to the follow-up.

The patients may withdraw from the study, if they decide to do so, at any time and for any reason. Patients who have been withdrawn from the study cannot be re-included in the trial.

6.3.1 Follow-up procedure for withdrawn patients

All withdrawn patients should be recorded by the Investigator in the appropriate pages of the CRF when considered as confirmed. If possible, the patients should be assessed using the procedure planned for the end of study visit.
If an event causing dropout corresponds to the definition of a Serious AE/ADR, the investigator carries out the pharmacovigilance procedure described.
For patients considered lost to follow-up, the CRF must be filled in up to the last visit performed. The Investigator should make every effort to re-contact and to identify the reason why the patient failed to attend the visit and to determine his/her health status.
In case of death autopsy will be strongly encouraged for evaluation of pathology and presence of transplanted cells.

6.3.1.1 Natural study termination

At the end of total follow-up.

6.3.1.2 Premature Termination or Suspension of study

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause or if any of the stopping criteria described in this protocol occurs. Circumstances that may warrant termination or suspension include, but are not limited to:

- determination of unexpected, significant, or unacceptable risk to participants;
- demonstration of efficacy that would warrant stopping;
- insufficient compliance to protocol requirements;
- data that are not sufficiently complete and/or evaluable;
- determination of futility;
- reconsideration of the risk/benefit ratio.

Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to <investigator, funding agency, the sponsor and regulatory authorities>.
If the study is prematurely terminated or suspended, the PI will promptly inform the Institutional Review Boards (IRBs) and/or Independent Ethics Committees (IECs), will provide the reason(s) for the termination or suspension and will instruct additional procedures to be followed in order to ensure that adequate consideration is given to the protection of the patient's interests.
For patients who have received the ATIMP, a long term safety follow-up it is required by law.
Study may resume once concerns about safety, protocol compliance, data quality are addressed and satisfy the sponsor, IRB and/or CA.

7 Treatment Plan

7.1 Experimental Treatment/Drug

The protocol will consist of a total of four treatment cohorts (TC), labeled from A to D, each of which receiving a single escalating dose of allogenic hNPCs injected intrathecally, as it follows:

-TC-A: $0.7x10^6 \pm 10\%$ cells/kg of body weight;
-TC-B: $1.4x10^6 \pm 10\%$ cells/kg of body weight;
-TC-C: $2.8x10^6 \pm 10\%$ cells/kg of body weight;
-TC-D: $5.7x10^6 \pm 10\%$ cells/kg of body weight.

The intrathecal injection of hNPCs will be performed in a hospitalized setting.
The patients will be left in Trendelenburg position for about 30 minutes and monitored for at least 24 hours. All patients will receive normal saline solution (500 cc).
Body temperature, blood pressure, heart rate and oxygen saturation will be measured at baseline and after 15, 60, 180 minutes and then before discharge. Any toxicity will be treated at the discretion of the attending neurologist.
All patients will receive an i.v. dose of **methylprednisolone** (125 mg) immediately prior to lumbar puncture.
The injections of cells will be performed with lumbar puncture in lateral decubitus position removing about 10 cc of CSF that will be used for chemical-physical, infectivological analysis and OBs/ BBB analysis. 2 CSF ml will be stored for further research analysis. CSF will be replaced with the 10 cc of suspension solution containing hNPCs (at 2 ml/min injection rate). The injection of stem cell will be performed in about 5 minutes. In case of traumatic lumbar puncture with haematic CSF, the injection of hNPCs will not be performed. A second attempt will be performed after 15 ± 3 days.

7.2 Immunosuppression:

Human NPCs have been shown to be relatively low immunogenic (e.g., no expression of MHC-I molecules and low levels of MHC-II molecules). However, as they are human in origin their transplantation will be handled like other organ transplant in order to prevent the unexpected rejection (if any) of transplanted hNPCs and we have planned to put our patients on long term immune suppression. The immunosuppression will be applied in agreement with previous studies with hNPCs in Amyotrophic Lateral Sclerosis and in children affected by neuronal ceroid lipofuscinosis and and Pelizaeus-Merzbacher disease. In all these studies the patients a long lasting immunosuppression was applied in order to guarantee the engraftment of stem cell. Data deriving by these studies confirmed that immunosuppression was well tolerated by the majority of patients and no opportunistic infections were reported till now.
According to our hematologist guidelines the patients will receive and oral prednisolone as explained below.
Tacrolimus (FK506) is a macrolide lactone with potent immunosuppressive activity 100 times that of cyclosporine by weight. The molecular mechanism of action is mediated via an inhibition of the phosphorylase activity of calcineurin by drug-immunophilin complex, resulting in the inhibition of IL-2 gene expression.
There are many studies now showing significant efficacy of tacrolimus in GVHD prevention in both allogeneic HSCT.
FK506: The initial oral dose of tacrolimus will be 0.05 mg/kg twice daily from day-7. Tacrolimus, if tolerated, will be given and gradually tapered till week 96.

