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EXPLORING IN VIVO THE ENERGETIC ORIGIN OF NEURODEGENERATION IN MULTIPLE SCLEROSIS: A ULTRA-HIGH FIELD SODIUM IMAGING, PHOSPHORUS SPECTROSCOPY AND DIFFUSION-WEIGHTED SPECTROSCOPY STUDY

THE ENERGETIC ORIGIN OF NEURODEGENERATION IN MS /ENERGYSEP

VERSION N°0.8 of the 03/10/2019

CONFIDENTIAL

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HISTORY OF THE VERSIONS OF THE PROTOCOL

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LIST OF ABREVIATIONS

23Na	Sodium
31P MRS	Phosphorus spectroscopy
ADC	Apparent diffusion coefficient
AE/SAE	Adverse event / Serious adverse event
ANSM	National agency for the safety of medications and health products
ARC	Clinical research associate
ATP	Adenosine triphosphate
BI	Investigator's Brochure
BICAMS	Brief International Cognitive Assessment for Multiple Sclerosis
CENIR	Centre de Neuroimagerie de Recherche
CIC	Centre d'investigation clinique
CNIL	Commission Nationale de l'Informatique et des Libertés
CNS	Central nervous system
CPP	Comité de Protection des Personnes
Cr	Creatine
CRF	Case report form/ Cahier observation
CV	Curriculum vitae
DIR	Double inversion-recovery
DM	Medical device
DW-MRS	Diffusion-weighted spectroscopy
EDSS	Expanded Disability Status Scale
FLAIR	Fluid attenuated inversion recovery
MFIS	Modified Fatigue Impact Scale
MR	Reference method
MRI	Magnetic resonance imaging
MRS	Magnetic resonance spectroscopy
MS	Multiple sclerosis
MSR	Motor-sensory region
MSSS	Multiple Sclerosis Severity Scale
MTR	Magnetization transfer ratio
NAA	N-acetyl aspartate
NIFC	Notice d'information et formulaire de consentement
NODDI	Neurite orientation dispersion and density imaging

PCr	Phosphocreatine
PMS	Pprogressive multiple sclerosis
PP-MS	Primary progressive MS
PRC	Pôle Recherche Clinique de l'ITMO Santé Publique de l'Inserm
RCP	Résumé des caractéristiques du produit
RIPH	Recherche impliquant la personne humaine
RQRC	Réglementation et qualité en recherche clinique
RR-MS	Relapsing remitting MS
RS-fMRI	Resting-state functional MRI
SP-MS	Secondary progressive MS
T	Tesla
tCr	Total creatine-phosphocreatine
VRB	Fichier national des volontaires (volontaires recherche nationale)

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1. RESEARCH JUSTIFICATION

1.1 Multiple Sclerosis: a disabling chronic demyelinating disease

Multiple Sclerosis (MS) is the most common human demyelinating disorder of the central nervous system. It affects one in one-thousand people in western countries, where it represents the main cause of non-traumatic neurological disability in young adults.
This pathology is usually considered as the consequence of an auto-immune attack against central nervous system (CNS) myelin, occurring in genetically predisposed individuals in response to an unknown environmental aggression. The occurrence of demyelinating plaques is the result of inflammatory cells entering the CNS and attacking myelin antigens.
From a clinical point of view, the disease course is most commonly relapsing-remitting, with a series of clinical relapses followed by full or partial remission, the interval amongst these being highly variable. This is the form of onset in 85% of cases. Approximately 10-15% of patients present a progressive form from the beginning, in which neurological symptoms progress inexorably [1]. This progressive form may sometimes be characterised by overlapping relapses. Lastly, after a variable period of relapses and remissions, the disease can evolve into a secondary progressive form of neurological handicap. Despite being considered for a long time as a purely inflammatory demyelinating pathology, it is nowadays clear that a neurodegenerative component is present from the early phases of MS and plays a major role in the occurrence and progression of permanent neurological signs. The physiopathology of this degenerative damage is not fully understood, but its importance has been highlighted by the epidemiological studies showing that the frequency of inflammatory relapses does not influence the progression of neurological signs once the stage of persistent disability is reached [2]. Moreover, immunomodulatory or immunosuppressive treatments, although effective on the inflammatory component of the disease - as evaluated by the frequency of newly occurring white matter lesions in MRI (magnetic resonance imaging) and clinical relapses - are poorly effective on disability progression [3]. Understanding the sequence of pathological events underlying neurodegeneration is therefore essential to develop effective treatments for prevention and accumulation of clinical disability in both relapsing and progressing MS patients.

1.2 Energy dysregulation is a key process in the physiopathology of neurodegeneration

Several mechanisms have been suggested to play a major role in the physiopathology of neurodegeneration in MS, among which: inflammatory demyelination, excitotoxicity, oxidative stress and ionic channel dysfunction [4]. Each of these mechanisms is potentially implicated in inducing an energy dysregulation. Moreover, the notion that early neuronal energy failure may play a key role in axonal degeneration is being more and more acknowledged. In these physiological conditions, myelin sheath integrity not only allows efficient saltatory conduction of the action potential, but it also helps to maintain the environment for neuronal survival by assuring the correct amount of cellular energy.

Nodal regions are enriched with voltage-gated sodium channels (VGSC), whilst sodium/potassium pumps (Na+/K+ ATPases) are abundantly located in the internodal and juxta-paranodal regions [7]. Similarly, mitochondria are not uniformly distributed along the axon and their metabolism is, at least partly, ensured by the surrounding oligodendrocytes and their myelin sheathes [8]. Inadequate energy level of the glial environment is essential to allow axonal energy metabolism. Indeed, glial cells provide metabolites to neurones such as creatine-phosphocreatine (Cr+PCr) and lactate, which are used by mitochondria as substrates to produce ATP, a process which is modified during demyelination [9].

Following myelin disruption and inflammation, multiple adaptive changes force the neurone to enter a temporary state of "virtual hypoxia" [10]. Indeed, the loss of axonal coverage determines the VGSC redistribution along the axon and the re-expression of NaV1.2 [11]. Furthermore, demyelination induces mitochondrial proliferation along damaged axons, together with a complex for hyperactivity in order to support the sodium/potassium pump. These compensatory mechanisms initially overcome the conduction block resulting from demyelination, but in such conditions transmembrane electrochemical gradients are maintained at the cost of an increased energy demand.

This supplementary energy demand leads to a dysequilibrium between energy demand and supply, called "neuronal virtual hypoxia". In MS energetic distress is worsened by the inflammatory environment, which affects mitochondrial function and is associated with a cell structural deformation [13]. This condition of energy dysfunction is potentially reversible in the first stages of the disease [13] but, if not reversed after a certain time it induces a cascade characterised by a mitochondrial function failure, free radical production and sodium/potassium ATPase dysfunction. Ionic pump dysfunction induces an accumulation of inter-cellular sodium with an inversion of calcium flux operated by the $Na+/Ca^{2+}$ pump. Calcium intracellular influx eventually leads to an irreversible neuronal degeneration [5,14]. Therefore, identifying in-vivo the early phase of neuronal energy dysfunction proceeding neuro-axonal death and understanding its temporal relationship with reversible neuronal degeneration is essential for the development of therapies aimed at reverting this degenerative process and maintain long term neuronal integrity.

1.3 Imaging energy dysregulation: state of the art

The optimisation over the past few years of innovative techniques based on magnetic resonance (MR) such as sodium MRI (23Na), phosphorus spectroscopy (31P-MRI) and diffusion-weighted spectroscopy (DW-MRS), have enabled the production of promising data which allow the clarification of different aspects of the initial phase of energy dysregulation in MS.

Several studies using 23Na MRI have shown an increase of the concentration of total sodium in the brain of patients with MS, which is correlating with clinical disability [15,16]. More recently, more sophisticated techniques to quantify 23Na concentration have been proposed, such as "triple quantum filtered (TQF) imaging", which allows the differentiation between intracellular and extracellular sodium [17], thus providing unique information on the early phase of energy dysregulation. Due the relatively weak sensibility of 23Na MRI, high-field MRI is particularly suited for TQF. The team lead by professor Matilde Inglese has recently applied 7 tesla TQF imaging in MS, showing that patients with a relapsing/remitting form of the disease (RR-MS) have increased intracellular sodium concentration compared to healthy volunteers. This reflects the pathological intracellular accumulation of 23Na as a consequence of Na+ K+ ATpase dysfunction [18].

31-MRS allows the direct measurement of the concentration in the brain of phosphocreatine (PCr) and adenosine triphosphate (ATP), which is the main energy currency of cells. 31P-MRS at 3T has shown that MS patients present higher PCr levels in the normal-appearing white matter when compared to healthy controls [19,20]. 21P-MRS has also shown that beta-ATP percentage was higher in RR-MS and lower in progressive forms, reflecting a "relatively high energy state" in the early phases of the disease, with a reduction in the progressive phase, leaving room for axonal degeneration [21].

Important technical processes have been reached through the successful generation of 31P-MRS data at 7T [22].

DW-MRS gives in-vivo measurements of the diffusion properties of creatine (Cr) + PCr. In a recent study using DW-MRS at 3T, we found a reduced Cr+PCr diffusivity in the normal-appearing white matter and the thalami of patients with MS compared with healthy controls, reflecting a reduction of energy reserved in neurons and glial cells [23]. Two alternative hypotheses could explain the observed reduction of Cr+PCr diffusivity: an altered intracellular transport of creatine, in particular from oligodendrocytes to neurones from one to another, or a reduced PCr consumption due to an altered function of creatine kinase B [24].

Overall, these results confirm the strong potential of combining these different techniques (23Na MRI, 31PMRS and DW-MRS) in order to obtain innovative information on each aspect of the pathogenic cascade linking energy dysregulation to neuro-axonal degeneration in MS.

1.4 Rationnel of the research

Two major questions on the key relationship between energy dysregulation following demyelination and neurodegeneration remain unanswered:

(i) can brain energy dysregulation measured at a given time predict the future development of neurodegeneration?
(ii) to what extent and for how long can demyelinated neurones bear this state of "virtual hypoxia", before suffering irreversible damage leading to neuronal death? The answer to this last question will allow us to identify the temporal "window" in which a therapeutic intervention could revert the pathogenic cascade produced by demyelination and prevent the development of neurodegeneration and clinical disability.

One of the reasons why these question have not yet been answered is the lack of specific and trustworthy markers of the energy dysregulation phase in vivo.

Imaging measurements able to capture in a selective way the energy dysregulation leading to axonal dysfunction are essential to identify the sequence of events underlying neurodegeneration. They could provide information on the individual energy reserves, on the definition of the temporal delay for targeted therapeutic intervention, and the identification of patients that could potentially benefit from myelin repair and neuroprotective treatments.

2. OBJECTIVES

2.1 Main objective

The main objective of this project is to explore the relationship between the MRI-derived parameters reflecting energy dysregulation in the motor sensory regions (MSR) at study entry, and the parameters of neurodegeneration in the MSR after 24 months in RR-MS and PMS patients compared to healthy volunteers, through a combination of 23Na quantitative MRI, 31P-MRS and DW-MRS, and measurements of cortical thickness.

