Clinical Study Protocol

Clinical Study Protocol Number	MS200136_0041
Title	Evaluation of cl**IN**ical re**C**overy after a **R**elapse: a pilot study ass**E**ssing the neuronal effects of D-**A**spartate in RR-M**S** subjects treated with Int**E**rferon beta 1a 44 mcg TIW (INCREASE)
Phase	II
IND Number	Not applicable
EudraCT Number	Not applicable
Coordinating Investigator	PPD
Sponsor	Merck Serono S.p.A. Medical Responsible: PPD Via Casilina, 125 00176 Rome, Italy Phone number: PPD Fax number: PPD
Sponsor Legal Representative in the European Union	Merck KGaA Frankfurter Strasse 250 64293 Darmstadt, Germany
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[redacted]

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List of Abbreviations

9HPT	9 Hole PEG Test
25-FWT	25-foot Timed Walk
AE	Adverse Event
ALT	Alanine Aminotransferase
ANOVA	Analysis of Variance
AP	Alkaline Phosphatase
AST	Aspartate Aminotransferase
BMI	Body Mass Index
BUN	Blood Urea Nitrogen
CI	Confidence Interval
CNS	Central Nervous System
CPK	Creatin phosphokinase
c.p.m	Counts per minute
CRO	Contract Research Organization
D-asp	D-aspartate
DMT	Disease Modifying Therapy
ECAR	Extracellular Acidification Rate
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
EDSS	Expanded Disability Status Scale
ET	Early Termination
FACS	Fluorescence Activated Cell Sorting
FDI	First Dorsal Interosseus
FSS	Functional Systems Scale
GCP	Good Clinical Practice
Gd+	Gadolinium enhanced
GMP	Good Manufacturing Practice
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IFN	Interferon
IP	Investigational Product
iTBS	Intermittent Theta Burst Stimulation
ITT	Intention to Treat
i.v.	Intravenous
LLN	Lower Limit of Normal
LTD	Long Term Depression
LTP	Long Term Potentiation
mAbs	Monoclonal Antibodies
MCP-1	Monocyte Chemoattractant Protein-1
MedDRA	Medical Dictionary for Regulatory Activities
MEP	Motor Evoked Potentials
MFIS	Modified Fatigue Impact Scale
MPO	Myeloperoxidase
MRI	Magnetic Resonance Imaging

MS	Multiple Sclerosis
NMDA	N-methyl-D-aspartate
NMDA	N-methyl-D-aspartate Receptor
NTR	Neurotrophins Receptor
OCR	Oxygen Consumption Rate
OPG	Osteoprotegerin
PBLs	Peripheral Blood Lymphocytes
PP	Per Protocol
PPMS	Primary Progressive Multiple Sclerosis
PT	Preferred Term
RBCs	Red Blood Cells
RPMI	Roswell Park Memorial Institute
RR-MS	Relapsing Remitting Multiple Sclerosis
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAS	Statistical Analysis Software
SDT	Symbol Digit Modalities Test
sLep-R	soluble Leptin Receptor
SOC	System Organ Class
sICAM-1	Soluble Intercellular Adhesion Molecule-1
sTNF-R	Soluble Tumour Necrosis Factor Receptor
SUSAR	Suspected Unexpected Serious Adverse Reaction
TEAE	Treatment-emergent Adverse Event
TIW	Three Times a week
TMS	Transcranial Magnetic Stimulation
ULN	Upper Limit of Normal
WBCs	White Blood Cells

1 Synopsis

Clinical Study Protocol Number	200136_0041
Title	Evaluation of cl**IN**ical re**C**overy after a **R**elapse: a pilot study ass**E**ssing the neuronal effects of D-**A**spartate in RR-M**S** subjects treated with Int**E**rferon beta 1a 44 mcg TIW (INCREASE)
Study Phase	II
IND Number	Not applicable
FDA covered study	No
EudraCT Number	Not applicable
Coordinating Investigator	PPD [redacted]
Sponsor	Merck Serono S.p.A. Via Casilina, 125 00176 Rome, Italy
Sponsor Legal Representative in the European Union	Merck KGaA Frankfurter Strasse 250 64293 Darmstadt, Germany
Study centers/countries	The study is planned to be performed in approximately 18 sites in Italy
Planned study period (first patient in-last patient out)	First patient in: December 2017 Last patient out: June 2020

Objectives:

Primary objective

The primary objective of the study is to evaluate the improving spontaneous recovery from the clinical deficits at the time of an acute relapse in relapsing remitting multiple sclerosis (RR-MS) patients already receiving interferon (IFN) beta 1a 44mcg three times a week (TIW) with the D-asp (versus placebo) as add-on therapy.

Secondary objectives

The secondary objectives of the study are:

- To evaluate the long term recovery in MS related disability following treatment with D-asp;
- To evaluate whether D-asp induces an effect in improving spontaneous recovery of the cognitive functions and fatigue after relapse;
- To evaluate whether D-asp enhances long term potentiation (LTP) induction, by using transcranial magnetic stimulation (TMS);
- To evaluate whether D-asp is able to influence the immune-phenotype and immune-metabolic response of lymphocytes in MS patients independently by relapse;
- To evaluate the safety and tolerability of D-asp as add-on therapy in RR-MS patients already receiving IFN beta 1a 44mcg TIW.

Methodology: This Phase II study will be a prospective, parallel group, double blind, balanced randomised block, placebo-controlled study. A number of 120 patients with MS presenting with a relapse or not will be randomised to receive either D-asp 2660mg once daily or placebo as add-on to conventional therapies (IFN beta-1a 44mcg TIW for patients without relapse and IFN beta-1a 44mcg TIW plus methylprednisolone i.v. for patients with relapse, for 5 consecutive days at 1000mg once daily).

The study plan will include five visits in total. An Early Termination (ET) visit will be performed in case of treatment discontinuation. The patients will be assessed at Screening (Visit -1, Day -5/-3), Baseline (Visit 0, Day 0-Week 0), and at Week 8 (Visit 1), Week 12 (Visit 2) and Week 24 (Visit 3, end of treatment). A ± 3-day window is allowed for the planned days of the visits.

The TMS will be performed only in the scheduled 40 patients without relapse enrolled in the MS centre of the PPD), which will follow a separate randomisation. Immune-phenotype and immune-metabolic response of lymphocytes will be evaluated in 80 patients, which will include 40 patients with relapse enrolled in the sites of Rome, Naples and Pozzilli, and 40 patients without relapse enrolled in the site of Pozzilli. This distribution will allow to measure immunological parameters in a balanced way among patients with D-asp and placebo. Patients that will perform immunological parameters will be randomised separately from those recruited in the other centres (which will enrol only patients with relapse that will be randomised to receive D-asp/placebo, and will not perform immunological assessments).

The duration of the whole study for a patient will be less than 25 weeks, which include 3-5 days of screening period and 24 weeks of treatment period.

Planned number of patients: A total number of 120 patients (80 with relapse and 40 without relapse) will be randomised (1:1) to receive D-asp or placebo.

Primary endpoints:

The primary endpoint of the study will be the proportion of patients with recovery in their MS related disability measured at 8 weeks through the Functional Systems Scale (FSS) of the EDSS (Visual, Brainstem, Pyramidal, Cerebellar, Sensory, Bowel/Bladder and Ambulation score). The subgroup of 40 patients without relapse will not be evaluated for the primary endpoint.

Secondary endpoints:

The secondary endpoints of the study will be:

- Proportion of patients with recovery in their MS related disability measured at 12 and 24 weeks through the Functional Systems Scale (FSS) of the EDSS (Visual, Brainstem, Pyramidal, Cerebellar, Sensory, Bowel/Bladder and Ambulation score);
- MS related disability and cognitive impairment measured through the 25-foot timed walk, the 9 hole PEG test, the Symbol Digit Modalities Test and the Low Contrast Letter visual Acuity Test during a clinical relapse and at 8, 12 and 24 weeks;
- Fatigue, measured through the Modified Fatigue Impact Scale (MFIS) and the Fatigue Severity Scale, at baseline during a clinical relapse and 8, 12 and 24 weeks after the onset of the relapse;

- LTP through TMS at baseline and 8, 12 and 24 weeks after the beginning of treatment, in patients without relapse;
- Immune-metabolic response of the lymphocytes in treated patients *in vitro* (glycolysis, mitochondrial respiration, fatty acids oxidation and circulating adipocytokines).

Pharmacokinetics: Not applicable

Safety endpoints:

- Incidence, severity, and relationship to treatment of treatment-emergent adverse events (TEAEs);
- Incidence, severity, and relationship to treatment of Serious Adverse Events (SAEs);
- Clinically significant changes in routine haematology, chemistry and urinalysis laboratory tests;
- Use of concomitant medications for the whole study period;
- Physical examination including recording of body mass index (BMI), waist circumference, vital signs (blood pressure and heart rate);
- Premature termination and reasons for premature termination from treatment.

Diagnosis and key inclusion and exclusion criteria:

The study will be conducted in 80 consecutive patients with MS relapse in a cohort of RR-MS patients and in 40 RR-MS patients without relapses, both already in treatment with IFN beta-1a 44mcg TIW for at least 6 months and less than 10 years before the screening visit.

Inclusion criteria

All patients (with or without MS relapse) must fulfil all of the following criteria to be eligible for this study:

- Males and females patients between 18 and 55 years of age;
- Patients with RR-MS, according to the revised McDonald Criteria (2010);
- Patients with an expanded disability status scale (EDSS) score between 0 and 3 before screening visit and before relapse;
- Patients receiving treatment with IFN beta 1a 44mcg TIW for at least 6 months but for no more than 10 years before the screening visit;
- Female patients must be neither pregnant nor breastfeeding and must lack childbearing potential as defined by either:
  - Postmenopausal or surgically sterile, or
  - Using a highly effective method of contraception for the duration of the study.

  Furthermore, female patients must not have been pregnant from at least three months prior to enter in the study;
- Patients willing and able to comply with the protocol for the total duration of the study;
- Patients able to understand the purposes and the risks of the study;
- Patients have signed the appropriate written informed consent form, approved by the Independent Ethics Committee (IEC), prior to the performance of any study activities.

MS Patients with relapse must fulfil the additional following criterion to be eligible for this study:

- Deterioration of at least one step in a relevant FSS (Visual, Brainstem, Pyramidal, Cerebellar,and Ambulation score) or an increase in EDSS of 1 point or more compatible, according to physician's judgment, with the therapy prosecution.
- Relapse started within maximum 5 days before the inclusion in the study.

MS Patients without relapse must fulfil the additional following criterion to be eligible for this study:

- Clinically stable RR-MS.

Exclusion criteria

All MS patients (with or without relapse) will be excluded from this study if they meet any of the following criteria:

- Patients with diagnosis of primary progressive MS (PP-MS);
- Patient have any disease other than MS that could better explain his/her signs and symptoms;
- Patients with any comorbidity with diseases that might alter synaptic plasticity (e.g. Parkinson Disease, Alzheimer Disease, Stroke);
- Patients receiving concomitant treatment with drugs that may alter synaptic plasticity (e.g. cannabinoids);
- Patients with history or presence of any unstable medical condition (e.g. tumour or infection, e.g. chronic infection or severe life threatening infection within the last 6 months);
- Patients who have received any corticosteroids therapy within 3 months prior to the screening;
- Patients with any concomitant disease that may require chronic treatment with systemic corticosteroids or immunosuppressive agents during the course of the study;
- Patients who have received any immunosuppressive agents other to corticosteroids, as monotherapy or combination therapy within 3 months prior to the screening visit;
- Patients with history or currently active primary or secondary immunodeficiency;
- Patients with inadequate liver function, defined by alanine aminotransferase (ALT) > 3 x upper limit of normal (ULN), or alkaline phosphatase (AP) > 2 x ULN, or total bilirubin > 2 x ULN if associated with any elevation of ALT or AP;
- Patients with inadequate bone marrow reserve, defined as a white blood cell count less than 0.5 x lower limit of normal (LLN);
- Patients with moderate to severe renal impairment;
- Patients unable to complete an MRI (contraindications for MRI include but are not restricted to weight ≥140 kg, pacemaker, cochlear implants, presence of foreign substances in the eye, intracranial vascular clips, surgery within 6 weeks of entry into the study, coronary stent implanted within 8 weeks prior to the time of the intended MRI, etc);

- Patients with contraindication to gadolinium (Gd) can be enrolled into the study but cannot receive Gd contrast dyes during their MRI scans;
- Patients receiving supplements that, in the Investigator's opinion, may affect the evaluation of fatigue;
- Patients with any known contraindications or hypersensitivity to D-aspartate or any excipient;
- Patients with any other significant disease that in the Investigator's opinion would impede study assessments or endanger the patient;
- Female patients with positive pregnancy test at baseline or patients with active project of pregnancy during the study;
- Patients with legal incapacity or limited legal capacity;
- Patients have participated in any other investigational study within 8 weeks before the screening visit.

Investigational Product: dose/mode of administration/ dosing schedule:

D-asp will be delivered at the dose of 2660mg once a day in form of oral solution preparation.

D-asp will be administered as add-on to conventional therapies (IFN beta-1a 44mcg TIW for patients without relapse and IFN beta-1a 44mcg TIW plus methylprednisolone i.v. for patients with relapse, for 5 consecutive days at 1000mg once daily).

Non Investigational Product: the prescription of IFN beta-1a in the individual patients will be totally independent from his/her inclusion in the study.

Reference therapy: dose/mode of administration/dosing schedule:

Placebo will be delivered once a day in form of oral solution preparation. Placebo will be administered as add-on to conventional therapies, as for D-asp.

Planned study and treatment duration per patient: 3-5 days of screening period followed by 24 weeks of treatment period

Statistical methods:

Sample size

The primary endpoint in the part of the study focused on relapsed MS patients is defined as the proportion of patients who, following a disease relapse associated with a worsening in their MS related disability, return to baseline status within 8 weeks. Disability will be assessed through the FSS of the EDSS (Visual, Brainstem, Pyramidal, Cerebellar, Sensory, Bowel/Bladder and Ambulation score).

Data from observational studies indicate that approximately 50% of the patients return to baseline EDSS within 2 months with standard treatments. Based on the fact that usually, in clinical studies where a rigid intention-to-treat (ITT) approach is used, a lower rate of responses/recoveries is observed as compared to observational studies, the expected rate of recovery at 2 months in the control group is set at 40%. With 40 patients per arm, the study will be able to detect with 80% power, at the 0.05 alpha level (1-sided), an increase in the proportion of recovery at 2 months from 40% to 70%.

Statistical analysis

The following analysis sets will be considered:

1) ITT Population: all patients enrolled into the study and assigned to the randomised treatment;

2) Per-protocol Population (PP): all patients treated according to protocol and satisfying inclusion/exclusion criteria, with absence of major protocol violations and adequate compliance with study medication;

3) Safety population: all patients who have received at least one dose of the planned study treatment.

Descriptive statistics (for continuous variables) or frequency count (for categorical data) will be summarized by each time point.

Demographic and clinical characteristics at baseline will be summarized as frequency distributions (for categorical variables) and descriptive statistics of mean, standard deviation, median, minimum and maximum (for continuous variables).

The efficacy analysis will be performed in the ITT population. In order to assess the robustness of the results, the analysis of the primary endpoint will be repeated in the PP population.

The comparison between the proportions of recoveries at 8 weeks in patients treated with D-asp and patients treated with placebo will be based on descriptive statistics (difference, relative risk and/or odds ratio) and on the chi-square test.

A set of exploratory analyses is planned. First, a multiple logistic regression model will be fitted to the data with recovery at 8 weeks as the dependent variable and age, sex, current disease modifying therapy (DMT) use, disease course, severity of relapse, and random assignment as covariates, to adjust for imbalances between the treatment groups.

Second, the time to recovery will be computed for each patient and included as the dependent variable in a multivariate Cox model, with all the above mentioned variables as covariates. Patients not recovered will be censored at last observation.

All continuous or semiquantitative variables will be tested for normality and homogeneity of variance by using Kolmogorov Smirnov and Levene tests. To evaluate for differences after treatment, between groups, repeated measures analysis of variance (ANOVA) will be used in case of parametric variables, and Mann-Whitney and Kruskal Wallis test will be used in case of non-parametric variables. For repeated measures, ANOVA sphericity will be tested by using Mauchly's test and results will be corrected in case of need, by using Greenhouse-Geisser method. Significance level will be set at $p<0.05$.

Analyses of safety variables will be performed on the safety population. Safety data will be summarized descriptively.

Table 1 Schedule of Assessments

	Screening	Baseline	On treatment visits		End of treatment	Early termination
Assessments	Day -5/-3	Day 0, Week 0	Week 8 (± 3 days)	Week 12 (± 3 days)	Week 24 (± 3 days)	
VISIT	VISIT -1	VISIT 0	VISIT 1	VISIT 2	VISIT 3	VISIT ET[A]
Written informed consent[B]	X					
Demographic data	X					
Medical History/Present Conditions	X					
History of MS/MS treatment	X					
Previous and concomitant medications	X[C]	X	X	X	X	X
Physical Examination[D]	X	X	X	X	X	X
Neurological examination	X	X	X	X	X	X
Recording of contraceptive method	X[E]					
Haematology, Blood chemistry, Urinalysis[L]	X		X		X	X
Pregnancy test	X[F]	X[G]				
Administration of EDSS	X	X	X	X	X	X
Inclusion and Exclusion criteria	X	X				
Administration of 25-FWT; 9HPT; Low Contrast Letter Visual Acuity Test; Symbol Digit Modalities Test	X	X	X	X	X	X
Administration of MFIS and Fatigue Severity Scale	X	X	X	X	X	X
Randomisation		X				
Neurophysiological assessments[H]		X	X	X	X	X
Immunological assessments[I]		X	X	X		X
Brain MRI[L]	X	X			X	X
12-lead ECG[L]	X				X	X
IP dispensing		X	X	X		
IP returning and compliance			X	X	X	X
Adverse Events[M]	X	X	X	X	X	X
Reason for study termination						X

25-FWT = 25-foot Timed Walk; 9HPT = 9 Hole PEG Test

A - Early Termination (ET) Visit is applicable for the patient that withdraws from the study at any time: they will undergo all assessments requested at the ET Visit as soon as possible after injection.

B - Prior to performing any study assessment (not part of the patient routine medical care).

C - Includes medications in the last 12 weeks

D - Includes height, weight, body mass index (BMI), waist circumference, vital signs (blood pressure and heart rate)

E - Only for female patient: if the patient is post-menopausal the age at menopause will be documented

F - Only for female patient: serum pregnancy test is not required if the patient is post-menopausal or surgically sterilized

G - Only for female patient: urine pregnancy test is not required if the patient is post-menopausal or surgically sterilized, or if a serum pregnancy test was performed within 7 days before Baseline (Visit 0)

H - To be performed at the centre of PPD), only in patients without relapse

I – Immunological assessments will include immune phenotype and immunometabolic analysis. The blood sampling must be done before receiving corticosteroids in the scheduled 80 patients with (40 patients) or without relapse (40 patients) enrolled in the sites of Rome, Naples and Pozzilli

L - Whenever it will be available according to local clinical practice

M - Recording of Adverse Events will start at signature of informed Consent

2 Sponsor, Investigators and Study Administrative Structure

This clinical study will be sponsored by Merck Serono S.p.A., Via Casilina 125, 00176 Rome, Italy.