A pharmacological surveillance of FK506 through drug level for consequent dose adjustment will be performed.
In order to maintain a target of 5-10 ng/ml, whole blood levels of tacrolimus will be obtained:
- two times a week until the therapeutic range was reached (first samples on day -4 and day -1);
- weekly till week 4 after hNPCs injection;
- at week 8, 12, 24, 36, 48 and then every 24 weeks till the end of follow-up.

Tacrolimus will be started day -7 before hNPCs injection as immunosuppressive medication to prevent the rejection of the cells, and it is expected that the patients will take the drug for the end of the follow-up.
Dose modifications of tacrolimus were dependent primarily on serum creatinine and the blood levels of tacrolimus. A dose reduction of at least 25% was mandatory for patients with a creatinine elevated to greater than 2.0 times baseline and at least a 50% reduction for patients with a creatinine greater than 3 times baseline.

Oral prednisone (deltacortene) 50 mg tapering of 5 mg every five days as follows dosage:
37,5 mg, 25mg, 12,5 mg and 5 mg.
Any or all the immunosuppressive agents may be reduced or stop at the discretion of the investigator if it is suspected or determined that the immunosuppressive agent is the cause of toxicity and if symptoms cannot be managed adequately with symptomatic treatment.
Anti-infective prophylaxis will be administered for the duration of FK506/tacrolimus therapy with acyclovir (1 tablet-800 mg twice daily), and co-trimoxazole (1 tablet-800 mg/160 mg, three times/week. Folic acid (1 tablet of 5 mg) will be administered with co-trimoxazole, as supportive therapy to reduce co-trimoxazole myelotoxicity three times a week.

7.3 Advanced Therapy Investigastional Medicinal Product definition

The Drug Product (DP) can be classified as an Advanced Therapy Investigastional Medicinal Product (ATIMP) belonging to the class of Cell Therapy Medicinal Products.

The following definitions shall apply throughout this study.

The Starting Materials is the Master Cell Bank (MCB) originated from cryopreserved non-immortalized human fetal neural precursor cells (hNPCs) BI-0194-008 cell line obtained from a single 10-12 weeks post-conception (wpc) human fetus and manufactured as defined in the Investigastional Medicinal Product Dossier (IMPD).

The Drug Substance (DS) will consist of Working Cell Bank (WCB) obtained after expansion of MCB as defined in IMPD.

The DP will be constituted by thawed WCB-hNPCs re-suspended in their final formulation medium as defined in IMPD.

Medicinal product manufacturing and release will be performed at “Laboratorio di Terapia Cellulare e Genica Stefano Verri - ASST - Monza, Ospedale San Gerardo” (later also “Laboratorio Verri”), as described in the IMPD. Production will be performed according to GMP (Good Manufacturing Practice) certified facility authorized by Italian Ministry of Health according to D.L.vo 166/2003 for the production of sterile medicinal products, the production of specific me-

dicinal products obtained with particular technologies (i.e. cell therapy), and for primary packaging operations.

7.4 Preparation of the drug product

When a new patient, enrolled in the clinical protocol, is ready for treatment, cryopreserved WCB vials will be thawed. Freezing medium will be discarded and cells re-suspended in 10 ml of injection medium and transferred to primary package(s).

7.5 ATIMP packaging and labelling

The Medicinal Product will be labelled with a unique identification code of the patient and of the product, released by Laboratorio di Terapia Cellulare e Genica Stefano Verri, according to cGMP.

7.6 ATIMP supply, storage and accountability

The ATIMP is produced at 37° C by "Laboratorio Verri" and it will be injected into patient's CSF at physiological body temperature. The DP will be shipped from "Laboratorio Verri" to OSR with a dedicated courier at controlled temperature (25°C ± 5°C) monitored with a data logger. The ATIMP will be released to an Investigator or another member of the investigating team designated by Sponsor or Principal Investigator. The person receiving the DP should control the data logger to check the temperature and report any non-compliance. Then the ATIMP will be transferred in a biohazard container at room temperature to the clinical ward for injection, according to OSR internal operating procedures.
Any non-compliance related to the delivery and / or transport of the drug will be managed according to OSR internal operating procedures.
The ATIMP will kept at ambient or room-air temperatures (max 37°C) while waiting for patient's preparation for intrathecally injection.
The accounting of the ATIMP will be recorded in the ATIMP log in the Trial Master File (TMF).
After injection the CRN will send to the OSR Pharmacy by fax the documentation certifying the receipt of the ATIMP, injection of ATIMP and waste disposal.
The PI is responsible for the accountability and traceability of the product.