2.2 Secondary objectives

The secondary objectives of this research will be:
(i) To compare MRI-derived metrics of energy dysregulation between patients and controls in the whole brain and the MSR at study entry.
(ii) To define the correlations between MRI-derived metrics of energy dysregulation and cortical demyelination at study entry, as well as cortical myelination after 12 months.
(iii) To study the correlation between cortical 23Na MRI at study entry and the evolution of cortical volume over 24 months.
(iv) To study the correlations between MRI-derived metrics of energy dysregulation and early axonal damage a well as axonal-dendritic density in the whole brain and in the MSR: 1) at study entry; 2) over the follow-up period of 24 months.
(v) To study the relationship between MRI-derived metrics of energy dysregulation and the changes of brain connectivity at study entry, at 12 and 24 months.
(vi) To establish the correlations between MRI-derived metrics and serum neurofilaments measured at study entry, at 12 and 24 months.
(vii) To establish the contribution of MRI-derived metrics of energy dysregulations to clinical disability and to cognitive dysfunction, at study entry and at 24 months.

3. EVALUATION CRITERIA

3.1. Main evalution criterion

Energy dysregulation in the MSR will be evaluated on the following parameters:
1) the levels of total, intracellular and extracellular sodium quantified through 23Na MRI in the whole brain;
2) ATP and PCr concentrations measured through 31P MRS in a voxel centred on the left MSR;
3) the ADC of tCr (Cr + PCr) measured through DW-MRS in a voxel centred on the left MSR.

Neurodegeneration after 24 months will be evaluated by the measurements of the following parameters:
1) MSR cortical thickness after 24 months;
2) Change in MSR cortical thickness between study entry and 24 months measured with Freesurfer (https://surfer.nmr.mgh.harvard.edu).

3.2. Secondary evaluation criteria

(i) The difference of MRI-derived metrics of energy dysregulation between patients and controls at study entry will be evaluated with 23Na MRI in the whole brain, and with 23Na MRI, 31P MRS and DW-MRS in the MSR;
(ii) Cortical demyelination at study entry will be measured by cross-sectional cortical magnetisation transfer ratio (MTR) [25-26], and cortical remyelination after 12 months will be evaluated by the measurement of cortical MTR change over 12 months [27];
(iii) The evolution of cortical volume over 24 months will be measured with Freesurfer (https://surfer.nmr.mgh.harvard.edu) and SIENA (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki/SIENA)[28];
(iv) Early axonal damage and axonal-dendritic density will be quantified through the NODDI-derived metrics [29];
(v) changes in brain connectivity at study entry at study entry and after 12 and 24 months will be measured by resting-state functional MRI (RS-fMRI);
(vi) Serum neurofilaments, measured at study entry and at 12 and 24 months, will be used as a biomarker of acute and chronic neuronal lesions [30,31];

(vii) neurologic diability will be evaluated by the Expanded Disability Status Scale (EDSS) [32], the Multiple Sclerosis Severity Scale (MSSS) [33], the Modified Fatigue Impact Scale (MFIS ou EMIF-SEP) [34] an the Jamar hydraulic hand dynamometer; neuropsychologic dysfunction will be evaluated by the Brief International Cognitive Assessment for Multiple Sclerosis (BICAMS) [35].

4. CONCEPTION OF THE RESEARCH PROJECT

4.1 Type of research project

This is a monocentric research project on human beings with physiopathologic pourposes (no health products involved), with a duration of 24 months, whose aim is to explore the role of energy dysregulation in neurodegeneration, which is the main pathologic subastrate of clinical progression in patients with MS. Our aim is to investigate the relationship between energy dysfunction and neuro-axonal damage in patients with MS compared to healthy controls, using a combination of imaging methods and clinical, cognitive and biological tests.
A group of relapsing-remitting (RR-MS, n=20) and progressive (primary progressive - PP-MS - or secondary progressive - SP-MS, n=20) patients with MS will be evaluated and compared to a group of age- and gender-matched healthy volunteers (n=15).

Given that the techniques used in the study involve imaging methods with the injection of contrast agents, the research will be classified as interventional research of category 1, according to the Jardé Law.

4.2 Methodology of the research

This is a non-randomized, controlled, open label clinical-radiological research.

Patients will be selected from the cohorts followed at the Department of Neurology of Saint-Antoine Hospital and the Department of Nervous System Pathologies of the Pitié-Salpêtrière Hospital. They will receive a first information on the research during the consultation, and the information form may be delivered on this occasion.
Healthy volunteers will be selected from the volunteers of the Center of Clinical Investigation (CIC) of the Pitié-Salpêtrière Hospital.

All participants will be included at the CIC of the Brain and Spine Insitute (ICM) – Pitié-Salpêtrière Hospital.

A group of RR-MS (n=20) and progressive (PP-MS or SP-MS, n=20) patients will be studied and compared to a group of healthy volunteers (n=15), age- and gender-matched (but without a 1:1 matching between patients and controls). The choice of matching a sample of 40 patients to a sample of 15 healthy volunteers is based on the calculation of the minimum number of observations per group needed to detect a between-group difference in the evaluation criteria, using a multivariable linear regression model.

Group matching will be performed during the research through the inclusion of healthy volunteers matching one or more patients as for age and gender. It will be validated by descriptive statistics (mean, median and standard deviation).

Each patient and healthy volunteer will undergo four visits:

Visit 1 (inclusion visit) - D0 **: ICM (CIC/CENIR) (30-minute clinical visit + 65-minute 3 T MRI)**

- information, verification of inclusion/exclusion criteria, signature of the informed consent form
- neurological evaluation (EDSS, MRC scores) and testing of fatigue (MFIS, Jamar hydraulic hand dynamometer) to assess the level of physical impairment
- neuropsychological tests (BICAMS) to evaluate the level of cognitive impairment
- pregnancy test for all women of child-bearing age
- 3T MRI - with gadolinium injection for patients only (see 8.1 for administration procedures)
- with DW-MRS sequences to quantify tCr et NAA (N-acetyl aspartate) diffusivity in a voxel centered on the MSR, and with sequences for volumetric, structural and functional analyses (65 min)
- blood sampling (4 mL) to search for neurofilaments as biological markers of neurodegeneration.

Visit 2 - M1**: NeuroSpin (CEA Center in Saclay, Gif-sur-Yvette, France) (120 minutes)**

- Urinary pregnancy test for all women of child-bearing age
- 7T MRI including a whole brain 23Na MRI and a 31P MRS centered on the MSR voxel for sodium, ATP and PCr quantification (1h20min)

Visit 3 - M12 **: ICM (CIC/CENIR) (15-minute clinical visit + 65-minute 3 T MRI)**

- neurological evaluation (EDSS, MRC scores) and testing of fatigue (MFIS, Jamar hydraulic hand dynamometer) to assess the level of physical impairment
- pregnancy test for all women of child-bearing age
- 3T MRI - using the same protocole of V1 - with gadolinium injection for patients only (see 8.1 for administration procedures) - with DW-MRS sequences to quantify tCr et NAA (N-acetyl aspartate) diffusivity in a voxel centered on the MSR, and with sequences for volumetric, structural and functional analyses (65 min)
- blood sampling (4 mL) to search for neurofilaments as biological markers of neurodegeneration.

Visit 4 - M24 **: ICM (CIC/CENIR) (30-minute clinical visit + 65 minute 3 T MRI)**

- neurological evaluation (EDSS, MRC scores) and testing of fatigue (MFIS, Jamar hydraulic hand dynamometer) to assess the level of physical impairment
- neuropsychological tests (BICAMS) to evaluate the level of cognitive impairment
- pregnancy test for all women of child-bearing age
- 3T MRI - using the same protocole of V1 - with gadolinium injection for patients only (see 8.1 for administration procedures) - with DW-MRS sequences to quantify tCr et NAA (N-acetyl aspartate) diffusivity in a voxel centered on the MSR, and with sequences for volumetric, structural and functional analyses (65 min)
- blood sampling (4 mL) to search for neurofilaments as biological markers of neurodegeneration.

Exams will be performed on multiple sites:

- Inclusion, neurological and neuropsychological evaluation, testing of fatigue, blood sampling and pregnancy test will be done at the CIC of the Pitié-Salpêtrière Hospital
- 3T MRI and gadolinium injection will be done at the Centre de Neuroimagerie de Recherche (CENIR) of the ICM - Pitié-Salpêtrière Hospital
- 7T MRI and urinary pregnancy test, when necessary, will be done at NeuroSpin (CEA Center of Saclay, Gif-sur-Yvette, France) within 4 weeks after inclusion.

4.3 Provisional calendar for the research

Subject recruitment will be performed over a 18-month period. The duration of participation in the research will be of 24 months for all the subjects.

Each subject will undergo 4 scans, of about 120 min for the 7 T MRI and 65 min each for the 3T MRI. Participants are allowed to participate into other research projects at the same time and no exclusion period is given.

5. SUBJECT SELECTION

5.1 Study populations

Two groups of patients will be included, evaluated and compared to a group of healthy volunteers:

- **Group I:** Patients with MS:
  - Group Ia: 20 patients presenting a RR form with a disease duration of less than 10 years;
  - Group Ib: 20 patients presenting a progressive form (primary or secondary), with a duration of the progressive phase of less than 10 years.

- **Group II:**
15 healthy subjects.

We will include up to 5 additional subjects per group (I and II), so 10 participants in total.

5.2 Inclusion criteria

- (Group Ia) **RR-MS patients with a disease duration of less than 10 years:**
  - 18-55 years
  - clinically defined RR-MS according to the 2017 revised McDonald's criteria (MS diagnostic criteria 2017)
  - disease duration <10 years
  - ability to understand the research objectives and the procedure details, and to sign the informed consent
  - affiliation with the French National Health Insurance, Universal Medical Coverage (CMU) or any equivalent

- (Group Ib) **Patients with progressive MS (primary or secondary) of less than 10 years**:
  - 18-55 years
  - clinically defined progressive MS according to the 2017 revised McDonald's criteria
  - disease duration <10 years from the beginning of the progressive phase
  - ability to understand the research objectives and the procedure details, and to sign the informed consent
  - affiliation with the French National Health Insurance, Universal Medical Coverage (CMU) or any equivalent

- (Group II) **Healthy Volunteers** :

- 18-55 years (matched with patients)
- no known general pathologies
- ability to understand the research objectives and the procedure details, and to sign the informed consent
- affiliation with the French National Health Insurance, Universal Medical Coverage (CMU) or any equivalent

5.3 Non-inclusion criteria

- (Groups Ia and Ib) **Patients with MS** :

- Pregnant or breastfeeding women*
- Last infusion of cyclophosphamide, mitoxantrone or methylprednisolone realized less than 1 month before inclusion
- last clinical relapse less than one month before inclusion
- severe cardiac, pulmonar, hepatic, hematologic renal, gastrointestinal disease, or cancer
- contraindications to MRI: claustrophobia, pace-maker implant, any surgical ferromagnetic clips, ocular impants, any intraocular or intracranial metallic fragments, any metallic objects able to concentrate the radiofrequency field, cochlear implants, cardiac or brain stimulators, any tattoos or permanent makeup on the face, renal failure (exclusion criterion for gadolinium injection)
- patients not willing to be informed of any possible cerebral malformations incidentally discovered at the MRI exam
- severe renal failure (clearance of creatinine < 30ml/min) **
- history of allergic reactions to gadolinium salts
- any other chronic neurological disorders associated
- persons deprived of liberty by law or by administrative decision
- Persons under legal protection

- (Group II) **Healthy volunteers :**

- Pregnant or breastfeeding women*
- severe cardiac, pulmonar, hepatic, hematologic renal, gastrointestinal disease, or cancer
- contraindications to MRI : claustrophobia, pace-maker implant, any surgical magnetic clips, ocular impants, any intraocular or intracranial metallic fragments, any metallic objects able to concentrate the radiofrequency field, cochlear implants, cardiac or brain stimulators, any tattoos or permanent makeup on the face
- person not willing to be informed of any possible cerebral malformations incidentally discovered at the MRI exam
- any other chronic neurological disorders associated
- persons deprived of liberty by law or by administrative decision
- Persons under legal protection

*a pregnancy test will be realized for all women of child-bearing age
** creatinine blood levels will be verified on a biological sample of less than 1 year before inclusion

5.4 Recruitment strategy

We plan to include in the research 40 patients with MS, of which 20 RR-MS and 20 progressive MS patients, and 15 age- and gender-matched healthy volunteers.

