



# Efficacy of interferon beta-1b as a first-line treatment in patients with Radiologically Isolated Syndrome

A CONTROLLED, RANDOMIZED, MULTICENTER, DOUBLE-BLIND CLINICAL TRIAL

## FINAL DEGREE PROJECT

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## **ABBREVIATIONS LIST**

**BBB:** Blood Brain Barrier

**CDMS:** Clinical Definite Multiple Sclerosis

**CIS:** Clinically Isolated Syndrome

**CNS:** Central Nervous System

**CSF:** Cerebrospinal Fluid

**DIS:** Dissemination in space

**DIT:** Dissemination in time

**DMT:** Disease-Modifying Therapy

**EBV:** Epstein-Barr Virus

**EDSS:** Extended Disability Status Scale

**FDA:** Food and Drug Administration

**FS:** Functional Systems

**Gd:** Gadolinium

**HLA:** Human Leukocyte Antigen

**HR:** Hazard Ratio

**IFN  $\beta$ -1b:** Interferon beta-1b

**IgG:** Immunoglobulin G

**MHC:** Major Histocompatibility Complex

**MRI:** Magnetic Resonance Imaging

**MS:** Multiple Sclerosis

**MOG:** Myelin Oligodendrocyte Glycoprotein

**PPMS:** Primary Progressive Multiple Sclerosis

**QoL:** Quality of Life

**RIS:** Radiologically Isolated Syndrome

**RISC:** Radiologically Isolated Syndrome Consortium

**RPMS:** Relapsing Progressive Multiple Sclerosis

**RR:** Relative Risk

**RRMS:** Relapsing Remitting Multiple Sclerosis

**SPMS:** Secondary Progressive Multiple Sclerosis

**TCR:** T-Cell Receptor

## **ABSTRACT**

**TITLE:** Efficacy of interferon beta-1b as a first-line treatment in patients with Radiologically Isolated Syndrome.

**BACKGROUND:** Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS), and it's the most common demyelinating disease of young adults. The onset of MS ranges from 10 to 59 years, but the majority of cases occur between the ages of 20 and 40 years. Radiologically Isolated Syndrome (RIS) is defined as incidental Magnetic Resonance Imaging (MRI) identified white matter anomalies within the central nervous system (CNS) suggestive of MS in healthy people without typical signs and symptoms associated with CNS demyelination and with normal neurological findings, after exclusion of other possible aetiologies. Although some individuals did not exhibit progression over a lengthy follow-up period, most patients will progress clinically or radiologically in the initial years of the follow-up (83.3%), so all patients with RIS should be considered as having a high risk of developing MS. Thus, for many patients, RIS represents the earliest visible manifestation of demyelinating disease, or a preclinical stage of MS. Among the different treatments approved for the treatment of relapsing forms of MS, the one with the best efficacy is interferon beta-1b (IFN  $\beta$ -1b), because it reduces relapse rates, decreases disability progression and also reduces clinical activity in the MRI. That's why this drug would be the best option for treatment of RIS patients, specifically the Betaseron presentation, which is the one that has more clinical experience. A statistical significant and clinically relevant result in this study would change the management of these patients, thus avoiding many new cases of MS and reducing economic impact of this disease in our society.

**AIMS:** To determine the efficacy of IFN  $\beta$ -1b on reducing the proportion of RIS patients experiencing a first clinical event of MS.

**DESIGN:** A controlled, randomized, multicentre, double-blind and placebo-controlled clinical trial conducted between September 2015 and April 2019.

**METHODS:** The clinical trial will enroll 522 patients with RIS. Patients will be randomized to receive 1 ml of subcutaneous IFN  $\beta$ -1b at doses of 0.25 mg or 1 ml of placebo every other day for 18 months. During the treatment period and 6 months more (24 months), clinical assessments, laboratory analysis as well as MRI will be performed. The main outcome is the proportion of patients with RIS experiencing a first clinical event of MS in each group, identified by anamnesis and neurological exploration based in functional systems, and secondary outcomes include radiological progression (defined by the prevention of new MRI lesions or reducing or avoiding the enlargement of pre-existing lesions on MRI), disability progression (measured by the EDSS) and description of adverse events.

**KEYWORDS:** Radiologically Isolated Syndrome; Multiple Sclerosis; Interferon beta-1b.

# 1. INTRODUCTION

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## 1.1 BACKGROUND

### 1.1.1 MULTIPLE SCLEROSIS

#### 1.1.1.1 EPIDEMIOLOGY

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of the central nervous system (CNS), and it's the most common demyelinating disease of young adults (1–7). This disease is the leading cause of non-traumatic neurological disability in young adults in North America and Europe (1,2).

It is estimated that more than two million people worldwide is affected by MS (1,2,7). However, its incidence rates and prevalence vary considerably between regions and populations, due to differential genetic predispositions and different environmental risk factors that modulate the risk of MS at the population level (1). In the high risk areas the incidence ranges from 0.1 to 0.2%, and areas of low prevalence have incidence rates much lower than 0.1% (8). Individuals from regions above 40° latitude within the western hemisphere generally have a higher risk of MS than other regions (8). The regions where the prevalence of MS is higher are northern Europe and continental north America and Australasia, and those with low prevalence are Orient, Arabian peninsula, Africa, continental South America or India (9). Europe is considered a high prevalence region for MS (defined by Kurtzke as a prevalence  $\geq 30/100,000$ ), containing more than half of the global population of people diagnosed with MS (1).

There is a north-to-south gradient in the occurrence of this disease in the northern hemisphere and a south-to-north gradient in the southern hemisphere (3,4), with MS being much less common in people living near the equator (6), but this latitude gradient is disappearing due to an increase in incidence in southern regions of risk areas (8). The geographic variation of MS can be partially explained by differences in genetic predisposition, but also by the amount of sunlight, which is also a potential risk factor (8).

It is observed that the incidence of MS in different parts of the world has increased over time, especially in Europe and the Mediterranean Basin (6), probably as a result of an increase in the incidence of MS among women, but also because an improved diagnosis due to the increased availability of magnetic resonance imaging (MRI), increased awareness and better symptomatic treatments leading to improved life expectancy (1,6,9,10).

The onset of MS ranges from 10 to 59 years (3), but the majority of cases occur between the ages of 20 and 40 years (only in rare cases the onset is before age 10 or after age of 59 years) (1,3,4,8). The peak incidence of MS is around age 24 and returns to baseline by age 60 (8). The average age of onset is 32.6 years in Spain (11). Women are more commonly affected than men, in a ratio of approximately 1,5-2:1, but it can increase to 3:1 for MS with childhood onset (1,3,4,8,9). It can be explained by the phenomenon that women, especially during childbearing years, are more likely to have autoimmune diseases (8). The incidence also varies in different ethnic populations and by geographical location (4). The male: female ratio among patients with MS ranges from 2.4:1 in Spain (11).

#### *1.1.1.2 ETIOLOGY*

MS is an etiologically complex disease, in which genetic predisposition, environmental events and other factors, like infectious agents, are involved in its development (4,8,10,12). It likely involve multiple genes, so it presents a polygenic inheritance (6,8). Other characteristics include heterogeneity, incomplete penetrance and temporal changes (8). The primary cause of MS is yet unknown (7).

Genetic susceptibility is overwhelmingly the most important determinant of MS pathogenesis (10,13). Family members of MS patients have a higher risk of developing the disease than individuals without a family history (4). If a family member is affected by MS, the risk for his/her cousins to develop the disease increases in proportion to the shared genetic information between themselves and the affected person (11). The familial recurrence rate is around 15% (9). First degree relatives and especially daughters of affected mothers carry the highest risk (4). It is known that this mainly results from coinheritance of susceptibility genes rather than shared exposure to a common environmental trigger (9), so the familial aggregation of MS is genetic (3).

Susceptibility to MS is associated with certain Major Histocompatibility Complex (MHC) genes or haplotypes, T-cell Receptor (TCR) chain genes and other genetic loci, which exert moderate but the most significant effects in susceptibility, and there is no single major susceptibility gene for MS (4). Susceptibility to MS is associated with particular Human Leukocyte Antigen (HLA) haplotypes within defined populations (4), and is determined by several genes acting independently or epistatically. It is demonstrated an association between the class II MHC alleles DR2, DR15 and DQ6 and their corresponding genotypes (4,8). Over 99% of individuals seem genetically incapable of developing MS, regardless of what environmental exposures they

experience (10). Nevertheless, the contribution of specific genes to MS susceptibility seems only modest (10). Moreover, because genetic susceptibility seems so similar throughout North America and Europe, environmental differences principally determine the regional variations in disease characteristics (10).

Among the environmental factors, vitamin D deficiency and Epstein-Barr virus (EBV) infection were the only ones for which causal links with MS were confirmed (10,11). Vitamin D deficiency (at least to some degree) is anticipated in the large majority of individuals living in low-sun exposure regions, and EBV is an extremely prevalent pathogen in human populations (10).

There is increasing evidence that infection with the EBV plays a role in the development of MS (7). EBV is a ubiquitous human herpesvirus that has the unique ability to infect, activate and latently persist in B lymphocytes for the lifetime of the infected individual (7). There is higher frequency of EBV and higher serum anti-EBV antibody titers in MS patients compared with controls, and studies have shown that MS patients are almost universally seropositive for EBV (7). The risk of developing MS is extremely low among individuals not infected with EBV but increases sharply in them following EBV infection, with an estimated mean interval of 5.6 years between primary EBV infection and onset of MS (7). However, infection with EBV is not sufficient to cause MS, and also it is not essential (7,10). A clinical history of infectious symptomatic mononucleosis before the age of 18 increases the risk of MS, with a RR of 2.3 (7,9,10).

Prevalence of MS increases with increasing latitude, and sunlight exposure decreases with increasing latitude, so it seems that sunlight protects against the development of MS (7). Considering that the ultraviolet component of sunlight is essential for the synthesis of vitamin D in the skin, it was proposed that vitamin D protects against MS (7). Epidemiological studies showed that higher vitamin D intake and higher serum levels of 25-hydroxyvitamin D are associated with a reduced risk of developing MS (7).

There is no definitive precipitating factor for either MS onset or the worsening of symptoms (3). Proposed precipitating factors include diet, heavy metals, trauma, and pregnancy (the last one with a threefold higher risk in the three to six months after term than during pregnancy) (3,9). New episodes of demyelination are more likely to occur following viral exposure, particularly upper respiratory (adenovirus) and gastrointestinal infections (4,9,12). Clinical exacerbations of MS are three times more likely to occur at the time of acute systemic infection with a wide variety of viruses and bacteria (7,12).

### 1.1.1.3 PATHOGENESIS

MS is a neurodegenerative autoimmune disease that affects the CNS, mainly the white matter tissue, producing plaques or lesions in the brain and spinal cord (8). The white matter tissue contains nerve fibres, which transmit electrical signals throughout the nervous system (8).

The primary cause of damage of the CNS is inflammation (12). The pathogenesis of MS occurs in two distinct phases, an initial inflammatory autoimmune phase with a relapsing-remitting disease course followed by a progressive neurodegenerative phase in which axonal loss and permanent neurological disability occur (5). Another hypothesis is that the different forms of MS represent different types of pathology with relapsing-remitting disease classified as an inflammatory demyelinating disease and primary-progressive disease as a neurodegenerative demyelinating disease (5). The different disease courses observed are explained by the differences in whether pathogenesis is initiated by axonal loss or demyelination and in the ratios of inflammation, demyelination, remyelination and neurodegeneration in individual patients (5).