7.7 NIMPs packaging and labelling

NIMPs will be provided by the local pharmacy, according to institutional guidelines.

7.8 NIMPs supply, storage and accountability

NIMPs will be delivered by courier to the responsible of pharmacy. The drugs has to be stored following the drug-specific SPC with access only to authorised personnel. The absolute amounts of drug administered per day and per patient have to be documented according to institutional guidelines.
The PI is responsible for supply, storage and accountability of the products.

7.9 Further information, emergency procedures

No other special information is necessary.

7.10 Methods of assigning patients to treatment groups

The protocol will consist of a total of four TC (from A to D), each of which receiving a single escalating dose of hNPCs injected intrathecally as it follows:

- TC-A: $0.7x10^6$ ± 10% cells/kg of body weight;
- TC-B: $1.4x10^6$ ± 10% cells/kg of body weight;
- TC-C: $2.8x10^6$ ± 10% cells/kg of body weight;
- TC-D: $5.7x10^6$ ± 10% cells/kg of body weight.

The trial will start with TC-A and will go through the subsequent enrolment of patients to be included in TCs from B to D.
Each cohort will consist of three patients at minimum. However in case of safety issue the numerosity of each TC will be increased to six patients, as explained below.
We define a DLT any adverse event of grade 3 possibly related to hNPCs or to the procedure. However we will not consider a DLT a prolonged hospitalization due to post lumbar puncture headache if not associated to any meningeal sign. We will not considered DLT adverse events related to concomitant treatments (tacrolimus and steroids)
After the inclusion of the first patient of the TC we will wait at least 14 days to treat the second patient. The same interval time will be used for all the further patients within the same TC. After the inclusion of all the planned patients within a TC, we will wait at least 3 months after the inclusion of the last patient before switching to the higher TC.
In case of none DLT within the TC, we will wait at least 3 months after the inclusion of the last patient and then we will move to the higher TC.
In case of 1 DLT within the TC, the cohort will be extended to 6 patients. If another DLT will be observed, the current dosage will be considered excessive ad the immediate lower dosage will be considered MTD.
The safety monitoring board will review all safety date in the case of an AE ≥ 3 evaluated "possibly related to hNPC" by investigators and in any case before the shifts between TCs.

This will be repeated for every TC up to the end of the study.

7.11 Concomitant medication accepted

A concomitant therapy is any drug or substance administered from the time of the subject's written consent until the subject's End of Study Visit.
Concomitant treatment with any of the following is allowed:

- Medications necessary for the treatment of AEs
- Any medications used to treat MS symptoms such as spasticity, bladder impairment, pain, or depression
- Short courses of iv high-dose corticosteroids as a rescue medication for exacerbations of MS
- Corticosteroids that are administered by non-systemic routes (e.g., topical, inhaled)

Any treatment which are continued during the study and/or initiated or changed during the study must be recorded in source data and in the CRF.

7.12 Prohibited Concomitant Therapy

Concomitant treatment with any of the following is *not* allowed while receiving study treatment, unless as otherwise described in this protocol:

- Any non-study drug treatments directed toward the treatment of MS such as immunomodulatory treatments or chronic immunosuppressant therapy except tacrolimus
- Any investigational product, including investigational symptomatic therapies for MS and investigational therapies for non-MS indications

-Systemic steroid therapy including, but not limited to, oral corticosteroids (e.g., prednisone) or periodic (e.g., monthly) treatment with IV methylprednisolone, except for protocol-defined treatment of relapses as described above and course of oral prednisolone at the above mentioned dosage in the first 25 days after hNPCs injection.

Subjects who receive any of these restricted treatments may be required to permanently discontinue study treatment.

7.13 Other medical procedures

All the routinely procedures used in MS patients are allowed.

8 Overall design

8.1 Sample size

Will be enrolled max 24 patients.

8.2 Protocol schedule and timelines

Anticipated start of recruitment:	**April, 2017**
Anticipated stop of recruitment:	**March 2018** or **study termination**
End of total follow-up:	**When all subjects have completed the 96 weeks follow up period or terminated the study**
Draft statistical report	3-4 months after the end of total follow-up
Integrated final report	6 months after the end of total follow-up

8.3 Study Procedure and Study Flow-chart

8.3.1 Enrolment phase

Is the process of informed consent signature (described in the ethical consideration chapter). Starting from the signature of the informed consent by the patient or patients' legal tutors, the subjects will be enrolled in the clinical trial. When the enrolment is confirmed the patient screening number will be assigned consecutively in increasing order starting with '001'. The list will be filed with the investigator's file. After the enrolment of a patient in the study, the investigator sends the enrolment module to the principle investigator by fax or email to clinical research monitor.