Patients will be selected from the cohorts followed at the Department of Neurology of Saint-Antoine Hospital (B Bodini, B Stankoff) and the Department of Nervous System Pathologies of the Pitié-Salpêtrière Hospital (GHPS, C Lubetzki).
They will receive a first information on the research during the consultation, and the information form may be delivered on this occasion.
Healthy volunteers will be selected from the volunteer registry of the Center of Clinical Investigation (CIC) of the Pitié-Salpêtrière Hospital.

All participants will be included at the CIC of the Brain and Spine Insitute (ICM) – Pitié-Salpêtrière Hospital.

6. PRACTICAL REALIZATION OF THE PROTOCOL

Table belows shows the calendar of all the visits in the research protocol:

	V1 D0	**V2 M1**	**V3 M12**	**V4 M24**
Signature of the informed consent form	**X**			
Neurological evaluation EDSS score MRC score	**X**		**X**	**X**
Neuropsychological tests BICAMS	**X**			**X**
Fatigue assessment MFIS scale Jamar hydraulic hand dynamometer	**X**		**X**	**X**
7T MRI		**X**		
3T MRI	**X***		**X***	**X***
Urinary pregnancy test for all women of child-bearing age	**X**	**X**	**X**	**X**
Blood sampling	**4 mL**		**4 mL**	**4 mL**

(*) with gadolinium injections for patients, without gadolinium injcetion for healthy volunteers

In case of technical problems (e.g. with the MRI scanner), the participant will be recalled to complete the rest of the exam (within one month, if possible).

Travel expenses for all participants will be covered for visits 1, 3, 4 (to reach the CIC) and for visit 2 (to reach Saclay) upon presentation of proof of purchase. Transport can also be organized bt the research team at CIC. Meal costs at each visit will also be covered.

6.1 Visit N°1

6.1.1. Inclusion, information, and consent to participate

This visit will take place for all subjects at the CIC of Pitié-Salpêtrière Hospital.
With prior agreement, patients and healthy volunteers will receive a detailed information and wll benefit of a reflection period of at least 24 hours.
They will then sign, during the inclusion visit, the informed consent form if they accept to participate in the research. Then, a general medical examination will be performed to check the eligibility, inclusion and exclusion criteria.

The exams performed at the inclusion are the following:
- for patients and healthy volunteers, the investigator will verify by interview and medical examination (heart rate, pulmonary auscultation, abdominal exam, blood pressure, height and weight) that the participant fulfills all the inclusion criteria and that no exclusion criteria exist
- for patients: clearance of creatinine must be present in the medical file and dated within the last year (see inclusion criteria 5.3) to rule out severe renal failure
- for healthy volunteers: recording of medical history and current treatments, general and neurolgical examination
- for patients: recording of medical history and current treatments, general and neurolgical examination
For both patients and healthy volunteers, the date of inclusion and of study exit, if applicable, will be recorded in the medical file.

6.1.2. Neurological evaluation

A complete neurological examination will be performed to record any neurological signs and symptoms:

- EDSS score: quantification of clinical diability in patients with MS. The EDSS is the most widely used and internationally validated MS disability, which justifies its use in clinical and research studies. The score ranges from 0 (absence of disability) to 10 (death due to MS) and is based on the integration of the following functional systems: pyramidal, sensitive, cerebellar, sphyncters, cognitive, visual and brainstem.

- MRC strength score: muscle strength is evaluated on the basisi of the score developed by the Medical Research Council, which is largely used in clinical practice. This score ranges from 5 to 0 and includes 6 categories: normal (5), good (4), fair (3), poor (2), traces (1) or zero.

- the MFIS, or EMIF-MS: the MFIS is a short version of the FIS, which includes 40 items and which can induce fatigue due to its length. MFIS total score is obtained by the sum of 21 items (maximum score is 84).

- a test with Jamar hydraulic hand dynamometer: this is an indispensable tool to measure in an objective way grip strength. The hydraulic system consists of a scale to measure hysometric grip strength from 0 to 90kg.

6.1.3. Neuropsychological tests

The objective is to quantify the reduction in specific cognitive domains usually affected in pattients with MS, such as working memory, attention, visuo-spatial and executive functions. Patients and healthy volunteers will undergo the BICAMS neurological evluation, including three tests:

- the Simbol Digit Modalities Test (SDMT): this test evaluates the information processing speed and attention and is helpful in detecting cognitive problems in MS (Deloire et al, 2006).

- the California Verbal Learning Test-II (CVLT-II) : this test measures episodic memories and allows a qualitative evaluation of learning abilities (Delis et al., 1987).

- the Brief Visuospatial Memory Test-Revised (BVMT-R): to evaluate memory and visual learning.

6.1.4. Blood sampling

A boold test will be performed to quantify serum neurofilament light and heavy chains, which reflect the degree of neuro-axonal damage.
A boold sampling of 4 mL will be realized using an anticoagulant tube (SST tube) to isolate serum by centrifugation and dose neurofilaments.
Blood samples will be taken within one hour to the DNA and Cell Bank of the ICM, located at the Biological Resource Platform (PRB) of Pitié-Salpêtrière Hospital (U1127, ICM, Bank Chiefs: operation chief Sylvie FORLANI, scientific chief Pr Alexis BRICE), together with the ENERGYSEP sampling form.
Serum will be stored at the DNA and Cell Bank. A part of 0.2 mL will be sent over to Dr Kuhle's lab to dose neurofilaments (Hebelstrasse 20, Department Biomedizin, Laboratory 302, 4031 Basel, Switzerland). Transport will be made by express delivery, in conditions that maintain sample freezing, in a single shipment, once all the samples have been collected. The rest of the serum (1.3 mL) will be stored in the Bank.

6.1.5. 1st 3T brain MRI

The 3T brain MRI (patients and healthy volunteers) will be done at the CENIR, Pitié-Salpêtrière.

All subjects will fill in the CENIR security checklist before the exam, to exclude the presence of any contraindications to the MRI (Annex 19.8), and a pregnancy test will be performed to women of child-bearing age.

Image acquisition

Images will be acquired with a 3T MAGNETOM Trio System (Siemens, Erlangen, Germany). The total duration of the exam is of about 65 minutes.

Acquisition protocol will include the following sequences:

1) A **T1-w 3D MP2RAGE** anatomic reference sequence, TR/TE 5000/1,99 ms, TI1 700 ms, TI2 2500 ms, voxel size of 1,0x1,0x1,0 mm³ (8,22 min). This sequence will be used to co-register all the other images, to place the diffusion spectroscopy voxel and to segment grey and white matter.

2) A **DW-MRS**, to measure energy dysfunction and axonal damage by quantifying the concentration and diffusion properties of Cr and NAA in the MSR. DW-MRS data will be acquired with a single-voxel semi-LASER technique from the same region where the 31P spectroscpy voxel will be palced, the left MSR (volume of interest, VOI =30(AP)x15(RL)x8(FH) mm3) with 6 optimized directions et 4 b values (b = 350, 1000, 2500, 4000 s / mm), TR = 3000 s, TE = 120 ms (13 min).

3) A **T2-w sequence**, TR/TE 4500/14 ms, voxel size of 1,0x1,0x1,0 mm3 (5,2 min), and a **FLAIR sequence**, TR/TE 5000/399 ms, 1 800 ms, voxel size of 1,0x1,0x1,1 mm3 (5,02 min), to measure white matter lesion load.

4) A **multi-shell diffusion-weighted sequence (DWI)**, TR/TE 4000/87,8 ms, b values: [0, 1500] 60 directions, [0, 700] 33 directions, [0, 300] 9 directions, voxel size of 2,0x2,0x2,0 mm3 (8 min), to extract the NODDI-derived metrics.

5) a **3D gradient-echo magnetization transfer ratio (MTR) sequence**, with (MTon) and without (Mtoff) magnetization gradient, voxel size of 1,3x1,3x1,3 mm3 (10 min) to accurately measure cortical myelin changes [36,37].

6) A **resting-state functional MRI (RS-fMRI)**, TR/TE 2100/25 ms, FA 90° and voxel size of 2.0x2.0x2.0 mm3 (7.06 min), to evaluate the changes of functional connectivity;

7) A **T1-w sequence with gadolinium injection**, TR/TE 650/14 ms, voxel size of 1,0x1,0x3,0 mm3 (4,14 + 4,14 min). This MRI will consist, only for patients, in an injection of a contrast agent commonly used in MS, the gadolinium, to visualize active lesions. The gadolinium salt used will be the DOTAREM (Guerbet Lab).

Gadolinium injection procedure is detailed in Paragraph 8.1 and is in agreement with the RCP.

Study exits and adverse events will be recorded.

Contraindications and adverse events

- Metallic objects are to be removed
- A urinary pregnancy test will be performed for all women of child-bearing age
- Patients will receive gadolinium injection, wich can in rare cases induce allergic reactions (Paragraph 8.1)
- Patients and healthy volunteers will remain still and not move on the MRI bed for a 65-minute time
- Given the noise produced by the image acquisition procedure, a set of headphones will be proposed. The patient or healthy volunteer will be alone in the exam room but will be able to communicate with the techincal personnel via a microphone.

6.2 Visit N°2

6.2.1. 7T brain MRI

The 7T brain MRI (patients and healthy volunteers) will be performed at NeuroSpin (CEA Center of Saclay, Gif-sur-Yvette, France).