MS is primarily a T cell-mediated autoimmune disorder, that involves a dysregulation of the immune system (4,8,14). Autoimmune responses against myelin proteins, which are degraded, are responsible for the pathology in the CNS (4,8). The primary target autoantigen in MS has yet to be definitively identified, but other minor myelin components, such as Myelin Oligodendrocyte Glycoprotein (MOG), may play a primary role in disease initiation (4). The myelin sheath insulates axons allowing for quicker nerve impulses between cells (8). Thus, the demyelinating process leads to the retardation or complete blockage of signal pathways in the CNS (8).

Inflammation and demyelination in MS are the result of autoreactive cell-mediated and humoral responses to myelin proteins, caused by a failure of self-tolerance, or by molecular mimicry, whereby activated T cells specific for microbes traverse the Blood Brain Barrier (BBB) and cross-react with myelin proteins due to structural similarities, breaking the self-tolerance (4). This autoimmunity to myelin proteins in humans is probably triggered by microbes (viruses and bacteria) which have structural homologies with myelin antigens, which is called molecular mimicry (4,5). It occurs an infiltration of immune cells into the CNS, localized myelin destruction and loss of oligodendrocytes, which nourish the nerve cells (8). Consequently, scar tissue, called sclerosis, is formed in various areas in the CNS, which gives the attribute of "multiple" (8).

The pathological hallmarks of MS are lesions of both white and grey matter in the central nervous system (11). It was considered that myelin-specific activated CD4+ T lymphocytes migrate from blood to brain tissue, crossing the BBB, bind to antigenic peptides presented by

antigen presenting cells in the brain, clonally expand and secrete pro-inflammatory cytokines which stimulate microglia, macrophages and astrocytes and recruit B cells, ultimately resulting in attack to oligodendrocytes, destroying myelin, and axons (5,11). MS lesions include breakdown of the BBB, multifocal inflammation, demyelination, oligodendrocyte loss, reactive gliosis and axonal degeneration (2). Neurons die due to loss of myelin protection, direct toxic action of immune cells, diminution of trophic support, metabolic changes and altered signalling (11).

For the MS diagnosis, it's necessary the identification of multiple foci of demyelination in the CNS of patients clinically diagnosed with MS (2). These lesions can be found anywhere in the CNS, although optic nerve, periventricular areas, spinal cord, and subpial gray matter are especially prone to demyelination (2). Patients have white matter lesions detectable by MRI (4). More than 90% of patients have oligoclonal immunoglobulin G (IgG) bands in their cerebrospinal fluid (CSF), with some specificity for myelin proteins (4).

Loss of axons is generally accepted as the main determinant of unremitting or permanent clinical disability (5,14). Inflammatory-mediated white matter demyelination is an underlying cause of axonal loss during early stages of MS (2). Chronic demyelination during progressive MS may lead to loss of axons (2). Axonal loss occurs early in the course of the disease in MS, but because of compensatory mechanisms within the CNS, it remains clinically silent until a threshold level of axonal loss is achieved and the compensatory resources exhausted (5). Cortical demyelination also plays an important role during the progressive stages of MS (2). Increased cortical atrophy has been shown to be associated with increased disability progression (2).

#### *1.1.1.4 CLINICAL COURSE*

MS is a clinically complex disease, with diverse clinical expressions and variable clinical courses among affected individuals, and also variable rates of disability accumulation (3,4,8,15). The heterogeneity in severity (it can range from a fulminating disorder to an asymptomatic condition), neurological symptoms, multiple phenotype presentations, rate of onset, pattern of symptoms and degree of disability are highly variable among individuals, where a continuum between slow mild onset and rapid acute onset is possible (3,4,8). This clinical variability is shown in extreme debilitation for some patients, where others conduct their daily lives with no dramatic changes (4,8). Clinical phenotype and course of MS are age dependent (15). The reasons for this neurological variability remain unknown, but it seems that the lesion burden does not necessarily correlate with the amount or intensity of disability (8).

The course of MS may be considered as the expression of two clinical phenomena, relapses of acute neurological symptoms, which end with a partial or complete remission, and progression, which refers to the steady and irreversible worsening of symptoms and signs over  $\geq 6$  months (15). They take place two biological activities: inflammation and degeneration; relapses are mainly the expression of acute, focal, disseminated and recurrent inflammation occurring within the CNS; progression and accumulation of disability correlate with the early, diffuse, chronic and progressive axonal loss, which is the hallmark of the neurodegenerative process in MS (15). For each clinical episode, there is an average of 10 new MRI lesions (15).

The patients experience an acute focal neurologic dysfunction which is not characteristic, followed by partial or complete recovery (11). These acute episodes with diverse signs and symptoms will then recur throughout their lives, with periods of partial or complete remission and clinical stability in between (11). Either relapsing or progressive disease may be characterized by severity of signs and symptoms, frequency of relapses, rate of worsening, residual disability, and impairment (16).

An attack (relapse or exacerbation) is defined as the occurrence, the recurrence or the worsening of symptoms reported by patients or objectively observed signs of neurological dysfunction, typical of an acute inflammatory demyelinating event in the CNS, with duration of at least 24 hours, in the absence of fever or infection (15). Symptoms occurring within a month were considered as part of the same relapse (15). A new attack should be documented by contemporaneous neurological examination (17). On the other hand, paroxysmal symptoms consist of multiple episodes occurring over not less than 24 hours (17). Regardless of lesion location, the great majority of initial attacks are associated with full or partial recovery (18). Recovery from the first neurological episode was classified as complete when the irreversible score after the episode was 2 or less on the Kurtzke Extended Disability Status Scale (EDSS); incomplete when this score was 3 or more (15).

The most commonly observed clinical course is a biphasic disease course (in the 85% of MS patients), initially characterized by a series of acute episodes of neurological disability (relapses) followed by partial or full recovery (remissions), which often become progressive over time, a condition called "secondary progressive", characterized by progressive neurological decline (2,3,12,15,18). This transition occurs in 60-80% of cases within 2 decades (2,18). About 80% of patients have such relapsing-remitting type of MS (RRMS) in the beginning, which after 10 or more years is followed by progressive clinical disability with or without superimposed relapses and remissions (SPMS) (11). To date, there are no clear clinical, imaging, immunologic, or pathologic criteria to determine the transition point when RRMS converts to SPMS; the

transition is usually gradual (16). It is thought to occur when axonal loss exceeds the compensatory capacity of the CNS, and additional axonal loss results in steady progression of permanent neurological disability (2). The most important outcome measures in the treatment of patients with early RRMS may be the prevention and/or attenuation of the progressive course (18).

In about 10-20% of the patients the disease is progressive from the beginning and they exhibit a decline or a steady worsening in neurological function without recovery that lasts for at least 6 months, sometimes with superimposed relapses and remissions (PPMS, PRMS) (2,11,15). Disability is defined as irreversible when a given score persisted at least 6 months, excluding transient worsening of disability related to relapses (15). The proportion of cases with superimposed relapses during progression is 40% (15). The progression is independent of relapses either preceding the onset of relapse-free progression or subsequent to it (18). The development of progression is the main determinant of prognosis because relapses are what can be partially suppressed with currently available treatments (18). In patients who may have begun to progress and continue to relapse, the progression of disability is not attributable either to lack of recovery from the last relapse or to the underlying progression (18). Although MS is not usually a fatal disease, disability and decreased quality of life (QoL) are common, that's why the economic cost of this disease is staggering (3).

Neurological impairment in the patients caused by the disease is quantified by the EDSS score: EDSS score from 0.0 to 2.5 (no or few limitations in mobility), EDSS 3.0 to 5.5 (moderate limitations in mobility), EDSS 6.0 to 7.5 (walking aid or wheelchair necessary), EDSS 8.0 to 9.5 (confined to bed) and EDSS 10 (death) (11).

Disease activity detected by clinical relapses or imaging (gadolinium-enhancing lesions or new or unequivocally enlarging T2 lesions) as well as progression of disability can be meaningful additional descriptors in either relapsing or progressive disease (16). An additional modifier of disease course is whether there is clinical evidence of disease progression, independent of relapses, over a given period of time in patients who have a progressive disease course (PPMS or SPMS) (16). Progressive disease does not progress in a uniform fashion and may remain relatively stable over periods of time (16).

The majority of MS patients have a monosymptomatic onset (frequently motor or sensory), but polysymptomatic onset can also occur (3). Onset symptoms include sensory symptoms (numbness, tingling), visual disturbances (optic neuritis, diplopia), spasticity, weakness, fatigue, ataxia and intention tremor, and bowel and/or bladder disturbances (3).

Patients can manifest with a heterogeneous group of symptoms (4,8,12,20). Symptoms for this disorder involve impaired vision (caused by optic neuritis, like unilateral or bilateral visual loss, diplopia, blurred vision, eye pain, and jerky eye movements), motor problems (partial or full paralysis, muscle weakness, stiffness, slurred speech, spasticity and twitching muscles or tremors), sensory loss or distortions (numbness, especially in the extremities, loss of awareness, facial pain, electric shocks, sensitivity to heat, a tightness around the torso or stomach, sensations of burning or prickling). Ataxia, nausea, vertigo, stuttering and loss of the ability to produce rapidly alternating movements are symptoms of impaired coordination and balance. Bowel/bladder problems include urgency, incontinence, retention, and sexual impotence. Moreover, some MS patients suffer from considerable cognitive impairment, experiencing short-term or long-term memory loss, difficulties in concentration, depression, mood swings, dementia and anxiety. Other symptoms include fatigue, sleeping disorders, and epileptic seizures (4,8,12,20). The most common of these symptoms include visual problems, spasticity, numbness/tingling, bowel/bladder/sexual dysfunction, depression, and fatigue (8).

The MS clinical course spectrum includes four categories (ANNEX 1):

- **Relapsing remitting multiple sclerosis (RRMS):** It is the classical relapsing and remitting disease (21) and the most common clinical form, seen in 80% of patients (11). It is characterized by relapses or attacks followed by either partial or total recovery of symptoms (8,15). Biologically, we can find focal areas of inflammation and demyelination, which resolve themselves over time leading to recovery (8). Thus, the damage done by inflammation is at least partially reversible (8).
- **Secondary progressive multiple sclerosis (SPMS):** It is the second most common type of MS, accounting for about 30% of cases (8). It has the initial relapses of RRMS, which over the course of the disease are replaced by progressive disability (8,15). Individuals with SPMS begin with reversible disability, but for unknown reasons, axonal degeneration occurs leading to irreversible damage, which presents clinically as progressive disability (8). The interval between clinical onset and onset of progression is highly variable (19). SPMS is diagnosed retrospectively by a history of gradual worsening after an initial relapsing disease course, with or without acute exacerbations during the progressive course (16). The course of SPMS is characterized by poor response to immunomodulatory treatments and an absence of new inflammatory demyelinating lesions as measured by MRI and histopathology (2).
- **Primary progressive multiple sclerosis (PPMS):** It occurs in approximately 10% of cases (2,8). It is characterized by progressive disability from the onset with no distinct relapses

and no remitting stages, although occasional plateaux and temporary improvements are allowed (8,15,19). The mean age of onset is approximately 38 years, and men are relatively more common in PPMS (1:1.3), but this ratio falls with advancing age of onset (21). Thus, individuals with this clinical subtype have irreversible damage that causes a slow or stepwise progression to increased disability with little to no symptomatic relief (8). Patients with a worse prognosis are those which have multiple systems involved at onset and those having rapid early progression (21). Progressive phase of MS is an age-dependent degenerative process, at least in part, because tract-specific chronic axonal loss is the pathological correlate of progression, so it probably begins long before clinical symptoms develop (18). PPMS represents a distinct, noninflammatory or at least less inflammatory pathologic form of MS, but it likely does not have pathophysiological distinct features from relapsing forms of MS that have entered a progressive course (16). We also find a relatively low rate of MRI activity (21).