The study will be conducted at approximately 18 sites in Italy, with competitive enrolment. Sites will be Academic or Hospital Neurological Departments.

The Coordinating Investigator (PPD) represents all Investigators for decisions and discussions regarding this study, consistent with the International Conference on Harmonisation (ICH) Topic E6 Good Clinical Practice (GCP; hereafter referred to as ICH GCP). The Coordinating Investigator will provide expert medical input and advice relating to study design and execution and is responsible for the review and signoff of the clinical study report.

Signature pages for the Protocol Lead and the Coordinating Investigator as well as a list of Sponsor responsible persons are in Appendix I.

The administrative structure of the study will be as follows:

- PPD is the CRO appointed for study management, monitoring and data management for this study.
- For this study, a steering committee will be organized. The role of the Study Steering Committee is to provide overall supervision of the study and ensure that it is being conducted in accordance with the study protocol, principles of GCP and the relevant regulations. The Study Steering Committee should agree the study protocol and any protocol amendments and provide advice to the investigators on all aspects of the study. Decisions about continuation or termination of the study or substantial amendments to the protocol are under the responsibility of the Study Steering Committee. Members of the Study Steering Committee will be: PPD

3 Background Information

Multiple sclerosis

Multiple Sclerosis (MS) is a chronic, inflammatory, demyelinating disease of the central nervous system (CNS) characterized, in its most frequent form, by a relapsing-remitting course (Compston and Coles, 2002). After a relapse, remyelination processes occur, that are followed by restoration of neuronal function (Irvine and Blakemore, 2008) and remission of the clinical deficit (Duncan et al, 2009). However, remyelination is frequently incomplete, especially in the advanced stages of the disease (Patrikios et al, 2006).

When remyelination and neuronal repair fail, irreversible damage progressively accumulates in the CNS, as shown by magnetic resonance imaging (MRI) studies (De Stefano at al, 1998; Trapp

et al, 1999). However, even if it may seem obvious that patients with new CNS lesions show a deteriorated clinical state, the association between radiological and clinical aspects is usually limited for MS. This lack of association is usually addressed as the clinico-radiological paradox (Barkhof, 2002). An additional important mechanism responsible for clinical recovery after a relapse in MS patients is synaptic plasticity (Schirmer et al, 2013).

Synaptic and adaptive plasticity

The nervous tissue has the capacity to potentiate or depotentiate interneuronal transmission at the level of synapses in a long lasting way (Bliss and Lomo, 1973). Increasing the efficiency of synaptic transmission, a phenomenon called long term potentiation (LTP), may potentially compensate the loss of synaptic inputs on surviving neurons following a brain damage, and restore their function.

Furthermore, LTP is also capable to drive the formation of new cerebral circuits (structural plasticity), with compensatory and adaptive utility (Singer et al, 2011; Zepeda et al, 2013). Indeed classic chemical neurotransmitters, apart from their activity as mediators of bioelectric conduction, also operate as neurotrophic factors (Wolff et al, 1978). When released by neurons, neurotransmitters induce or suppress axonal and dendritic outgrowth (Wolff and Wagner, 1983; reviewed in Mattson, 1988 and Wolff and Missler, 1993), spinogenesis (Shi et al, 1999; Maletic-Savatic et al, 1999; Richards et al, 2005) and synaptogenesis (Chang et al, 1991; Toni et al, 1999; Lamprecht and LeDoux, 2004).

Moreover, recent evidence suggests that, apart from its compensatory role, LTP induction may also affect survival of neuronal cells. Indeed, the processes leading to synaptic plasticity and neuronal survival share the same cellular receptors and intracellular signalling pathways. As example, activation of glutammate N-methyl-D-aspartate receptors (NMDAR) or of neurotrophins receptors (NTR) plays an essential role in the regulation of both long-term synaptic potentiation/depotentiation and neuronal survival/death induction. The presence of specific NMDAR (containing the GluN2A vs the GluN2B subunits) or NTR subtypes (type TrkB vs p75NTR), is associated with either neuronal survival or death. It was also observed that intracellular signalling molecular pathways leading to NMDAR-dependent LTP also promote neuronal survival, while those leading to long term depression (LTD) promote neuronal death (i.e. PI3K-Akt vs. PTEN/GSK3b and MAPK-ERK1/2 vs. p38) (Bartlett and Wang, 2013).

Long term potentiation and clinical recovery in humans

Long term potentiation can be measured non invasively and painlessly, in awake humans through transcranial magnetic stimulation (TMS) (*Hallet, 2007*). TMS uses high intensity, brief duration, magnetic fields that, when applied on the scalp, can activate neurons within a small focal region of the cerebral cortex, through electromagnetic induction. If delivered repetitively TMS can induce plastic modifications of cortical excitability. If these modifications are induced in the motor cortex, they can be measured by recording the motor evoked potentials (MEP) from the muscles represented within that cortical region. Either an increase or decrease of the MEP amplitude, lasting after the end of repetitive TMS, indicates that LTP- or LTD-like respectively, plastic changes of motor cortex excitability occurred.

TMS studies showed that LTP influences clinical expression of a brain damage. For example, in patients affected by Parkinson Disease, presenting with asymmetric clinical expression, TMS-induced LTP is greater in the cerebral hemisphere corresponding to the less affected side of the

body (Kojovic et al, 2012). A further study reported that after acute stroke patients showing greater LTP induction by TMS had a greater clinical recovery at six months (Di Lazzaro et al, 2010).

In MS patients also, a greater TMS-induced LTP correlates with the amount of clinical recovery 3 months after a relapse. In particular, the probability of presenting a full recovery of the relapse-related symptoms is significantly reduced in those patients showing a reduced or absent LTP at the time of a relapse (Mori et al, 2014). Moreover, a further study reported that in primary progressive MS (PP-MS) patients, a condition characterized by the ineluctable progression of MS-related disability without periods of remission or recovery, TMS-induced LTP is absent or reduced in comparison to RR-MS and healthy controls (Mori et al, 2013). These studies support the idea that the reduced capacity of inducing synaptic plasticity is associated to a reduced capacity of recovery from a clinical deficit after brain damage.

Furthermore, interferon (IFN) beta-1a is the only disease modifying therapy that was associated with an improvement of cortical plasticity and cognitive deficits in patients with inflammatory activity, as showed by the presence of gadolinium-enhancing (Gd+) lesions at brain MRI (Mori et al, 2012).

Long term potentiation and D-aspartate

At the synaptic level, LTP is mainly regulated by the NMDAR (Malenka and Nicoll, 1993). Animal models showed that the induction of compensatory plasticity in surviving neurons through NMDAR stimulation might limit the clinical manifestations of neuronal damage (Centonze et al, 2007; Li et al, 2007; Yaka et al. 2007; Molina-Luna et al, 2009). It has been thus hypothesized that NMDAR agonists may enhance LTP induction in RR-MS patients.

The NMDAR glutamate receptor agonist NMDA is synthesized by methylation of D-aspartate (D-asp) through the D-aspartate methyltransferase enzyme (D'Aniello et al, 2000). D-asp also acts as a classic neurotransmitter as demonstrated by the observation that its biosynthesis, degradation, absorption and release all occur in the pre-synaptic neurons, and that its release determines a response in the post-synaptic neurons (Ota et al, 2012). D-asp indeed binds the NMDAR at the L-Glu binding site (Fagg and Matus, 1984).

D-Aspartate: Tolerability and Safety

D-aspartic acid is a normal constituent of all animal tissues at variable concentrations depending on the type of tissue, and is present in all most common foods. The salified for of D-aspartic acid (D-aspartate–D-asp) is the physiological form present in animal and vegetal tissues.

D-asp expression in the CNS is very abundant during the embryonic and early life stages, while it significantly reduces during adulthood. The peak concentration of D-asp in the last embryonic life had suggested that it may promote the gene expression that forms the basis of the nervous development. This hypothesis is confirmed by the significantly increased presence of racemase, which synthesizes D-asp in the brain (Wolosker et al, 2000). In mice deprived of the gene that codes the racemase protein, and therefore with inhibited production of D-asp, brain nerve cells growth with impaired synaptic contacts (Kim et al, 2010). Moreover, it has been demonstrated that D-asp has an important role in neurotransmission (D'Aniello et al, 2011). As confirmation of its role in CNS, D-asp levels seem to be particularly low in the brains of schizophrenic (Errico et al, 2013) and Alzheimer (Fisher et al, 1991) patients.

Recent studies indicate that D-asp enhances LTP induction in rodents (Errico et al, 2008; Errico et al, 2011). Preliminary results from a study performed at the Laboratory of non-invasive Brain Stimulation, MS centre, Tor Vergata University Hospital, Rome, showed that after 2 weeks of D-asp 2660mg per day, oral intake TMS-induced LTP is enhanced in patients affected by progressive forms of MS.

D-asp is commercialized as food supplement and generally used at a dose varying between 2g and 3g per day. It is one of the ingredients of a food supplement marketed by Merck under the tradename GENADIS in Italy from 2011 and in Spain from 2013.

D-asp is indicated in patients with reduced endogenous synthesis of the enzyme racemase, and in patients that, due to dietary habits, introduce a low amount of D-asp.

The safety and tolerability of D-asp has been investigated in animal models and in humans. In animals in which D-asp was administered via intraperitoneal route, D-asp activated antioxidative, cytoprotective effects in the kidney and enhanced caspase levels, indicative of apoptosis in brain and heart tissues (Burrone et al, 2010). It also aggravated nephritis in rats induced by Staphylococcus aureus bacteria (Koyuncuoglu et al, 1988). In rats administered for 28 days with D-asp or its enantiomer L-aspartic acid, no pathological changes in the organs were observed and no signs of subacute toxicity (liver, kidney) were found (Schieber et al, 1997).

In humans, D-asp is well tolerated and no adverse reactions have been reported during D-asp use in infertility. In patients with oligo-asthenozoospermia (D'Aniello et al, 2012), a daily dose of 2660 mg of D-asp given for up to 3 months did not cause adverse effects or abnormal changes in laboratory parameters in treated patients.

In resistance trained men (Melville et al, 2015), treatment with a daily dose of 6g of D-asp decreased levels of total testosterone and free testosterone, without any concurrent change in other hormones measured, whereas 3g of D-asp had no significant effect on either testosterone markers. These results confirmed finding of a previous study (Willoughby and Leutholtz, 2013), in which resistance trained mean received 3g/day of D-asp or placebo for 28 days. The gonadal hormones were unaffected by D-asp supplementation and were not associated with the observed increases in muscle strength and mass. Therefore, at the dose provided, D-asp supplementation is ineffective in upregulating the activity of the hypothalamo-pituitary-gonadal axis and has no anabolic or ergogenic effects in skeletal muscle.

As further confirmation of the safety profile of D-asp, a product containing D-asp (DADAVIT), marketed in 2005, contains an amount of D-asp of 3120 mg. The recommended daily dose is of one unit (i.e. a dose higher than that used in this study). During the period of commercialisation more than 10.000 units have been sold every year, and no reports of adverse effects related with the use of the product have been notified.

Rationale for the study

Given the potential role of LTP compensating denervation and hinder neuronal death after a brain damage, this study was designed to test whether enhancing LTP by D-asp intake may favour spontaneous recovery from the clinical deficits following an acute relapse in patients affected by RR-MS already in treatment with subcutaneous IFN beta 1a 44mcg Three Times a week (TIW) according to clinical practice.

It has been hypothesized that dietary supplement of D-asp (2660mg per day) in patients affected by RR-MS may favour spontaneous clinical recovery by enhancing LTP in synergy with IFN beta 1a, after a clinical relapse.

The study will also include the *ex-vivo* and *in-vitro* assessment of the immune-phenotype and immune-metabolic response of lymphocytes. It is well known that immune and nervous system interact (Pavlov and Tracey, 2015) through a bidirectional communication. Specifically, neurotransmitters can affect immunity as well as immunity can affect neurotransmission. In the context of D-asp it is well known that NMDAR are expressed by immune cells, including T lymphocytes (Boldyrev et al, 2012). Thus the effect of D-asp on synaptic plasticity could be either direct or indirect through the modulation of immune cell activity, cytokine production and killing of myelin cells. In addition, given the D-asp aminoacidic nature, specific immunometabolic effects of D-asp on T cells can be hypothesized, particularly in the context of modulation of the inflammatory response by T cells and the enhancement of regulatory T cell activity. Given these premises, it will be crucial to investigate also the possible effects of D-asp on immunity in terms of effector and regulatory type responses.

Based on the above premises, this study will help understanding how the metabolic changes induced by D-asp may influence the immune responses towards triggering immunological tolerance and facilitate LTP. Therefore, the influence of D-asp on the proliferative capacity of Treg cells that play a protective role against the disease, will be evaluated *in vitro*; indeed their expansion index, which recently showed to be a reliable predictive marker of disease progression (Carbone et al, 2014) will be measured *in vitro* during treatment with D-asp.

Moreover, given that D-asp induces the augmentation of the number of dendritic spines and favors survival of neurons (Errico et al, 2008; Errico et al, 2011; Errico et al, 2013), brain atrophy will also be assessed in a subgroup of patients by mean of brain MRI, whenever possible and according to clinical practice.

Refer to the latest Investigator's Brochure (IB) for further information about the nonclinical and clinical programs and Guidance for the Investigator.

This clinical study will be conducted in compliance with the clinical study protocol, ICH GCP, and any additional applicable regulatory requirements.

Based on the available nonclinical and clinical data to date, the conduct of the study specified in this protocol is considered justifiable.

4 Study Objectives

4.1 Primary Objectives

The primary objective of the study is to evaluate the improving spontaneous recovery from the clinical deficits at the time of an acute relapse in relapsing remitting multiple sclerosis (RR-MS) patients already receiving interferon (IFN) beta 1a 44mcg TIW with the D-asp (versus placebo) as add-on therapy.

4.2 Secondary Objectives

The secondary objectives of the study are:

- To evaluate the long term recovery in MS related disability following treatment with D-asp;

- To evaluate whether D-asp induces an effect in improving spontaneous recovery of the cognitive functions and fatigue after relapse;
- To evaluate whether D-asp enhances LTP induction, by using transcranial magnetic stimulation (TMS);
- To evaluate whether D-asp is able to influence the immune-phenotype and immune-metabolic response of lymphocytes in MS patients independently by relapse;
- To evaluate the safety and tolerability of D-asp as add-on therapy in RR-MS patients already receiving IFN beta 1a 44mcg TIW.

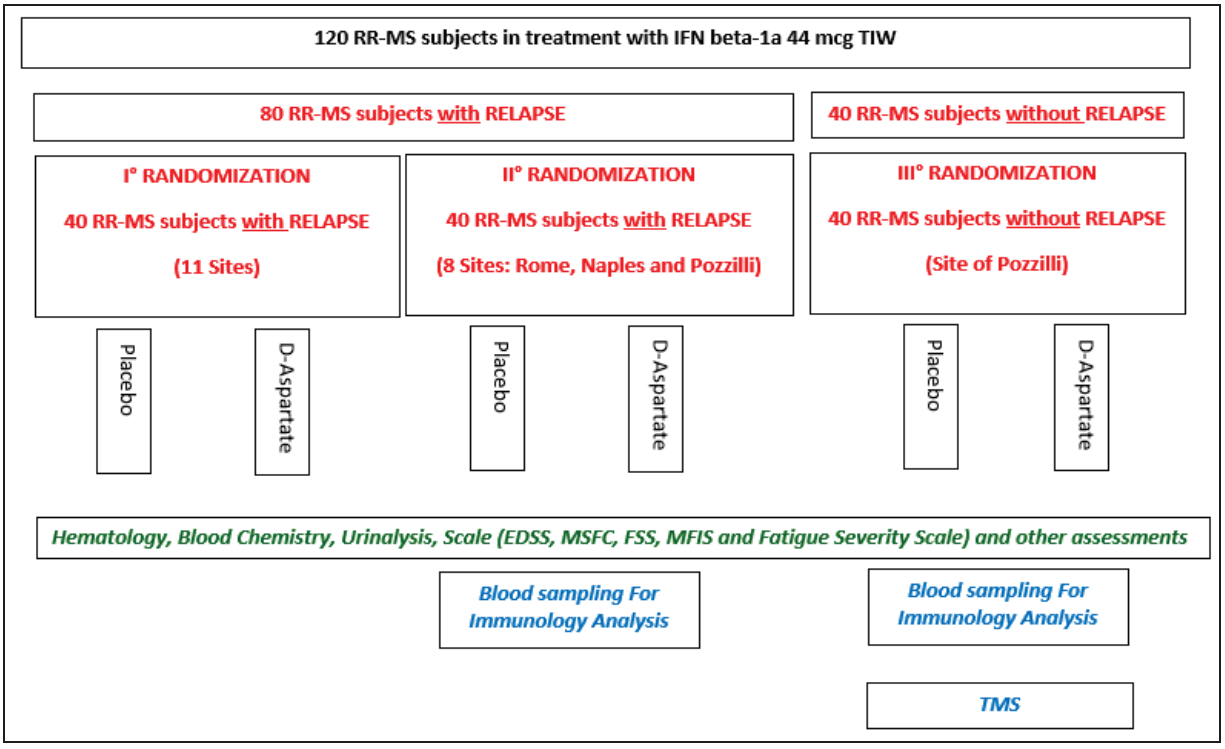
5 Investigational Plan

5.1 Overall Study Design and Plan

This Phase II study will be a prospective, parallel group, double blind, balanced randomised block, placebo-controlled study. The study is expected to start on December 2017 (first patient in) and to end on December 2020 (Clinical Study Report).

The study will be conducted according to the plan presented in Figure 1.

Figure 1. Study plan



Note: the first randomisation (from a specific randomisation list) will involve 10 sites, which will not be involved neither in the immunological analyses nor in TMS; the second randomisation (from a specific randomisation list) will involve only the sites of Rome, Naples and Pozzilli (Isernia), which will collect the blood samples for the immunology analyses, and the third randomisation (from a specific randomisation list) will involve site of Pozzilli for patients without relapse, which will perform the TMS.

The study will be carried out in approximately 18 investigational sites in Italy.

Patients will be recruited from the following 3 MS centres of the 5 Steering Committee members reported in Section 2: PPD

and from other approximately 16 qualified MS centres that will participate to the study.

A number of 120 MS patients presenting with a relapse or not will be randomised to receive either D-asp 2660mg once daily or placebo as add-on to conventional therapies (IFN beta-1a 44mcg TIW for patients without relapse and IFN beta-1a 44mcg TIW plus methylprednisolone i.v. for patients with relapse, for 5 consecutive days at 1000mg once daily).

The TMS will be performed only in the scheduled 40 patients without relapse enrolled in the MS centre of the PI

Immune-phenotype and immune-metabolic response of lymphocytes will be evaluated in 80 patients, which will include 40 patients with relapse enrolled in the sites of Rome, Naples and Pozzilli, and 40 patients without relapse enrolled in the site of Pozzilli. This distribution will allow to measure immunological parameters in a balanced way among patients with D-asp and placebo. Patients that will perform immunological parameters will be randomised separately from those recruited in the other centres (which will enrol only patients with relapse that will be randomised to receive D-asp/placebo and will not perform immunological assessments).