Those protocol phases are foreseen after signature of the informed consent:

- *Screening/baseline phase*, during which the conditions required by the clinical protocol for patients' inclusion/exclusion will be assessed (screened subject). The duration will be of three weeks (+/- 3 working days). At the end of the screening phase, the inclusion/exclusion criteria of the study will be evaluated (enclosed subject).
- *Pre-treatment phase:* carried from the end of the screening phase to the day before the start of treatment.
- *Treatment phase*: The intrathecally administration of hNPCs will be performed on day 0. Profilactical treatment with Tacrolimus will be performed for 96 weeks starting from day -7 whereas oral corticosteroids will be administered for the first 4 weeks.
- *Follow-up phase*: 96 weeks after the experimental drug administration day 0.

At the end specify if the end of total follow-up or single patient follow-up.

At the end of the total follow-up, for all patients enrolled and treated in the study is planned a long term follow up, in accordance with the national applicable laws and the international guidelines.

8.3.2 Screening Phase

All subjects must sign informed consent prior to conduct any study specific procedure. All the inclusion criteria and none of the exclusion criteria listed above respectively, must be satisfied. Following consent, all these evaluations will be performed within the screening phase three weeks (+/- 3 working days):

- Record of general medical and MS history with concomitant medication revision
- Inclusion Criteria and Exclusion criteria evaluation
- Physical examination
- Record of vital signs (blood pressure, heart rate, O_2 saturation, temperature, weight, height)
- Hematological visit
- Haematological blood tests comprehensive of:
  Complete blood count*, extensive clinical laboratory evaluations**,
  *Hemoglobin, Platelets, Leukocytes, Neutrophils, Lymphocytes, Monocytes
  **PCR, LDH, Total Protein, albumin, IgG, IgA, IgM, AST, ALT, GGT, ALP, Na+, K+, Ca++, BUN, uric acid, Total Bilirubin, Direct Bilirubin, Glucose, creatinine, creatinine clearance, ferritin, Serum Iron, transferrin, transferring saturation, INR, PTT
- Infections screening: HBs-Ag, HBs-Ab, HBe-Ag, HBc-IgG, HBc-IgM, HBV-DNA, HCV-Ab, HCV-RNA, HIV-Ab, VZV-IgG, VZV-IgM, HSV1-IgG, HSV1-IgM, EBV-IgG, EBV-IgM, EBV-DNA, CMV-IgG, CMV-IgM, CMV-DNA, Toxo-IgG, Toxo-IgM, HSV2-IgG, HSV2-IgM, Galactomannan detection
- Vitamin B12 and folats evaluation
- TSH evaluation
- Blood group
- Serum pregnancy test (if applicable)
- Immunological analysis
- Electrocardiogram
- Chest Rx
- Abdominal ultrasound
- Neurological examination comprehensive of EDSS scale, T25FW, 9HPT, SDMT
- Neurophysiological assessment (OCT, VEP, SEP, MEP)
- Brain and Spinal cord MRI

After the patient inclusion the investigator sends the enrolment module to the clinical research module. When the inclusion is confirmed the patient definitive number will be assigned as follow:

Patient number for monocentric trial (UPN)					
Screening number			**Inclusion number**		
0	**0**	**1**	**0**	**0**	**1**

8.3.3 Pre-treatment phase

This phase will start after the ending of all the procedure of the screening/baseline phase and it will end the day before the treatment phase (day-1):

Day 1 of pretreatment phase (Friday):

- Inclusion/exclusion criteria revision
- Record of vital signs (blood pressure, heart rate, O_2 saturation, temperature, weight, height)
- AEs, SAEs record
- Tacrolimus administration at 0.05 mg/kg twice daily
- Acyclovir, co-trimoxazole and pholates from the beginning of tacrolimus as prophylactic medication
- Haematological evaluation
- Physical examination
- Pharmacological surveillance of FK506 through blood sample will be performed after three (Monday) and seven days (Friday). This procedure will be performed till the tacrolimus will reach the therapeutical range. Haematological blood test comprehensive of: complete blood cell count, blood chemistry and urin analysis: PCR, AST, ALT, GGT, Na+, K+, Ca++, Total Bilirubin, Direct Bilirubin, Glucose, creatinine, creatinine clearance, LDH, PT, PTT (will be performed seven days after the beginning of tacrolimus)

Day -1

- Neurological examination comprehensive of EDSS scale
- Vital signs
- AEs, SAEs record
- Relapse Assessment
- Concomitant medication revision
- Haematological blood examination: complete blood cell count, blood chemistry: will be performed if not performed within seven days
- Prednisone (Deltacortene) administration (50 mg)

At the end of all these assessment, patients will be assigned to one of the four different cohort of treatment (A-D) (see chapter Methods of assigning patients to treatment groups).