- **Progressive relapsing multiple sclerosis (PRMS):** It occurs in nearly 5% of cases and is characterized by progressive disability from the onset of symptoms who later in their course develop clear acute superimposed attacks or relapses, with or without full recovery (8,15,19). The criterion for progressive disease is continuing deterioration for at least one year without substantial remission or exacerbation, regardless the rate of deterioration (19). Relapses in PPMS occur in 27.8% of patients within two or three decades after onset, with mild and remitting relapses; these patients become then PRMS patients (19). Relapses occur in the first ten years in half the cases, but in the other half they occur at intervals from onset of up to 20 years or longer (15,19). These exacerbations are generally mild, often discrete, in extraspinal locations and are characterized by good recovery, the degree of which seems unrelated to the duration of the disease (15,19).

#### *1.1.1.5 DIAGNOSTIC*

The diagnosis of MS is based mainly in clinical grounds, although MRI of the CNS and other paraclinical studies can support, supplement, or even replace some clinical criteria (8,16,17). The clinical phenotype may be assessed based on current status and historical data, although this can be a dynamic process and the subtype on initial assessment may change over time (16). Currently, there is neither a single clinical feature nor a sole diagnostic test than can confirm the diagnosis of MS (22). Diagnostic criteria for MS include clinical and paraclinical laboratory

assessments used to provide more accurate diagnoses (8), as well as the demonstration of dissemination of lesions in space (DIS) and dissemination in time (DIT) to exclude alternative diagnoses (17).

Nowadays, diagnosis is routinely confirmed by MRI, the diagnostic test with 95% sensitivity (11). MRI is the most effective paraclinical tool to define DIS and DIT for the diagnosis of MS in patients with at least one clinical event consistent with a demyelinating disease after exclusion of alternative possibilities, although MRI analysis alone is not enough to confirm the diagnosis of MS (22). Annual brain MRI scanning for activity in relapsing forms of MS is useful; however, there is no consensus on how frequently to scan progressive patients (16). MRI scans are used to examine the brain and spinal cord for lesions, both active and old (8).

A definite diagnosis of MS requires two different areas of the CNS being affected by inflammation in the form of lesion or plaque formation with two separate occurrences of an attack, usually described as neurological dysfunction (8). Before a definite diagnosis of MS can be made, at least one attack must be corroborated by findings on neurological examination, visual evoked potential (VEP) response in patients reporting prior visual disturbance, or MRI consistent with demyelination in the area of the CNS implicated in the historical report of neurological symptoms (17). Anatomical correlates of acute relapse can often be visualized by MRI scanning by location (19).

Other paraclinical exams are the following. Neurologic exams are performed to investigate coordination, strength, reflexes, and sensation (8). Various evoked potential tests are used to measure nerve response to stimulation (8). A spinal tap or lumbar puncture is performed to look at the CSF for various abnormalities, such as the number and type of white blood cells, glucose levels, and the levels of various proteins (antibodies and immunoglobins) (8). Positive CSF findings (elevated IgG index or two or more oligoclonal bands) can be important to support the inflammatory demyelinating nature of the underlying condition, to evaluate alternative diagnosis, and to predict Clinical Definite Multiple Sclerosis (CDMS) (17). Although these findings support an MS diagnosis there are still no specific biomarkers to confirm the diagnosis (17). Medical histories are collected along with various blood tests to rule out other disorders (8).

There are six criteria required to give a positive diagnosis (8):

1. Objective abnormalities must be present causing dysfunction in the CNS.
2. These abnormalities must involve the white matter long tracts.
3. Two or more areas of the CNS must be affected.

4. The clinical pattern must either involve two or more separate episodes, each lasting 24 hours and at least 30 days apart, or a slow or step-wise progression of disability over 6 months and an abnormal spinal fluid screen, in which CSF would contain oligoclonal bands and increased production of IgG.
5. The age of onset should be between the ages of 10 and 60.
6. The symptoms experienced cannot be attributed to another neurological disease.

With advancing technologies, the criteria for MS were updated to integrate MRI into the diagnostic scheme (8). If clinical evidence does not support dissemination of lesions in both time and space, then MRI can be used to provide evidence for this (8).

The McDonald Criteria (ANNEX 2) have resulted in earlier diagnosis of MS with a high degree of both specificity and sensitivity, allowing for better counselling of patients and earlier treatment (17). Criteria for MS diagnosis should therefore be applied only when patients have experienced a typical Clinically Isolated Syndrome (CIS) suggestive of MS or symptoms consistent with CNS inflammatory demyelinating disease (or progressive paraparesis/cerebellar/cognitive syndrome in the case of suspected PPMS) (17). CIS presentations can be monofocal or multifocal, and typically involve the optic nerve, brainstem/cerebellum, spinal cord or cerebral hemispheres (17).

Essential for diagnosis of its relapsing-remitting form is dissemination of clinical episodes in time (two or more episodes) and space (more than one focal lesion) (11). DIS demonstrated by MRI was based on the Barkhof/Tintoré criteria (17) (ANNEX 3). DIS can be demonstrated with at least one T2 lesion in at least two of four locations considered characteristic for MS and as specified in the original McDonald criteria (juxtacortical, periventricular, infratentorial and spinal cord), with lesions within the symptomatic region excluded in patients with brainstem or spinal cord symptoms (17). MRI evidence required for DIT is the appearance on new T2 lesion on a scan compared to a reference on baseline scan performed at least 30 days after the onset of the initial clinical event (17).

Once a person has been diagnosed with MS, the severity of disability must be quantified (8). Kurtzke EDSS measurements were developed as a method for measuring disability in eight functional systems (FS): pyramidal, cerebellar, brain stem, sensory, bowel and bladder, visual, cerebral, and other (8). The EDSS, while an excellent attempt at quantifying MS disability, has its drawbacks (8). It is a subjective measurement that can change frequently even during a single exam (8). EDSS measurements do not assess disease duration or the difference in rates of disease progression (8).

### 1.1.2 RADIOLOGICALLY ISOLATED SYNDROME

The increasing use of MRI over the past decade in the diagnostic work-up of pathological conditions has contributed to the uncovering of asymptomatic brain pathologies and incidental identification of abnormalities in the CNS (23,24). Incidental MRI findings are relatively common in the brains of asymptomatic subjects, increasing with age and the use of high-resolution MRI sequences (23). The MRI detection in the brain of asymptomatic subjects of white matter lesions suggestive of MS is a frequent incidental MRI finding, increasing with age, in subjects with a history of psychiatric disorders and asymptomatic first-degree relatives of MS patients (23).

Structural neuroimaging studies of patients with an initial clinical event suggestive of MS usually reveal other brain or spinal lesions, which were asymptomatic at the time of MS diagnosis, suggesting the existence of a presymptomatic period (22). Moreover, during the course of established MS new asymptomatic lesions often appear, confirming this subclinical activity (22).

RIS, a term first introduced in 2009, defined a cohort of individuals routinely encountered in clinical practice who are at risk for future demyelinating events (24,25). RIS is defined as incidental MRI identified white matter anomalies within the CNS suggestive of MS in healthy people without typical signs and symptoms associated with CNS demyelination and with normal neurological findings, after exclusion of other possible aetiologies (16,22,24,26,27). These subjects reveal unanticipated brain spatial dissemination of MRI lesions highly suggestive of MS (23).

Symptoms that led to the first MRI scan included mainly primary or migraine headache and depression unrelated to a typical inflammatory demyelinating CNS syndrome (22,24).

The proposed diagnostic criteria for RIS include brain MRI to establish anatomic DIS in the absence of a better explanation or a clinical history of inflammatory demyelinating disease of the CNS (22) (ANNEX 3).

The natural history or evolution of RIS remains incompletely understood (22,24). RIS patients may develop clinical symptoms, converting to either RRMS or PPMS (22). The median time to the first clinically defining event (CIS) was 5.4 years (24). Alternatively, patients may show progression in the MRI lesions without any objective clinical symptoms, which is called radiological progression, or they may even show stabilized brain abnormalities in subsequent imaging examinations (22). Radiologic progression is defined as the presence of new T2 focal abnormality, gadolinium enhancement or enlargement of pre-existing lesions in longitudinal

follow-up scans (22,24). With a median follow-up of 2.7 years, radiologic progression rate was documented in 50-60% of patients (22,24).

Although some individuals did not exhibit progression over a lengthy follow-up period, most patients will progress clinically or radiologically in the initial years of the follow-up (83.3%), so all patients with RIS should be considered as having a high risk of developing MS (22). Thus, a RIS patient, in spite of not showing obvious clinical signs or symptoms suggestive of MS, should be followed prospectively (16). Some of these individuals followed clinically and by serial imaging will develop DIT by MRI, and some have clinical disease-defining events after several years (17).

Individuals with RIS are more likely to progress to symptomatic MS: approximately, two-third of individuals show radiological progression, and one-third of individuals develop neurological symptoms during mean follow-up periods of up to 5 years since the identification of their abnormal MRI (26). The conversion rate to MS in patients with RIS at baseline was 1.5%/month; showing evidence that individuals with RIS are at increased risk of developing definite MS (26). The conversion rate from RIS to MS was 65% after a mean follow-up of 5.3 years and 88% after a mean follow-up of 14.1 years (24,26). Thus, for many patients, RIS represents the earliest visible manifestation of demyelinating disease, or a preclinical stage of MS (27). Clinical conversion following RIS most commonly occurs during the initial years (22).

There is a predominance occurrence of RIS in women, but the rate of conversation to CDMS is higher in males as compared to that of females (22).

The mechanisms underlying the increased risk of MS in patients with RIS are not clear (23). While focal and diffuse macroscopic brain tissue damage is by and large similar between RIS and RRMS, the subtle myelin/axonal damage can be much milder in RIS subjects than in RRMS patients (23). This might be explained by a different degree of demyelination between the two groups, being milder in RIS patients, possibly due to a more beneficial response to the demyelinating insult occurring in the RIS subjects (23). The evidence that this milder tissue damage occurs in brain regions that are clinically relevant to MS might provide a plausible biological explanation for the lack of clinical manifestations existing in RIS subjects (23,26).

Gadolinium enhancement in brain lesions reflects disturbances of the BBB (22). Disruption of the BBB is an early event in the development of inflammatory lesions in MS and a predictor of the occurrence of relapses (22). Patients whose baseline MRI scans exhibited gadolinium enhanced lesions had a substantially increased risk of developing new lesions (22). Furthermore,

the presence of contrast-enhancing lesions on the initial MRI signifying RIS constituted a significant factor in increasing the risk of DIT on subsequent brain MRI scans (24).