Transfer of participants to Neurospin can be autonomous or organized by the research team of the CIC, upon demand. Travel costs will be covered under presentation of proof of purchase.
Subjects will be received by the health personnel of the CEA. Before data acquisition, a physician of the NeuroSpin platform will check the absence of contraindications to the high-field MRI and will fill in a checklist for all subjects (CEA safety checklist, Annex 19.9). A member of the research team (B. Bodini, V. Ricigliano, R. Porciuncula Baptista) will be present during the acqusitions to deal with any problems which may occur.

A urinary pregnancy test will be performed for all women of child-bearing age (time for results: 30 minutes).

Image acquisition

Images will be acquired with a 7T MAGNETOM Siemens System. Total duration of the exam, excluding subject placement, is of about 120 minutes, dividend in 2 sessions (75 minutes in the morning for 1H sequences and 31P spectroscpy, and 45 minutes in the afternoon for 23Na MRI). A lunch break will be done between the two sessions, and meal costs will be covered.
This sysytem does not have a CE marking yet and isn't therefore a commercial product. It is an experimental system for research on humans: imaging biomedical applications by MRI and MRI spectroscopy. The system has been entirely developed in agreement with the EU rules detailed in the EN 60601-2-33 norm, which defines the reglementary limits on mechanics, electrics and subject protection (gradient cululation, SAR). Any hardware or software updates on this machine, made by Siemens or in the context of the collaboration between Siemens and the CEA, will be in agreement with the EN 60601-2-33 norm.
For 23Na MRI and 31P spectroscopy, double resonance radiofrequency coils will be used: a 23Na/1H birdcage coil by Rapid Biomedical GmbH and a 31P/1H multielement coil by Resonance Research Inc., respectively.

The acquisition protocol will include the following sequences:

1) A **T1-w 3D MP2RAGE** anatomic reference sequence, TR/TE 6000/2,89 ms, TI1 800 MS, TI2 2700 MS, voxel size 0,8X0,8X0,8 mm3 (9,38 min), acquired as anatomic reference for the 1H MRI, the 23Na MRI and the 31P MRS.

2) A **T2** * sequence, TR 27 ms, TE 2,71, 5,61, 8,51, 11,41, 14,31, 17,21, 20,11 ms, voxel size of 0,8x0,8x0,8 mm3 (12,06 min), used to quantify iron accumulation.

3) A **DIR sequence**, TR/TE 8500/320 ms, TI1 450 ms, TI2 3300 ms, voxel size of 0,8x0,8x0,8 mm3 (10,56 min) to identify cortical lesions.

4) A **FLAIR** sequence, TR/TE 8000/290 ms, TI 2300 ms, voxel size of 0,8x0,8x0,8 mm3 (11,30 min), to evaluate white matter lesion load.

5) **3D single quantum (SQ) and triple quantum filtered (TQF) sequences of 23Na MRI**. For the SQ 23Na MRI we will use a 3D echo-gradient sequence (TR=150 ms, TE=6,8 ms, flip angle=90°, vision field=240x240 mm2, matrix=48x48, voxel size=5x5x5 mm33, 50 min); for the TQF 23Na MRI we will use a 3D modified echo-gradient sequence with a new B0-adjusted phase-cycle scheme in 12 steps (TR=150 ms, TE=6,8 ms, flip angle=90°, vision field=240x240x240mm3, matrix 30x30x24, voxel size 8x8x10 mm3, t1=6,8 ms, t2=150 ms, 2 means). B0 and B1 corrections will be applied to SQ et TQF images using the method

described in Fleysher et al., 2013 [36]. 23Na MRI will be used to quantifiy total, intracellular and extracellular sodium in the MSR and in the whole brain.

6) a **31P spectroscopy**, TR/TE=1200/2 ms, voxel size of 12x12x12 mm3 placed in the left MSR (20 min) to measure ATP and PCr levels inside the selected voxel.

Contraindications and details

- Metallic objects are to be removed
- An acustic protection with ear plugs and/or headphones will be systematically used for all subjects to reduce sequence noise
- Urinary pregnancy test: this test will be performed upon arrival for all women of child-bearing potential
- The subjects will remain remain still and not move on the MRI bed for the 75-minute session of the morning and the 45-minute session of the afternoon
- The subjects will be installed in the MRI scanner with their head on a rigid support.

6.3 Visit N°3

This visit will take place for all subjects at the CIC of Pitié-Salpêtrière Hospital.

6.3.1. Neurological evaluation

A complete neurological examination identical to that of Visit N°1 will be performed to track any neurological signs and symptoms.

6.3.2. Blood sampling

A blood test identical to that of Visit N°1 will be performed to dose serum neurofilament light and heavy chains.

6.3.3. 2nd 3T brain MRI

The 3T brain MRI (patients and healthy volunteers) will be done at the CENIR, Pitié-Salpêtrière.

All subjects will fill in the CENIR security checklist before the exam, to exclude the presence of any contraindications to the MRI (Annex 19.8), and a pregnancy test will be performed to women of child-bearing age.

Contraindications and adverse events

- Metallic objects are to be removed
- A urinary pregnancy test will be performed for all women of child-bearing age
- Patients will receive gadolinium injection, wich can in rare cases induce allergic reactions (Paragraph 8.1)
- Patients and healthy volunteers will remain still and not move on the MRI bed for a 65-minute time
- Given the noise produced by the image acquisition procedure, a set of headphones will be proposed. The patient or healthy volunteer will be alone in the exam room but will be able to communicate with the techincal personnel via a microphone.

6.4 Visit N°4

This visit will take place for all subjects at the CIC of Pitié-Salpêtrière Hospital.

6.4.1. Neurological evaluation

A complete neurological examination identical to that of Visit N°1 will be performed to track any neurological signs and symptoms.

6.4.2. Neuropsychological tests

Neuropsychological tests identical to those of Visit N°1 will be performed to quantifty the degree of cognitive impairment.

6.4.3. Blood sampling

A blood test identical to that of Visit N°1 will be performed to dose serum neurofilament light and heavy chains.

6.4.4. 3rd 3T brain MRI

The 3T brain MRI (patients and healthy volunteers) will be done at the CENIR, Pitié-Salpêtrière.

All subjects will fill in the CENIR security checklist before the exam, to exclude the presence of any contraindications to the MRI (Annex 19.8), and a pregnancy test will be performed to women of child-bearing age.

Contraindications and adverse events

- Metallic objects are to be removed
- A urinary pregnancy test will be performed for all women of child-bearing age
- Patients will receive gadolinium injection, wich can in rare cases induce allergic reactions (Paragraph 8.1)
- Patients and healthy volunteers will remain still and not move on the MRI bed for a 65-minute time
- Given the noise produced by the image acquisition procedure, a set of headphones will be proposed. The patient or healthy volunteer will be alone in the exam room but will be able to communicate with the techincal personnel via a microphone.

6.5 End of research

6.5.1. End of the research on subjects

The end of the research will occur 1 year after the last vist and exam of the last subject included. This delay will give time to complete all the analyses before the redaction of the final result report.

6.5.2. Rules for permanent or temporary stop of a part or of the totality of the research

In agreement with the article R1123-26 of the "Code de la santé publique", if inclusions have not started within the two years following the Ethics Committee approval, the authority approval will be considered null and the research project will need to be re-submitted for prorogation. This prorogation request will be accompanied by a letter justifying the delay on the initial provisional calendar.
During the course of the research, if the rhythm and the number of inlcusions appear insufficient, Inserm could decide to end the research if no other solutions can be proposed.

The sponsor or the competent authorities can interrupt the research for any other justified reasons (major deviation from the protocol not allowing to grant the safety of participants, data quality and research results).

The research can be temporarily or permanenty stopped:
- upon the decision of the Principal investigator, the Sponsor or the Competent Authority;
- in case of the presence of information compromising the realization of the research for safety reasons;
- in case of publication of new scientific data questioning the utility of the research;
- in case of severe adverse events potentially harming subjects' health and not clearly defined as being in relation with the research;
- in case of the absence of inclusions 2 years after the official start date of the research;
- in case of appearance of multiple severe adverse events.

6.5.3. Conditions for study exit (upon decision of the participant following consent withdrawal)

Each participant has, at any time, the right to withdraw his consent and ask the Investgator to drop out of the research.
If the subject wishes to prematurely stop his participation in the protocol, the data regarding the subject acquired before consent withdrawal will be exploited, unless the subject opposes. In this case, his data will be destroyed.
In case of consent withdrawal or of study exit upon decision of the Investgator, 10 subjects can be replaced.

6.5.4. Follow-up arrangements at the end of the research

No follow-up of subjects is planned at the end of the research.

6.5.5. Arrangements in case of premature exit from the research

In case of premature exit from the study, no arrangements are planned.

The reasons of premature exit will be specified in the observation logs. If they are related to the occurrence of a severe adverse event, a declaration form will be completed.

6.5.6. Arrangements in case of significant results concerning participants' health (information, follow-up, treatment)

In case of discovery of significant abnormalities other than that/those known by the patient during the course of the research, the physician indicated by the participant will be contacted and the participant will be referred to him/her.

In case of incidental discovery during the MRI exam of a brain abnormality other than that/those known (stroke, leukoaraiosis known at the inclusion), the subject will be informed by the Investigator and will contact his/her neurologist, with the Investigator's help, in order to define the strategy to follow.

7. LOGISTIC ORGANIZATION OF THE RESEARCH

This research will be carried on by teams already collaborating on multiple imaging research projects in the field of MS, which supports its feasibility. Each team is internationally known for its expertise in the the domain conferred to it in this project:

i) Department of Neurology of GHPS (Catherine Lubetzki) and Service of Neurology of Saint Antoine Hospital (B Stankoff, C Giannesini):
- patient selection

ii) CIC, GHPS (led by Pr Jean-Christophe Corvol, Neurologists involved in the project: Pr Bruno Stankoff, Pr Benedetta Bodini, Dr Vito AG Ricigliano):
- Recrutement of healthy volunteers
- Inclusion of patients and healthy volunteers
- Neurological examination performed by one of the Investigator Neurologists
- blood sampling by the nourses
- planning of visits and MRI exams
- Organization of transfers to the ICM and to Neurospin in Saclay
- Creation of the eCRF and recording of clinical data in the eCRF
- Data management

iii) CENIR, ICM (led by Pr Stéphane Lehericy, collaborator in this project: Dr Francesca Branzoli):
- 3T MRI acquisition
- 3T MRI processing
- gadolinium handling and tracking

iv) NEUROSPIN Center, Saclay (led by Dr Stanislas Dehaene, chief of the URC: Lucie Hertz Pannier, collaborators in this project: Dr Fawzi Boumezbeur, Renata Porciuncula Baptista):
- 7T MRI acquisition
- 7T MRI processing

v) Team « Mécanisme de la myélinisation et de la remyélinisation », ICM (Bruno Stankoff-Catherine Lubetzki):
- Data analysis will be coordinated by the ICM (B Bodini, B Stankoff), in collaboration with the CEA team (F Boumezbeur for the analysis of anatomical sequences at 7T, 23Na and 31P MRS data analysis)
- Data management will be under the responsibility of the ICM team led by B Stankoff, in collaboration with the platform Iconics

vi) Team led by Dr Matilde Inglese, Departement of Neurology, Neuroscience and Radiology, Icahn School of Medicine, Mount Sinai (New York, US):
- methodological support by Dr M Inglese and Dr Lazar Fleyscher for the technical setting, acquisition and quantification of 23Na at 7T. No data transfer (neither clinical, biological nor imaging) of subjects towards the USA is planned in this study (see Paragraph 11.3).

vii) CRB DNA & Cell Bank-ICM, U1127, operative chief: Sylvie FORLANI

- serum extraction, storage of bioogical samples, sample shipping.

viii) Dr Jens Kuhle, Department Biomedizin, Laboratory 302, 4031 Basel, Switzerland:
- neurofilament dosage (without transfer of any personal data regarding participants)

ix) Dr Raffaele Palladino, Department of Primary Care and Public Health, School of Public Health, Imperial College of London, London, UK:
- statistical analysis (without transfer of any personal data regarding participants), see Paragraph 11.3.