8.3.4 Treatment Phase

On day 0 patients will undergo pharmacological surveillance of FK506 through blood sample.
IV Methylprednisolone at the dosage of 125 mg will be administer one hour prior to lumbar puncture.
They will received intrathecally hNPCs through lumbar puncture in an hospitalized setting and the patients will be monitored for at least 24 hours. Before the injection, hNPCs will be thawed and DMSO will be carefully washed out; hNPCs will be than resuspended in 10 cc of saline solution.
CSF examination cohprensive of chemicophisical and infectivological will be performed.

Body temperature, blood pressure, heart rate and oxygen saturation will be measured at baseline and after 15, 60, 180 minutes and then before discharge. Any toxicity will be treated at the discretion of the treating neurologist.

8.3.5 Follow-up Phase

The follow-up phase will have a duration of 96 weeks. However the patients we'll be followed for the further 6 years at least.
Patients will attend control visits weekly for the first 4 weeks after hNPCs administration and then at 8 and 12 weeks. Starting from 12 weeks visits will be performed every 12 weeks. The procedures of each visit are described in details in the flow chart.
During the follow-up phase patients will receive tacrolimus, acyclovir, co-trimoxazole and folates for the entire follow-up (till week 96) and oral prednisone for 4 weeks.

The patient will undergo:
- Neurological visit with EDSS at week 4, 12, and then every scheduled visit
- T25FW, 9-HPT, SDMT at week 24, 48, 72, 96
- Haematological visit at week 4, 12, and then every scheduled visit
- General physical examination at every scheduled visit
- Record of vital signs at every scheduled visit
- AEs, SAEs record- Relapse Assessment at every scheduled visit
- Concomitant medication revision at every scheduled visit
- **Haematological blood tests comprehensive of**:
  Complete blood count, extensive clinical laboratory evaluations will be performed at week 4, 12, 24, 48 and 96
  Complete blood tests and biochemistry, urinanalisys evaluation will be performed at week 2, 8, 36, 60, 72 and 84
  Complete blood count evaluation will be performed at week 16, 20, 30, 42, 54, 66, 78 and 90
- Infectivological screening: CMV-DNA, EBV-DNA, Galactomannan detection will be performed at week 4, 12, 24, 48 and 96
- Immunological analysis: will be performed at week 4, 12, 24, 48 and 96
- Whole blood levels of tacrolimus will be obtained as reported in the specific section, in order to maintain a target of 5-10 ng/ml.
- CSF examination will be performed at week 12. During this visit the patient will be admitted to the hospital in the morning and will be discharged the day after (24 hours of observation after procedure). Complete chemico-physical evaluation, oligoclonal bands detection, CSF Cell evaluation, virological screening, ddPCR assays.
- Electrocardiogram, Chest Xray will be performed at visit week 48 e 96 and abdominal ultrasound only at visit 96
- Brain and spinal cord MRI at week 12, 24, 48 and 96
- Neurophysiological assessment (OCT, VEP, SSEP, MEP) will be performed at week 24, 48 and 96
- Administration of MsQuol54 will be performed at 12, 24, 48, 72 and 96 weeks

Additional visits will be planned if necessary. Specifically unscheduled visits will be performed in case of new or worsening symptoms related to the disease to exclude a relapse or in case of any adverse event not related to the disease.

END OF STUDY VISIT:
This visit will be performed 8 week after the end of treatment visit.