RIS should not be considered a MS subtype since clinical evidence of demyelinating disease (a current criterion for MS diagnosis) is lacking and MRI findings alone may be nonspecific (16). However, RIS may raise the suspicion of MS, depending on the morphology and location of detected MRI lesions (16). Changes on brain imaging that are highly suggestive of demyelinating pathology carry the greatest risk of future MS clinical symptoms (16). The changes that are highly suggestive of demyelinating pathology based both upon their location and morphology in the CNS are periventricular geography, involvement of the corpus callosum, ovoid, well-circumscribed and homogeneous lesions (24).

The findings that enhance the likelihood of an eventual MS diagnosis are the presence of asymptomatic spinal cord lesions, infratentorial lesions, gadolinium-enhancing lesions, the total number of lesions and positive CSF findings, and they are predictors of clinical or radiological progression (16,22–24).

From the largest study done with RIS patients, the following data could be concluded. The mean age at from the time of the first brain MRI revealing anomalies suggestive of MS was 37.2 years (25). Clinical events were identified in 34% of individuals within a 5-year period from the first brain MRI study (25) (ANNEX 4). Of those who developed symptoms, 9.6% fulfilled criteria for PPMS (25). Age below 37 years old, sex (male) and lesions within the cervical or thoracic spinal cord were identified as the most independent predictors for the development of a first clinical event (25). These data provide supportive evidence that a meaningful number of RIS subjects evolve to a first clinical symptom (25). Mean age of the patients who progressed to definite MS was younger than in patients with RIS who did not progress to MS. This finding is consistent with reports that MS may occur as early as the third decade of life (26). A younger age at RIS diagnosis was associated with an increased risk of developing an initial symptomatic event with an estimated risk of developing an event decreasing by 2% for every year additional year of age (25). The 5-year risk of developing a first clinical event for RIS subjects with both spinal cord involvement and younger age was 58% (25). Risk also appeared to be higher with the presence of multiple risk factors (25).

CSF profiles were suggestive of MS in 67% of subjects in a cohort (IgG index  $\geq 0.7$  or  $\geq 2$  unique oligoclonal bands not observed in the periphery) (24).

Increasing age appeared to be important in reducing the risk for symptom development, a plausible observation given a greater degree of disease activity in younger patient groups, suggesting an opportune age window for symptom development (25).

In a sample of RIS subjects, approved Disease-Modifying Therapies (DMT) for MS were introduced prior to the development of a first clinical episode with a mean treatment duration of 3.2 years (25). The 5-year risk of developing a first clinical event for RIS subjects who were exposed to DMTs was 45% as compared to 31% for RIS subjects who did not receive treatment (25).

### 1.1.3 TREATMENT

The availability of DMT has revolutionized the care of patients with the relapsing forms of MS, according to (12). These medications help control the underlying disease process, probably by decreasing immune mediated inflammation, although they do not cure the disease or reverse the damage that has occurred with prior events (12). In general, for them, the effects of these agents appear more potent when they are given to patients before more severe widespread damage and disability have occurred (12).

Currently, there are several therapeutic options for MS with disease-modifying properties (11). The main mechanism of injury in MS appears to be inflammation, and there are currently 8 Food and Drug Administration (FDA) approved agents to help control MS (12). These agents for relapsing forms of MS target different parts of the immune system, with the end goal of decreasing and avoiding further inflammation (12). No agents were FDA approved for the primary progressive version of MS (12), although recently a new drug has been approved. FDA approved agents include four preparations of interferon beta (Avonex, Rebif, Betaseron and Extavia), glatiramer acetate (Copaxone), mitoxantrone (Novantrone), natalizumab (Tysabri) and fingolimod (Gilenya), the first oral medication approved (12). Moreover, there are several drug undergoing phase II and III trials (12). Immunomodulatory therapy with IFN  $\beta$ -1b or -1a, glatiramer and natalizumab shows similar efficacy; in a resistant or intolerant patient, the most recently approved therapeutic option is mitoxantrone, according to (11). Glatiramer acetate has shown almost the same efficacy as IFN  $\beta$ , and is used mostly when therapy with IFN  $\beta$  is no longer possible, due to emergence of neutralizing antibodies against it (11). Natalizumab is used in cases which are resistant to treatment with both IFN  $\beta$  and glatiramer, due to serious adverse effects recorded in a few patients (11). Available therapies for MS patients, while effective during the relapse phase, have little benefit for progressive MS patients (2).

IFN  $\beta$  was first approved by the FDA for MS treatment on 1993 (12). It has been shown to reduce relapse rate, especially if given early in the course of the disease (11), decrease disability progression, and MRI evidence of disease activity (12). The clinical efficacy of IFN  $\beta$  is greater in RRMS than in SPMS (11,12). The exact mechanism of how IFN  $\beta$  affects MS is uncertain, however several potential pathways have been postulated (12) (ANNEX 5). Among these mechanisms, inhibition of T cell activation and proliferation as well as reduction in matrix metalloproteinase activity may play an important role (12). Another mechanism, proposed by (11), is that IFN  $\beta$ 1b binds to specific receptors on surface of immune cells, changing the expression of several genes and leading to a decrease in quantity of cell-associated adhesion molecules, inhibition of MHC class II expression and reduction in inflammatory cells migration into the CNS. IFN  $\beta$  has other immunologic effects: it reduces the production of proinflammatory cytokines and induces the production of anti-inflammatory cytokines by increasing suppressor T cell activity (12).

Different preparations of IFN  $\beta$  showed similar efficacy in the majority of clinical trials, with a slight dominance of IFN  $\beta$ -1b (11). There are four IFN  $\beta$  products available on the market (12). IFN  $\beta$ -1a (Avonex and Rebif) are recombinant peptides produced in Chinese hamster ovary cells and are identical to natural human interferon-beta (12). The Avonex formulation is given intramuscularly once a week and the Rebif formulation is given subcutaneously 3 times per week (12). IFN  $\beta$ -1b products (Betaseron and the identical Extavia) are recombinantly produced by Escherichia coli bacteria (12). It differs from the IFN  $\beta$  made endogenously in humans as it has a single amino acid substitution and is not glycosylated (12). IFN  $\beta$ 1b is administered via subcutaneous injection every other day and is titrated to a target dose over 6 weeks (12). All four formulations have in common that they bind to the same type I interferon receptor expressed on human cells (12). Neutralizing antibodies can negate the benefits of these agents, and its rate formation varies between the different interferon beta products, with IFN  $\beta$ -1b having the highest rate (12).

The efficacy of IFN  $\beta$ -1b in RRMS is higher than that of IFN  $\beta$ -1a, and similar to the efficacy of glatiramer acetate (11). Higher efficacy and similar safety compared with other drugs of the same class, mean that IFN  $\beta$ -1b has a significant segment of the drug market for MS (11). These facts promote IFN  $\beta$ -1b as one of the most important drugs in the spectrum of immunological therapies for this debilitating disease, according to (11).

In recommended doses IFN  $\beta$ -1b causes the following frequent adverse effects (frequency is given in parenthesis): injection site reactions (redness, discoloration, inflammation, pain, necrosis and non-specific reactions) (85%), insomnia (31%), influenza-like syndrome (fever, myalgia and rigors) (34%), asthenia (34%), headache (32%), myalgia (26%), hypoesthesia (26%),

nausea (16%), paresthesia (16%), myasthenia (11%), chills (8%), depression (8%), back pain (5%), increased liver enzymes (11%), lymphopenia (11%), fever (5%), and pain in extremities (3%), as well as fatigue and thrombocytopenia (11,12). Patients receiving IFN  $\beta$ -1b perceive depression, influenza-like reactions and pain due to injection site reactions as most disturbing (11). During treatment with IFN  $\beta$ -1b, a number of patients develop neutralizing antibodies (11,12). (12) Recommends that patients should have a complete blood cell count and hepatic function tests prior to starting IFN  $\beta$  therapy and periodically thereafter.

When starting an IFN  $\beta$ -1b therapy, a treatment discontinuation rate ranging from 14%–44% could be expected (11). However, proposed options to improve adherence are administering the drug subcutaneously by auto-injectors, gradually increasing the dose at the start of the treatment, using ibuprofen prophylactically and administering the drug in the evening (11). The patients with MS dependent on a wheelchair are at increased risk to become non-adherent to the treatment due to the adverse effects of IFN  $\beta$ -1b (11).

About 7% of patients during treatment with IFN  $\beta$ -1b develop auto-antibodies, primarily against thyroid and hepatic structures; however, emergence of the auto-antibodies was not linked to thyroid or liver function alterations (11). Although there are no published studies of interactions between IFN  $\beta$ -1b and other drugs, there are reports that IFNs reduce the activity of hepatic cytochrome P450-dependent enzymes (11). Therefore, one should be careful when using IFN  $\beta$ -1b in combination with drugs which are metabolized by the cytochrome P450 system, and whose therapeutic index is narrow (11).

After at least 1 year of IFN  $\beta$ -1b therapy, about one fifth of the patients with MS develop tolerance to this drug, manifested as an increase in the relapse rate (11). This process correlates well with emergence of neutralizing antibodies produced by the patient's immune system, which then bind to IFN  $\beta$ -1b, preventing its action (11). This tolerance spontaneously abates after several years of continuous treatment, coinciding with disappearance of neutralizing antibodies from the patients' serum (11). Therefore, a finding of neutralizing antibodies against IFN  $\beta$ -1b in serum of MS patients is not an indication for discontinuing therapy with this drug (11).

After 2 years of treatment, IFN  $\beta$ -1b reduces the risk of development of CDMS from 45% (with placebo) to 28% (with IFN  $\beta$ -1b) (11). It also reduces relapses for 34% and makes 31% more patients relapse-free (11). In secondary-progressive disease annual rate of progression is 3% lower with IFN  $\beta$ -1b (11).

In a observational study, it was detected a large and clinically important survival advantage associated with randomization to early IFN  $\beta$ -1b treatment at either dose (250 $\mu$ g and 50 $\mu$ g) compared to placebo, and benefits as determined by both clinical and MRI outcomes (13). Patients originally randomly assigned to IFN  $\beta$ -1b 250 $\mu$ g showed a significant reduction in all-cause mortality over the 21-year period compared with placebo, with a hazard ratio (HR) of 0.532 (13). The hazard rate of death at long-term follow-up by Kaplan-Meier estimates was reduced by 46.8% among IFN  $\beta$ -1b 250 $\mu$ g treated patients (46.0% among IFN  $\beta$ -1b 50 $\mu$ g-treated patients) compared with placebo (13). Thus, early use of IFN  $\beta$ -1b improves survival in patients with MS (13). This study provides Class III evidence that early treatment with IFN  $\beta$ -1b is associated with prolonged survival in initially treatment-naive patients with RRMS (13).

According to (12), the unknown aetiology, probable disease heterogeneity, individual patient response and immune system complexity and medication toxicities will continue to provide challenges for clinicians treating MS. It also says that to date there is no cure for MS, and medications which decrease immunologic functions may have significant risks (12).

Because of the considerable cost of IFN  $\beta$ -1b therapy its cost/effectiveness is still an open issue, which depends on duration of therapy, an accurate estimate of long-term benefit and prices of health services in health care settings (11).