8. ADMINISTERED PRODUCTS (medications, medical products, treatments…)

The only chemical product, administered in patients only, in the contex of this research will be the gadolinium, routinely used in MS as contrast agent for the 3T MRI.

8.1. Gadolinium

Gadolinium is not an experimental treatment in this research. The type of product used in the research is the Dotarem®, Guerbet Lab.

Indeed, Dotarem® is used according to its indication for diagnostic purposes in the context of a brain 3T MRI to detect MS active demyelinating lesions. It will be administered following the RCP at the dose of 0,1 mmol/kg, equivalent to 0,2 mL/kg. Dotarem®'s RCP is given in Annex 19.10. Dotarem® must not be administered to patients with severe renal failure (DFG < 30mL/min/1,73m^2). The product is administered via an intravenous injection in the forearm. After administration, the subject will need to stay under observation for at least 30 minutes due to the possible adverse events related to the product. The subject will be informed of a possible retarded effect up to 7 days after administration. Dotarem® provisioning, distribution, tracking and elimination will be taken care of by the CENIR.

Gadolinium's most frequent adverse events are the following: headache, paresthesias, pain and hot or cold sensation at the injection site, nausea, vomiting, hypersensibility reactions (cutaneous rush, pruritus). Cases of systemic nephrogenic fibrosis (SNF) have been reported after the injection of contrast agents containing gadolinium in patients suffering from an acute or chronic severe renal failure (clearance of creatinine < 30 mL/min/1,73m^2).

8.2 Authorized treatments before and during the research

All treatments are authorized before and during the research, respecting the indications contained in Paragraph 8.3.

8.3. Unauthorized treatments before and during the research

In the month before each visit, the following treatments are not authorized:
cyclophosphamide, mitoxantrone or methylprednisolone, because they affect gadolinium enhancement during the MRI.
These 3 treatments, administered intravenously on a monthy base, are not interrupted, but visits are to be programmed in order not to have the MRI performed within one month after last infusion.

8.4. Treatments to be taken with precaution before and during the research

The concomitant medications to be taken into account are the following: betablockers, vasoactive substances, angiotensin converting enzyme inhibitors, angiotensin receptor antagonists. These medications reduce the efficacy of the cardiovascular compensatory mechanisms regulating blood pressure: the radiologist must be informed before gadolinium injection and must have resuscitation equipment available.

9. BIOLOGIC SAMPLE COLLECTION

4 mL of whole blood will be collected in a 5 mL SST tube to dose serum neurofilament light and heavy chains. The peripheral vein sampling will take place at the CIC.
A collection of biological samples (sera) to dose neurofilaments will be made up with the blood smples of visits V1, V3, V4.
Biological samples and all the associated data (clinical, imaging) can be shared with institutional or industrial national and international teams within and outside Europe, in particular in the case of collaborations in international consortia, in a research setting on MS or any other neurological disorders, unless the patient opposes him/herself.
In case of transfer of data or biological samples to national or international teams, within or outside Europe, the Sponsor will take the necessary administrative and reglemetary steps to allow this transfer.

9.1 Nature of samples

Blood samples drawn at visits V1, V3, V4 :

- 4 mL of whole blood collected at each visit in SST tubes without anticoagulant (maximum capacity 5 mL) to isolate the serum.

9.2 Conditions of blood sampling

At each V1, V3, V4 visit, a blood volume of 4 mL will be collected in a 5 mL SST tube to specifically dose serum neurofilament light and heavy chains. The peripheral vein sampling will take place at the CIC.

Blood samples will be taken within one our at the DNA & Cell Bank of the ICM (Institut du Cerveau et de la moelle épinière, U1127) located in the Biological Resource Platform (CRB) of Pitié-Salpêtrière Hospital.

9.3 Coding and labeling procedures

The samples collected at the CIC will be labeled with the ID code of each subject followed by the aliquot number.

Biological sample coding procedures are described in Paragraph 13 (CONFIDENTIALITY).

9.4 Sample treatment

At the CRB, blood samples will be kept at room temperature for at least 30 minutes to allow coagulation, then centrifuged to isolate and store the serum.

- serum isolation: this step will be made by centrifugation of the SST (5 ml) tubes for 10 min at 2000 rpm at a temperature of 4°C.

- sample aliquoting: the supernatant corresponding to the serum (about 1.5 mL) will be transferred into polypropylene cryotubes for immediate storage in a -80°C freezer. Aliquots willl be made up of an aliquot of 0.2 mL, 2 aliquots of 0.5 mL and one aliquot of the remaining volume.

The material at the bottom of each tube will be destroyed. The 0.2 mL aliquot will be sent over for neurofilament dosage once all samples have been collected, while the others will be stored at the CRB.

9.5 Transport

All the blood samples collected at the CIC will be brought to the CRB by a nurse of the CIC within one hour, together with the blood sampling form (Annex 19.11) contaning data that match those on the tube.

An amount of serum of 0.2 mL will be sent over to Dr Kuhle's lab for neurofilament dosing (Hebelstrasse 20, Department Biomedizin, Laboratory 302, 4031 Basel, Switzerland), in tubes labeled with the date of sampling (any subject's personal data will be put on the tube). The shipment to Switzerland will be made by a specialized service for biologic sample transport. Once all the samples have been collected, they will be shipped all together, using all the precautions that will allow to keep them frozen.
Once in Switzerlan, single sample tracking will be granted by the labeling code (date of sampling).
The samples shipped to Switzerland will be entirely used to dose neurofilaments and no further storage will be done.

9.6 Storage

Serum samples will be stored at the CRB in polypropylene cryotubes at a themperature of -80C, for a period of 10 years. The duration of storage is justified by the need to use them for a long-term follow-up of energy dysfunction, in order to identify biologic pronostic markers correlating with individual clinical course.

9.7 Storage security conditions

At the PRB (Biological Resource Platform of Pitié-Salpêtrière hospital), where the DNA & Cell Bank is located, access to the room with lab freezers is secured through a badge and is only reserved to the authorized personnel.
Lab freezers are secured as well: continuous temperature monitoring throught the software Oceasoft, with phone alert (in case of any problems) to the Bank personnel on call during night-hours and weekends. This allows a continuous surveillance with 7/7, 24h/24 intervention, if necessary. Moreover, a backup freezing system is available on site in case of technical problems and need to sample transfer. Climatization and power supply of freezers depends on the Pitié-Salpêtrière hospital and uses its emergency circuits in case of necessity.

9.8 Informatic management of data associated with the samples

See Paragraph 11 : DATA COLLECTION
Informatic management of data associated with the samples is done at the Bank by means of encrypted Excel spreasheets. A database software is being implemented to allow an integrated management of all data.

Bank informatic files are subjected to a strict security procedure, run by the ICM IT Direction (DSI): access restricted to authorized people (password, read-only or edit access), dedicated server, double saving of data on servers and on Bank computers every day. All people accessing the data have signed a confidentiality agreement.

The data associated with the samples stored at the bank in the context of the research are:

- subject-related data: diagnosis, code of subject;
- sample-related data: date and hour, quality, type of sample;
- data obtained after sample treatment to extract the serum: sample amount, treatment method, date and hour of freezing;
- follow-up and quality-control data on incoming and outgoing samples;
- storage data: sample location in freezers, amount, storage temperature;
- distribution data: amount shipped, date, recipient.

An informatic follow-up file of received samples in the context of the research is sent to the Principal Investigator on a frequency agreed by both parts.

9.9 Tracking conditions

Continuous tracking will be assured in the following ways:

- Upon arrival ath the ICM DNA & cell Bank, the correspondence between the subject code indicated on the tubes and on the blood sampling form, as well as the conformity of documents (consent form) and tubes (type, amount collected…) are checked.
- samples are then checked-in on 2 separate informatic platforms (sample treatment spreadsheet and research follow-up spreadsheet).
- Biologic samples are identified at the Bank via automatically printed labels with the subject code and the treatment date.
- All the treatments performed on samples, as well as storage and distribution are tracked in an informatic platform and/or in lab notebooks.
  Traking will be strengthened through informatic management of databases. This will be associated to sample identification via barcodes, readable by electronic scanners. This strategy will reduce the risk of error only to the step of manual check-in upon arrival of the samples at the Bank.

9.10 Storage quality system

The DNA & Cell Bank has a Quality Management System (SMQ) following the NF S96-900 norm (Qualité des CRB – Système de management d'un CRB et qualité des ressources biologiques). This system was certified by the Afnor in 2009 and is reevaluated yearly. (See valid certificate, Annex 19.12).

In the respect of the laws and rules that apply, the Bank SMQ focuses on assuring quality of collections and services, satisfying the needs of the parts involved in research projects, and of the users in particular. All what can affect the activity and management of the Bank is organized in 9 interactive, operational, management and support processes. The SMQ

allows the management of these processes through a continuous surveillance and improving. All is based on a series of validated documents (operational procedures and protocols) for the preparation, storage and distribution of biologic samples.

The specific quality documents for this protocol correspond to those available at the Bank. The Bank also fulfills all the reglementary needs and has all the authorizations needed for these activities.

10. VIGILANCE OF THE RESEARCH

10.1 Definitions

10.1.1. Adverse Event (AE)

Any harmful manifestations occurring in a person participating in a biomedical research, being it related or not to the research or the studied product.

10.1.2. Udesired effect

Undesired event occurring in a person participating in a research involving human beings, when the event is related to the resarch (e.g. to the procedures, methods or practical acts needed in the research) or to the studied product.

10.1.3. Serious Adverse Event (SAE)

Any adverse events/undesired effects is defined as "serious" if it:

- causes death;
- is life-threatening (event during which the participant is at risk of death at the moment of the event; this does not correspond to an event which could have hypothetically caused death had it been more severe);
- need of hospitalization or prolongement of hospitalization;
- produces a handicap or any severe or durable disabilities;
- iduces a congenital abnormality or malformation;
- is an undesired clinical or biological effect of grade 4;
- is an "important medical event" (event considered by the Investigator as medically important, able to threaten the subject or to produce the need of intervention, to prevent any of the abovementioned characteristics/consequences. Examples: intensive care in the hospital emergency department or at home for allergic bronchospasm, epilepsy, clotting problems);
- is an “event of special interest".