The patient will undergo:
- Neurological visit with EDSS
- Haematological visit
- General physical examination
- Record of vital signs
- AEs, SAEs record- Relapse Assessment
- Concomitant medication revision
- Haematological blood tests_comprehensive of: Complete blood tests and biochemistry, urinanalisys evaluation
- Infectivological screening: CMV-DNA, EBV-DNA, Galactomannan detection
- Administration of MsQuol54

UNSCHEDULED VISIT:
In case of clinical, neurological deterioration or every systemic new symptom not due to MS an unscheduled visit will be performed. During this visit the following procedure might be performed, at investigator discretion:
- complete neurological and general examination
- Haematology and biochemistry examination complete of ESV e CRP, CMV DNA, EBV DNA will be determinate every 24 weeks
- Chest Xray
- Brain and spinal cord MRI with gadolinium
- Lumbar puncture

MS RELAPSE VISIT
The assessment, management and reporting of MS relapse is made by the Primary Treating Physician.
MS relapse definition: appearance of a new neurological abnormality or worsening of previously stable or improving pre-existing neurological abnormality, separated by at least 30 days from onset of a preceding clinical demyelinating event (McDonald et al. 2001). The abnormality must have been present for at least 24 hours and occurred in the absence of fever (< 37.5°C) or known infection. MS relapse is defined as: any perceived change in symptoms or signs that persist for ≥ 48 hours in the absence of flu or fever and that is accompanied by objective changes on EDSS functional subscores (FSS) that leads to either a 1.0 point change in EDSS (for baseline EDSS 3 - 5.0) or a 0.5 change (for baseline EDSS ≥ 5.5) together with FSS changes of 2 points in any one FSS or 1 point in any 2 FS. Only relapses meeting the protocol definition will be considered as measure of efficacy.
Diagnosing MS relapses during the study: Patients will be instructed to immediately contact the Primary Treating Physician if he/she develops new or re-occurring or worsening neurological symptoms. If a patient reports new neurological symptoms or worsening of previous symptoms, an unscheduled visit is to be planned as soon as possible, ideally within 7 days. During this visit, the treating physician will first assess whether the new/worsening neurological abnormality is consistent with the definition of MS relapse above.
If so, the standard neurological examination (for the EDSS score) should be performed by the Independent EDSS Rater. If there is any doubt in the opinion of the treating physician, the default must always be to refer the case to the Independent EDSS Rater to perform an EDSS rating.

Confirmation of MS relapse: the definition of a confirmed MS relapse is one accompanied by a clinically relevant change in the EDSS performed by the Independent EDSS Rater, i.e. an increase of at least 0.5 points on the EDSS score, or an increase of 1 point on two FSs or 2 points on one FS, excluding changes involving bowel/bladder or cerebral FS compared to the previous available rating (the last EDSS rating that did not occur during a relapse).

9 Ethical and Regulatory Considerations

9.1 Regulatory and ethical compliance

This clinical study was designed, shall be implemented, conducted and reported in accordance with:

- the ethical principles laid down by the 18th World Medical Assembly (Helsinki, 1964) and all applicable amendments laid down by the World Medical Assemblies;
- the International Conference on Harmonization (ICH) Harmonized Tripartite Guidelines for Good Clinical Practice (GCP);
- all international laws and regulations;
- all national laws and regulations of the country(ies) in which the clinical trial is performed;
- all international or national applicable guidelines.

9.2 Responsibilities of IRB/IEC/REB

The protocol, the patient information sheet, the data protection declaration form, the informed consent/assent form and all proposed clinical trial documents must be reviewed and approved by a properly constituted Institutional Review Board/Independent Ethics Committee/Research Ethics Board (IRB/IEC/REB) before study start.

It is mandatory to obtain the written and dated approval/favorable opinion, signed by the chairman with (IRB/IEC/REB) composition. The clinical trial (study number, clinical trial protocol title and version number), the documents reviewed (clinical trial protocol, Informed Consent Form, Investigator's Brochure, investigator's CV, etc.), the list of voting members and their qualifications and the date of the review should be clearly stated on the written ethics committee approval/favorable opinion.

9.3 Responsibilities of Competent Authority(ies)

The protocol and all proposed clinical trial documents must be reviewed and approved by Competent Authority(ies) of the country(ies) in which the clinical trial is performed before study start. It is mandatory to obtain the written and dated authorization, signed by the chairman of CA of the country(ies) in which the clinical trial is performed before study start.

9.4 Responsibilities of the Investigator

Prior to study start, the investigator is required to sign a protocol signature page confirming his/her agreement to conduct the study in accordance with these documents and all of the instructions and procedures found in this protocol. The investigator undertake to perform the clinical trial in accordance with this clinical trial protocol, ICH/Good Clinical Practice and the applicable regulatory requirements. The investigator is required to ensure compliance with all procedures required by the clinical trial protocol and with all study required procedures. The investigator agrees to provide all information requested in the Case Report Form (CRF) in an accurate and legible manner and to give access to all relevant data and records to Sponsor monitors, auditors, Clinical Quality Assurance, IRBs/IECs/REBs and regulatory authorities as required.