## **1.2 JUSTIFICATION**

Much effort has been devoted to attempting to correctly identify and predict the clinical evolution of RIS subjects, in view of the growing consensus for an early DMT in patients diagnosed with MS (23). Until now, treatment in RIS failed to demonstrate a benefit in extending the time to the onset of the first symptomatic event (25).

The findings of these studies have important implications for clinical practice. RIS is a preclinical stage of MS. Patients with incidental findings of brain abnormalities in their initial MRI studies are at substantial risk for the development of MS (26). MS can course with diverse clinical forms. However, in final phases of MS, most of patients have a decreased QoL and are affected by different grades of disability. Furthermore, this disease means an enormous expenditure for our sanitary system, either in sanitary resources, as well as in health professionals and indirect costs (derived from absenteeism due to the impairment caused by the disease). Thus, it results interesting to reduce the RIS progression to CDMS, either for the patients QoL as well as for the economy of our sanitary system.

The initiation of prophylactic treatment for RIS is controversial (26). Currently, there is not any indication for treatment of patients with RIS. Although treatment with DMT is generally not recommended for individuals with RIS because many may never develop MS, a recent review noted that about 10% of the reported RIS population is treated (26). It is not known whether early treatment with DMT (as for patients with confirmed MS) improves symptom-free survival for individuals with RIS (26). For patients with abnormal brain MRI findings, one must balance their risk of developing MS with the potential side-effects and cost of DMT (26). Treatment may be appropriate, but the evidence is insufficient, partly because of the lack of controlled trials of treatments and partly because the long-term prognosis is unknown (26). Randomized controlled trials designed to test the efficacy of early initiation of DMT in relapsing MS, and to determine whether such treatment delays the conversion of RIS to MS, will aid in defining the role of DMT in the high-risk RIS population (26). That's why new approaches in the treatment of RIS may be developed (25).

Among the different treatments approved for the treatment of relapsing forms of MS (RRMS, PRMS, SPMS), the one with the best efficacy is IFN  $\beta$ -1b, because it reduces relapse rates, decreases disability progression and also reduces clinical activity in the MRI. That's why this drug would be the best option for treatment of RIS patients, specifically the Betaseron presentation, which is the one that has more clinical experience. The objective of this study is to measure the efficacy of IFN  $\beta$ -1b in patients with RIS on reducing the proportion of subjects experiencing a first clinical event of MS and avoiding radiological progression as well as disability progression. A statistical significant and clinically relevant result in this study would change the management of these patients, thus avoiding many new cases of MS and reducing economic impact of this disease in our society.

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### 3. HYPOTHESIS

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Treatment with IFN  $\beta$ -1b reduces the relapse rates in patients with diagnosed MS. RIS is a preclinical stage of MS which share the typical demyelinating pathogenesis with this disease, so the administration of IFN  $\beta$ -1b will avoid the development of a first clinical event in patients with RIS.

IFN  $\beta$ -1b treatment will avoid the development on new lesions in the MRI scan and will avoid the enlargement or reduce the pre-existing lesions in the MRI scan in RIS patients.

IFN  $\beta$ -1b treatment will reduce the disability progression and the frequency of relapses in patients with RIS.

### 4. OBJECTIVES

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#### 4.1 PRIMARY ENDPOINT

1. To determine the effect of IFN  $\beta$ -1b on reducing the proportion of RIS patients experiencing a first clinical event of MS.

#### 4.2 SECONDARY ENDPOINTS

2. To study whether treatment with IFN  $\beta$ -1b improves radiologically progression in RIS patients by:
  - a. Preventing the occurrence of new lesions in the brain and spinal cord MRI scan.
  - b. Reducing the size or avoiding the enlargement of pre-existing lesions in the brain and spinal cord MRI scan.
3. To assess whether the IFN  $\beta$ -1b treatment in RIS patients modifies disability progression measured by the EDSS.
4. Check the level of safety and tolerability of IFN  $\beta$ -1b as a first-line treatment in RIS patients, assessed by the presence of side effects in this population.

## 5. MATERIALS AND METHODS

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### 5.1 STUDY DESIGN

This study will assess the efficacy of IFN  $\beta$ -1b as a first-line treatment in patients with RIS. This trial is an interventional, prospective and multicentre study. The allocation of patients to each treatment (placebo or IFN  $\beta$ -1b) will be randomized. The intervention model will be parallel assignment in 2 groups and the masking will be double blind, so the study participants as well as the researchers will not know treatment assignments. Patients will be randomized in a ratio of 1:1 to receive placebo or IFN  $\beta$ -1b. So, using this design, there is a control group with which to compare the results on the primary variable obtained by the group treated with the study drug.

The treatment duration will be 18 months from the last patient recruited, and the duration of the study will be a period of time of 44 months. For those patients who complete the treatment period, a follow up extension after end of treatment will have a duration of 6 months in order to observe and detect potential side effects and assure security of subjects.

### 5.2 STUDY POPULATION

The study population includes patients diagnosed with RIS between 18 and 65 years according to diagnostic criteria from Okuda in 2009 (ANNEX 3). All patients met Barkhof criteria for DIS on baseline brain MRI scans. The reason why people below the age of 18 years are excluded is that the administration of IFN  $\beta$ -1b is not tested in these patients. In addition, patients over 65 years old are excluded because the probability of developing MS in these range of years is very low.

Written informed consent must be acquired from all study subjects.

### 5.3 INCLUSION AND EXCLUSION CRITERIA

#### 5.3.1 INCLUSION CRITERIA

1. People between 18 and 65 years old.
2. Patients must have a confirmed diagnosis of RIS according to the criteria for RIS of 2009, with initial brain MRI studies revealing incidental anomalies suggestive of demyelinating disease.

3. Patients may accept the purpose and the risks of the study and sign the informed consent.
4. Patients who are able to cooperate in the study.

### 5.3.2 EXCLUSION CRITERIA

1. Patients with history of remitting clinical symptoms consistent with neurologic dysfunction of the CNS lasting more than 24 hours prior to CNS imaging with anomalies suggestive of MS.
2. Diagnosis of MS in any disease course.
3. Diagnosis of CIS.
4. Patients in treatment for RIS.
5. Patients who cannot be subjected to repeated examinations with MRI.
6. History of hypersensitivity or severe side effects of gadolinium (MRI contrast).
7. Immunosuppressed patients or patients receiving any immunosuppressive treatment.
8. History of hypersensitivity or severe side effects of IFN  $\beta$  natural or recombinant.
9. Decompensated liver disease.
10. Severe renal insufficiency.
11. Severe depression and/or suicidal ideation.
12. Women who are pregnant or who have desire to be pregnant.
13. Women of childbearing potential not using contraception.
14. Women who are in lactation.

## 5.4 SAMPLE

### 5.4.1 SAMPLE SIZE

Accepting an alpha risk of 0.05 and beta risk lower than 0.2 in a bilateral contrast, 261 subjects are needed in the exposed group (treatment with IFN  $\beta$ -1b) and 261 in the non-exposed (placebo) to detect a minimum relative risk (RR) of 0.66 if the rate of RIS patients who turns into MS in unexposed group is 0.36 (calculated accepting a rate of conversion to MS of 1,5% per month and a duration of the assessment of subjects of 24 months, and accepting a reduction of relapses of 34% with treatment with IFN  $\beta$ -1b). It has been estimated rate of 10% withdrawals. Poisson approximation was performed.

#### 5.4.2 SAMPLING METHOD

It is not possible to obtain a sample from our study population because it is too scarce and we need to recruit all eligible patients who met the inclusion and exclusion criteria to reach a sufficient number of sample. Thus, our sampling method will be a non-probabilistic consecutive sampling, which will recruit all subjects meeting the inclusion and exclusion criteria until we reach the number of 522 subjects.

The recruitment of patients will be initially done by UNIEM (Unitat de Neuroimmunologia i Esclerosi Múltiple) from Hospital Josep Trueta of Girona, performing a formally request to RISC Research Network (Radiologically Isolated Syndrome Consortium Research Network) to ask for the participation of all subjects registered in this consortium, because the number of RIS patients is very limited and we need the participation of the maximum number of subjects possible to achieve a sample that can detect statistically significant differences in the results between the two groups.

RISC Research Network includes five different participating countries (France, Turkey, Italy, Spain and United States of America) with the aim to collect the clinical and radiological characteristics of patients with RIS, and involves the participation of 22 centres (4 from USA, 14 from France, 2 from Italy, 1 from Turkey and 1 from Spain) (ANNEX 6). With the participation of this consortium we will collect all necessary subjects to perform this trial. As the hospital Josep Trueta of Girona is not included in the RISC Research Network, the inclusion in the consortium will be requested as this hospital is the project promoter.

To perform the recruitment in each country, a visit screening will be performed in different centres in order to check inclusion and exclusion criteria and propose the participation in the trial. The trial will be multicentre, so the assignment of patients in each arm, the treatment and the successive follow-up visits where different variables will be collected will be performed in each centre where patients are registered. The analysis of MRI, however, will be centralized in Hospital Josep Trueta.

In the screening visit, a neurologist will assess whether the participants meet the inclusion and the exclusion criteria of the study. The information sheet and informed consent will be given to them.

Sample recruitment will take place during 6 months.

Before the randomization and the administration of the therapy some procedures must be done in the screening visit:

- To collect a detailed clinical history of the patient and obtain a comprehensive neurologic evaluation.
- To assure that the participant fulfil strictly inclusion and exclusion criteria.
- To collect the study variables prior to the administration of medication.

## 5.5 RANDOMIZATION AND MASKING

The patients will be randomized using a 1:1 randomization ratio between two groups:

- Group 1: Nearly 261 patients. Recombinant IFN  $\beta$ -1b (Betaferon) 0.25 mg subcutaneous every other day. It contains 0.25 mg per ml of solution.
- Group 2: Nearly 261 patients. Placebo (physiological saline serum) 1ml subcutaneous infusion every other day.

In order to assure blinding procedures, one neurologist will be the examining and another one will be the treating neurologist. The examining neurologist will perform the tasks of neurological examination, assessing the punctuation on the EDSS, and the evaluation of FS, as well as a direct anamnesis in order to detect any clinical abnormality suggestive of a demyelinating event. The treating neurologist will perform the tasks related to the identification of any possible side effect due to the administration of IFN  $\beta$ -1b, the collection of any concomitant medication took during the study, the removal, if necessary, of the treatment, and also a direct anamnesis to detect, again, any clinical symptom probably due to a demyelinating event.

To assure the masking, both IFN  $\beta$ -1b and placebo will have the same presentation in a bottle and the administration will be subcutaneous. Each subject is anonymous, and can be identified in a database introducing the code that define each subject, which is formed by the identification number of the centre, the number of the study and finally the number of the patient. Once randomization of subjects into each arm of treatment is done in a computerized way, each centre will receive the treatment for every patient into a bottle, which will be labelled with the identification code of the subject. Neither the examining nor the treating neurologist know which treatment is receiving each subject. Only by introducing the identification code of the subject in the database which contains the results of the randomization, the assignment into treatment with IFN  $\beta$ -1b or with placebo could be known. Neither the treating nor the examining neurologists will have access to this database. Only when data analysis were performed, the analyst researcher will have access to this database.