10.1.4. Unexpected severe adverse events (USAEs)

Any undesired effect whose nature, severity or evolution are not in agreement with the information related to the practical acts and methods used during the research. To define the unexpected nature of each effect, it is suggested to refer to the information described in the protocole, in particular to the practical acts and methods of the research, and to the reference document summarizing the chracteristics Dotarem®.

10.1.5. New safety fact

A new fact is defined as any new safety data potentially leading to a re-assessment of the risk/benefit ratio of the research or of the studied product, to a modification of its use in the research, in the documents of the research or to the interruption/modification of the research protocol, or of similar research protocols.

Examples: a serious adverse event that might be associated to the procedures of the reseach and alter the development of the research, any reccommendations of the surveillance committee, if they are relevant for participants' safety.

10.2 Investigator's responsibilities

10.2.1. Notification of non-serious AE

The Investigator has to note in the observation logs any biological or clinical AE, except for those occurred before the execution of the practical acts and methods of the research. If a clinical or biological AE is present upon inclusion, only its worsening has to be notified.

10.2.2. Notification of SAE to the Sponsor

10.2.2.1. What to declare?

The Investigator must notify to the Sponsor any serious adverse events except for those indicated in the protocol or in the Investigator's booklet as not requiring an immediate notification.

EXCEPTIONS: SAE not requiring an immediate notification to the Sponsor:

- Some hospitalizations;
- Day-hospital admissions; the participant in the research is admitted to a day-hospital; the participant in the research is admitted for a duration of less than 24 hours for a non-severe medical condition (see severity criteria above);
- Hospitalization for medical or surgical treatment planned before the research;
- Hospitalization for social or administrative reasons;
- Hospitalization pre-defined by the protocol;
- pre-existing pathology or pathology discovered before the first administration of the research treatment, and not worsening;
- Biological event of grade 4 discovered between the signature of the consent form and the first administration of the research treatment.

The Investigator transfers to the Sponsor all important documents related to the SAE (e.g.: copy of lab test results, report of hospital exams describing the SAE) after having anonymized them by putting the participant ID code used in the contex of the research.

The Investigator must follow-up participant having experienced a SAE until the complete resolution of the AE, its stabilization to a degree considered acceptable by the investigator, or the return to the before-event state, even if the partcipant has dropped out of the research (when the event is probably related to the research).

10.2.2.2. When to declare?

The SAE to be declared and any important documents related to the SAE must be trasferred by the investigator to the Sponsor immediatly or within 24 hours maximum starting form the moment the investigator became aware of the event.

The Investigator checks that all the informations concerning the follow-up are transferred to the Sponsor within 7 days.

All SAEs must be declared, if tey occur in a participant in the research, during the entire duration of the research, from the signature of the consent form till the end of the participant's follow-up.

SAEs occurring up to 4 weeks after the end of the participant's follow-up must be declared when they are related to the research.

10.2.2.3. How to declare?

Initial notification comes in a detailed written report, known as "Déclaration initiale d'événement indésirable grave" (found in annex of the observation logs or in the SAE section of the eCRF).
Initial notification must contain at least all the following elements: participant's ID, notifier, treatment of the research, adverse event.

Initial notification is followed-up by all the detailed supplementary information in the form "Déclaration complémentaire d'événement indésirable grave" (found in annex of the observation logs or in the SAE section of the eCRF) in order to folow-up the evolution of the case or to complete the information.

The Investigator fills in and sends the SAE declaration form dated and signed, as well as all the other anonymized documents related to the SAE (e.g. copy of lab test results, report of hospital exams) to:

ITMO Santé Publique – Pôle de Recherche Clinique (PRC)
Réglementation et Qualité et recherche clinique (RQRC)
Fax : 01 44 23 67 10
E-mail : pharmacovigilance.prc@inserm.fr

10.2.3. SAE related to a medication

Serious or unexpected AE related to Dotarem®, which is the only produc taken by participants during the 3T MRI (patients only), will be declared by the Investigator to the competent authorities following the reglementation that applies (http://social-sante.gouv.fr/grands-dossiers/signalement-sante-gouv-fr en France); he notes this declaration in the observation logs and sends a copy to the Sponsor.

10.2.4. Evaluation of AE and SAE

AE
The Investigator must detail the AE and give, if possible, the medical diagnosis. The date of occurrence of the AE must precede (or coincide with) the date of the occurrence of its worsening. In case of medical or surgical procedure (e.g.: surgery, endoscopy, tooth extraction, transfusion...), the Investigator must notify the event having led to such procedure.

Intensity
The intensity of all AE (severe and not severe) must be evaluated by the Investigator following the scale below, and reported in the observation logs.

Grade 1	Mild	mild or transient impairment, without limitation of usual daily activity; does not require medical intervention or treatment.
Grade 2	Moderate	partial limitation of usual daily activity; a medical intervention or treatment is not always needed.
Grade 3	Severe	limitation of usual daily activity; a medical intervention or treatment is needed, possible hospitalization.
Grade 4	Life-threatening	very limited activity; needs medical intervention and treatment, almost always in a hospital setting.

Severity
The Investigator evaluates the severity of any AEs (see chapter "Definition of SAE"). Death for known cause of a participant in the research must be notified as an evolution of an AE and not as an AE. If the cause of death is unknown, the death must be notified as "death of unknown cause".

Causality relationship
The Investigator must evaluate the causality relationship between the SAE and the research (with the procedure, the practical acts or methods needed in the research) and/or the associated treatments taken by the participant in the research. All the SAE, for which the Investigator or the Sponsor think that the causality relationship is likely, are to be considered as SAE.

The expected or unexpected nature
The evaluation of the expected or unexpected nature is usually performed by the Sponsor. The expected/unexpected nature of a SAE is evaluated in the light of the information described in the protocol, regarding in particular the practical acts and methods performed during the research.

Evaluation of the AE
The evaluation of the AE at the moment of its notification must be tracked in the SAE initial declaration form. Any modifications of the evolution of the event (resolution, worsening...) must be reported in a SAE supplementary declaration form.

10.2.5. Notification of pregnancy

The Investigator must notify to the Sponsor, immediatly or within 48 hours maximum starting form the moment he became aware of it, all the pregnancies occurred in the participants.

Initial notification must be made in a detailed written report, using the form "Recueil des données initiales de grossesse" (found in annex of the observation logs). This form must contain the expected delivery date, the contact of the midwife and the maternity clinic for the delivery if the pregnancy is not interrupted.

The Investigator must follow the pregnancy until its term or its interruption and must notify its issue to the Sponsor using the "Recueil de l'issue de grossesse" form (found in annex of the observation logs).

Attention:
If the issue of the pregnancy fulfills one of the severity criteria (e.g.: **congenital abnormality or malformation, fetal death, voluntary or therapeutic interrpution of pregnancy, abortion requiring hospitalization...**), the Investigator must notify it to the Sponsor following the rules of the SAEs.

The Investigator fills in and sends the "Recueil de l'issue de grossesse" form dated and signed to:

ITMO Santé Publique - Pôle de Recherche Clinique (PRC)
Réglementation et Qualité et recherche clinique (RQRC)
Fax : 01 44 23 67 10
E-mail : pharmacovigilance.prc@inserm.fr

10.2.6. Potential risks of the research and conduct to adopt in case of undesired event

The acts specifically performed in this research are:

- A 3 Tesla MRI exam without gadolinium injection for healthy volunteers, at Visit 1, Visit 3, Visit 4
- A 3 Tesla MRI exam with gadolinium injection for patients, at Visit 1, Visit 3, Visit 4
- A 7 Tesla MRI exam without gadolinium injection at Visit 2 for all participants
- a blood sampling to dose serum neurofilaments (4 mL) at Visit 1, Visit 3, Visit 4.

If all the usual precautions concerning the practical acts of the research are respected, no major risks related to the research are expected.

- MRI: there is no risk related to the MRI if the contraindications to this exam are respected:

- Pregnancy
- Pacemaker neurosensorial stimulator of ICD
- Clips on brain aneurysms or vascular malformations
- Cochlear implants
- Ocular or brain ferromagnetic objects
- Metallic prosthesis or clips
- Neurosurgical ventriuloperitoneal derivation valvs
- Tattoos or permanent makeup of eyelids or lips
- Implanted pumps (insulin, chemioterapy drugs, morphine…)

- Gadolinium (Dotarem®) administration: Contraindication is a known allergy to gadolinium or severe renal failure (DFG < 30mL/min/1,73m^2). Following the RCP of the Dotarem® (Annex 19.10), the most frequent undesired effects observed with gadolinium use are : paresthesias, headache, cold or hot sensation or pain in the site of injection, and hypersensitivity reactions (nausea, vomiting, pruritus, cutaneous rush).
Other adverse events, rarer but more severe, can also occur with Dotarem® injection. They include systemic nephrogenic fibrosis, coma, convulsions, cardiac arrest, respiratory arrest.
In case of allergic reactions to the Dotarem®, antihystaminic medications and/or steroid drugs will be administered by the medical personnel. In case of anaphylactic shock, an injection of norepinephrine will be administered by the medical personnel.
Upon participant's request, the full list of adverse events included in the summary of the characteristics of the product Dotarem® will be delivered.
In case of claustrophobia at the moment of the MRI, short-term sedative/anxiolytic medications may be proposed to the participants.

Contrast agent injection is also associated to: infusion placing in the forearm, which causes a pain similiar to that of a blood sampling. A local hematoma can occur. At the moment of the injection, the vein can be damaged under the pressure and the product can diffuse around the injection site (extravasation, very painful). In rare cases, if the product is injected in large amounts in the muscle, a skin necrosis can occur.

- Blood sampling through a venous catheter (about 4 mL): possible occurrence of a vagal reaction or of a local heamtoma.

10.3 Responsabilities of the Sponsor

10.3.1. Recording and evaluation of SAE

The Sponsor keeps detailed registers of all the SAEs notified by the Investigator.

The Sponsor evaluates the causality relationship between the SAE and the research. In the absence of information on the causality from the Investigator, the Sponsor should consult him and encourage him to express his opinion on this subject.
The causality relationship established by the Investigator must not be minimized by the Sponsor. If the judgments on the causality relationship differ between the Investigator and the Sponsor, both are mentioned in the declaration of the case to the competent national authority and to the specific ethics committee.
All the SAEs, for which the the Investigator and the Sponsor believe that the causality relationship is likely, are considered as SAEs.
The evaluation of the expected or unexpected nature of the SAE is usually performed by the Sponsor, in the light of the information described in the protocol, concerning in particular the practical acts and methods of the research. If information on the expected/unexpected nature of the event have been previded by the Investigator in the notification form, they must be taken into account by the Sponsor.

10.3.2. Declaration of safety data to the competent national authorities and to the ethics committee

10.3.2.1. Declaration of *unexpected severe adverse events (USAEs)*

All the USAEs will be declared by the Inserm-ANRS Vigilance service to the ANSM with the following delays:
- no delay, starting from the day the Sponsor becomes aware of them, for the USAEs causing death or threatening participants'life
- within 15 days, starting from the day the Sponsor becomes aware of them, for all other USAEs.