Determinations of immunological parameters will be performed in the laboratory IEOS-CNR in Naples on fresh blood.

The study plan will include five visits in total. An Early Termination (ET) visit will be performed in case of treatment discontinuation.

The patients will be assessed at Screening (Visit -1, Day -5/-3), Baseline (Visit 0, Day 0-Week 0), and at Week 8 (Visit 1), Week 12 (Visit 2) and Week 24 (Visit 3). A ± 3-day window is allowed for the planned days of the visits.

Clinical assessments and cognitive impairment (EDSS, 25-foot Timed Walk [25WFT]; 9 Hole PEG Test [9HPT]; SDT; Low Contrast Letter Acuity test; MFIS and FSS) will be performed in all patients at screening (Visit -1, Day -5/-3), baseline (Visit 0, Day 0-Week 0), and at Week 8 (Visit 1), Week 12 (Visit 2) and Week 24 (Visit 3) from the first day of treatment with D-asp/placebo, as well as at the ET visit in case of treatment discontinuation. In the case of occurrence of a relapse, clinical assessments will be recorded during the inpatient ward access by neurologist's experts in the diagnosis and treatment of MS.

Neurophysiological assessments (LTP and recruitment curve) will be performed at the centre of the PPD only in patients without relapse, at baseline (Visit 0, Day 0-Week 0), and at Week 8 (Visit 1), Week 12 (Visit 2) and Week 24 (Visit 3) from the first day of treatment with D-asp/placebo, as well as at the ET visit in case of treatment discontinuation.

Immune-phenotype and immunometabolic analysis will be performed in patients enrolled in the centres of Rome, Naples and Pozzilli in samples obtained at baseline (Visit 0, Day 0-Week 0) before receiving corticosteroids, and after 8 (Visit 1) and 12 (Visit 2) weeks from the first day of treatment with D-asp/placebo, as well as at the ET visit in case of treatment discontinuation.

Brain MRI will be performed according to local clinical practice at screening (Visit -1, Day -5/-3), baseline (Visit 0, Day 0-Week 0) or previous MRI will be evaluated if available and 24 (Visit 3) weeks from the first day of treatment with D-asp/placebo, as well as at the ET visit in case of treatment discontinuation, whenever it will be available.

Adherence to treatment will be assessed after 8 (Visit 1), 12 (Visit 2) and 24 (Visit 3) weeks from the first day of treatment with D-asp/placebo, as well as at the ET visit in case of treatment discontinuation.

The occurrence of adverse events (AEs) will be monitored over the total study duration.

Physical examination including recording of vital signs will be performed at screening (Visit -1, Day -5/-3), baseline (Visit 0, Day 0-Week 0), and after 8 (Visit 1), 12 (Visit 2) and 24 (Visit 3) weeks from the first day of treatment with D-asp/placebo, as well as at the ET visit in case of treatment discontinuation.

A serum pregnancy test will be performed in childbearing potential females within 7 days before screening (Visit -1, Day -5/-3). A urine pregnancy test will be performed at baseline (Visit 0, Day 0-Week 0), unless the patient is postmenopausal or surgically sterilized, or if a serum pregnancy test was performed within 7 days before Baseline (Visit 0).

Standard haematology, biochemistry and urine safety tests will be performed according to local clinical practice at screening (Visit -1, Day -5/-3), baseline (Visit 0, Day 0-Week 0), and after 8 (Visit 1), 12 (Visit 2) and 24 (Visit 3) weeks from the first day of treatment with D-asp/placebo, as well as at the ET visit in case of treatment discontinuation.

A standard 12-lead ECG will be performed according to local clinical practice at screening (Visit -1, Day -5/-3) and after 24 (Visit 3) weeks from the first day of treatment with D-asp/placebo, as well as at the ET visit in case of treatment discontinuation.

The duration of the whole study for a patient will be less than 25 weeks, which include 3-5 days of screening period and 24 weeks of treatment period.

5.2 Discussion of Study Design

This Phase II study will be conducted according to a prospective, parallel group, double blind, placebo-controlled study.

The use of a comparative double blind design with the inclusion of a placebo arm is aimed at minimizing the risk of biases in the evaluation of the effects of D-asp. Moreover, the use of a placebo control arm was considered as appropriate for a reliable assessment of the efficacy of the active treatment.

The study is focused on changes in spontaneous recovery after relapse potentially due to D-asp. The expected results of the study are: 1) greater clinical recovery 8 weeks after relapse onset (evaluated as a recovery to the EDSS baseline); 2) enhancement of TMS-induced LTP; and 3) decreased cerebral atrophy in the active treatment group compared to placebo.

The rationale for the study is described in Section 3. A treatment period lasting for 24 weeks is considering adequate for the assessment of the objectives of the study.

The proposed study endpoints are widely accepted as clinically relevant and have been used in numerous clinical studies in patients with RR-MS. The rationale for the *ex-vivo* and *in-vitro*

assessment of the immune-phenotype and immune-metabolic response of lymphocytes is detailed in Section 3.

5.2.1 Inclusion of Special Populations

Not applicable.

5.3 Selection of Study Population

The study will be conducted in 80 consecutive MS patients with relapse in a cohort of RR-MS patients and in 40 RR-MS patients without relapses, both already in treatment with IFN beta-1a 44mcg TIW for at least 6 months and less than 10 years before the screening visit.

Relapse is defined as the occurrence of new or worsening neurological symptoms attributable to MS, which must persist for >24 hours and should not be attributable to confounding clinical factors (e.g., fever, infection, injury, adverse reactions to medications) and immediately preceded by a stable or improving neurological state for least 30 days.

Only persons meeting all the inclusion criteria and none of the exclusion criteria may be enrolled into the study as patients. Prior to performing any study assessments not part of the patient's routine medical care, the Investigator will ensure that the patient or the patient's legal representative has provided written informed consent following the procedure described in Section 9.2.

5.3.1 Inclusion Criteria

All patients (with or without relapse) must fulfil all of the following criteria to be eligible for this study:

- Males and females between 18 and 55 years of age;
- Patients with RRMS, according to the revised McDonald Criteria (2010) (Polman et al, 2011);
- Patients with an expanded disability status scale (EDSS) score between 0 and 3 before screening visit (Kurtzke, 1983) and before relapse (if applicable);
- Patients receiving treatment with IFN beta 1a 44 mcg TIW for at least 6 months but for no more than 10 years before the screening visit;
- Female patients must be neither pregnant nor breastfeeding and must lack childbearing potential as defined by either:
  - Postmenopausal or surgically sterile, or
  - Using a highly effective method of contraception for the duration of the study.

  Furthermore, female patients must not have been pregnant from at least three months prior to enter in the study;
- Patients willing and able to comply with the protocol for the total duration of the study;
- Patients able to understand the purposes and the risks of the study;

- Patients have signed the appropriate written informed consent form, approved by the Independent Ethics Committee (IEC), prior to the performance of any study activities.

Patients with relapse must fulfil the additional following criterion to be eligible for this study:

- Deterioration of at least one step in a relevant functional system scale (FSS) (Visual, Brainstem, Pyramidal, Cerebellar and Ambulation score) or an increase in EDSS of 1 point or more compatible, according to physician's judgment, with the therapy prosecution;
- Relapse started within maximum 5 days before the inclusion in the study.

Patients without relapse must fulfil the additional following criterion to be eligible for this study:

- Clinically stable RR-MS.

5.3.2 Exclusion Criteria

All patients (with or without relapse) will be excluded from this study if they meet any of the following criteria:

- Patients with diagnosis of primary progressive MS (PP-MS);
- Patients have any disease other than MS that could better explain his/her signs and symptoms;
- Patients with any comorbidity with diseases that might alter synaptic plasticity (e.g. Parkinson Disease, Alzheimer Disease, Stroke);
- Patients receiving concomitant treatment with drugs that may alter synaptic plasticity (e.g. cannabinoids);
- Patients with history or presence of any unstable medical condition (e.g. tumor or infection, e.g. chronic infection or severe life threatening infection within the last 6 months);
- Patients who have received any corticosteroids therapy within 3 months prior to the screening;
- Patients with any concomitant disease that may require chronic treatment with systemic corticosteroids or immunosuppressive agents during the course of the study;
- Patients who have received any immunosuppressive agents other to corticosteroids, as monotherapy or combination therapy within 3 months prior to the screening visit;
- Patients with history or currently active primary or secondary immunodeficiency;
- Patients with inadequate liver function, defined by alanine aminotransferase (ALT) > 3 x upper limit of normal (ULN), or alkaline phosphatase (AP) > 2 x ULN, or total bilirubin > 2 x ULN if associated with any elevation of ALT or AP;
- Patients with inadequate bone marrow reserve, defined as a white blood cell count less than 0.5 x lower limit of normal (LLN);
- Patients with moderate to severe renal impairment;
- Patients unable to complete an MRI (contraindications for MRI include but are not restricted to weight ≥140 kg, pacemaker, cochlear implants, presence of foreign substances in the eye,

intracranial vascular clips, surgery within 6 weeks of entry into the study, coronary stent implanted within 8 weeks prior to the time of the intended MRI, etc). Patients with contraindication to Gd can be enrolled into the study but cannot receive Gd contrast dyes during their MRI scans;

- Patients with contraindication to gadolinium (Gd) can be enrolled into the study but cannot receive Gd contrast dyes during their MRI scans;
- Patients receiving supplements that, in the Investigator's opinion, may affect the evaluation of fatigue;
- Patients with any known contraindications or hypersensitivity to D-asp or any excipient;
- Patients with any other significant disease that in the Investigator's opinion would impede study assessments or endanger the patient.
- Female patients with positive pregnancy test at baseline or patients with active project of pregnancy during the study;
- Patients with legal incapacity or limited legal capacity;
- Patients have participated in any other investigational study within 8 weeks before the screening visit.

5.4 Criteria for Initiation of Study Treatment

Patients that fulfil all of the inclusion criteria and none of the exclusion criteria at Screening (Visit -1, Day -5/-3) will enter the 3-5 days of screening phase. All inclusion and exclusion criteria will be checked again at Baseline (Visit 0, Day 0-Week 0). All patients still eligible at the Baseline visit (Visit 0, Day 0-Week 0) will be randomised to receive treatment with D-asp or matched placebo.

5.5 Criteria for Subject Withdrawal

5.5.1 Withdrawal from Study Therapy

A patient must be withdrawn from IP if any of the following occur:

- Patient withdraws consent;
- Patient is lost to follow up;
- Participation in another clinical study;
- Occurrence of an exclusion criterion which is clinically relevant and affects the patient's safety, if discontinuation is considered necessary by the Investigator and/or Sponsor;
- Therapeutic failure requiring urgent additional drug;
- Any events that unacceptably endanger the safety of the patient;
- Occurrence of adverse events, if discontinuation of study drug is desired or considered necessary by the Investigator and/or the patient;
- Occurrence of pregnancy;

- Serious intercurrent illness or significant worsening of intercurrent illness;
- MS-related neurological events requiring steroid treatment (in this case, an ET visit will be conducted, during which a blood sample will be taken before the start of the steroid treatment);
- Patients without relapse at entry in the study that will relapse during the 24-week treatment phase.

If a patient prematurely withdraws from the IP at any time during the study, he/she will undergo all assessments requested at the ET visit (Section 7.1) as soon as possible after his/her last injection. In any case, the appropriate electronic Case Report Form (e-CRF) section including the reason(s) for withdrawal from the IP must be completed.

5.5.2 Withdrawal from the Study

Patients may withdraw from the study at any time without giving a reason.

Patient must be withdrawn from the study if a patient withdraws the informed consent. Withdrawal of consent will be considered as a withdrawal from the study.

Patient must be also withdrawn from the study if he/she participates in another clinical study.

If a patient has failed to attend scheduled study assessments, the Investigator must determine the reasons and the circumstances as completely and accurately as possible.

If a patient fails to return for assessments, attempts should be made to contact the patient to determine whether or not the reason for not returning is an adverse event. Likewise if a patient declares his/her wish to discontinue the study, e.g., for personal reasons, an attempt should be made to establish whether or not the true reason is an adverse event (bearing in mind the patient is not obliged to state his/her reasons).

If a patient prematurely withdraws from the study at any time, he/she will undergo all assessments requested at the ET visit (Section 7.1) as soon as possible after his/her last injection. In any case, the appropriate e-CRF section including the reason(s) for withdrawal from the study must be completed.

The patient who has withdrawn early will receive other appropriate treatment, in accordance with the study site's standard of care and generally accepted medical practice and depending on the patient's individual medical needs.

5.6 Premature Termination of the Study

The clinical study may be terminated prematurely or suspended at the request of Health Authorities or if new safety or efficacy information leads to an unfavourable risk benefit judgment for any IP. The Sponsor may discontinue the study if it becomes unjustifiable for medical or ethical reasons, for poor enrolment, or because of discontinuation of clinical development of an IP or withdrawal of an IP or comparator from the market for safety reasons.

Health Authorities and Independent Ethics Committees (IECs) will be informed about the discontinuation of the study in accordance with applicable regulations.

5.7 Definition of End of Study

This study is considered closed when all the e-CRFs are satisfactorily completed and the database is finally locked.

A clinical study protocol may not be considered closed as long as:

- Any patient is still receiving any IP;
- Visits specified by the protocol are still taking place;
- Procedures or interventions according to the protocol are still being undertaken in any patient;
- The post-treatment follow up period, defined in the clinical study protocol as being part of the study, has not yet been completed for any patient.

6 Investigational Product and Other Drugs Used in the Study

The term "Investigational Product" (IP) refers to an active substance or a placebo being tested or used as a reference therapy in a clinical study, including products that have a marketing authorization but are formulated, packaged, or administered differently from the authorized form, used for an unauthorized indication, or used to gain further information about the authorized form.

6.1 Description of the Investigational Product

In this study, the term "Investigational Product" (IP) refers to D-asp or placebo.

Investigational Product

D-asp is commercialized and approved in Italy for its use in humans as a dietary supplement.

Underlying therapy

D-asp 2660mg or placebo will be administered once daily as add-on to conventional therapies (IFN beta-1a 44mcg TIW for patients without relapse and IFN beta-1a 44mcg TIW plus methylprednisolone i.v. for patients with relapse, for 5 consecutive days at 1000mg once daily).

Patients and investigators involved in both clinical and neurophysiological and MRI assessments will be blinded to patients' group allocation.

Draft Labels of IP are inserted in Appendix VI.

6.2 Dosage and Administration

D-asp at the dose of 2660mg or placebo will be administered once a day for 24 weeks in the form of identical oral solution preparations. The IP (D-asp or placebo) should be administered preferably after the meal.

The content of a sachet should be cleared out in an empty glass. Then approximately 100 ml of still water should be added and the product should be mixed until the effervescence is ended. The product should be administered immediately after the preparation.

Placebo will be undistinguishable from D-asp in terms of packaging, size, colour, weight, appearance, taste and mode of administration.

6.3 Assignment to Treatment Groups

Patients presenting with a relapse and meeting the eligibility criteria during a screening period of 3-5 days will be randomised in a 1:1 ratio via e-CRF to:

- D-asp,
- Placebo.

It is planned to randomise 80 patients with relapse. Randomisation will be stratified according to age (<30 years vs. ≥ 30 years) and site location (Rome+Naples+Pozzilli vs. the other sites).

Patients without a relapse will be recruited only at the Pozzilli site and will be randomised in a 1:1 ratio via e-CRF to:

- D-asp,
- Placebo.

It is planned to randomise 40 patients without a relapse. Randomisation will be stratified according to age (<30 years vs. ≥ 30 years)

The randomisation list will be in a balanced block design and will be centralized. In each site, eligible patients will be sequentially assigned to the next study number as they present themselves for the study, starting from the lowest number provided for each centre. If a patient discontinues from the study, the patient number will not be reused, and the patient will not be allowed to re-enter the study.

It will be the responsibility of the Investigator (or designee) to explain, and make sure patients fully understand any appropriate treatment related information.

6.4 Non-investigational Medicinal Products to be Used

Treatment with the IP (D-asp 2660mg once daily or placebo) will be administered as add-on to conventional therapies (IFN beta-1a 44mcg TIW for patients without relapse and IFN beta-1a 44mcg TIW plus methylprednisolone i.v. for patients with relapse, for 5 consecutive days at 1000mg once daily).

The prescription of IFN beta-1a in the individual patients will be totally independent from his/her inclusion in the study.

IFN beta-1a will be prescribed exclusively following the indications approved by the Italian Regulatory Authorities, and following the clinical routine of each participating site.

IFN beta-1a will not supplied by the Sponsor and the Investigator should use commercially available sources.

6.5 Concomitant Medications and Therapies

All concomitant medications taken by the patient during the study, from the date of signature of informed consent are to be recorded in the appropriate section of the e-CRF, noting the name, dose, duration and indication of each drug. Nondrug interventions and any changes to a concomitant medication or other intervention should also be recorded in the e-CRF.

6.5.1 Permitted Medicines

The investigator and/or study personnel will record all concomitant medications taken by the patient during the study from the date of signature of informed consent.

Any medications other than those excluded by the protocol, which are considered necessary for the patient's welfare, and which do not interfere with the study medication, may be given at the discretion of the investigator. Administration of all concomitant medications must be reported in the appropriate section of the e-CRF along with dosage information, dates of administration and reasons for use. If the study drug is discontinued, all continuing medication taken must be recorded in the e-CRF until the end of the study. The following conditions should be addressed by the investigator with particular attention, documenting specifically the reasons for the administration of a concomitant medication for the following cases:

- For a MS related condition (e.g. pain, fatigue or weakness, bladder dysfunction, spasticity, etc.);
- For a medical condition already reported in the patients Medical History (e.g. any form of pain, especially lumbar pain and headache, depression, etc.) and/or in the Family History (primary headache, insomnia etc.);
- For prophylactic use (e.g. flu-like symptoms, neuroprotection, etc.).

6.5.2 Prohibited Medicines

The following medicines are prohibited for the total study duration, i.e. from the screening visit to the end of treatment or ET visit:

- Any concomitant treatment for MS, i.e. other IFN therapy to IFN beta 1a 44mcg TIW, glatiramer acetate, dimethyl fumarate, fingolimod, teriflunomide, natalizumab, alemtuzumab, mitoxantrone;
- Concomitant treatment with drugs that may alter synaptic plasticity (e.g. cannabinoids);
- Any corticosteroids therapy (apart from the supportive therapy with methylprednisolone i.v. for patients with relapse for 5 consecutive days at 1000mg once daily);
- Any other immunosuppressive agents other to methylprednisolone i.v. for patients with relapse for 5 consecutive days at 1000mg once daily, given as monotherapy or combination therapy.

If a prohibited concomitant drug is administered during the study, the patient should be discontinued from the study.

6.5.3 Other Interventions

Any unplanned diagnostic, therapeutic or surgical procedure performed during the study period must be recorded in the concomitant procedure section of the e-CRF, including the date, indication, description and outcome of the procedure(s).