9.5 Patient information and informed consent

All patients should be informed to the fullest extent possible about the study, in language and terms they are able to understand. The investigator (according to applicable regulatory requirements), or a person designated by the investigator, and under the investigator's responsibility, should fully inform the patient of all pertinent aspects of the clinical trial. The patient must be given ample opportunity to decide whether or not to participate in this study and to ask questions concerning this. It must also be made clear to the patient that he/she can withdraw from the study at any time without giving reasons and that he/she will not be in any way disadvantaged by this.

Prior to a patient's participation in the clinical trial, he/she MUST give consent freely and consciously.

Eligible patients may only be included in the study after providing written (witnessed, where required by law or regulation), IRB/IEC/REB-approved informed consent or, if incapable of doing so, after such consent has been provided by a legally acceptable representative of the patient. In cases where the patient's representative gives consent, the patient should be informed about the study to the extent possible given his/her understanding. If the patient is capable of doing so, he/she should indicate assent by personally signing and dating the written informed consent document or a separate assent form.

Informed consent/assent must be obtained before conducting any study-specific procedures (i.e. all of the procedures described in the protocol). The process of obtaining informed consent/assent should be documented in the patient source documents. The date when a patient's informed consent/assent was actually obtained will be captured in their CRFs. Any Informed Consent will be part of Investigator's file and retained with it. A copy of the signed and dated written Informed Consent Form must be provided to the patient.

9.6 Minimizing distress

Repeated invasive procedures may be painful or frightening. This trial is designed for minimized the discomfort, the studies are designed and conducted by investigators experienced in the treatment of patients.
The protocols and investigations was been designed specifically for the MS population (not simply re-worked from adult protocols) and approved by an IEC expert in patients.
Practical considerations to ensure that participants' experiences in clinical studies are positive and to minimize discomfort and distress include the following:

- Personnel knowledgeable and skilled in dealing with the MS population and its needs, including skill in performing MS procedures
- A physical setting with furniture, play equipment, activities, and food appropriate for MS
- The conduct of studies in a familiar environment such as the hospital or clinic where participants normally receive their care
- Approaches to minimize discomfort of procedures, such as:
- Topical anesthesia to place IV catheters
- Indwelling catheters rather than repeated venipunctures for blood sampling
- Collection of some protocol-specified blood samples when routine clinical samples are obtained

9.7 Minimizing Risk

However important a study may be to prove or disprove the value of a treatment, participants may suffer injury as a result of inclusion in the study, even if the whole community benefits. Every effort are made to anticipate and reduce known hazards. Investigators should be fully aware before the start of a clinical study of all relevant preclinical and clinical toxicity of the medicinal product. To minimize risk in clinical studies, those conducting the study should be properly trained and experienced in studying the, including the evaluation and management of potential adverse events.

In designing studies, every attempt are made to minimize the number of participants and of procedures, consistent with good study design. Mechanisms should be in place to ensure that a study can be rapidly terminated should an unexpected hazard be noted.

10 Data management

10.1 Definition of source data and source documents

Source Data: All information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents (original records or certified copies).
Source Documents: Original documents, data, and records (e.g., hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate copies, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories and at medico-technical departments involved in the clinical trial).
All parameters asked for in the case report form (CRF) should be documented in the source documents (see the follow scheme).

Parameter	Source document
Medical history / patient's eligibility	- patients' charts
Therapy	- patients' charts
Disease status	- laboratory reports - imaging reports
Adverse events	- patients' charts - laboratory reports - doctor's letters - nurses documentation
Physical examination	- patients' charts
Survival	- patients' charts, autopsy report if available

Table 3 – List of parameters and its source

For the following parameters, the CRF might be used as source document:
Specify the parameters

10.2 Documentation of data in the case report form (CRF)

All relevant data collected during the study for all of the patients enrolled in the study shall be entered in the CRF by the responsible investigator or someone authorised by him in a timely manner so that they are clear and legible. The physician shall confirm the completeness, correctness and plausibility of the data by his signature with the date. The entries shall be made with black ballpoint pen.
The properly filled in CRF will remain in the trial centre, in case of on-line web CRF a copy of all pages are conserved in the trial centre.
All the health workers, involved in the data management, have attended a specific course on use of CRF.

10.3 Data management

The data will be recorded in the CRF designed for this study. All CRF will be checked for completeness, plausibility and compliance with the ICH guidelines and the institutional SOPs.

10.4 Record keeping

The investigator shall arrange for the retention of the patient identification list, the signed informed consent forms and the signed data protection declaration for at least 15 years after the completion or discontinuation of the study. Patient files and other source data shall be kept for the maximum period of time permitted by the hospital.
The sponsor will keep essential documents according to ICH-GCP, chapter 8.