If the Sponsor receives new supplementary information regarding a USAE already declared, this new information will be declared within 8 days after reception.

10.3.2.2. Declaration of new safety facts

If a new fact regarding the research can potentially harm participants' safety, the Sponsor and the Investigator adopt urgent safety measures to protect participants against an immediate danger.
The Sponsor informs with no delay the competent national authority on the new facts and on the measures taken.

The Vigilance service writes a report that is addressed within 15 days to the competent national authority and the specific ethics committee.

If the new fact leads to a substantial modification of the research, the Sponsor will send, within 15 days following the adoption of urgent measures, a request of authorization of substantial modifications to the competent national authorities, and a request of notice to the specific ethics committee.

10.3.2.3. Submission of the annual safety report

The Sponsor must make, once a year for the entire duration of the research, a safety report. This report will be written by the Inserm-ANRS Vigilance service, with the assisitance of the research project chief, and with the Investigator's validation. This includes:
- the list of all suspected SAEs and USAEs occurred in the research during the period concerned;
- a recap table of all the SAEs;
- the list of deaths;
- a short and critical analysis of participants' safety.

The annual safety report is transferred by the Sponsor to the competent national authorities and the specific ethics committee within 60 days following the anniversary of the first authorization of the research.

If no new safety information has been added in the research during the year period concerned, the Sponsor sends to the ANSM a letter to inform that, in the absence of new safety data, the Sponsor is not transferring the annual safety report for the current year.

11. COLLECTION AND PROCESSING OF DATA

Personal data of the research participants will be processed with the unique purpose of carrying out the research. This processing includes the management of participants' data, the collection, quality check and statistical analysis of data collected during the research.

11.1 Description of collected data

The software used for the development of the electronic case report form (CRF) is Red Cap. This is a free application for clinical data collection, which allows data tracking and personalized secure access. The observation logs is in Annex 19.13.

The electronic case report form (eCRF) will include the data described below, according to the calendar of the visits:

- demographic and clinical data: age (entire number), gender, date of first symptomes, date of diagnosis, mode of onset, progressive for of MS, total number of relapses, date of last relapse, date of EDSS 3: this information will be used as confounding factors in statistical analyses ad will allow the verification of the absence of exclusion criteria
- personal track history of patients and healthy volunteers, to verify the absence of exclusion criteria
- history of all MS disease-modifying treatments/concomitant medical treatments of patients and healthy volunteers: this information will be taken into account in the statistical analyses as possible confounding factors influencing the response to main and secondary evaluation criteria. Indeed, some treatments (e.g. Gilenya) have

shown neuroprotective effects, influencing the evolution of neurodegeneration (in particular, changes in cortical thickness).

- verification of inclusion and exclusion criteria
- neurological evaluation: EDSS and functional scores, motor assessment, in order to test for correlations beween MRI results and motor disability
- education level, to standardize the results of neuropsychological tests
- Results of neuropsychological tests and fatigue assessment (BICAMS: SDMT, CVLT-II, BVMT-R / MFIS Jamar hydraulic hand dynamometer), in order to test for correlations beween MRI results, cognitive impairment and fatigue
- date of brain MRI exams, notification of gadolinium injection: MRI data (3T and 7T MRI) will allow sodium, ATP and PCr quantification, as well as brain volumetric, structural and functional analysis. Gadolinium injection will identify the presence of active inflammatory lesions.
- biological sampling: extracted data will allow to dose serum neurofilaments as markers of neurodegeneration.
- AE/SAE collection.
  In case of participant's premature drop out, a page of end of the study will be added, with mention of the reason for study exit.

11.2 Source data

The source data are the information featuring in the original documents, or in authenticated copies of these documents, which pertain to clinical exams, observations or other activities carried out for research involving human beings and which are required to assemble and evaluate the research. The documents in which the source data are saved are called source documents, irrespective of the media (paper, digital…).

The data sources in this research project are:

- The medical file of the patient or their copy as well as the research file, created specifically for the research project for healthy volunteers
- Neuropsychological tests
- Imaging data
- Checklists for MRI contra-indications
- Blood test results

Medical data concerning the past disease history, treatments and neurological or fatigue assessments will be entered directly into the eCRF, and will therefore constitute source data.

11.3 Data circuit

The data resulting from medical files and neurological exams will be entered into the case report form as and when obtained by the Principal Investigator (B. Bodini) or by collaborating neurologists (B. Stankoff, V. Ricigliano). During input, the data is identified by an identification code, as defined in paragraph 13.1.

Medical data particularly concerning the past disease history, treatments and neurological or fatigue assessments will be entered directly into the eCRF, as and when they are obtained, and will therefore constitute source data.

The data resulting from neuropsychological evaluations (scores obtained from tests, questionnaires, scales) will be collected on paper for handover by neuropsychologists before being entered into the eCRF as and when by the ARC of the Neuroscience CIC.

MRI acts (3T by the ICM team / 7T by the NeuroSpin team) and biological samples (i.e. taken/not taken) will be noted in the observation logs as and when obtained by the Investigator and/or the ARC.

Raw imaging data from CENIR will be saved anonymously on the Lustre-ICM archiving server (acquired by CENIR personnel and automatically transferred to the archiving server). The transfer to this backup server requires a single login and password (restricted access, attributed only to CENIR technicians). Only the Principal Investigator, the scientific responsibles and the system administrators may consult this server.

Prof. Stankoff's ICM team (the investigator or the person in charge of analyses), along with Dr. Francesca Branzoli, will extract parameters (continuous values), resulting from MRI data saved on the server, and enter them in the format of an Excel spreadsheet named « images 3T » (or bi3). The image database, not holding any directly-identifying information, will be protected by a password, known only to the Principal Investigator.

Raw imaging data from NeuroSpin will be saved anonymously on the internal archiving server at 7 T (acquisition by the personnel of NeuroSpin and transferred automatically to the archiving server). The transfer to the backup server requires a single login and password (restricted access, attributed only to NeuroSpin technicians). Only Dr. Boumezbeur and the system administrator may consult this server.
Dr. Boumezbeur's team (the person in charge of the analyses) will extract the parameters (continuous values), resulting from MRI data saved on the server, and enter them in the format of an Excel spreadsheet named « images 7T » (or bi7). The image database, not holding any directly-identifying information, will be protected by a password, known only to Dr. Boumezbeur and the Principal Investigator.

The team lead by Dr. Inglese will provide methodological support necessary for the implementation of imaging sequences and the quantification of 23Na at 7T (for example, technical support for the creation of codes, calibration and the realisation of the 23Na imaging), yet will not have access to the data to identify the participants. No subject's clinical, biological or imaging data is intended to be transferred to the USA in the research project.
MRI 7T data from NeuroSpin (bi7) which will have been anonymised will be transferred as and when on a secured external hard drive, for which the access will be limited by a password known only those working on the project.
The transfer of this data to Prof. Stankoff's ICM team will allow for multi-modal statistical analysis.

The file containing dosage results of neurofilaments by Dr. Kuhle will be transferred to Prof. Stankoff's ICM team, once all of the samples have been analysed, as an encrypted email attachment. A password will be required to read the file (known only to those working on the project).
The samples being uniquely labelled with the date when they were taken, no identifying data will be shared.

At the end of the research, in preparation for statistical analysis, the Principal Investigator will be in charge of creating a « complete » Excel database by integrating the clinical, biological,

3T and 7T (bi3 and bi7) data. The participants will be identified within this new database by their identification code.
This base will be protected by a password known only to the Investigator and stored on the secured ICM servers, in collaboration with the Iconics platform.
This platform is a data depository which will be in charge of the creation of databases, processing, storage and delivery of the data.

The management of data will be under the responsibility of the CIC and the data processing under the responsibility of the ICM, B. Bodini and B. Stankoff's team, in collaboration with the Iconics platform and the Imperial College of London (Dr. Palladino) for statistical analysis.
The anonymised Excel database will be transferred as an encrypted email attachment. A password will be required to read the file (known only to those working on the project). The data will be transferred respecting the regulation in vigour.
Dr. Palladino will analyse the data collected using several programmes (for example, Stata 14).

Conservation of data

Flat files (text or binary) containing raw data, transformed and analysed data will be stored on the computer servers managed respectively by the ICM and NeuroSpin IT departments.

High capacity NAS will be used, integrated into a secure infrastructure (power supply, regular backup, local and remote replication). The data within the database will be stored on a high-performance SAN, benefitting from the same security norms.

In compliance with regulation, the data will be kept in the research team's IT systems for 20 years. The duration of conservation is justified by the possibility of very-long-term longitudinal follow-up of the energetic dysfunction in order to extract prognostic markers from imaging in correlation with the individual's clinical evolution.
They will then be electronically archived for a duration compliant with the regulation in vigour.

11.4 Conservation of research documents

Documents relating to the research are archived in compliance to the regulation in vigour.

All research documents are archived in a secure room accessible only to CIC personnel within the Neuroscience CIC at La Pitié-Salpêtrière.

- The protocol
- Information and consent forms
- The list of correspondence codes of participants
- MRI security questionnaire
- Evaluation scales

The data resulting from imaging will be saved encoded on ICM servers and/or external hard drives.
No removal or destruction of such data can be conducted without prior agreement of the sponsor. At regulatory term of archiving, the sponsor will be consulted for its destruction.

All data, documents and reports are subject to audit or inspection.

12. STATISTICAL ANALYSIS OF DATA

12.1 Statistical analysis responsibility

Statistical analysis will be conducted by Dr R. Palladino, PhD, Department of Primary Care and Public Health, School of Public Health, Imperial College of London, London, UK, in collaboration with the ICM Team « mécanismes de la myélinisation et de la remyélinisation dans le SNC » (Mechanisms of Myelinisation and Remyelinisation in the CNS).

12.2 Calculation of sample size

No antecedent research has evaluated the association between energetic parameters and cortical thickness of the MSR.
By using a multivariable linear regression model, a sample size of 55 subjects (20 RR-MS, 20 PMS and 15 healthy volunteers) would be sufficient to detect an increase of 10% of the adjusted square of the linear correlation coefficient (R^2) when a combination of energetic parameters (total intra and extracellular sodium, ATP, tCr concentration, tCr diffusion) are included in the model. This calculation was based on the following hypotheses: a p-value equal to 0.05, a strength of 80% and an initial R^2 equal to 0.3 in the regression model partially adjusted for the covariates of the research (age, gender and disease form).

In order to account for drop-outs, we anticipate including up to 5 extra subjects per group (I and II), for a total of 10 participants.

12.3 Description of the statistical analysis plan

12.3.1. Analysis of the main criterion

Statistical analysists will be carried out in Stata 13 (Stata Corportation, College Station, Texas, United States of America). The values where $P<0.05$ will be considered as statistically significant.

A multivariable linear regression model will be used to explore the relationship between energetic parameters measured in the MSR at inclusion (total intra and extracellular sodium concentration, ATP, PCr, NAA concentrations, PCr and NAA diffusivity) – each considered separately or assembled – and the cortical volume of the MSR at 24 months as well as the changes in cortical volume of the MSR between the beginning of the research and 24 months.