6.5.4 Special Precautions

Not applicable. There are no known special precautions to be taken with respect to D-asp.

6.5.5 Management of Specific Adverse Events or Adverse Drug Reactions

Not applicable. There are no known specific adverse events or adverse drug reactions related with the administration of D-asp.

6.6 Packaging and Labelling of the Investigational Product

All IPs will be packaged and labelled in accordance with all applicable regulatory requirements and Good Manufacturing Practice (GMP) Guidelines.

6.7 Preparation, Handling, and Storage of the Investigational Product

The active IP will be made available in sachets, each containing 2660mg of D-asp. The product from the market (marketed by Giellepi SpA) will be used in the study.

Each sachet of active IP will contain D-asp and the following excipients: saccharose, sodium carbonate, citric acid, carragenine, Arabic gum, acesulfame K. Each sachet of placebo will contain the excipients only.

The total weight of each sachet of D-asp or placebo will be 7 grams.

The IP should be stored and kept in a cool and dry place, and protected from light.

6.8 Investigational Product Accountability

The Investigator (or a designee) is responsible for ensuring IP accountability, including reconciliation of drugs and maintenance of records.

- Upon receipt of IP, the responsible person will check for accurate delivery and acknowledge receipt by signing or initialling and dating the appropriate documentation and returning it to the location specified. A copy will be archived for the Investigator Site File.
- Investigational product dispensing will be recorded on the appropriate drug accountability forms so that accurate records will be available for verification at each monitoring visit.
- Study site IP accountability records will include the following:
  - Confirmation of IP receipt, in good condition and in the defined temperature range.
  - The inventory of IP provided for the clinical study and prepared at the site.
  - The use of each dose by each patient.
  - The disposition (including return, if applicable) of any unused IP.
  - Dates, quantities, batch numbers, units numbers, expiry dates, and the individual patient study numbers.

The Investigator site should maintain records, which adequately document that patients were provided the doses specified in this protocol, and all IPs provided were fully reconciled.

Unused IP must not be discarded or used for any purpose other than the present study. No IP that is dispensed to a patient may be re-dispensed to a different patient.

A Study Monitor will periodically collect the IP accountability forms.

6.9 Assessment of Investigational Product Compliance

IP will be distributed to patients at the Baseline visit (Visit 0, Day 0-Week 0), and at Week 8 (Visit 1) and Week 12 (Visit 2). Patients will be instructed to bring with them the unused IP at Week 8 (Visit 1), Week 12 (Visit 2) and Week 24 (Visit 3), or at the ET visit in case of treatment discontinuation, in which the adherence to the IP will be evaluated.

The patient's compliance to IP will be evaluated on the basis of the count of the used IP, defined as the difference between the number of units of IP dispensed at the Baseline visit (Visit 0, Day 0-Week 0), and at Week 8 (Visit 1) and Week 12 (Visit 2), and the number of unused units of IP returned at Week 8 (Visit 1), Week 12 (Visit 2) and Week 24 (Visit 3), or at the ET visit in case of treatment discontinuation.

The evaluation of the compliance in the overall treatment period will be done using the following formula:

Compliance (%) = [Total number of administered doses/Total number of scheduled doses] x 100

The total number of scheduled doses will be calculated on the basis of the extent (days) of exposure of each patient. A range between 80-120% will be taken as reference limits for a satisfactory level of compliance.

6.10 Blinding

The realization of the double blind design will be made possible by the realization of matched placebo that was identical to the active IP (D-asp) in terms of size, appearance, smell and taste.

The Randomisation List will not be available to the study centres, monitors, project Statisticians or to the project team at sponsor and/or CRO. All investigators and all other individuals directly involved in this study will remain blinded to the treatment assignment of D-asp or placebo until the analysis of the primary endpoint has been completed.

All breaks of the study blind must be adequately documented.

6.11 Emergency Unblinding

The study blind may be broken for an individual only if knowledge of the IP is essential for clinical management of the patient. The Investigator must promptly explain the reason for any unblinding of an IP to the Sponsor without revealing the result to any Sponsor employee except the designated Drug Safety representative (using the Emergency Unblinding Notification Form). The Investigator must record the date of unblinding and the reason in the e-CRF. Contact information for breaking the blind in an emergency is given on the patient emergency card provided to each patient (see Section 9.4).

Under certain circumstances, Drug Safety may be required to unblind the treatment assignment for an individual patient following a serious adverse event (SAE) or other serious events; for example, if an expedited regulatory report is required. See Section 7.4 for further details on expedited reporting and SAEs.

6.12 Treatment of Overdose

An overdose is defined as any dose greater than the highest daily dose included in a clinical study protocol or planned for an individual patient enrolled in the study. Even if it does not meet other criteria for an SAE, any overdose must be recorded in the study medication section of the e-CRF and reported to Drug Safety in an expedited manner using the SAE Report Form, and following the procedure in Section 7.4.

No known symptoms of overdose have been described with the use of D-asp.

6.13 Medical Care of Subjects after End of Study

The Sponsor will not provide any additional care to patients after they leave the study because such care should not differ from what is normally expected for patients with RR-MS.

7 Study Procedures and Assessments

7.1 Schedule of Assessments

The schedule of the Study Procedures and Assessments is reported in Table 1. Procedures and Assessments to be performed at each study visit are described below.

A study initiation visit must be conducted prior to the commencement of any study activities at the site.

General Instructions

Prior to performing any study assessments not part of the patient's routine medical care, the investigator will ensure that the patient has provided written informed consent according to the procedure described in Section 9.2.

Therefore, each potentially eligible patient will be informed of the study's objectives and overall requirements. Before conducting any of the pre-entry tests not performed routinely in the patient's treatment, the Investigator will explain the study fully to the patient using the Informed Consent Form. If the patient is willing to participate in the study, he/she will be requested to give written informed consent after being given sufficient time to consider his/her participation and the opportunity to ask for further details. The Informed Consent Form will be signed and personally dated by both the patient and the Investigator/Co-Investigator. A copy of the signed form will be provided to the patient and the original will be retained with the source documents. Although nursing staff may be involved in describing the study to a patient, the Investigator/Co-Investigator must participate in discussions with the patient and must sign and personally date the Informed Consent Form.

Patients that will take part in the Neurophysiological assessments (patients without relapse enrolled in the centre of PPD) and in the immune-phenotype and immunometabolic analysis (patients enrolled in the centres of Rome, Naples and Pozzilli) will sign a further specific Informed Consent Form.

A screening log will be completed for all patients who sign the Informed Consent Form but do not subsequently enter the study. Patients will be identified by their initials and dates of birth; in addition, each patient's gender and reasons for exclusion from the study will be recorded.

Patients meeting the eligibility criteria during the 3-5 days of Screening period will be assessed at baseline (Visit 0, Day 0-Week 0), and after 8 (Visit 1), 12 (Visit 2) and 24 (Visit 3) weeks from the first day of treatment with D-asp/placebo. The Baseline visit (Visit 0) will take place within 3-5 days from the screening visit (Visit -1). A ± 3-day window is allowed for the planned days of the post-baseline visits.

An ET visit will be arranged for the patients that withdraw from the study at any time: they will undergo all assessments requested at the ET Visit as soon as possible after the study discontinuation.

Screening Period (Visit -1, Day -5/-3)

A complete pre-study evaluation (Screening visit, Visit -1) will be performed within 3-5 days prior to Baseline to include:

- Written Informed Consent prior to conducting any protocol assessments;
- Demographics data;
- Medical history/present conditions;
- History of MS, date of first attack, date of MS diagnosis, number of clinical relapses, date of the last clinical relapse;
- Previous (in the last 12 weeks) and concomitant medications for the treatment of MS and other concomitant diseases will be recorded;
- Physical examination including height, weight, body mass index (BMI), waist circumference, blood pressure, heart rate;
- Neurological examination;
- For female patients, the contraceptive method used will be recorded, or, alternatively, if applicable, the age at menopause will be documented;
- A blood sample will be collected for haematology and blood chemistry tests, according to local clinical practice;
- A urine sample will be collected for routine urinalysis, according to local clinical practice;
- Serum pregnancy test, if applicable (is not required if the patient is postmenopausal or surgically sterilized);
- Administration of the EDSS;
- Administration of the 25-FWT, the 9HPT, the Symbol Digit Modalities Test and the Low Contrast Letter visual Acuity Test;
- Administration of the MFIS and of the Fatigue Severity Scale;
- Review of Inclusion/Exclusion Criteria;
- A 12-lead ECG will be performed whenever possible, according to local clinical practice;
- A brain MRI will be performed whenever possible, according to local clinical practice;
- Adverse events occurred from the signature of informed consent will be recorded;

A patient who fails to meet the protocol-specified criteria for study entry during the screening period is a screen failure. The date at which it was determined will be collected on the study entry e-CRF.

Should a patient be withdrawn from the study, his/her identification number will not be reallocated.

Once all procedures and assessments for this visit are completed, an appointment for the Baseline visit (Visit 0, 3-5 days after the Screening visit) will be made.

Baseline Visit (Visit 0, Day 0-Week 0)

The following procedures and assessments will be performed at this Visit:

- Confirmation that Inclusion/Exclusion Criteria are satisfied;
- Any change in concomitant medications will be recorded;
- Physical examination including height, weight, BMI, waist circumference, blood pressure, heart rate;
- Neurological examination;
- A urine pregnancy test will be performed in female patients to exclude pre-existing pregnancy (is not required if the patient is postmenopausal or surgically sterilized or if a serum pregnancy test was already performed within 7 days before the Baseline visit);
- Administration of the EDSS;
- Administration of the 25-FWT, the 9HPT, the Symbol Digit Modalities Test and the Low Contrast Letter visual Acuity Test;
- Administration of the MFIS and of the Fatigue Severity Scale;
- Patients satisfying all eligibility criteria will be randomised to the assigned treatment group;
- IP will be dispensed to patients with directions for use. Patients will be reminded to return the unused IP at the next visit;
- Neurophysiological assessments (LTP and recruitment curve) will be performed in patients without relapse enrolled at the centre of the PPD
- A blood sample will be collected (before receiving corticosteroids) for immune-phenotype and immunometabolic analysis in patients enrolled in the centres of Rome, Naples and Pozzilli;
- A brain MRI will be performed whenever possible, according to local clinical practice;
- Adverse events occurred from the previous visit and/or during this visit will be recorded;

Once all procedures and assessments for this visit are completed, an appointment for the next visit will be made.

Visit 1 (Week 8)

The following procedures and assessments will be performed at this Visit:

- Any change in concomitant medications will be recorded;
- Physical examination including height, weight, BMI, waist circumference, blood pressure, heart rate;
- Neurological examination;
- A blood sample will be collected for haematology and blood chemistry tests, according to local clinical practice;
- A urine sample will be collected for routine urinalysis, according to local clinical practice;
- Administration of the EDSS;
- Administration of the 25-FWT, the 9HPT, the Symbol Digit Modalities Test and the Low Contrast Letter visual Acuity Test;
- Administration of the MFIS and of the Fatigue Severity Scale;
- Neurophysiological assessments (LTP and recruitment curve) will be performed in patients without relapse enrolled at the centre of the PPD
- A blood sample will be collected for immune-phenotype and immunometabolic analysis in patients enrolled in the centres of Rome, Naples and Pozzilli;
- IP will be returned by patients and compliance to therapy will be evaluated.
- New IP will be dispensed to patients. Patients will be reminded to return the unused IP at the next visit;
- Adverse events occurred from the previous visit and/or during this visit will be recorded;

Once all procedures and assessments for this visit are completed, an appointment for the next visit will be made.

Visit 2 (Week 12)

The following procedures and assessments will be performed at this Visit:

- Any change in concomitant medications will be recorded;
- Physical examination including height, weight, BMI, waist circumference, blood pressure, heart rate;
- Neurological examination;
- Administration of the EDSS;
- Administration of the 25-FWT, the 9HPT, the Symbol Digit Modalities Test and the Low Contrast Letter visual Acuity Test;
- Administration of the MFIS and of the Fatigue Severity Scale;
- Neurophysiological assessments (LTP and recruitment curve) will be performed in patients without relapse enrolled at the centre of the PPD
- A blood sample will be collected for immune-phenotype and immunometabolic analysis in patients enrolled in the centres of Rome, Naples and Pozzilli;

- IP will be returned by patients and compliance to therapy will be evaluated.
- New IP will be dispensed to patients. Patients will be reminded to return the unused IP at the next visit;
- Adverse events occurred from the previous visit and/or during this visit will be recorded;

Once all procedures and assessments for this visit are completed, an appointment for the next visit will be made.

Visit 3 (End of treatment, Week 24)

The following procedures and assessments will be performed at this Visit:

- Any change in concomitant medications will be recorded;
- Physical examination including height, weight, BMI, waist circumference, blood pressure, heart rate;
- Neurological examination;
- A blood sample will be collected for haematology and blood chemistry tests, according to local clinical practice;
- A urine sample will be collected for routine urinalysis, according to local clinical practice;
- Administration of the EDSS;
- Administration of the 25-FWT, the 9HPT, the Symbol Digit Modalities Test and the Low Contrast Letter visual Acuity Test;
- Administration of the MFIS and of the Fatigue Severity Scale;
- Neurophysiological assessments (LTP and recruitment curve) will be performed in patients without relapse enrolled at the centre of the PPD
- A 12-lead ECG will be performed whenever possible, according to local clinical practice;
- A brain MRI will be performed whenever possible, according to local clinical practice or previous MRI will be evaluated if available;
- IP will be returned by patients and compliance to therapy will be evaluated;
- Adverse events occurred from the previous visit and/or during this visit will be recorded.

Early Termination (ET) Visit

The procedures scheduled for Visit 3 (end of treatment, Week 24) plus immunological assessments (blood sample collection for immune-phenotype and immunometabolic analysis) will be performed in case of study discontinuation. Reasons for early termination will be reported in the e-CRF.

7.2 Demographic and Other Baseline Characteristics

At screening, the following demographic data will be collected: date of birth, sex (gender), race, ethnicity.

The patient's demographic and medical characteristics including age, history of previous illness, concomitant illness at study initiation, previous treatment for illness treated in the study, concomitant treatment maintained (including oral contraceptives or hormone replacement therapy), treatments stopped at entry into the study period (or changed at study initiation) will be collected at the Screening Visit.

The inclusion/exclusion criteria (Section 5.2.1 and 5.2.2) will be confirmed at the Baseline Visit.

7.3 Efficacy Assessments

EDSS

The Functional Systems Scale (FSS) of the EDSS (Kurtzke, 1983) (Visual, Brainstem, Pyramidal, Cerebellar, Sensory, Bowel/Bladder and Ambulation score) will be used to assess recovery in MS related disability.

Each of the FSS is an ordinal clinical rating scale ranging from 0 to 5 or 6.

Scoring procedures of EDSS and FSS are described in Appendix II.

Functional assessments

The 25-FWT, the 9HPT,, the Symbol Digit Modalities Test and the Low Contrast Letter visual Acuity Test (Polman and Rudick, 2010; Drake et al, 2010) will be used to assess MS disability.

Scoring procedures of the Functional assessments are described in Appendix III.

Fatigue

Fatigue will be evaluated through the MFIS and the Fatigue Severity Scale at baseline during a clinical relapse and 8, 12 and 24 weeks after the onset of the relapse.

The MFIS is a modified form of the Fatigue Impact Scale (Fisk et al, 1994) based on items derived from interviews with MS patients concerning how fatigue impacts their lives. This instrument provides an assessment of the effects of fatigue in terms of physical, cognitive, and psychosocial functioning. The full-length MFIS consists of 21 items. The total score for the MFIS is the sum of the scores for the 21 items. Individual subscale scores for physical, cognitive, and psychosocial functioning can also be generated by calculating the sum of specific sets of items.

The Fatigue Severity Scale is a self-report questionnaire designed to assess disabling fatigue in all individuals (Krupp et al, 1989). The Fatigue Severity Scale consists of 9 questions, uses a 7-point Likert scale ranging from strongly disagrees to strongly agree.

Scoring procedures of the MFIS and of the Fatigue Severity Scale are described in Appendix IV and Appendix V, respectively.

Generally, to maximize data consistency, the same physician should evaluate the same patient on the same assessments at approximately the same time of day, whenever possible. The assessments must be performed without consulting the results of the prior visit.

Details on the immune-phenotype and immunometabolic analysis performed in patients enrolled in the centres of Rome, Naples and Pozzilli are given in Section 7.6.

Details on neurophysiological assessments performed in patients without relapse enrolled at the centre of the PPD are given in Section 7.7.

7.4 Assessment of Safety

The safety profile of the IP will be assessed through the recording, reporting and analysis of baseline medical conditions, AEs, physical examination findings including vital signs, laboratory tests and ECG.

Comprehensive assessment of any apparent toxicity experienced by each patient will be performed from the time of giving informed consent and throughout the study. The investigator will report any AEs, whether observed by the Investigator or reported by the patient (see Section 7.4.1.2). The reporting period for AEs is described in Section 7.4.1.3.

7.4.1 Adverse Events

7.4.1.1 Adverse Event Definitions

Adverse Event

An AE is any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product, regardless of causal relationship with this treatment. An 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 product, whether or not considered related to the medicinal product.

For surgical or diagnostic procedures, the condition/illness leading to such a procedure is considered as the AE rather than the procedure itself.

The Investigator is required to grade the severity of each AE.

Qualitative Severity Scale:

Investigators must assess the severity of AEs according to the Qualitative Toxicity Scale, as follows:

Mild: The subject is aware of the event or symptom, but the event or symptom is easily tolerated.

Moderate: The subject experiences sufficient discomfort to interfere with or reduce his or her usual level of activity.

Severe: Significant impairment of functioning: the subject is unable to carry out his or her usual activities.

Investigators must also systematically assess the causal relationship of AEs to IP treatment using the following definitions. Decisive factors for the assessment of causal relationship of an AE to the IP treatment include, but may not be limited to, temporal relationship between the AE and the IP treatment, known side effects of IP treatment, medical history, concomitant medication, course of the underlying disease, study procedures.

Unrelated: Not reasonably related to the IP treatment. AE could not medically (pharmacologically/clinically) be attributed to the IP/study treatment under study in this clinical study protocol. A reasonable alternative explanation must be available.

Related: Reasonably related to the IP treatment. AE could medically (pharmacologically/clinically) be attributed to the IP/study treatment under study in this clinical study protocol.

Abnormal Laboratory Findings and Other Abnormal Investigational Findings

Abnormal laboratory findings and other abnormal investigational findings (for example, on an ECG trace) should not be reported as AEs unless they are associated with clinical signs and symptoms, lead to treatment discontinuation or are considered otherwise medically important by the Investigator. If a laboratory abnormality fulfils these criteria, the identified medical condition (for example, anaemia, increased ALT) must be reported as the AE rather than the abnormal value itself.

Serious Adverse Events

An SAE is any untoward medical occurrence that at any dose:

- Results in death.
- Is life-threatening. (Note: The term "life-threatening" refers to an event in which the subject is at risk of death at the time of the event, not an event that hypothetically might have caused death if it was more severe.)
- Requires inpatient hospitalization or prolongs an existing hospitalization.
- Results in persistent or significant disability or incapacity.
- Is a congenital anomaly or birth defect.
- Is otherwise considered to be medically important. (Note: Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered as SAEs when, based upon appropriate medical judgment, they may jeopardize the subject or may require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.)