10.5 Independent Data Safety and Monitoring Committee

A DSMC is involved (specify the essential items of DSMC SOPs).

11 Statistical considerations

Descriptive statistics (including means, standard deviations, and medians for continuous variables and proportions for discrete variables) will be used to summarize data as appropriate. A statistical analysis plan (SAP) with detailed statistical methods and techniques used for data analysis will be prepared before conducting the analysis.

12 Quality assurance and quality control

12.1 Definitions

Audit: A systematic and independent examination of trial related activities and documents to determine whether the evaluated trial related activities were conducted, and the data were recorded, analyzed and accurately reported according to the protocol, sponsor's standard operating procedures (SOPs), Good Clinical Practice (GCP), and the applicable regulatory requirement(s).
Monitoring: The act of overseeing the progress of a clinical trial, and of ensuring that it is conducted, recorded, and reported in accordance with the protocol, Standard Operating Procedures (SOPs), Good Clinical Practice (GCP), and the applicable regulatory requirement(s).
Quality Assurance (QA): All those planned and systematic actions that are established to ensure that the trial is performed and the data are generated, documented (recorded), and reported in compliance with Good Clinical Practice (GCP) and the applicable regulatory requirement(s).
Quality Control (QC): The operational techniques and activities undertaken within the quality assurance system to verify that the requirements for quality of the trial-related activities have been fulfilled.

12.2 Monitoring

A comprehensive quality control can be ordered in the form of monitoring. It might include checking the whole course of the study, the management of documentation, the management of data, the management of subject enrolled the management of experimental drug and the management of biological sample. The Sponsor of the study will be in charge for monitoring.
The investigators will assure the monitoring of the clinical study to assure conformance to protocol as well as the completeness, correctness and plausibility of the completed case report forms (CRF).

12.3 Deviation from study protocol.

Every deviation from the trial protocol must be specified and documented separately for each patient. The investigator must consult with the monitor and discuss the type and extent of deviation as well as the possible consequences for further participation of the patient in the study. If the evaluability of a patient is questionable the coordinating investigator will be consulted.

12.4 Audits and inspections

If necessary a comprehensive quality control could be ordered in the form of an audit. It might include checking the whole course of the study, the documentation, statistical analysis and the investigators.
The Sponsor guarantees the availability for the inspections from the regulatory agencies.

12.5 Reference institutes/extramural review board (ERB)

No reference institute or extramural review board is planned.

13 Formalities/Regulatory aspects

13.1 Legal regulations and guidelines

This study will be conducted in conformance with the regulations of the latest versions of the current applicable laws: the last version of "Declaration of Helsinki" and the principles of good clinical practice (ICH-GCP).

13.2 Patient insurance

The Sponsor certifies that it has taken out a liability insurance policy which covers the liability of the Investigator and his/her co-workers and which is in accordance with local laws and requirements. An abstract copy of insurance certificate will be archived in Trial Master File.

13.3 Financial aspects

Fondazione Italiana Sclerosi Multipla (FISM), Fondazione CARIPLO and Associazione Amici del Centro Sclerosi Multipla Onlus (ACeSM) are financial sponsors of this clinical trial.
Agreements (contracts) between Hospital San Raffaele (via Olgettina, 60 – Milano) and financial sponsors assigned a grant to Neuroimmunology Unit.
This grant covers the costs of the present clinical trial.

13.4 Final study report

The Sponsor will be responsible for preparing a Final Clinical Study Report.

13.5 Declaration on the property of the data

The Sponsor of this trial declares that:

- ظ (First option) All the data deriving from this protocol belong to the Sponsor.

13.6 Publication policy

No specific rules are determined.

14 Feasibility declaration

The clinical trial feasibility is evaluated based on five principal items: Potential enrolment, Budget, Required staff, Facility, Supplies defined as following:

Potential enrolment: we estimate an enrolment of 24 patients per year, based on patient diagnosed or referred to our centre in the past 10 years.
Budget: the protocol has a dedicated reasonable (grant/financing/donation), managed by hSR grant office.
Required staff: our team is fully qualified, and trained, dedicated research staff is present to coordinate the trial. The PI has adequate time and scheduling availability to devote to the overall supervision of the trial. Sub-investigators in number and qualified adequate to assist PI. Ancillary or specialty staff (pharmacy, labs, diagnostics, etc) involved in the trial is adequate in number and qualification.
Facility: adequate clinic and office space is available. The following special equipment is present.
Supplies: The Sponsor will supply (Case Report Forms, source documents, electronic consent template, packaged lab kits, pre-paid shipping, drugs, etc.).

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