12.3.2. Analysis of secondary criteria

Multiple regression models are used to study the relation between the extracted energetic parameters at the beginning of the research and:
- MTR-derived demyelination/remyelination
- total cortical volume
- NODDI-derived metrics
- Functional MRI-derived connectivity
- Serum neurofilaments
- Clinical metrics (EDSS, MSSS, MFIS) and neuropsychological test results.

Age, sex and disease duration are included as confounding factors at each step of the analysis.

13. CONFIDENTIALITY

13.1 Respect of individuals' confidentiality

Only the health professional who leads the research project in one of the centres can retain the link between the coded identity of those who consent to the research and their forenames and surnames.

The confidentiality of the participants will be respected by attributing an identification code, defined as follows :

- Research acronym (ENERGYSEP)
- C/P according to the inclusion group (respectively [Healthy] Control and Patient)
- Subject number
- Initials of the surname and forename of the participant

13.2 Respect of confidentiality vis-à-vis

Information concerning subjects will remain confidential.
Subjects' consent will remain under guarded protection.

Access to clinical data and sources will be given directly in the case of monitoring, INSERM sponsor audits and inspections lead by administrative competent authorities.

14. COMMUNICATION

14.1 Publication of the results

All of the data collected during this research are the property of the research Sponsor and cannot under any circumstances be communicated to a third party without written permission from the Sponsor.

The results will be published after final analysis under the form of scientific articles in peer-reviewed journals, and will be presented during national and international conferences. Any publication or communication (oral or written) is subject to mutual agreement between the Principal Investigator, the scientific responsibles, the Sponsor and will respect international recommendations: "Uniform Requirements for Manuscripts Submitted to Biomedical Journals".

Any publication must follow the present rules in the publication charter defined by AVIESAN. The source of funding, authorisations of competent authorities, consent of participants must be included in the acknowledgements according to the suggested model below:

/Ethics statement /*/This study is part of clinical trial ****C19-20 sponsored by Inserm. It was granted approval by local Ethics Committee or "Comité de Protection des Personnes" on ---****DATE**---, authorized by the French authorities (****ANSM** ****NB**), and registered in a public trials registry (****CT XXXX**).*

All study participants gave their informed, written consent to participation, in line with French legal guidelines.

14.2 Final report

Once the research project has ended, a final report of the results will be constructed within one year following the end of the research.

The final research report is a written document, sufficiently detailed for full comprehensions of the research proceedings and to result in an objective judgement on the quality of the research data. It is written by the Principal Investigator in collaboration with the biostatistical of this research and submitted to all investigators for their approval. Once consensus is obtained, the final version is endorsed by signature of each of the Investigators/scientific responsibles and made available to Inserm. It will only be addressed to ANSM if requested.

This report includes a summary of the results written according to the competent authority's reference guide. Inserm will validate and transmit the summary to the competent authority and to the Ethics Committee according to the terms established per type of research. The document must be transmitted within one year following the end of the research project.

14.3. Access to subjects' overall results data

Following this research project, the participants have the right to be informed of the overall results of this research project, by the Principal Investigator or by his designated representative who will have collected their agreements.

The articles and abstracts resulting from this research project will be communicated to the participants should they wish.

14.4. Access to subjects' health data during and after the research project

The subjects may be informed at any time of health data that concerns them, upon simple request.

Any significative clinical anomaly detected in the results of the exam or analysis will be communicated to the subject and to the doctor chosen prior by the participant.

The subject participating in research is informed of their right to the information in the possession of the Investigator (or if necessary, the physician or a qualified representative) concerning their health, during or after the research project. Exceptionally, in the best interests of an unhealthy participant, if diagnosis of their illness does not permit divulgation, the Investigator may, in respect of their trust, withhold certain information related to this diagnosis.

No information of medical character will be conveyed to a participant by any research personnel who is not a qualified doctor.

14.5. Press communication

The articles and abstracts but also the oral communications resulting from this research project will be addressed to the Pôle Recherche Clinique (Clinical Research Centre) and to the Département de l'information scientifique et de la communication (DISC) (Communication and Scientific Information Department) prior to publication.

15. PROTECTION OF SUBJECTS

15.1. Ethical justification of the protocol

There is no direct individual benefit for participating in this research project, although the fulfillment of the research project will allow a better understanding of the mechanisms of energy dysfunction in primary progressive MS.

There are no foreseen risks related to the research project if all contraindications for the MRI exams are respected. The risks linked to the research project are indicated in article 10.2.6.

15.2 Adequacy of the place of research

In compliancy with the article L1121-13 of the Code de la santé publique (French public health code), the research imaging centre (CENIR) of the ICM and the CEA, where the healthy volunteers and patients will be included in the study and followed, disposes of human resources, material and techniques suitable for the research project and compatible with the obligations of security for those who consent to the research.
The implemented security measures will be respected through the research project.

15.3 Ethical and reglementary provisions

The research project will be carried out in the respect of the French law in vigour, notably the provisions relating to research projects including human beings stated in articles L 1121-1 and following the Code de la Santé Publique (French national public health code), the WMI Declaration of Helsinki, as well as Good Clinical Practice and the present protocol.

The Investigator commits to carry out the research project in compliance with these ethical and reglementary provisions. The Investigator is aware that all documents as well as all data relating to the research project may be audited and inspections carried out in respect of professional secrecy and without being subjected to opposition by medical privacy. The Investigator recognises that the results of the research project are the property of Inserm, Sponsor of the research project.

15.4 Comité de Protection des Personnes, CPP (Ethics Committee)

Before carrying out the research project, the Sponsor will submit the project for approval by a CPP designated at random under the conditions stated in the article L. 1123-14 of the Code de la Santé Publique (French civil public health code) and the Sponsor will provide all necessary information to the committee.
The research project can only commence once Inserm has been informed of the approval without reserve delivered by the CPP regarding the protocol submitted and subject to approbation of any other authorisation necessary for the project. The approval will carry the title and the protocol number attributed by the Sponsor, the documents assessed, as well as the date of assessment and the list of members of the CPP having participated.

The Sponsor will inform the CPP of any ulterior amendments.

15.5 ANSM (French National Drug Security Agency)

No research project listed under the first article of the Code de la Santé Publique (French public health code) can be carried out without authorisation of the competant authority. Research may only commence upon authorisation by the ANSM for Inserm to do so without reserve, with regards to the protocol submitted and subject to approbation of the Ethics Committee and any other necessary authorisation for the project

15.6 Cnil (French national IT and freedom commission)

This research project is lead with compliance to the reference methodology MR 001 certified by the French national IT and freedom commission (Cnil) on the 3rd of May 2018 and to which Inserm commits itself to compliancy (certificate of receipt n° 2211062 v 0 of the 15th January 2019).

15.7. Insurance

Inserm, as sponsor, is contracted to public liability insurance under number XXX, in compliance with French legal and reglementary provisions on 1° category research excluding health products.

The certificate of insurance pertaining to the present protocol is represented in Annex 19.3.

15.8 VRB file

Participation of healthy volunteers under the present protocol is registered on the national file of those consenting to research involving human beings. Enrolled patients are not excluded from participating in another clinical trial during the research.

Healthy volunteers participating in this research will receive a compensation for the sum of 400 euros.
This compensation will be transferred in proportion to the subject's participation: 100 euros for the inclusion and 3T MRI (ICM CENIR, visit 1), 100 euros for the 7T MIR (Saclay, visit 2), 100 euros for the 3T MRI (ICM CENIR, visit 3) and 100 euros for the 3T MRI (ICM CENIR, visit 4). No compensation is intended for patients.

Travel expenses will be covered for patients and healthy volunteers for visits 1, 3, 4 (to the CIC) and for visit 2 (to Saclay) upon presentation of proof of purchase. Transport can also be organised by the research team (CIC), upon request.

Meals at each visit will also be covered.

Accommodation fees will not be covered.

15.9 Terms and conditions for gathering consent

Written informed consent from each person who agrees to participate in the research project must be obtained by the Investigator, who is registered with the French Medical Association and declared to the Sponsor as an Investigator, before any acts be carried out in the research project, and any act must be compliant to regulation.

Information will be given both orally and written in the first part of the information and consent form (Annex 19.2). The information will be written in a clear and perfectly understandable language for the person, in compliance with the Code de la Santé Publique, article L 1122-1.

The consent of the person to participate in the research project is written in the second part of the information and consent form. This second part must be written in a clear and perfectly understandable language for the person who consents to the research project. It must contain all of the elements to which the person is consenting. The consent is given by the signature, the surname, forename and the date, handwritten in person by the person consenting to the research.

In addition, the Investigator who gathers the consent agreements will date and sign the box on the form reserved for them and ensures 1) that the form is exact and that no note or date is missing, 2) that one original copy of the document is returned to the patient. The Investigator keeps a second original copy in a safe place with controlled access.

The Investigator must ensure that the person who consents to the research project will have had the time to make their decision freely and will have read and understood the information and consent form.

The information and consent form is a document which will have been approved by the Ethics Committee prior to the implementation of the research, during the protocol examination.

In this research, there will be an information and consent form for the patients and an information and consent form for the healthy volunteers.

16. QUALITY ASSURANCE

16.1 Description

The guidelines of the research will be set according to Inserm standard operational procedures.
The research within the investigatory centres and the management of participants will be carried out according to protocol, the Helsinki declaration and Good Clinical Practice.

The Investigator is above all responsible for the quality of the research proceedings.
The role of quality assurance is to guarantee the safety of those who consent to research involving human beings and to ensure the credibility of the data issued from such research and their recognition by the medical and scientific community.

16.2 Monitoring (research quality control)

The representatives of the Sponsor will carry out visits in the investigatory centre according to the frequency of inclusions and to the level of risk that has been attributed to this protocol.
Inspections will be carried out by the sponsor CRA according to the procedures in vigour.
At the end of the study, a final visit will be carried out.
At the end of each visit, a report will be written by the CRA.

17. SUBSTANTIAL MODIFICATIONS OF THE PROTOCOL

Procedure of the Sponsor in relation to substantial modifications

Before any request, contact the Sponsor project leader to prepare for the amendment.
It is preferred that the amendment is approved by the steering committee or another research committee before submission to Inserm. It is also recommended to obtain assessment from the statistician where necessary.

Any research modification request pertaining to the research project as initially authorised must be submitted by the Principal Investigator to the Sponsor for their consideration.

The terms and conditions for submission are available on the Inserm intranet.

Following agreement, Inserm will implement the reglementary administrative procedures necessary for approbation of such substantial modifications by the CPP and/or the competent administrative authority.

18. REFERENCES

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19. ANNEXES

Synopsis du protocole
Formulaires d'information et consentement
Assurance
Avis du CPP
Autorisation ANSM
Liste des lieux de recherche
Récépissé de déclaration de conformité à la MR
Questionnaire de sécurité du CENIR
Questionnaire de sécurité du CEA
RCP du Dotarem®
Fiche de prélèvement
Certificat de qualité du CRB
Approvisionnement et traçabilité du Dotarem®