For the purposes of reporting, any drug interactions, medication errors, overdose, abuse, misuse, off-label use, occupational exposure, lack of efficacy reports or suspected transmission of an infectious agent via an IP is also considered an SAE, as described in Section 7.4.1.4.

Events that Do Not Meet the Definition of an SAE

Elective hospitalizations to administer, or to simplify study treatment or study procedures (for example, an overnight stay to facilitate chemotherapy and related hydration therapy application) are not considered SAEs. However, all events leading to unplanned hospitalizations or unplanned prolongation of an elective hospitalization (for example, undesirable effects of any administered treatment) must be documented and reported as SAEs.

Events Not to Be Considered as AEs/SAEs

Medical conditions present at the initial study visit that do not worsen in severity or frequency during the study are defined as Baseline Medical Conditions, and are not to be considered AEs.

However, if adverse signs or symptoms occur in association with disease progression then these should be recorded as AEs.

Adverse Events of Special Interest

There are no known or theoretical safety concerns related to the use of D-asp. Therefore, adverse events of special interest (AESI) are not defined in this study.

7.4.1.2 Methods of Recording and Assessing Adverse Events

At each study visit, the patient will be queried on changes in his/her condition. During the reporting period, any unfavourable changes in the patient's condition will be recorded as AEs, whether reported by the patient or observed by the investigator.

Complete, accurate and consistent data on all AEs experienced for the duration of the reporting period (defined below) will be reported on an ongoing basis in the appropriate section of the e-CRF. All SAEs must be additionally documented and reported using the appropriate Report Form as described in Section 7.4.1.4.

It is important that each AE report include a description of the event, its duration (onset and resolution dates (and times when it is important to assess the time of AE onset relative to the recorded treatment administration time), its severity, its causal relationship with the study treatment, any other potential causal factors, any treatment given or other action taken, including dose modification or discontinuation of the IP, and its outcome. In addition, serious cases should be identified and the appropriate seriousness criteria documented.

Specific guidance can be found in the e-CRF Completion and Monitoring Conventions provided by the Sponsor.

7.4.1.3 Definition of the Adverse Event Reporting Period

The AE reporting period for safety surveillance begins when the patient is initially included in the study (date of first signature of informed consent/date of first signature of first informed consent) and continues until the End of Study Visit.

Any SAE must be reported whenever it occurs, irrespective of the time elapsed since the last administration of IP.

7.4.1.4 Procedure for Reporting Serious Adverse Events, Adverse Events of Special Interest and Dose Limiting Toxicities

Adverse events related to Rebif (adverse drug reactions/serious adverse drug reactions) should be reported to the Sponsor. Other AEs should be recorded in the CRF/patient file and reported spontaneously according to local regulations.

Serious Adverse Events

In the event of any new SAE occurring during the reporting period, the Investigator must immediately (within a maximum of 24 HOURS after becoming aware of the event) inform the Sponsor or its designee in writing. All written reports should be transmitted using the SAE Report Form, which must be completed by the Investigator following specific completion instructions.

In exceptional circumstances, an SAE (or follow-up information) may be reported by telephone; in these cases, a written report must be sent immediately thereafter by fax or e-mail. Names, addresses, and telephone and fax numbers for SAE reporting will be included in the study-specific SAE Report Form.

Relevant pages from the e-CRF may be provided in parallel (for example, medical history, concomitant drugs). Additional documents may be provided by the Investigator, if available (for example, laboratory results, hospital report, autopsy report). In all cases, the information provided on the SAE Report Form must be consistent with the data about the event recorded in the e-CRF.

The Investigator must respond to any request for follow-up information (for example, additional information, outcome, final evaluation, other records where needed) or to any question the Sponsor/designee may have on the AE within the same timelines as those noted above for initial reports. This is necessary to ensure prompt assessment of the event by the Sponsor or designee and (as applicable) to allow the Sponsor to meet strict regulatory timelines associated with expedited safety reporting obligations.

Requests for follow-up will usually be made via the responsible Monitor, although in exceptional circumstances the Global Drug Safety department may contact the Investigator directly to obtain further information or to discuss the event.

7.4.1.5 Safety Reporting to Health Authorities, Independent Ethics Committees and Investigators

The Sponsor will send appropriate safety notifications to Health Authorities in accordance with applicable laws and regulations.

The Investigator must comply with any applicable site-specific requirements related to the reporting of SAEs (particularly deaths) involving study patients to the IEC that approved the study.

In accordance with ICH GCP, the Sponsor/designee will inform the Investigator of "findings that could adversely affect the safety of patients, impact the conduct of the study or alter the IEC's approval/favourable opinion to continue the study." In particular and in line with respective regulations, the Sponsor/designee will inform the Investigator of AEs that are both serious and unexpected and are considered to be related to the administered product ("suspected unexpected serious adverse reactions" or SUSARs). The Investigator should place copies of Safety Reports in the Investigator Site File. National regulations with regard to Safety Report notifications to Investigators will be taken into account.

When specifically required by regulations and guidelines, the Sponsor/designee will provide appropriate Safety Reports directly to the concerned lead IEC and will maintain records of these

notifications. When direct reporting is not clearly defined by national or site-specific regulations, the Investigator will be responsible for promptly notifying the concerned IEC of any Safety Reports provided by the Sponsor/designee and of filing copies of all related correspondence in the Investigator Site File.

For studies covered by the European Directive 2001/20/EC, the Sponsor's responsibilities regarding the reporting of SAEs/SUSARs/Safety Issues will be carried out in accordance with that Directive and with the related Detailed Guidance documents.

7.4.1.6 Monitoring of Subjects with Adverse Events

AEs are recorded and assessed continuously throughout the study (see Section 7.4.1.3) and are assessed for final outcome at the End of Study Visit. All SAEs ongoing at the End of Study Visit must be monitored and followed up by the Investigator until stabilization or until the outcome is known, unless the patient is documented as "lost to follow-up". Reasonable attempts to obtain this information must be made and documented. It is also the responsibility of the Investigator to ensure that any necessary additional therapeutic measures and follow-up procedures are performed.

7.4.2 Pregnancy and In Utero Drug Exposure

Only pregnancies considered by the Investigator to be related to study treatment (for example, resulting from a drug interaction with a contraceptive medication) are considered to be AEs. However, all pregnancies with an estimated conception date during the period defined in Section 7.4.1.3 must be recorded by convention in the AE page/section of the e-CRF. The same rule applies to pregnancies in female patients and to pregnancies in female partners of male patients. The Investigator must notify the Sponsor/designee in an expedited manner of any pregnancy using the Pregnancy Report Form, which must be transmitted according to the same process as described for SAE reporting in Section 7.4.1.4.

Investigators must actively follow up, document and report on the outcome of all these pregnancies, even if the patients are withdrawn from the study.

The Investigator must notify the Sponsor/designee of these outcomes using the Pregnancy Report Form. If an abnormal outcome occurs, the SAE Report Form will be used if the patient sustains an event and the Parent-Child/Foetus Adverse Event Report Form if the child/foetus sustains an event.

Any abnormal outcome must be reported in an expedited manner as described in Section 7.4.1.4, while normal outcomes must be reported within 45 days after delivery.

In the event of a pregnancy in a patient occurring during the course of the study, the patient must be discontinued from study medication immediately. The Sponsor/designee must be notified without delay and the patient must be followed as mentioned above.

7.4.3 Clinical Laboratory Assessments

Blood and urine samples will be collected for the following clinical laboratory tests, following the timing noted in the Schedule of Assessments (Table 1). All samples should be clearly identified.

- Haematology: haemoglobin, hematocrit, red blood cells (RBCs) count, white blood cells (WBCs) count, and differential count (neutrophils, lymphocytes, monocytes, eosinophils, basophils), platelet count;
- Blood chemistry: sodium, potassium, calcium, blood urea nitrogen (BUN), creatinine, total bilirubin, total protein, albumin, aspartate aminotransferase (AST), ALT, AP, creatin-phophokinase (CPK), glucose, amylase, lipase;
- Urinalysis: specific gravity, pH, glucose, proteins, haemoglobin, ketones, bilirubin.

All the above listed tests will be analyzed in the site's local laboratory.

Blood and Urine samples will be collected at the time points indicated in the study schedule of assessments (Table 1).

The Sponsor should receive a list of laboratory normal ranges before shipment of study drug. Any change in laboratory normal ranges during the study should be forwarded to the Sponsor.

7.4.4 Vital Signs, Physical Examinations, and Other Assessments

Physical Examination including measurement of BMI and waist circumference and recording of vital signs (blood pressure and heart rate) will be performed at the time points indicated in the study schedule of assessments (Table 1).

A standard 12-lead ECG will be performed at the time points indicated in the study schedule of assessments (Table 1).

7.5 Pharmacokinetics

Not applicable.

7.6 Biomarkers

Immune-phenotype and immune-metabolic assessments

The immune-phenotype and immune-metabolic analysis will be performed in the scheduled 80 patients with relapse (40 patients) or without relapse (40 patients) enrolled in the sites of Rome, Naples and Pozzilli, in samples obtained at baseline (Visit 0, Day 0-Week 0), and after 8 (Visit 1) and 12 (Visit 2) weeks from the first day of treatment with D-asp/placebo, as well as at the ET visit in case of treatment discontinuation.

Samples and procedures

Blood samples should be taken between 08.00 and 11.00 AM in lithium-heparinised tubes. A blood volume of 15-ml should be taken to obtain a number of cells adequate for all determinations. After the sample and before the shipment to the central laboratory, blood samples should be kept at room temperature. If the external temperature is below 25 °C, shipment will be performed at room temperature. If the external temperature exceeds 25 °C, shipment will be performed at refrigerated temperature in appropriate containers.

Shipment to the central laboratory will be performed as soon as possible in order to allow receipt in the same day of the sample. Samples will be shipped to: PPD [redacted]. The laboratory should be alerted in the day before the shipment of the samples.

Ex vivo analyses

Blood samples (20-25 ml of heparinized blood) will be obtained from normal and RR-MS patients from the different study groups. A basal and overtime immune-metabolic profiling will be obtained for: immune cells on one side (immune phenotype) (CD3,CD4, CD8, CD19, NK, CD4CD25, CD4DR, CD4 Memory, CD4 Naive, CD8 Memory, CD8 Naive, CD14, $CD4^{+}FoxP3^{+}Ki67^{+}p\text{-}S6^{+}$, etc); plasma adipocytokines (circulating leptin [Lep]; soluble leptin receptor (sLep-R); soluble CD40L (sCD40L); soluble Intercellular Adhesion Molecule-1 (sICAM-1); monocyte chemoattractant protein-1 (MCP-1); myeloperoxidase (MPO); osteoprotegerin (OPG); resistin; and Soluble Tumour Necrosis Factor Receptor (sTNF-R).

The data will be collected and stored over time before and after D-asp treatment to perform subsequently multiple correlation matrices analyses to assess the effect of D-asp on these immune-metabolic variables which has been shown to have relevance in MS.

In vitro analyses

The following cellular functional analyses will be performed on a fraction of the same heparinized above blood sample:

- Seahorse studies: Analysis of the action of D-asp on the capacity to change the basal metabolism pre-post activation of T cells by measuring oxygen consumption rate (OCR) and extracellular acidification rates (ECAR), which reflect mitochondrial respiration glycolysis and lipid oxidation. Since the delicate balance between these metabolisms has been linked to immune tolerance, the capacity of D-asp treatment to switch the equilibrium among these different metabolisms will be assessed. From the same blood withdrawn (about 7 ml of the heparinized blood) for the immunophenotyping (see above) peripheral blood lymphocytes (PBLs) will be isolated by Ficoll gradient and ECAR/OCR over time in the different study groups will be measured on isolated cells activated with anti-CD3 antibody. These analyses will be performed with our Seahorse extracellular flux analyzer 96XFe.
- Treg cell expansion assay: The assay has been designed to meet user-friendly criteria and with relatively simple laboratory procedures able to reduce costs and standardize the procedures. On the final remaining fraction (10-15 ml heparinized blood) after Ficoll Hypaque gradient centrifugation, human Treg ($CD4^{+}CD25^{high}CD127^{-}$) and Tresp cells ($CD4^{+}CD25^{-}$) from study patients and healthy controls will be isolated at high level of purity (95-98% pure by FACS analysis). These Treg cells will be cultured for a micro-culture assay (minimum of $1x10^{4}$ cells/well) in round-bottom 96-well plates with Roswell Park Memorial Institute (RPMI) 1640 medium (final volume 100 ml) supplemented with 100 UI/ml penicillin and 100 μg/ml streptomycin and supplemented with either 5% autologous serum from patients/controls or commercially available 5% normal subjects AB human serum. After plating the cells, they will be stimulated for 3 days in the presence of anti-CD3/anti-CD28–coated Dynabeads alone (0.2 beads per cell) or also in the presence of anti-leptin neutralizing monoclonal antibodies (mAbs) at a final concentration of 20 μg/ml. [3H]thymidine (0.5 μCi per well) will be added to the cultures and harvested after 12 hours. Radioactivity will be measured with a betaplate scintillation counter. Data will be collected and stored to measure the Treg cell stimulation index, calculated as the ratio between counts per minute (c.p.m.) proliferation of Treg cells stimulated with anti CD3/anti CD28 and anti leptin neutralizing mAb and c.p.m. proliferation of Treg cells stimulated with anti CD3/CD28 alone.

Storage and analyses of samples will be handled according to the specifications as described in the specific Informed Consent Form.

7.7 Other Assessments

Neurophysiological assessment

Neurophysiological assessments (LTP and recruitment curve) will be performed at the centre of the PPD only in the scheduled 40 patients without relapse, at baseline (Visit 0, Day 0-Week 0), and after 8 (Visit 1), 12 (Visit 2) and 24 (Visit 3) weeks from the first day of treatment with D-asp/placebo, as well as at the ET visit in case of treatment discontinuation.

MEPs will be evoked through a figure-of-eight coil connected to a Magstim 2002 magnetic stimulator and recorded from the right first dorsal interosseus (FDI) muscle with surface cup electrodes. Coil position will be adjusted to find the optimal scalp site to evoke motor responses in the contralateral FDI. The intermittent theta-burst stimulation (iTBS) protocol will consist of 10 bursts, each burst composed of three stimuli at 50 Hz, repeated at a theta frequency of 5 Hz every 10 seconds for a total of 600 stimuli (200 s duration) will be delivered over the motor "hot spot" of the FDI through a Magstim Rapid2 stimulator. The effect of iTBS on corticospinal excitability will be quantified by measuring the amplitude of MEPs evoked by a constant intensity TMS pulse delivered over the motor "hot spot". Twentyfive MEPs will be collected before iTBS (baseline) and at two different time points (0 and 15 min) after the end of the stimulation procedure. Post iTBS MEP amplitudes will then be averaged and normalized to the mean baseline amplitude. A recruitment curve, resting and active motor thresholds and paired pulse measurements of intracortical inhibition and facilitation will also be recorded at each TMS experimental session to evaluate cortical functional integrity, and trans-synaptic transmission. MEP amplitudes elicited by single TMS pulses of increasing intensity (110, 130 and 150% of the RMT) will be measured to calculate recruitment curves of the motor cortex.

8 Statistics

8.1 Sample Size

The primary study endpoint in the part of the study focused on relapsed patients is defined as the proportion of patients who, following a disease relapse associated with a worsening in their MS-related disability, return to baseline status within 8 weeks. Disability will be assessed through the Functional Systems Scores of the EDSS (Visual, Brainstem, Pyramidal, Cerebellar, Sensory, Bowel/Bladder and Ambulation score).

Available data from observational studies (Hirst et al, 2012) indicate that approximately 50% of the patients return to baseline EDSS within 2 months with standard treatments. Based on the fact that usually, in clinical studies where a rigid intention-to-treat (ITT) approach is used, a lower rate of responses/recoveries is observed as compared to observational studies, the expected rate of recovery at 2 months in the control group is set at 40%. With 40 patients per arm the study will be able to detect with 80% power, at the 0.05 alpha level (1-sided), an increase in the proportion of recovery at 2-months from 40% to 70%. The 1-sided significance level is justified by the exploratory nature of this study, aimed at generating study hypotheses to be tested in subsequent studies.

A total of 120 patients will be randomised to include the 40 RR-MS patients without relapses. This subgroup of patients without relapse has been included to assess whether D-Aspartate enhances LTP induction, by means of TMS

Refer to Section 6.3 for procedures of randomisation.

Eligible patients at the end of the screening period will be randomised in a 1:1 ratio to D-asp or placebo. Randomisation will be stratified according to age (<30 years vs. ≥ 30 years) and site location (Rome+Naples+ Pozzilli vs. the other sites).

8.2 Endpoints

8.2.1 Primary Endpoints

The primary endpoint of the study will be the proportion of patients with recovery in their MS related disability measured at 8 weeks through the Functional Systems Scores of the EDSS (Visual, Brainstem, Pyramidal, Cerebellar, Sensory, Bowel/Bladder and Ambulation score). The subgroup of 40 patients without relapse will not be evaluated for the primary endpoint.

8.2.2 Secondary Endpoints

The secondary endpoints of the study will be:

- Proportion of patients with recovery in their MS related disability measured at 12 and 24 weeks through the FSS of the EDSS (Visual, Brainstem, Pyramidal, Cerebellar, Sensory, Bowel/Bladder and Ambulation score);
- MS-related disability and cognitive impairment measured through the 25-FWT, the 9HPT, the Symbol Digit Modalities Test and the Low Contrast Letter visual Acuity Test (Polman and Rudick, 2010; Drake et al, 2010) during a clinical relapse at 8, 12 and 24 weeks;
- Fatigue, measured through the MFIS (Fisk et al, 1994) and the Fatigue Severity Scale (Krupp et al, 1989), at baseline during a clinical relapse and 8, 12 and 24 weeks after the onset of the relapse;
- LTP through TMS at baseline and 8, 12 and 24 weeks after the beginning of treatment, in patients without relapse;
- Immune-metabolic response of the lymphocytes in treated patients *in vitro* (glycolysis, mitochondrial respiration, fatty acids oxidation and circulating adipocytokines).

8.2.3 Other Endpoints

The following safety parameters will be evaluated:

- Incidence, severity, and relationship to treatment of treatment-emergent adverse events (TEAEs);
- Incidence, severity, and relationship to treatment of SAEs;
- Clinically significant changes in routine haematology, chemistry and urinalysis laboratory tests;

[redacted]

8.3 Analysis Sets

Intention-to-treat (Full Analysis Set)

The intention-to-treat (ITT) population will include all patients enrolled into the study and assigned to the randomised treatment.

Per-protocol

The per-protocol (PP) population will include all patients who have been treated according to protocol and fulfil the following criteria:

- Compliance with all entry criteria;
- Absence of major protocol violations;
- Adequate compliance with study medication.

Safety

The safety population will include all patients who have received at least one dose of planned study treatment.

The number and percentage of the patients included in the analysis populations will be reported in a table showing the reason of exclusion for all patients enrolled into study.