## **5.6 VARIABLES. METHODS OF MEASUREMENT**

### **5.6.1 INDEPENDENT VARIABLE**

#### *5.6.1.1 VARIABLE A: TREATMENT WITH IFN $\beta$ -1b*

A dose of 0.25 mg of IFN  $\beta$ -1b via subcutaneous (1 ml) will be given every other day in group 1. Group 2 will receive a subcutaneous similar dose of placebo (1 ml) every other day. Neither the person who gives them the medication nor the participant will know which treatment is receiving.

This variable is expressed as a qualitative dichotomous variable.

The treatment will be given for 18 months.

### **5.6.2 DEPENDENT VARIABLES**

#### *5.6.2.1 VARIABLE B: EVIDENCE OF A FIRST CLINICAL EVENT*

A first clinical event is defined as the development of an acute neurological episode localized to the optic nerve, brainstem, cerebellum, spinal cord, or long sensory or motor tracts, lasting 24 hours followed by a period of symptom improvement or the onset of a clinical symptom with the temporal profile revealing at least a 12-month progression of neurological deficits (25). Every neurological event will be documented by evaluating the clinical description, localization within the CNS and the EDSS (25).

Each clinical event will be confirmed by the treating neurologist, based on the objective assessments by the examining neurologist. Subjects will be instructed to contact their investigator immediately if any symptoms suggestive of a MS exacerbation appear.

In order to recognize any neurological symptom suggestive of a demyelinating event, an exhaustive anamnesis, clinical evaluation and full neurologic examination, as well as an active search for symptoms attributable to MS, will be performed once every 3 months by the examining neurologist. In order to have objective criteria to perform the assessment, the evaluation based on FS and EDSS will be performed (ANNEX 7).

This variable is expressed as a qualitative dichotomous variable (presence or absence of a first clinical event).

### 5.6.2.2 VARIABLE C: RADIOLOGICAL PROGRESSION

In each patient 3 MRI explorations will be performed: basal, in 12 months and in 24 months.

The MRI will be done in each centre, but image analysis will be done in a centralized way in a single centre with technical and logistical capacity. This centre will be the Hospital Universitari Josep Trueta, located in Girona. Digital MRI explorations must be sent to this centre. Minimal conditions are the utilization of equipment with a field intensity of 1.5 tesla, with capacity to export digital images and anonymous.

Abnormalities within the brain or the spinal cord will be initially identified by a neuroradiologist on the formal interpretation and then examined and verified by a MS specialist to ensure DIS MRI criteria are met, and during the study to analyse any modification in the neuro-imaging studies. A qualitative analysis of the available brain imaging studies will be performed on all study participants (geographical location within the brain or spinal cord, supratentorial and infratentorial lesions, and morphology of lesions) and quantitative (number of T2 foci, presence or absence of gadolinium enhancement) (25).

This variable is expressed as a qualitative dichotomous variable for radiological progression, which is positive with the presence of at least the first or the second of the following features, and negative with the absence of both the first and the second of the following features:

- Occurrence of new lesions in the brain and spinal cord MRI scan.
- Enlargement of pre-existing lesions in the brain and spinal cord MRI scan.

Moreover, we will perform a description about whether pre-existing lesions have reduced its size or not.

**Table 1 MRI protocol for the diagnostic of radiological progression.**

<b>MRI PROTOCOL: MRI sequences required in the 3 plans (coronal, sagittal and transverse)</b>
Sequences enhanced in PD and T2 (Fast / turbo spin-echo, conventional spin-echo): 3mm thick contiguous slices covering the whole brain parenchyma with square pixels.
Sequences enhanced in T1 (spin-echo): 3mm thick contiguous slices covering the whole brain parenchyma with square pixels.
FLAIR sequences: held immediately after the administration of gadolinium contrast.
Sequences enhanced in T1 (spin-echo) after the administration of gadolinium contrast.

The following parameters will be evaluated to measure lesion activity:

- Asymptomatic spinal cord lesions (cervical and thoracic) and infratentorial lesions.
- Number of total and apparent active lesions (measured in T1 sequence by the enhancement with gadolinium).
- Number of new active lesions.
- Number of new lesions/augmented lesions in DP/T2.
- Volume of active lesions (T1Gadolinium).
- Percentage of lesions with enhancement in the basal study that are hypointense (black holes) in the final study.

Definitions, boundaries and characteristics of MRI are collected in the annex (ANNEX 8).

#### *5.6.2.3 VARIABLE D: DISABILITY*

Patients' disability progression will be assessed using EDSS (ANNEX 7). It is based on the assessment of different FS (visual, pyramidal, sensory, brainstem, bladder and bowel, mental, cerebellum and the ability to roam) together. The scale ranges from 0.0 (normal neurological exam) to 10.0 (death due to MS). Measurements 1.0 to 4.5 indicate a person who is fully ambulatory, while the 6.0 to 9.5 range indicates significant impairment (8).

This variable is a qualitative ordinal variable (increase in points in EDSS), but we will express it as a qualitative dichotomous variable, indicating the presence or absence of disability progression, defined as:

- Increase of 1 point in EDSS in 12 months or
- Increase of 0.5 points in EDSS in 6 months.

Disability will be assessed once every 3 months by the examining neurologist.

#### *5.6.2.4 VARIABLE E: INCIDENCE OF SIDE EFFECTS IN PATIENTS TREATED WITH IFN $\beta$ -1b*

They will be collected the most common side effects associated with interferon and any undercurrent disease recorded during the study. Are considered severe side effects those that require hospitalization, moderate those that require suspension of study treatment, and mild those that are transient or don't require the suspension of the medication.

Side effects will be collected by anamnesis by the treating neurologist in each visit (every three months). The neurologist will have to ask by direct questions and perform an active search for

the side effects of the IFN  $\beta$ -1b, in order not to forget any data. The expected side effects of IFN  $\beta$ -1b are collected in the technical slug of the drug, extracted from vademecum (ANNEX 9).

This variable is expressed as a qualitative dichotomous variable (presence or absence of each side effect).

**Table 2 Questions to ask to find any side effect of interferon beta-1b**

<b>Questions to ask to find any side effect of IFN beta-1b</b>
- <b>Redness, rash, discoloration, inflammation, pain or other reactions in the injection site.</b>
- <b>Problems to sleep, waking up very early in the morning, spending many time trying to fall asleep, waking up several times in the night.</b>
- <b>Fever, myalgia, rigors, chills, sweating, malaise.</b>
- <b>Infection, abscess.</b>
- <b>Lymphadenopathy (exploration).</b>
- <b>Fatigue.</b>
- <b>Dizziness.</b>
- <b>Headache.</b>
- <b>Loss of sensibility in any part of the body or a change in sensibility. Tingling.</b>
- <b>Nausea and vomiting.</b>
- <b>Back and extremities pain.</b>
- <b>Low mood, sadness, loss of desire to do things, having no desire to get out of bed.</b>
- <b>Anxiety.</b>
- <b>Conjunctivitis, abnormal vision.</b>
- <b>Earache.</b>
- <b>Palpitation, hypertension.</b>
- <b>Upper respiratory tract infection, sinusitis, increased cough, dyspnoea.</b>
- <b>Diarrhoea, constipation, abdominal pain.</b>
- <b>Urinary retention, incontinence or urinary frequency or urgency.</b>
- <b>Dysmenorrhea, menstrual disorders, vaginal bleeding, impotence.</b>
- <b>Chest pain, peripheral oedema.</b>

Moreover, the following analysis have to be performed:

- **Blood analysis:** it may include complete blood cell count (including leukocytes, neutrophils, lymphocytes and thrombocytes) and blood biochemical parameters, including glucose, liver function tests (AST (SGOT) and ALT (SGPT)) and renal function (creatinine). The normal values of blood cell count will be the corresponding of Hospital Josep Trueta. Specifically, absolute values of lymphocytes  $<200/\text{mm}^3$  require the suspension of treatment. The measurement of glucose will be random (it means not in fast), and normal values are considered those below 200 mg/dL. We will consider the upper limit of normal values of GOT and GPT about 40 UI/L; values greater than three to five times this upper limit indicate hepatotoxicity and requires stopping treatment. Normal levels of creatinine are between 0.6 and 1.1 mg/dl for women and 0.7 and 1.3 for men. An increased creatinine maintained in different determinations associated with signs of renal failure is an indication for stopping treatment.
- **A urine strip to detect proteinuria:** in adults, proteinuria is defined for the presence of urine proteins above 150 mg in 24 hours.
- **Measurement of the blood pressure:** before the measurement of the blood pressure, the subject must not have consumed caffeine or smoked any cigarette in the past 30 minutes and must not have done exercise. The subject must rest at least 5 to 10 minutes before the measurement. At the time of the measurement, the subject have to be sitting with both feet touching the ground, and the forearm should be supported at the level of the heart. The sphygmomanometer will be placed around the arm so that the bottom of this remains an inch above the elbow flexure. 3 measurements will be done at intervals of 1 minute. Levels below 120/80 mmHg are considered normal (absence of hypertension).

### 5.6.3 COVARIATES

These variables have to be taken into account to interpret the outcomes due to their influence on the development of a relapse of MS. Covariates with significant differences will be analysed with a multivariate analysis. We also want to take into account some other interest variables which be useful to describe our study population and that could be used for a deeper analysis in our research.

- **Age:** Age has shown an association with the risk of developing MS in patients with RIS, increasing the risk as more young is the patient, especially below the age of 37 (25). A younger age at RIS diagnosis was associated with an increased risk of developing an

initial symptomatic event with an estimated risk of developing an event decreasing by 2% for every year additional year of age (25). The age will be collected by clinical history.

- **Sex:** It has been demonstrated an elevated risk of developing MS in patients with RIS in males than in females in a ratio of nearly 2:1 (25). Sex will be recorded by clinical history.
- **Age at disease onset and duration of the disease:** this data will be collected by clinical history.
- **Demographic characteristics:** they will be collected by anamnesis and the poblational census.
- **Family history of MS:** it will be recorded by anamnesis and clinical history.
- **Detailed historical and clinical data:** collected by anamnesis and clinical history.
- **EDSS score at baseline:** collected by clinical history and also measured in the screening visit by the examining neurologist, as explained above.
- **Concomitant treatment:** we have to collect all concomitant treatment received by patients by direct anamnesis and clinical history.

## 5.7 ORGANIZATION AND PROCEDURES

### 5.7.1 SCREENING VISIT

During the screening visit, which will be performed during the first 4 months of the study, the following parameters must be collected:

- Check-up of the inclusion and exclusion criteria
- Elaboration of a detailed clinical history and collection of other demographic and clinical data (covariates)
- Neurologic examination, which must include EDSS and assessment of FS
- Brain and spinal MRI
- Blood test that will include blood cell count as well as liver and renal function
- Basal measure of study variables
- Concomitant treatment

### 5.7.2 BASAL VISIT

Basal visit will be done in the fifth and sixth month of the study, and this will be the first month of treatment. Hereinafter, we will denominate it as month 1. The treatment will have a duration of 18 months for each patient from basal visit. The following parameters will be evaluated:

- Revision of the inclusion and exclusion criteria
- Randomization of subjects into an interventional arm (IFN  $\beta$ -1b and placebo) with a proportion of 1:1
- Concomitant treatment
- Introduction of contraception in women of childbearing age

### 5.7.3 FOLLOW-UP ASSESSMENTS

During these visits in the Neurology area of each participating centre, which will be done with a periodicity of 3 months (in months 3, 6, 9, 12, 15 and 18), the following evaluations must be done:

- Anamnesis and neurological examination of the different functional FS aimed at recognizing any possible MS relapse
- Punctuation in the EDSS
- Evaluation of any possible side effect due to the administration IFN  $\beta$ -1b, by direct anamnesis and physical examination
- Concomitant medication
- Blood test: we will evaluate the parameters that can be modified by treatment with IFN  $\beta$ -1b, such as glucose, liver enzymes (including AST (SGOT) and ALT (SGPT)), renal function and complete blood cell count.
- Urine strip
- Measurement of blood pressure

These measures will be collected in visits 1 to 6 (corresponding to months 3, 6, 9, 12, 15 and 18).