The efficacy analysis will be performed in the ITT population. In order to assess the robustness of the results, the analysis of the primary endpoint will be repeated in the PP population.

The safety analysis will be performed in the safety population.

8.4 Description of Statistical Analyses

8.4.1 General Considerations

Methods described here represent the outline of the intended methods.

A Statistical Analysis Plan (SAP) will be produced before the database lock and will contain full details of all planned summaries, listings and analyses.

All summaries and listings will be performed using Statistical Analysis Software (SAS) System software (version 9.2 or later). Unless otherwise specified, all statistical hypotheses will be tested at the 5% significance level ($\alpha = 0.05$) against two-sided alternatives, and corresponding 95% confidence intervals (CI) will be reported as appropriate.

For endpoints measured over time, descriptive statistics (for continuous variables) or frequency count (for categorical data) will be summarized by each time point.

Demographic and clinical characteristics at baseline will be summarized as frequency distributions (for categorical variables) and descriptive statistics of mean, standard deviation, median, minimum and maximum (for continuous variables).

All collected variables will be tested for normality and homogeneity of variance by using Kolmogorov Smirnov and Levene tests.

8.4.2 Analysis of Primary Endpoint

The comparison between the proportions of recoveries at 8 weeks in patients treated with D-aspartate and patients treated with placebo will be based on descriptive statistics (difference, relative risk and/or odds ratio) and on the chi-square test.

A set of exploratory analyses is planned. First, a multiple logistic regression model will be fitted to the data with recovery at 8 weeks as the dependent variable and age, sex, current DMT use, disease course, severity of relapse, and random assignment as covariates, to adjust for imbalances between the treatment groups.

Second, the time to recovery will be computed for each patient and included as the dependent variable in a multivariate Cox model, with all the above mentioned variables as covariates. Patients not recovered will be censored at last observation.

8.4.3 Analysis of Secondary Endpoints

The comparison between the proportions of recoveries at 12 and 24 weeks in patients treated with D-aspartate and patients treated with placebo will be analysed as the main analysis of the primary endpoint.

To evaluate for differences after treatment, between groups in other secondary endpoints, repeated measures analysis of variance (ANOVA) will be used in case of parametric continuous variables, and Mann-Whitney and Kruskal Wallis tests will be used in case of non-parametric continuous variables. For repeated measures, ANOVA sphericity will be tested by using Mauchly's test and results corrected in case of need, by using Greenhouse-Geisser method. Significance level will be set at $p<0.05$.

8.4.4 Analysis of Safety and Other Endpoints

Treatment emergent adverse events (TEAEs) will be those that started on or after the IP intake. All TEAEs will be summarised by Medical Dictionary for Regulatory Activities (MedDRA) System Organ Class (SOC) and Preferred Term (PT). In tables showing the overall incidence of AEs, patients who experienced the same event on more than one occasion are counted only once in the calculation of the event frequency.

Descriptive summaries of laboratory data and vital signs will be provided for actual values and changes from baseline at each visit. Shift tables with reference to range of normal values will also be used in the analysis of laboratory data.

The results of physical examination and ECG will be analysis by descriptive statistics.

8.5 Interim and Additional Planned Analyses

Not applicable. No interim or other additional analyses are planned.

9 Ethical and Regulatory Aspects

9.1 Responsibilities of the Investigator

The Investigator is responsible for the conduct of the study at the site and will ensure that the study is performed in accordance with this protocol, the ethical principles outlined in the Declaration of Helsinki, ICH GCP, and any other applicable regulations. The Investigator must ensure that only patients who have given informed consent are included in the study.

9.2 Subject Information and Informed Consent

An unconditional prerequisite for each patient prior to participation in the study is written informed consent, which must be given before any study-related activities are carried out. Adequate information must therefore be given to the patient by the Investigator or an appropriate designee (if local regulations permit) before informed consent is obtained.

A subject information sheet must be prepared in the local language in accordance with ICH GCP and will be provided by the Sponsor for the purpose of obtaining informed consent. In addition to providing this written information to a potential patient, the Investigator or a designate will inform the patient verbally of all pertinent aspects of the study, using language chosen so that the information can be fully and readily understood by laypersons. The patient will be given sufficient time to read the information and the opportunity to ask questions and to request additional information and clarification.

Patients that will take part in the Neurophysiological assessments (patients without relapse enrolled in the centre of the PPD and in the immune-phenotype and immunometabolic analysis (patients enrolled in the centres of Rome, Naples and Pozzilli) will sign a further specific Informed Consent Form.

If permitted by national regulations, a person other than the Investigator may inform the patient about the study and sign the Informed Consent Form, as above.

After the information is provided by the Investigator, the Informed Consent Form must be signed and dated by the patient and the Investigator.

The signed and dated declaration of informed consent will remain at the Investigator's site, and must be safely archived so that the forms can be retrieved at any time for monitoring, auditing and inspection purposes. A copy of the signed and dated information and Informed Consent Form should be provided to the patient prior to participation.

Whenever important new information becomes available that may be relevant to informed consent, the Investigator will revise the subject information sheet and any other written information to be provided to the subjects and submit them to the IEC for review and opinion. Using the approved revised subject information sheet and other written information, the Investigator will explain the changes to the previous version to each study patient and obtain new written consent for continued participation in the study. The patient will be given sufficient time to read the information and the opportunity to ask questions and to request additional information and clarification about the changes.

9.3 Subject Identification and Privacy

A unique number will be assigned to each patient, immediately after informed consent has been obtained. This number will serve as the patient's identifier in the study as well as in the clinical study database. All patient data collected in the study will be stored under the appropriate patient number. Only the Investigator will be able to link study data to an individual patient via an identification list kept at the site. For each patient, original medical data will be accessible for the purposes of source data verification by the Monitor, audits and regulatory inspections, but patient confidentiality will be strictly maintained.

Data protection and privacy regulations will be observed in capturing, forwarding, processing, and storing patient data. Patients will be informed accordingly, and will be requested to give their consent on data handling procedures in accordance with national regulations.

Blood samples for the immune-phenotype and immunometabolic analysis will be stored for up to 10 years after study completion. During this time, the samples may be reanalyzed for newly identified markers or with new or improved technology. After 10 years, the samples will be destroyed or fully anonymized or a new IEC approval and informed consent will be requested to keep the samples for an additional period.

9.4 Emergency Medical Support and Subject Card

Patients will be provided with Emergency Medical Support cards supplied by the Sponsor for use during study participation in order to provide clinical study patients with a way of identifying themselves as participating in a clinical study and to give health care providers access to any information about this participation that may be needed to determine the course of medical treatment for the patient. The information provided on the Emergency Medical Support card may include the process for emergency unblinding (if applicable).

The first point of contact for all emergencies will be the clinical study Investigator caring for the affected patient. The Investigator agrees to provide his or her emergency contact information on the card for this purpose. If the Investigator is available when an event occurs, they will answer any questions. Any subsequent action (for example, unblinding) will follow the standard process established for Investigators.

In cases where the Investigator is not available, the Sponsor provides the appropriate means to contact a Sponsor physician. This includes the provision of a 24-hour contact number at a call centre, whereby the health care providers will be given access to the appropriate Sponsor physician to assist with the medical emergency and to provide support for the potential unblinding of the patient concerned.

9.5 Clinical Study Insurance and Compensation to Subjects

Insurance coverage will be provided. Insurance conditions shall meet good local standards, as applicable.

9.6 Independent Ethics Committee or Institutional Review Board

Prior to commencement of the study at a given site, this clinical study protocol will be submitted together with its associated documents (subject information sheet, informed consent for, e-CRF

and all other required documents) to the responsible IEC for its favourable opinion or approval, which will be filed in the Investigator Site File. A copy will be filed in the Sponsor Trial Master File at PPD

The IEC will be asked to document the date of the meeting at which the favourable opinion or approval was given and the members and voting members present. Written evidence of favourable opinion or approval that clearly identifies the study, the clinical study protocol version and the Subject Information and Informed Consent Form version reviewed should be provided. Where possible, copies of the meeting minutes should be obtained.

Amendments to this clinical study protocol will also be submitted to the concerned IEC, before implementation of substantial changes (see Section 10.5). Relevant safety information will be submitted to the IEC during the course of the study in accordance with national regulations and requirements.

9.7 Health Authorities

The clinical study protocol and any applicable documentation (for example, Investigational Medicinal Product Dossier, Subject Information and Informed Consent Form) will be submitted or notified to the Health Authorities in accordance with all local and national regulations for each site.

10 Study Management

10.1 Case Report Form Handling

Refer to the Manual of Operations for e-CRF handling guidelines.

The main purpose of the e-CRF is to obtain data required by the clinical study protocol in a complete, accurate, legible and timely. The data in the e-CRF should be consistent with the relevant source documents.

The Investigator or designee is responsible for ensuring that the data collected in the course of this study is accurate and documented appropriately on all applicable forms. They will then be processed, evaluated, and stored in anonymous form in accordance with applicable data protection regulations. The Investigator must ensure that the e-CRFs and any other associated documents forwarded to Sponsor or its designated organization contain no mention of any patient names.

The data will be entered into a validated database. PPD will be responsible for data processing, in accordance with the Sponsor's data management procedures. Database lock will occur once quality control and quality assurance procedures have been completed. PDF files of the e-CRFs will be provided to the Investigators at the completion of the study.

10.2 Source Data and Subject Files

Source data are defined as all data in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical study that are necessary for the reconstruction and evaluation of the study.

The Investigator must keep a file (medical file, original medical records) on paper or electronically for every patient in the study. It must be possible to identify each patient by using this patient file. This file will contain the demographic and medical information for the patient listed below and should be as complete as possible.

- Patient's full name, date of birth, sex, height, weight
- Medical history and concomitant diseases
- Prior and concomitant therapies (including changes during the study)
- Study identification, that is, the Sponsor study number for this clinical study, and patient number
- Dates for entry into the study (informed consent) and visits to the site
- Any medical examinations and clinical findings predefined in this clinical study protocol
- All AEs
- Date that the patient left the study including any reason for early withdrawal from the study or IP (if applicable).

All documents containing source data must be filed, including, but not limited to MRI scan images, ECG recordings, and laboratory results. Such documents must bear the patient number and the date of the procedure. If possible, this information should be printed by the instrument used to perform the assessment or measurement. As necessary, medical evaluation of such records should be performed; all evaluations should be documented, signed, and dated by the Investigator.

Electronic subject files will be printed whenever the Monitor performs source data verification. Printouts must be signed and dated by the Investigator, countersigned by the Monitor and kept in a safe place at the site.

10.3 Investigator Site File and Archiving

Upon initiation of the study, the Investigator will be provided with an Investigator Site File containing all necessary study documents, which will be completed throughout the study and updated as necessary. The file must be available for review by the Monitor, during Sponsor audits and for inspection by Health Authorities during and after the study, and must be safely archived for at least 15 years (or longer, per local requirements or as otherwise notified by the Sponsor) after the end of the study. The documents to be archived include the Subject Identification List and the signed subject Informed Consent Forms. If archiving of the Investigator Site File is no longer possible at the site, the Investigator must notify the Sponsor/designee.

All original patient files (medical records) must be stored at the site (hospital, research institute, or practice) for the longest possible time permitted by the applicable regulations, and/or as per ICH GCP guidelines, whichever is longer. In any case, the Investigator should ensure that no destruction of medical records is performed without the written approval of the Sponsor.

10.4 Monitoring, Quality Assurance and Inspection by Health Authorities

This study will be monitored in accordance with the ICH GCP, and any other applicable regulations. The site Monitor will perform visits to the study site at regular intervals.

The clinical study protocol, each step of the data capture procedure, and the handling of the data, including the final clinical study report, will be subject to independent Quality Assurance activities. Audits may be conducted at any time during or after the study to ensure the validity and integrity of the study data. Representatives of the Quality Assurance unit from the Sponsor or a designated organization, as well as Health Authorities, must be permitted to access all study documents and other materials at the site, including the Investigator Site File, the completed e-CRFs, all IP and IP accountability records, and the original medical records or files for each patient.

10.5 Changes to the Clinical Study Protocol

Changes to the clinical study protocol will be documented in writing. Substantive amendments will usually require submission to the Health Authorities and to the relevant IEC for approval or favourable opinion. In such cases, the amendment will be implemented only after approval or favourable opinion has been obtained.

Minor (non-substantial) protocol amendments, including administrative changes, will be filed by the Sponsor and at the site. They will be submitted to the relevant IEC or to Health Authorities only where requested by pertinent regulations. Any amendment that could affect the patient's agreement to participate in the study requires additional informed consent prior to implementation following the process as described in Section 9.2.

10.6 Clinical Study Report and Publication Policy

10.6.1 Clinical Study Report

After completion of the study, a clinical study report will be written by the Sponsor in consultation with the Coordinating Investigator following the guidance in ICH Topic E3.

10.6.2 Publication

The first publication will include the results of the analysis of the primary endpoints and will include data from all study sites that provided evaluable data. The Investigator will inform the Sponsor in advance about any plans to publish or present data from the study. Any publications and presentations of the results (abstracts in journals or newspapers, oral presentations, etc.), either in whole or in part, by Investigators or their representatives will require review by the Sponsor before submission. The Sponsor will not suppress publication, but maintains the right to delay publication in order to protect intellectual property rights.

Posting of data is planned and will occur 12 months after the last clinic visit of the final study patient or another appropriate date to meet applicable requirements.

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12 Appendices

Appendix I: Signature Pages and Responsible Persons for the Study

Signature Page – Protocol Lead

Study Title:	Evaluation of cl**IN**ical re**C**overy after a **R**elapse: a pilot study ass**E**ssing the neuronal effects of D-**A**spartate in RR-M**S** subjects treated with Int**E**rferon beta 1a 44 mcg TIW (INCREASE)
EudraCT Number:	Not applicable
Clinical Study Protocol Date / Version:	Protocol amendment 04 Jul 2017 / Version 2.0

Protocol Lead responsible for designing the clinical study:

I approve the design of the clinical study:

_____ _____

Signature Date of Signature

Name, academic degree: PPD

Function / Title: PPD

Institution: Merck Serono S.p.A.

Address: Via Casilina 125, 00176 Rome, Italy

Telephone number: PPD

Fax number: PPD

E-mail address: PPD

Signature Page –Coordinating Investigator

Study Title	Evaluation of cl**IN**ical re**C**overy after a **R**elapse: a pilot study ass**E**ssing the neuronal effects of D-**A**spartate in RR-MS**S** subjects treated with Int**E**rferon beta 1a 44 mcg TIW (INCREASE)
EudraCT Number	Not applicable
Clinical Study Protocol Date / Version	Protocol amendment 04 Jul 2017 / Version 2.0

I approve the design of the clinical study and I understand and will conduct the study according to the clinical study protocol, any approved protocol amendments, International Conference on Harmonisation Good Clinical Practice (Topic E6) and all applicable Health Authority requirements and national laws.

_____ _____

Signature Date of Signature

Name, academic degree: PPD

Function / Title: PPD

Institution: PPD

Address: PPD

Telephone number: PPD

Fax number: PPD

E-mail address: PPD

Signature Page – Principal Investigator

Study Title	Evaluation of cl**IN**ical re**C**overy after a **R**elapse: a pilot study ass**E**ssing the neuronal effects of D-**A**spartate in RR-M**S** subjects treated with Int**E**rferon beta 1a 44 mcg TIW (INCREASE)
EudraCT Number	Not applicable
Clinical Study Protocol Date / Version	Protocol amendment 04 Jul 2017 / Version 2.0
Center Number	
Principal Investigator	

I, the undersigned, am responsible for the conduct of the study at this site and affirm that I understand and will conduct the study according to the clinical study protocol, any approved protocol amendments, International Conference on Harmonisation Good Clinical Practice (Topic E6) and all applicable Health Authority requirements and national laws.

_____ _____

Signature Date of Signature

Name, academic degree:

Function / Title:

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Appendix II: Expanded Disability Status Score (EDSS)

neurostatus scoring

Definitions for a standardised, quantified neurological examination and assessment of Kurtzke's Functional Systems and Expanded Disability Status Scale in Multiple Sclerosis

EQUIVALENCE WITH PREVIOUS VERSIONS

This version of the neurostatus scoring guidelines is fully compatible with previous versions. Additional help is provided by clarifying some definitions and by introducing an ambulation score in order to reduce measurement noise. But these changes do not imply changes in scoring levels.

GENERAL GUIDELINES

To ensure unbiased EDSS assessment in controlled clinical trials, the EDSS rater should not inquire about the patients' condition except as necessary to perform the EDSS assessment. Patients must be observed to walk the required distance.
The functional system and EDSS scores should reflect the MS related deficits only. In case of doubt the examining physician should assume a relation to MS.
Temporary signs or symptoms that are not due to multiple sclerosis, e.g. temporal immobilisation after fracture of one limb, as well as permanent signs or symptoms that are not due to multiple sclerosis, e.g leg amputation after accident, will not be taken into consideration when assessing the FS scores and EDSS steps, but need to be noted in neurostatus and commented by adding "P" next to the respective field on the scoring sheet for permanent findings and "T" for temporary findings.

FUNCTIONAL SYSTEMS (FS)

A neurostatus score "signs only" is noted when the examination reveals signs of which the patient is unaware.
A score of 1 in a Functional System implies that the patient is not aware of the deficit and that the deficit or sign does not interfere with normal daily activities. However, this general rule does not apply to the Visual, Bowel/Bladder and Cerebral FS.

EXPANDED DISABILITY STATUS SCALE (EDSS)

The EDSS step should not be lower than the score of any individual FS, with the exception of the Visual and Bowel/Bladder FS before conversion.

EDSS steps from 0 up to 4.0 should not change compared to the previous examination, unless there is a change by one grade in at least one FS score.
EDSS steps from 0 up to 1.5 can only apply if ambulation is "unrestricted".
EDSS steps from 2.0 up to 5.0 are defined by the Functional System (FS) scores and/or walking range restriction. As an example, EDSS step 5.0 is possible with an unrestricted ambulation. EDSS steps from 2.0 up to 4.0 does only apply in individuals when at least "fully ambulatory" (able to walk ≥500 meters). If ambulation is assessed as "restricted" the pyramidal or cerebellar FS must be ≥2.
EDSS steps ≥ 5.5 are exclusively defined by the ability to ambulate, the assistance required or the use of a wheelchair.

1 VISUAL (OPTIC) FUNCTIONS

VISUAL ACUITY

The visual acuity score is based on the line in the Snellen chart at 20 feet (5 meters) for which the patient makes no more than one error, using best available correction. Alternatively, best corrected near vision can be assessed, but this should be noted and consistently performed during follow-up examinations. Switching from near to distance visual acuity measurements should be avoided in follow-up examinations.

VISUAL FIELDS

0 normal
1 signs only: deficits present only on formal (confrontational) testing
2 moderate: patient aware of deficit, but incomplete hemianopsia on examination
3 marked: complete homonymous hemianopsia or equivalent

SCOTOMA

0 none
1 small: detectable only on formal (confrontational) testing
2 large: spontaneously reported by patient

* DISC PALLOR

0 not present
1 present

NOTE

When determining the EDSS step, the Visual FS score must be converted to a lower score as follows:

Visual FS Score	6	5	4	3	2	1
Converted Visual FS Score	4	3	3	2	2	1

FUNCTIONAL SYSTEM SCORE

0 normal
1 disc pallor and/or small scotoma and/or visual acuity (corrected) of worse eye less than 20/20 (1.0) but better than 20/30 (0.67)
2 worse eye with maximal visual acuity (corrected) of 20/30 to 20/59 (0.67–0.34)
3 worse eye with large scotoma and/or moderate decrease in fields and/or maximal visual acuity (corrected) of 20/60 to 20/99 (0.33–0.21)
4 worse eye with marked decrease of fields and/or maximal visual acuity (corrected) of 20/100 to 20/200 (0.2–0.1);
grade 3 plus maximal acuity of better eye of 20/60 (0.33) or less
5 worse eye with maximal visual acuity (corrected) less than 20/200 (0.1);
grade 4 plus maximal acuity of better eye of 20/60 (0.33) or less
6 grade 5 plus maximal visual acuity of better eye of 20/60 (0.33) or less

* = optional part of the examination.

2 BRAINSTEM FUNCTIONS

EXTRAOCULAR MOVEMENTS (EOM) IMPAIRMENT

0 none
1 signs only: subtle and barely clinically detectable EOM weakness, patient does not complain of blurry vision, diplopia or discomfort
2 mild: subtle and barely clinically detectable EOM weakness of which patient is aware; or obvious incomplete paralysis of any eye movement of which patient is not aware
3 moderate: obvious incomplete paralysis of any eye movement of which patient is aware; or complete loss of movement in one direction of gaze in either eye
4 marked: complete loss of movement in more than one direction of gaze in either eye

NYSTAGMUS

0 none
1 signs only or mild: gaze evoked nystagmus below the limits of "moderate" (equivalent to a Brainstem FS score of 1)
2 moderate: sustained nystagmus on horizontal or vertical gaze at 30 degrees, but not in primary position, patient may or may not be aware of the disturbance
3 severe: sustained nystagmus in primary position or coarse persistent nystagmus in any direction that interferes with visual acuity; complete internuclear ophthalmoplegia with sustained nystagmus of the abducting eye; oscillopsia

TRIGEMINAL DAMAGE

0 none
1 signs only
2 mild: clinically detectable numbness of which patient is aware
3 moderate: impaired discrimination of sharp/dull in one, two or three trigeminal branches; trigeminal neuralgia (at least one attack in the last 24 hours)
4 marked: unable to discriminate between sharp/dull or complete loss of sensation in entire distribution of one or both trigeminal nerves

FACIAL WEAKNESS

0 none
1 signs only
2 mild: clinically detectable facial weakness of which patient is aware
3 moderate: incomplete facial palsy, such as weakness of eye closure that requires patching overnight or weakness of mouth closure that results in drooling
4 marked: complete unilateral or bilateral facial palsy with lagophthalmus or difficulty with liquids

HEARING LOSS

0 none
1 signs only: hears finger rub less in one or both sides and has lateralized Weber test but does not complain of any hearing problem
2 mild: as in 1 but is aware of hearing problem
3 moderate: does not hear finger rub on one or both sides, misses several whispered numbers
4 marked: misses all or nearly all whispered numbers

DYSARTHRIA

0 none
1 signs only
2 mild: clinically detectable dysarthria of which patient is aware
3 moderate: obv. dysarthria during ordinary conversation that impairs comprehensibility
4 marked: incomprehensible speech
5 inability to speak

DYSPHAGIA

0 none
1 signs only
2 mild: difficulty with thin liquids
3 moderate: difficulty with liquids and solid food
4 marked: sustained difficulty with swallowing; requires a pureed diet
5 inability to swallow

OTHER CRANIAL NERVE FUNCTIONS

0 normal
1 signs only
2 mild disability: clinically detectable deficit of which patient is usually aware
3 moderate disability
4 marked disability

FUNCTIONAL SYSTEM SCORE

0 normal
1 signs only
2 moderate nystagmus and/or moderate EOM impairment and/or other mild disability
3 severe nystagmus and/or marked EOM impairment and/or moderate disability of other cranial nerves
4 marked dysarthria and/or other marked disability
5 inability to swallow or speak

3 PYRAMIDAL FUNCTIONS

REFLEXES

0 absent
1 diminished
2 normal
3 exaggerated
4 nonsustained clonus (a few beats of clonus)
5 sustained clonus

Cutaneous Reflexes
0 normal
1 weak
2 absent

*Palmomental Reflex
0 absent
1 present

Plantar Response
0 flexor
1 neutral or equivocal
2 extensor

LIMB STRENGTH

The weakest muscle in each group defines the score for that muscle group. Use of optional functional tests (hopping on one foot and walking on heels/toes), is highly recommended in order to assess BMRC grades 3–5.

BMRC RATING SCALE

0 no muscle contraction detected
1 visible contraction without visible joint movement
2 visible movement only on the plane of gravity
3 active movement against gravity, but not against resistance
4 active movement against resistance, but not full strength
5 normal strength

FUNCTIONAL TESTS

*Pronator Drift (upper extremities) Pronation and downward drift:
0 none
1 mild
2 evident

*Position Test (lower extremities – ask patient to lift both legs together, with legs fully extended at the knee) Sinking:
0 none
1 mild
2 evident
3 able to lift only one leg at a time (grade from the horizontal pos. at the hip joints…°)
4 unable to lift one leg at a time

*Walking on heels/toes
0 normal
1 impaired
2 not possible

*Hopping on one foot
0 normal
1 6–10 times
2 1–5 times
3 not possible

LIMB SPASTICITY (AFTER RAPID FLEXION OF THE EXTREMITY)

0 none
1 mild: barely increased muscle tone
2 moderate: moderately increased muscle tone that can be overcome and full range of motion is possible
3 severe: severely increased muscle tone that is extremely difficult to overcome and full range of motion is not possible
4 contracted

GAIT SPASTICITY

0 none
1 barely perceptible
2 evident: minor interference with function
3 permanent shuffling: major interference with function

OVERALL MOTOR PERFORMANCE

0 normal
1 abnormal weakness (as compared to peers) in performing more demanding tasks, e.g. when walking longer distances, but no reduction in limb strength on formal (confrontational) testing
2 Reduction in strength of individual muscle groups at confrontational testing

FUNCTIONAL SYSTEM SCORE

0 normal
1 abnormal signs without disability
2 minimal disability: patient complains of motor-fatigability or reduced performance in strenuous motor tasks (motor performance grade 1) and/or BMRC grade 4 in one or two muscle groups
3 mild to moderate paraparesis or hemiparesis: usually BMRC grade 4 in more than two muscle groups;
and/or BMRC grade 3 in one or two muscle groups (movements against gravity are possible);
and/or severe monoparesis: BMRC grade 2 or less in one muscle group
4 marked paraparesis or hemiparesis: usually BMRC grade 2 in two limbs or monoplegia with BMRC grade 0 or 1 in one limb;
and/or moderate tetraparesis: BMRC grade 3 in three or more limbs
5 paraplegia: BMRC grade 0 or 1 in all muscle groups of the lower limbs;
and/or marked tetraparesis: BMRC grade 2 or less in three or more limbs;
and/or hemiplegia;
6 tetraplegia: BMRC grade 0 or 1 in all muscle groups of the upper and lower limbs

4 CEREBELLAR FUNCTIONS

HEAD TREMOR

0 none
1 mild
2 moderate
3 severe

TRUNCAL ATAXIA

0 none
1 signs only
2 mild: swaying with eyes closed
3 moderate: swaying with eyes open
4 severe: unable to sit without assistance

LIMB ATAXIA (TREMOR/DYSMETRIA AND RAPID ALTERNATING MOVEMENTS)

0 none
1 signs only
2 mild: tremor or clumsy movements easily seen, minor interference with function
3 moderate: tremor or clumsy movements interfere with function in all spheres
4 severe: most functions are very difficult

TANDEM (STRAIGHT LINE) WALKING

0 normal
1 impaired
2 not possible

GAIT ATAXIA

0 none
1 signs only
2 mild: problems with balance realized by patient and/or significant other
3 moderate: abnormal balance with ordinary walking
4 severe: unable to walk more than a few steps unassisted or requires a walking aid or assistance by another person because of ataxia

ROMBERG TEST

0 normal
1 mild: mild instability with eyes closed
2 moderate: not stable with eyes closed
3 severe: not stable with eyes open

OTHER CEREBELLAR TESTS

0 normal
1 mild abnormality
2 moderate abnormality
3 severe abnormality

NOTE

The presence of severe gait and/or truncal ataxia alone (without severe ataxia in three or four limbs) results in a Cerebellar FS score of 3.
If weakness or sensory deficits interfere with the testing of ataxia, score the patient's actual performance. To indicate the possible role of weakness make an "X" after the affected subsystems and Cerebellar FS score.

FUNCTIONAL SYSTEM SCORE

0 normal
1 abnormal signs without disability
2 mild ataxia and/or moderate station ataxia (Romberg) and/or tandem walking not possible
3 moderate limb ataxia and/or moderate or severe gait/truncal ataxia
4 severe gait/truncal ataxia and severe ataxia in three or four limbs
5 unable to perform coordinated movements due to ataxia
X pyramidal weakness (BMRC grade 3 or worse in limb strength) or sensory deficits interfere with cerebellar testing

5 SENSORY FUNCTIONS

SUPERFICIAL SENSATION (LIGHT TOUCH AND PAIN)

0 normal
1 signs only: slightly diminished sensation (temperature, figure-writing) on formal testing of which patient is not aware
2 mild: patient is aware of impaired light touch or pain, but is able to discriminate sharp/dull
3 moderate: impaired discrimination of sharp/dull
4 marked: unable to discriminate between sharp/dull and/or unable to feel light touch
5 complete loss: anaesthesia

VIBRATION SENSE (AT THE MOST DISTAL JOINT)

0 normal
1 mild: graded tuning fork 5–7 of 8; alternatively, detects more than 10 seconds but less than the examiner
2 moderate: graded tuning fork 1–4 of 8; alternatively, detects between 2 and 10 sec.
3 marked: complete loss of vibration sense

POSITION SENSE

0 normal
1 mild: 1–2 incorrect responses, only distal joints affected
2 moderate: misses many movements of fingers or toes; proximal joints affected
3 marked: no perception of movement, astasia

*LHERMITTE'S SIGN

Does not contribute to the Sensory FS score

0 negative
1 positive

*PARAESTHESIAE (TINGLING)

Does not contribute to the Sensory FS score

0 none
1 present

FUNCTIONAL SYSTEM SCORE

0 normal
1 mild vibration or figure-writing or temperature decrease only in one or two limbs
2 mild decrease in touch or pain or position sense or moderate decrease in vibration in one or two limbs;
and/or mild vibration or figure-writing or temperature decrease alone in more than two limbs
3 moderate decrease in touch or pain or position sense or marked reduction of vibration in one or two limbs;
and/or mild decrease in touch or pain or moderate decrease in all proprioceptive tests in more than two limbs
4 marked decrease in touch or pain in one or two limbs;
and/or moderate decrease in touch or pain and/or marked reduction of proprioception in more than two limbs
5 loss (essentially) of sensation in one or two limbs;
and/or moderate decrease in touch or pain and/or marked reduction of proprioception for most of the body below the head
6 sensation essentially lost below the head

6 BOWEL AND BLADDER FUNCTIONS

URINARY HESITANCY AND RETENTION

0 none
1 mild: no major impact on lifestyle
2 moderate: urinary retention; frequent urinary tract infections
3 severe: requires catheterisation
4 loss of function: overflow incontinence

URINARY URGENCY AND INCONTINENCE

0 none
1 mild: no major impact on lifestyle
2 moderate: rare incontinence occurring no more than once a week; must wear pads
3 severe: frequent incontinence occurring from several times a week to more than once a day; must wear urinal or pads
4 loss of function: loss of bladder control

BLADDER CATHETERISATION

0 none
1 intermittent self-catheterisation
2 constant catheterisation

BOWEL DYSFUNCTION

0 none
1 mild: no incontinence, no major impact on lifestyle, mild constipation
2 moderate: must wear pads or alter lifestyle to be near lavatory
3 severe: in need of enemata or manual measures to evacuate bowels
4 complete loss of function

* SEXUAL DYSFUNCTION

Male

0 none
1 mild: difficulty to maintain erection during intercourse, but achieves erection and still has intercourse
2 moderate: difficulty to achieve erection, decrease in libido, still has intercourse and reaches orgasm
3 severe: marked decrease in libido, inability to achieve full erection, intercourse with difficulty and hypoorgasmia
4 loss of function

Female

0 none
1 mild: mild lack of lubrication, still sexually active and reaches orgasm
2 moderate: dysparunia, hypoorgasmia, decrease in sexual activity
3 severe: marked decrease in sexual activity, anorgasmia
4 loss of function

NOTE

When determining the EDSS step, the Bowel and Bladder FS score must be converted to a lower score as follows:

Bowel and Bladder FS Score	6	5	4	3	2	1
Converted Bowel and Bladder FS Score	5	4	3	3	2	1

Sexual dysfunction can be documented but in general does not impact on FS score because of obvious difficulties in assessment by examining physician

FUNCTIONAL SYSTEM SCORE

0 normal
1 mild urinary hesitancy, urgency and/or constipation
2 moderate urinary hesitancy/retention and/or moderate urinary urgency/incontinence and/or moderate bowel disfunction
3 frequent urinary incontinence or intermittent self-catheterisation; needs enemata or manual measures to evacuate bowels
4 in need of almost constant catheterisation
5 loss of bladder or bowel function; external or indwelling catheter
6 loss of bowel and bladder function

7 CEREBRAL FUNCTIONS

° DEPRESSION AND EUPHORIA

0 none
1 present: Patient complains of depression or is considered depressed or euphoric by the investigator or significant other.

° Depression and Euphoria are documented on the scoring sheet but are not taken into consideration for FS and EDSS calculation.

DECREASE IN MENTATION

0 none
1 signs only: not apparent to patient and/or significant other
2 mild: Patient and/or significant other report mild changes in mentation. Examples include: impaired ability to follow a rapid course of association and in surveying complex matters; impaired judgement in certain demanding situations; capable of handling routine daily activities, but unable to tolerate additional stressors; intermittently symptomatic even to normal levels of stress; reduced performance; tendency toward negligence due to obliviousness or fatigue.
3 moderate: definite abnormalities on brief mental status testing, but still oriented to person, place and time
4 marked: not oriented in one or two spheres (person, place or time), marked effect on lifestyle
5 dementia, confusion and/or complete disorientation

+ FATIGUE

0 none
1 mild: does not usually interfere with daily activities
2 moderate: interferes, but does not limit daily activities for more than 50 %
3 severe: significant limitation in daily activities (> 50 % reduction)

+ Because fatigue is difficult to evaluate objectively, in some studies it does not contribute to the Cerebral FS score or EDSS step. Please adhere to the study's specific instructions.

FUNCTIONAL SYSTEM SCORE

0 normal
1 signs only in decrease in mentation; mild fatigue
2 mild decrease in mentation; moderate or severe fatigue
3 moderate decrease in mentation
4 marked decrease in mentation
5 dementia

8 AMBULATION

Unrestricted ambulation means the patient is able to walk a distance without assistance that is regarded as normal, compared with healthy individuals of similar age and physical condition. In this case the EDSS step can be anything between 0 and 5.0, depending on the FS scores.

Fully ambulatory means at least 500 meters of ambulation without assistance, but not unrestricted. The EDSS step can be anything between 2.0 and 5.0, depending on the FS scores. In this case, the pyramidal and/or cerebellar FS must be ≥ 2 to reflect this „restriction" of ambulation.

If ambulation is < 500 meters, the EDSS step must be ≥ 4.5 depending on the walking ranges provided by the ambulation score (see next page) and combination of FS scores. EDSS steps 5.5 to 8.0 are exclusively defined by the ability to ambulate and type of assistance required, or the ability to use a wheelchair.

If assistance is needed, the definitions of EDSS steps 6.0 or 6.5 include both a description of the type of assistance required when walking and the walking range. Assistance by another person is equivalent to bilateral assistance.

NOTE

The ambulation score represents both a description of walking range and the type of assistance required for ambulation. The score replaces the former use of several checkboxes (paragraph 8 ambulation on the scoring sheet) but does NOT introduce new definitions. The use of wheelchair can now be scored on the scoring sheet.
Please indicate the reported distance and time for the patient in the appropriate field on the scoring sheet, followed by the type of assistance and the walking distance measured during the assessment.

DISTANCE AND TIME REPORTED BY PATIENT

Maximal unassisted walking distance reported by patient (in meters) without rest or assistance and time required to walk max. distance according to patient (in minutes)

ASSISTANCE

0 Without help or assistance (allowing the use of an ankle foot orthotic device, without any other type of assistive device)
1 Unilateral assistance: one stick/crutch/brace
2 Bilateral assistance: two sticks/crutches/braces or assistance by another person
3 Wheelchair

DISTANCE

Measure the distance the patient is able to walk im meters.
Unassisted: observe the patient walking unassisted for a minimum distance of 500 meters and measure the time needed, if possible.
Assisted: observe the patient walking with the assistive device or help by another person for a minimum distance of 130 meters, if possible.