Moreover, every 12 months, we will perform an imaging study with the MRI protocol. Thus, we will perform it in screening visit, visit 4 and visit 8 (in months -2 to 0, 12 and 24), the last one 6 months after the last dose of medication.

A period of 6 months of follow-up will be done after the end of treatment in order to collect any additional side effect of the medication and ensure the safety of all patients. It will be collected:

- Clinical laboratories (every 3 months, visit 7 and 8 in months 21 and 24)
- Presence of side effects (every 3 months, visit 7 and 8 in months 21 and 24)
- MRI protocol (visit 8 in month 24)

**Table 3 Follow-up assessments.**

<b>FOLLOW-UP ASSESSMENTS</b>	<b>Screening Visit</b>	<b>Basal visit</b>	<b>Visit 1 (3 months)</b>	<b>Visit 2 (6 months)</b>	<b>Visit 3 (9 months)</b>	<b>Visit 4 (12 months)</b>	<b>Visit 5 (15 months)</b>	<b>Visit 6 (18 months)</b>	<b>Visit 7 (21 months)</b>	<b>Visit 8 (24 months)</b>
Checking-up of inclusion and exclusion criteria										
Elaboration of clinical history and collection of data (covariates)										
Randomization into two arms										
Introduction of contraception in women of childbearing age										
Neurologic examination (FS + EDSS)										
Brain and spinal MRI										
Blood test										
Urine strip										
Measurement of blood pressure										
Side effects										
Concomitant treatment										

## 6. STATYSTICAL ANALYSIS

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The results are expressed as percentages for categorical variables.

All independent and dependent variables in the study are categorical and we will express them in each group as proportions. Proportions were compared with the chi square test (used to compare two qualitative proportions).

In order to appreciate the association between the dependent and the independent variable and to adjust the effect of confusion, a multivariate analysis will be performed. When the appearance of a first clinical event and the administration of treatment with IFN  $\beta$ -1b or placebo are compared, a logistic regression analysis is used. When the variables to compare are the radiologic progression, disability progression and the appearance side effects with the administration of the treatment, the same model is used.

These analysis results in a generation of relative risks (RR) with 95% confidence interval (CI) and p values. Values of  $P < 0.05$  are considered statistically significant in all tests.

All statistical analysis will be carried out using the Statistical Package for Social Science (SPSS). To manage computed data, Microsoft Excel tool will be used. Analysis will be done in intention to treat.

## 7. ETHICAL CONSIDERATIONS

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All basic ethical principles will be respected according the World Medical Association Declaration of Helsinki - Ethical Principles for Medical Research Involving Human subjects (last actualization October 2013) (28), and it will also be conducted with the fulfilment of the protocol, in accordance with ethical and methodological aspects of Good Clinical Practice guidelines in the European Union.

The trial must be evaluated by the Clinical Research Ethics Committee (CEIC) of all centres participating in the study, and their approval must be obtained before initiating the study. AEMPS must also authorise the clinical trial.

The clinical trial will be performed in agreement with all national and international legal frameworks related to clinical trials of the countries in which the clinical trial will be performed, as well as any applicable guidelines. It will be conducted under the normative framework of these laws:

- “RD 223/2004 de 6 de febrero: ensayos clínicos con medicamentos”
- “RD 1591/2009 de 16 de octubre y 1616/2009 de 26 de octubre: investigación con productos sanitarios”
- “Ley 29/2006 de 26 Julio, de garantías y uso racional de los medicamentos y productos sanitarios”

The trial will be registered in ClinicalTrials.gov (<http://clinicaltrials.gov.com>) and in EUDRA-CT, as it is now recommended.

Prior to the beginning of the investigation, every subject participating in the clinical trial must be properly informed about the study to the fullest extent using language and terms they are able to understand in order to allow a fully knowledgeable decision. Patients will be informed on the aim, procedures, anticipated benefits, and potential hazards of the study. Patients will be given an information sheet (ANNEX 10) containing information about the study before they are included in the clinical trial. Prior to the participation in the clinical trial, the written informed consent (ANNEX 11) must be obtained and signed by the patient or by the patient’s legally acceptable representative and the investigator. A copy of the informed consent will be provided to the patient. It will also be explained to the participants that they are free to refuse entry into the study and to withdraw from the study at any time without prejudice to future treatment.

All the information and data collected from each patient during the course of the trial will be treated and used anonymously, preserving the confidentiality of the patient according to the

“Ley Orgánica 15/1999, de 13 de diciembre, de Protección de Datos de Carácter Personal”, in order to guarantee and protect the public liberties and fundamental rights of persons. Subjects will be identified by their unique identification numeric code instead of their names. Personal patient data (personal identity and all personal medical information) will be maintained in privacy. In any presentation of the results of this study at conferences or publications, the patient identities will remain confidential.

An insurance will be taken out by the sponsor in order to take the responsibility towards its members if any damage or serious side effect is suffered because of our intervention. The insurance policy will be in accordance with local laws and requirements in each country. Information regarding compensation, insurance and indemnity is addressed in the insurance policy.

All investigators will have to declare no conflict of interest in any of the aspects of the study.

The first ethical problem of this study is related with the characterization of RIS. As RIS constitutes a pathological condition which means an increased risk of developing MS, it is important to definite this syndrome as concisely as possible. This implies the realization of many diagnostic and laboratory tests, including the extraction of a CSF sample by lumbar puncture in order to detect oligoclonal bands that are suggestive of a primary inflammatory process of the CNS. As RIS consists in a pathological finding in healthy subjects who do not experience any sign or symptom, and the realization of a lumbar puncture in these subjects is not a necessary procedure to diagnose it (because the diagnostic of RIS do not include CSF findings, it only means an increased risk of converting to MS), the performing of this procedure is not essential. Furthermore, we have to balance the benefits and the potential risks that can lead the performing of an invasive procedure like this in healthy patients without any clinical findings. Thus, participants are not subject to the realization of a lumbar puncture in order to preserve the principle of non-maleficence.

Finally, another problem could be the ethical dilemma of treating for all life healthy patients with only radiological findings suggestive of the development of a pathology on the future, although not all patients will develop the disease in their lifetime. This has to be seen as an opportunity to avoid new cases of MS, a disease that, on the other hand, causes a great disability and a decrease of QoL in those suffering from it, so we are acting in favour of the principle of beneficence. The use of placebo is justified because any treatment is approved for these patients.

## 8. STUDY STRENGTHS AND LIMITATIONS

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To discuss about the limitations of the study we must make reference to bias, the type of study and the sample size. The concept of bias is the lack of internal validity or incorrect assessment of the association between an exposure and an effect in the target population in which the statistic estimated has an expectation that does not equal the true value (29). The most important biases are those produced in the definition and selection of the study population (selection bias), data collection (information bias), and the association between different determinants of an effect in the population (confounding) (29).

The selection bias is the error introduced when the study population does not represent the target population (29). Examples of selection bias that can occur in this trial are:

- Healthy worker bias: it consists in the participation of sicker subjects in the clinical trial than normal population when we are doing the recruitment of subjects.
- Referral bias: Our population, who are mainly outpatient subjects, may not represent the whole RIS population (inpatient subjects, primary health care subjects...).
- Healthcare access bias: when the patients admitted to an institution do not represent the cases originated in the community.
- Length-bias sampling: cases with diseases with long duration are more easily included in surveys. This series may not represent the cases originated in the target population, and usually have a better prognosis.
- Lack of accuracy of sampling frame: The most common bias is non-random sampling bias that can yield a non-representative sample in which a parameter estimate differs from the existing at the target population. We can solve it applying randomisation.
- Lack of intention to treat analysis: We will perform intention to treat analysis by including the results of those subjects who had not completed the assigned treatment period until the end of the study, but we have the final variable measurements of them. In randomised studies the analysis should be done keeping participants in the group they were assigned to. If non-compliant participants or those receiving a wrong intervention are excluded from the analysis, the branches of a randomised trial may not be comparable.
- Allocation of intervention bias: it occurs when intervention is differentially assigned to the population. It is more common in non-randomised trials. In randomised trials it is recommended concealment of the allocation sequence of intervention. If concealment is unclear or inadequate, larger estimates of treatment effects are reported.

- During study implementation, the three most common biases are the following:
  - o Losses/withdrawals to follow up: when they are uneven in both the exposure and outcome categories, the validity of the statistical results may be affected.
  - o Missing information in multivariable analysis: multivariable analysis selects records with complete information on the variables included in the model. It occurs when participants with complete information do not represent the target population.
  - o Non-response bias: when participants differ from non-participants.

Information bias results from the concept that measures are collected incorrectly, and occurs during data collection. Such exposure, disease and variables are measured with a certain sensitivity and specificity. The accuracy of a test is the ability to correctly diagnose patients, as applied to positive and negative results. The possible selection bias in our study are:

- Misclassification bias: It is originated when sensitivity and/or specificity of the procedure to detect exposure and/or effect is not perfect, that is, exposed/diseased subjects can be classified as non-exposed/non-diseased and vice versa.
- Detection bias occurs when there is lack of blind. In our study all individuals, researchers, evaluators and statistics must be blind.
- Observer/interviewer bias: the knowledge of the hypothesis, the disease status, or the intervention received can influence data recording (observer expectation bias). The means by which interviewers can introduce error into a questionnaire include administering the interview or helping the respondents in different ways, putting emphases in different questions, with gestures and so on.
- Reporting bias: participants can “collaborate” with researchers and give answers in the direction they perceive are of interest (obsequiousness bias).

The last three biases can be reduced using blinding, a procedure by which subjects ignore some important aspects of a research to avoid differential misclassification bias. In trials, blinding means that participants do not know the intervention they receive (participants blinding) and/or observers do not know the intervention received by participants (observer blinding), and/or data analysts do not know the labels of the groups to be compared.

- Compliance bias: in trials requiring adherence to intervention, the degree of adherence (compliance) influences efficacy assessment of the intervention.
- Hard vs soft endpoints: hard measures are those that provide more reliable results, and should be used instead of the measures whenever possible. Example of hard measures

used in this study are laboratory measures. However, most other variables collected are soft measures (like subjective symptoms, physical signs, disease events that are difficult to diagnose, some side effects of drugs such as rash and nausea), but we cannot omit them because the diagnostic of a relapse is clinical, and any hard measure can replace anamnesis and neurological examination.