AMBULATION SCORE

0 Unrestricted
1 Fully ambulatory
2 ≥ 300 meters, but < 500 meters, without help or assistance (EDSS 4.5 or 5.0)
3 ≥ 200 meters, but < 300 meters, without help or assistance (EDSS 5.0)
4 ≥ 100 meters, but < 200 meters, without help or assistance (EDSS 5.5)
5 Walking range < 100 meters without assistance (EDSS 6.0)
6 unilateral assistance, ≥ 50 meters (EDSS 6.0)
7 bilateral assistance, ≥ 120 meters (EDSS 6.0)
8 unilateral assistance, < 50 meters (EDSS 6.5)
9 bilateral assistance, ≥ 5 meters, but < 120 meters (EDSS 6.5)
10 Uses wheelchair without help; unable to walk 5 meters even with aid, essentially restricted to wheelchair; wheels self and transfers alone; up and about in wheelchair some 12 hours a day (EDSS 7.0)
11 Uses wheelchair with help; unable to take more than a few steps; restricted to wheelchair; may need some help in transferring and in wheeling self (EDSS 7.5)
12 essentially restricted to bed or chair or perambulated in wheelchair, but out of bed most of day; retains many self-care functions; generally has effective use of arms (EDSS 8.0)

9 EXPANDED DISABILITY STATUS SCALE

0 normal neurological exam (all FS grade 0)
1.0 no disability, minimal signs in one FS (one FS grade 1)
1.5 no disability, minimal signs in more than one FS (more than one FS grade 1)
2.0 minimal disability in one FS (one FS grade 2, others 0 or 1)
2.5 minimal disability in two FS (two FS grade 2, others 0 or 1)
3.0 moderate disability in one FS (one FS grade 3, others 0 or 1) though fully ambulatory; or mild disability in three or four FS (three/four FS grade 2, others 0 or 1) though fully ambulatory
3.5 fully ambulatory but with moderate disability in one FS (one FS grade 3) and mild disability in one or two FS (one/two FS grade 2) and others 0 or 1; or fully ambulatory with two FS grade 3 (others 0 or 1);or fully ambulatory with five FS grade 2 (others 0 or 1)
4.0 ambulatory without aid or rest for ≥500 meters; up and about some 12 hours a day despite relatively severe disability consisting of one FS grade 4 (others 0 or 1) or combinations of lesser grades exceeding limits of previous steps
4.5 ambulatory without aid or rest for ≥300 meters; up and about much of the day, characterised by relatively severe disability usually consisting of one FS grade 4 and combination of lesser grades exceeding limits of previous steps
5.0 ambulatory without aid or rest for ≥200 meters (usual FS equivalents include at least one FS grade 5, or combinations of lesser grades usually exceeding specifications for step 4.5)
5.5 ambulatory without aid or rest for ≥100 meters
6.0 unilateral assistance (cane or crutch) required to walk at least 100 meters with or without resting (see chapter 8, Ambulation)
6.5 constant bilateral assistance (canes or crutches) required to walk at least 20 meters without resting (see chapter 8, Ambulation)
7.0 unable to walk 5 meters even with aid, essentially restricted to wheelchair; wheels self and transfers alone; up and about in wheelchair some 12 hours a day
7.5 unable to take more than a few steps; restricted to wheelchair; may need some help in transferring and in wheeling self
8.0 essentially restricted to bed or chair or perambulated in wheelchair, but out of bed most of day; retains many self-care functions; generally has effective use of arms
8.5 essentially restricted to bed much of the day; has some effective use of arm(s); retains some self-care functions
9.0 helpless bed patient; can communicate and eat
9.5 totally helpless bed patient; unable to communicate effectively or eat/swallow
10 death due to MS

neurostatus scoring

Scoring Sheet for a standardised, quantified neurological examination and assessment of Kurtzke's Functional Systems and Expanded Disability Status Scale in Multiple Sclerosis

STUDY NAME

PERSONAL INFORMATION

Patient	
Date of Birth (04-Jun-1980)	– –
Centre Nr/Country	
Name of EDSS rater	
Date of Examination	– – 2 0

SYNOPSIS

1. Visual	[1]	Ambulation Score
2. Brainstem		
3. Pyramidal		EDSS Step
4. Cerebellar		
5. Sensory		
6. Bowel/Bladder	[1]	Signature
7. Cerebral		

1. VISUAL (OPTIC) FUNCTIONS

OPTIC FUNCTIONS	OD	OS
Visual acuity ☐ CC ☐ SC		
Visual fields		

Scotoma		
* Disc pallor		
FUNCTIONAL SYSTEM SCORE	→	[1]

2. BRAINSTEM FUNCTIONS

CRANIAL NERVE EXAMINATION	
Extraocular movements (EOM) impairment	
Nystagmus	
Trigeminal damage	
Facial weakness	

Hearing loss	
Dysarthria	
Dysphagia	
Other cranial nerve functions	
FUNCTIONAL SYSTEM SCORE	

3. PYRAMIDAL FUNCTIONS

REFLEXES	R	><	L
Biceps			
Triceps			
Brachioradialis			
Knee			
Ankle			
Plantar response			
Cutaneous reflexes			
* Palmomental reflex			

LIMB STRENGTH	R	L
Deltoid		
Biceps		
Triceps		
Wrist/finger flexors		
Wrist/finger extensors		
Hip flexors		
Knee flexors		

Knee extensors		
Plantar flexion (feet/toes)		
Dorsiflexion (feet/toes)		
* Position test UE, pronation		
* Position test UE, downward drift		
* Position test LE, sinking		
* Able to lift only one leg at a time (grade in °)	*	*
* Walking on heels		
* Walking on toes		
* Hopping on one foot		
SPASTICITY		
Arms		
Legs		
Gait		
OVERALL MOTOR PERFORMANCE		
FUNCTIONAL SYSTEM SCORE		

CC = corrected
SC = without correction
* = optional part of the examination
[1] = converted FS Score

4. CEREBELLAR FUNCTIONS

CEREBELLAR EXAMINATION	R	L
Head tremor		
Truncal ataxia		
Tremor/dysmetria UE		
Tremor/dysmetria LE		

Rapid alternating movements UE impairment		
Rapid alternating movements LE impairment		
Tandem walking		
Gait ataxia		
Romberg test		
Other, e. g. rebound		
FUNCTIONAL SYSTEM SCORE		

5. SENSORY FUNCTIONS

SENSORY EXAMINATION	R	L
Superficial sensation UE		
Superficial sensation trunk		
Superficial sensation LE		
Vibration sense UE		
Vibration sense LE		

Position sense UE		
Position sense LE		
* Lhermitte's sign		
* Paraesthesiae UE		
* Paraesthesiae trunk		
* Paraesthesiae LE		
FUNCTIONAL SYSTEM SCORE		

6. BOWEL/ BLADDER FUNCTIONS

Urinary hesitancy/retention	
Urinary urgency/incontinence	
Bladder catheterisation	

Bowel dysfunction		
* Sexual dysfunction		
FUNCTIONAL SYSTEM SCORE	→	[1]

7. CEREBRAL FUNCTIONS

MENTAL STATUS EXAMINATION	
* Depression	
* Euphoria	

Decrease in mentation	
* Fatigue	
FUNCTIONAL SYSTEM SCORE	

AMBULATION

Distance reported by patient (in meters)	
Time reported by patient (in minutes)	

Assistance	
Distance measured (in meters)	
AMBULATION SCORE	

* = optional part of the examination
[1] = converted FS Score
° Depression and Euphoria are not taken into consideration for FS and EDSS calculation.
* Because fatigue is difficult to evaluate objectively, in some studies it does not contribute to the Cerebral FS score or EDSS step. Please adhere to the study's specific instructions.

UE = upper extremities
LE = lower extremities

Standardised Neurological Examination and Assessment of Kurtzke's Functional Systems and Expanded Disability Status Scale
Slightly modified from J.F. Kurtzke, Neurology 1983;33,1444-52

Appendix III: Functional Assessments

TIMED 25-FOOT WALK

Did patient wear an AFO? ☐ Yes ☐ No

Was assistive device used? ☐ Yes ☐ No

Assistive device used *(mark one)*:

☐ Unilateral Assistance ☐ Cane ☐ Crutch

☐ Bilateral Assistance ☐ Cane ☐ Crutch ☐ Walker/Rollator

Trial 1

Time for 25-Foot Walk ☐☐☐☐ ☐ seconds

For a complete trial, record any circumstances that affected the patient's performance:

_____

_____

If trial was not completed *(mark one)*: Specify:

☐ Unable to complete trial due to physical limitations ➡ _____

☐ Other ➡ _____

Trial 2

Time for 25-Foot Walk ☐☐☐☐ ☐ seconds

For a complete trial, record any circumstances that affected the patient's performance:

_____

_____

If trial was not completed *(mark one)*: Specify:

☐ Unable to complete trial due to physical limitations ➡ _____

☐ Other ➡ _____

Did it take more than two attempts to get two successful trials? ☐ Yes ☐ No

If yes, please specify reasons(s) for more than two attempted trials:

_____

9-HOLE PEG TEST

DOMINANT HAND *(Check one):* Right ☐ Left ☐

DOMINANT HAND

Trial 1

☐☐☐ ☐ seconds

For a complete trial, record any circumstances that affected the patient's performance:

_____

_____

If trial was not completed *(mark one):*

☐ Unable to complete trial due to physical limitations ➡ Specify: _____

☐ Other ➡ _____

Trial 2

☐☐☐ ☐ seconds

For a complete trial, record any circumstances that affected the patient's performance:

_____

_____

If trial was not completed *(mark one):*

☐ Unable to complete trial due to physical limitations ➡ Specify: _____

☐ Other ➡ _____

Did it take more than two attempts to get two sucessful trials? ☐ Yes ☐ No
If Yes, please specify reason(s) for more than two attempted trials:

NON-DOMINANT HAND

Trial 1

☐☐☐ ☐ seconds

For a complete trial, record any circumstances that affected the patient's performance:

_____

_____

If trial was not completed *(mark one):*

☐ Unable to complete trial due to physical limitations ➡ Specify: _____

☐ Other ➡ _____

Trial 2

☐☐☐ ☐ seconds

For a complete trial, record any circumstances that affected the patient's performance:

_____

_____

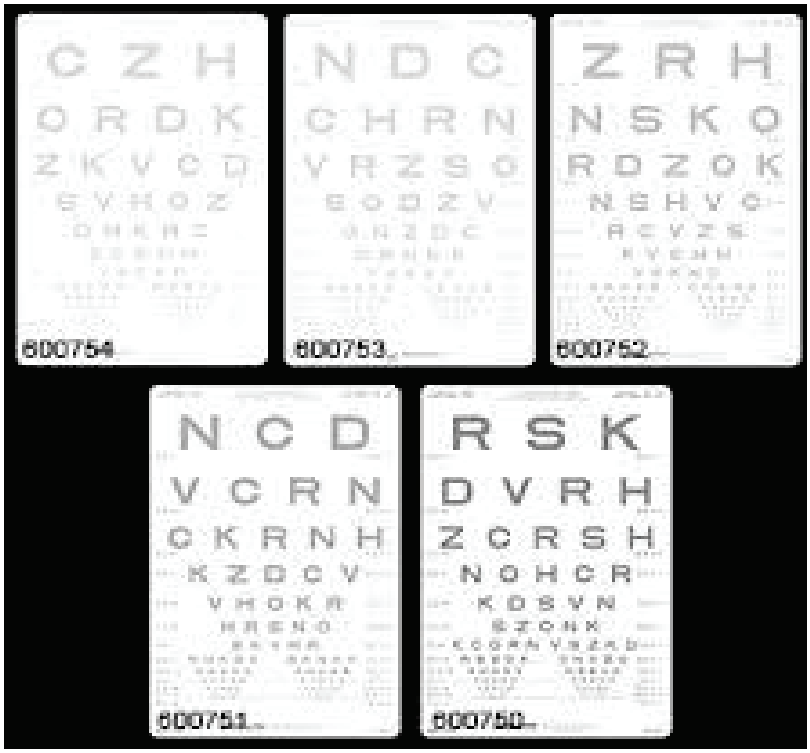
If trial was not completed *(mark one):*

☐ Unable to complete trial due to physical limitations ➡ Specify: _____

☐ Other ➡ _____

Did it take more than two attempts to get two sucessful trials? ☐ Yes ☐ No
If Yes, please specify reason(s) for more than two attempted trials:

Low Contrast Letter Visual Acuity



Symbol Digit Modalities Test

≥	±	«	∏	ж	ψ	Δ	o	↑
1	2	3	4	5	6	7	8	9

ψ	±	∏	ψ	±	o	≥	Δ	↑	ж	±	«	±	≥	Δ
6	2	4												

ж	Δ	↑	o	∏	«	Δ	↑	ж	±	«	«	«	ж	ψ

o	±	«	∏	ж	ψ	≥	o	±	≥	±	«	«	ψ	o

≥	∏	«	ψ	ж	±	Δ	o	↑	o	±	«	∏	ж	«

±	±	«	∏	ж	ψ	o	±	o	≥	±	«	∏	o	ψ

«	∏	«	Δ	«	∏	Δ	o	↑	Δ	«	«	Δ	ж	ψ

≥	±	«	±	ж	«	±	o	«	≥	±	±	∏	Δ	ψ

Appendix IV: Fatigue Severity Scale

PER CORTESIA SCRIVERE IN STAMPATELLO E SEGNARE IN QUESTO MODO ☒ DOVE RICHIESTO. DATARE E SIGLARE OGNI CORREZIONE

FATIGUE SEVERITY SCALE (FSS)

Le nove affermazioni che può leggere qui sotto tentano di indagare la severità dei sintomi legati alla fatica. Legga ogni affermazione e per ciascuna indichi uno dei numeri (da 1 a 7) considerando che 1 corrisponde ad un completo disaccordo con quanto affermato, mentre 7 indica un completo accordo con quanto affermato. Prenda come riferimento la settimana appena trascorsa.

	1	2	3	4	5	6	7
1. Le mie motivazioni sono state scarse quando ero affaticato.	☐	☐	☐	☐	☐	☐	☐
2. L'esercizio fisico ha aumentato il mio affaticamento.	☐	☐	☐	☐	☐	☐	☐
3. Mi sono affaticato facilmente.	☐	☐	☐	☐	☐	☐	☐
4. La fatica ha interferito con la mia attività fisica.	☐	☐	☐	☐	☐	☐	☐
5. La fatica mi ha causato frequenti problemi.	☐	☐	☐	☐	☐	☐	☐
6. La fatica mi ha impedito una attività fisica sostenuta.	☐	☐	☐	☐	☐	☐	☐
7. La fatica ha interferito con lo svolgimento di alcuni compiti e responsabilità.	☐	☐	☐	☐	☐	☐	☐
8. La fatica è stata fra i tre sintomi più disabilitanti.	☐	☐	☐	☐	☐	☐	☐
9. La fatica ha interferito con il mio lavoro, la famiglia e la vita sociale.	☐	☐	☐	☐	☐	☐	☐

Appendix V: Modified Fatigue Impact Scale (MFIS)

MFIS		MAI	RARAMENTE	QUALCHE VOLTA	SPESSO	QUASI SEMPRE
1	sono stato meno vigile	0	1	2	3	4
2	ho avuto difficoltà a prestare attenzione per lunghi periodi di tempo	0	1	2	3	4
3	Non sono stato in grado di pensare in modo lucido	0	1	2	3	4
4	Sono stato maldestro e scoordinato	0	1	2	3	4
5	Sono stato smemorato	0	1	2	3	4
6	Ho dovuto rallentare la mia attività fisica	0	1	2	3	4
7	Sono stato meno motivato a fare qualsiasi cosa richieda uno sforzo fisico	0	1	2	3	4
8	Sono stato meno motivato a partecipare ad attività sociali	0	1	2	3	4
9	Sono stato limitato nella mia capacità di fare cose fuori di casa	0	1	2	3	4
10	Ho avuto problemi a compiere sforzi fisici per lunghi periodi	0	1	2	3	4
11	Ho avuto difficoltà nel prendere decisioni	0	1	2	3	4
12	Sono stato poco motivato nel compiere qualsiasi cosa richieda pensare intensamente	0	1	2	3	4
13	Sento i miei muscoli molto deboli	0	1	2	3	4
14	Sono stato male fisicamente	0	1	2	3	4
15	Ho avuto problemi a portare a termine compiti che richiedano riflessione	0	1	2	3	4
16	Ho avuto difficoltà nell'organizzare i miei pensieri svolgendo incarichi a casa o al lavoro	0	1	2	3	4
17	Ho avuto maggiori difficoltà del solito a concludere compiti che richiedano uno sforzo fisico	0	1	2	3	4
18	La mia capacità di ragionamento è risultata piuttosto rallentata	0	1	2	3	4
19	Ho avuto problemi di concentrazione	0	1	2	3	4
20	Ho limitato le mie attività fisiche	0	1	2	3	4
21	Ho avuto necessità di riposarmi più spesso del solito o per periodi più lunghi	0	1	2	3	4

Appendix VI: Draft labels of Investigational Product

Patient Kit: TERTIARY packaging

Studio: INCREASE (MS 200136_0041) **KIT N°: XXXX**

Paziente N°: _____

Centro: _____

Indirizzo: _____

Sperimentatore: _____

Questo box contiene:

6 scatole ognuna contenente 32 bustine di D-Aspartato 2660mg o Placebo, polvere per soluzione orale.

Lotto: AAA/AA Data di scadenza: MM/YYYY

Assumere per via orale 1 bustina al giorno, sciolta in 100ml di acqua.

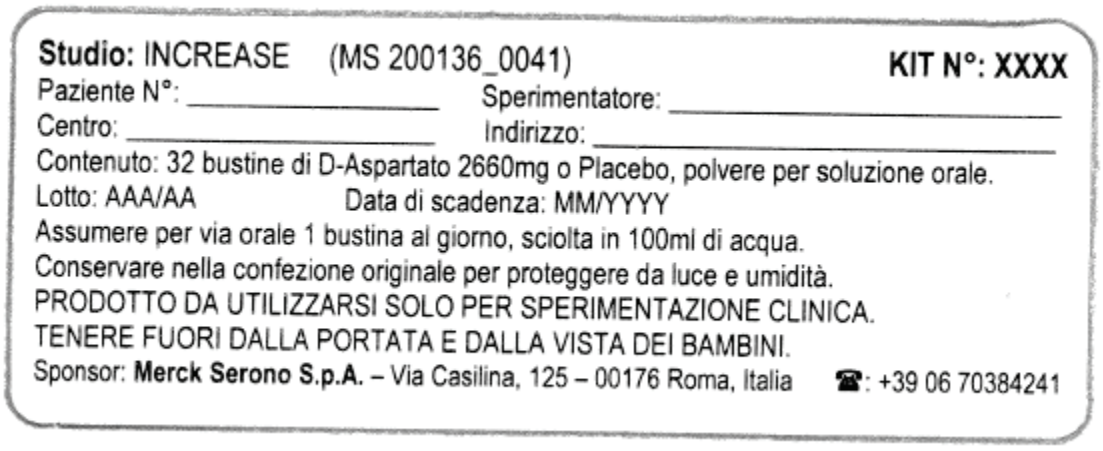
Conservare nella confezione originale per proteggere da luce e umidità.

PRODOTTO DA UTILIZZARSI SOLO PER SPERIMENTAZIONE CLINICA.

TENERE FUORI DALLA PORTATA E DALLA VISTA DEI BAMBINI.

Sponsor: **Merck Serono S.p.A.** – Via Casilina, 125 – 00176 Roma, Italia
☎: +39 06 70384241

Treatment box: SECONDARY packaging


Studio: INCREASE (MS 200136_0041) **KIT N°: XXXX**
Paziente N°: _____ Sperimentatore: _____
Centro: _____ Indirizzo: _____
Contenuto: 32 bustine di D-Aspartato 2660mg o Placebo, polvere per soluzione orale.
Lotto: AAA/AA Data di scadenza: MM/YYYY
Assumere per via orale 1 bustina al giorno, sciolta in 100ml di acqua.
Conservare nella confezione originale per proteggere da luce e umidità.
PRODOTTO DA UTILIZZARSI SOLO PER SPERIMENTAZIONE CLINICA.
TENERE FUORI DALLA PORTATA E DALLA VISTA DEI BAMBINI.
Sponsor: **Merck Serono S.p.A.** – Via Casilina, 125 – 00176 Roma, Italia ☎: +39 06 70384241

Sachet: PRIMARY packaging


Studio: INCREASE (MS 200136_0041)
KIT N°: XXXX
D-Aspartato 2660mg o Placebo,
polvere per soluzione orale.
Assumere per via orale 1 bustina al giorno,
sciolta in 100ml di acqua.
Lotto: AAA/AA
PRODOTTO DA UTILIZZARSI SOLO PER
SPERIMENTAZIONE CLINICA.
Conservare nella confezione originale per
proteggere da luce e umidità.
Sponsor: Merck Serono S.p.A.