Confounding consists in the existence of variables that can distort the relationship between the dependent variable and independent variable. It occurs when a variable is a risk factor for an effect among non-exposed persons and is associated with the exposure of interest in the population from which the effect derives, without being affected by the exposure or the disease (in particular, without being an intermediate step in the causal pathway between the exposure and the effect) (29). Confounding can be neutralised at the design stage of a research (for example, by randomisation) and/or at the analysis, given that the confounders have been measured properly (29). Randomisation consists in random assignment of individuals in an experimental study in either the treatment group or the placebo group. All confounders are equally distributed between the groups if the sample size is sufficiently large (both measured and unmeasured factors). The goals of randomisation are to avoid confounding and selection bias (29).

In order to minimize the possibility of bias, the following actions will be performed:

- Randomisation
- Blinding process
- Using, when possible, hard measures (laboratory tests, MRI)
- Train investigators to collect the different measures and standardize
- Collecting data of reliability of measures
- Performing sensitivity analysis
- Using the same mechanism of measurement of the event in the exposed and unexposed group

The type of study is a clinical trial, so the level of causality is high. It is the study design with more evidence. Moreover, it is a multicentre study, so we can generalize the findings to the general population. However, it is the type of study with higher costs.

Regarding to the sample size, the limitation in this case is not the sample size, but the organization to achieve all necessary subjects, so there is a logistical difficulty. RIS is a very rare syndrome and the number of patients among the world is scarce. To detect the minimal clinically relevant difference on the rate of RIS patients experiencing a first clinical event suggestive of MS

between the treated group and the group receiving placebo, the sample needed are 261 subjects in each arm, a total of 522 subjects. It means that the research study must be multicentre and international, which can be possible with the participation of the RISC (Radiologically Isolated Syndrome Consortium). With the participation of the centres attached to this consortium, we are able to contact people with this syndrome and perform the successive follow-up visits and imaging examinations.

Another limitations of our investigation are:

- The high costs of conducting a clinical trial.
- The lack of results from other diagnostic studies (neuropsychiatric testing and lumbar puncture) that may detect the presence of clinical deficits not appreciated on routine neurologic evaluation.
- The side effects of the study drugs can impair the blinding process and induce a procedure bias.
- Intervention will be applied by the patient following our instructions. Thus, we do not know if they will do it correctly and if the exact dose needed will be administered. This can interfere in our results.

## 9. WORK PLAN

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### 9.1 RESEARCH TEAM

The research team will be composed by:

- The study coordinator or principal investigator (PI) of the main centre (Josep Trueta Hospital).
- A monitor (M, which will act as a link between the PI and researchers of each participating hospital).
- Two neurologists from neuroimmunology/multiple sclerosis survey (N), the exploring neurologist (EN), and the treating neurologist (TN). They will work as the main researchers for each hospital (two for centre). All of them will be blinded to the treatment groups. They will also perform data collection and handle entering data into the database.
- A neuroradiologist (NR).
- A nurse (Nu, one for each centre).
- A statistical specialist (SS).

### 9.2 STUDY CHRONOGRAM (ANNEX 12)

The study will be achieved in 44 months (3 years and 8 months) and will be organized according to the following 4 phases:

#### *PHASE 1: PREPARATION AND COORDINATION (4 MONTHS)*

Prior to the first meeting, the IP and N will have conducted a literature search to prove the importance of the study (2 months).

#### Meeting 1: Study research proposal and evaluation by ethical committees (2 months)

(PI, M, N and CEICs of each hospital): An organization meeting will be held initially. Coordination of all centres and researchers teams before starting the pilot experiment will be performed.

- The protocol has to be discussed and evaluated by the members of the study to make sure that it has been fully understood and will be followed according to what's been established, and make sure that all centres agree with the procedures. Explanation of the project design and execution plan, the system and procedures of patient's selection

(inclusion and exclusion criteria), data recruitment and management and central data monitoring. Details will be discussed in order to ensure homogeneity in all items. The timeline will be examined and the methods of data collection will be shared in the database.

- The team will discuss about the most suitable communication system that will be used through the trial. Among all the study, regular feedback will be provided to each participating centre and adequate methods of communication will be established between the monitor (M) and the neurologists (EN and TN) of each hospital.
- Final drafting of the definitive protocol and coordination and training of all the research team.
- The protocol has to obtain the ethical approval by all participating hospital ethical committees (CEIC).

#### Eligibility of collaborating centres (2 months)

(PI, M): Approach and selection of centres participating in the study, checking the quality of the staff according to the needs for the trial. Every centre will select one EN, one TN and one Nu. Collaborating centres have to meet the following criteria in order to ensure the appropriateness of their participation:

- Previous experience of research.
- Existence of a neuroimmunology/multiple sclerosis department.
- Neurologists specialized in the diagnosis of MS.
- Presence of patients diagnosed of RIS registered in the service.
- Availability of MRI of 1.5 tesla and other relevant information (variables described in the protocol) in the clinical history of patients.

#### Pilot data extraction (1 month)

(PI, M, N): A first extraction of data will be done during pilot experiment.

#### Meeting 2 (1 month)

(PI, M, N): A second in person meeting will be done after pilot experiment to detect problems, mistakes and possible failure of coordination. Proposal of changes.

#### *PHASE 2: FIELD WORK AND DATA COLLECTION (30 MONTHS)*

#### Subjects recruitment, randomization in the two study groups and data collection (6 months)

(SS, EN, TN and NR): Patients will be proposed to take part in the study by the neurologists involved in the study in each centre. Patients will be included from each hospital until the sample

size is achieved. Non-probability consecutive sampling will be performed in the participating centres. Every patient diagnosed with RIS will be approached at the participating centres. The neurologists will perform the explanation of the purposes of the study and will inform about all the procedures to both groups of participants. Then, each participant will be given the information sheet and the informed consent. At least six months will be required to collect all the information from the medical records, considering they will have to be collected from different sources and hospitals. Study variables will be collected by the N, and MRI will be assessed by the NR. The nurse (Nu) will explain how to perform self-injectable medication.

- Screening visit (4 months): Checking up of the inclusion and exclusion criteria, elaboration of a detailed clinical history and collection of other demographic and clinical data (covariates), and collection of study variables.
- (SS): Preparation of randomization: design of statistical software to carry out the probability sampling.
- Basal visit (2 months): Revision of the inclusion and exclusion criteria, randomization of patients into an interventional arm (IFN  $\beta$ -1b and placebo) with a proportion of 1:1, collection of concomitant treatment and introduction of contraception in women of childbearing age.

#### Data collection (18 months)

(EN): Anamnesis and neurological examination of the different functional systems aimed at recognizing any possible MS relapse, punctuation in the EDSS.

(TN): Evaluation of any possible side effect due to the administration of IFN  $\beta$ -1b, collection of concomitant medication.

(NR): analysis of MRI from each subject.

(Nu): performing of the blood test, urine strip, measurement of blood pressure.

#### Evaluation of correct data collection (18 months)

(PI, M): Data will be entered in the database simultaneously with the trial development. A regularly evaluation and validation of the data collected in each centre will be required in order to control its evolution, thus defining and classifying the information obtained using the guidelines described in the variables section of this protocol, and also a checking if the following of the protocol is performed. Within all this period the monitor will perform controls in all the hospitals to ensure adequate data collection.

#### Teleconference meetings (24 months)

(PI, M, EN and TN): Quarterly meetings will be held for the collection of information from the different centres. This teleconference meetings will be done to review and evaluate the recruiting, the quality and homogeneity of data collected and to discuss the progress of the study. The first one will be at the beginning of the recruitment period and the second one in the end of that phase. The following meetings will take place quarterly during all data collection period.

#### Follow-up period (6 months)

(EN, TN and NR): A period of 6 months of follow-up will be done after the end of treatment in order to collect any additional side effect of the medication and ensure the safety of all patients, and a final MRI will be done.

#### *PHASE 3: DATA ANALYSIS AND FINAL EVALUATION (4 MONTHS)*

##### Meeting 3: Statistical analysis and analysis, interpretation and discussion of results (4 months)

(SS, PI, N): Once interventions are completed, all data collected in the database will be analysed using the appropriate statistical test. At least four months will be required to perform the statistical analysis exposed, to interpret the results from the statistical analysis performance, and to discuss them with all participating centres in order to achieve a conclusion of the outcomes. The statistical analysis of the results obtained will be done by the SS.

#### *PHASE 4: ARTICLE PUBLICATION AND SCIENTIFIC DIFFUSION OF THE RESEARCH FINDINGS (6 MONTHS)*

(PI, N): The principal researchers of each centre will write and edit a final report with the results and conclusions, and findings will be ultimately published. They will assist to conferences to disseminate findings at national and international level. As a multicentre clinical trial is performed, each investigator can be responsible for local dissemination. We will send the article to different neurologic journals and magazines for its official publication.

## 10. BUDGET

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The budget will be calculated for each study participant and adjusted for estimated total sample size. The study drugs will be provided by the pharmaceutical company.

Materials used in daily clinical practice and visits carried out in centers attached are not considered additional costs of the study.

**Table 4 Budget required for each participant.**

Description	Amount	Cost	Total cost
Brain MRI with contrast	3	177 €	531 €
Blood tests	9	45 €	405 €
Urine strip	9	20 €	180 €
			<b>TOTAL 1.116 €</b>

**Table 5 Total study cost.**

Description	Cost
522 participants	582.552 €
Statistical analysis (40h x 20€/h)	800 €
Article scientific revision and publication	1.500 €
MS international meeting	1.000 €
In person meetings (travels and diet)	4.000 €
<b>TOTAL STUDY COST 589.852 €</b>	

## 11. CLINICAL AND HEALTH SYSTEM IMPACT

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MS is one of the most common causes of disability among young people (6). Currently, there is not any cure for this disease yet, leaving both patients and their caregivers with the challenge of living with a chronic medical condition that affects their health-related QoL (6). MS follows a long and unpredictable course, and often leads to substantial disability accumulated over time (30). Moreover, MS largely occurs in people of working age, so it may have an adverse impact on employment status, work productivity, and health-related QoL (30).

Research has shown that certain individuals (women and those diagnosed before age of 35), certain disease courses (RRMS with long intervals between relapses and complete recovery from relapses), and certain disease symptoms (sensory symptoms during relapses instead of motor symptoms) have better prognosis (8). However, the majority of patients perceive at least some degree of impairment in most domains as early as the first year of disease (especially sensory symptoms and fatigue), with the severity of impairment increasing with disease duration across all domains, although the patterns of disability accumulation differ (20). The disease impact transcends ambulatory and motor functions, which are the focus of traditional disability assessment scales in MS (such as the EDSS), and extends to nearly every neurologic domain (20).

The survival in MS is shortened (14). Compared to the general population, MS patients live some 5–7 years less (14). The standardized mortality ratio is 2–3 times higher for patients with MS than people without MS in the general population, with differences emerging as early as 2–10 years after MS diagnosis (13). In comparison with the general population, MS patients have a higher risk for death from all causes except cancer, and the mortality rate due to cardiovascular disease is significantly higher than in general population (31). Suicide is more frequent during the first few years after diagnosis (31).

The development of a progressive course is by far the most deleterious event in the case of an MS patient (14). Individuals with progressive subtypes of MS, particularly the PPMS subtype, have a more rapid decline in function (32). In the PPMS, supportive equipment (such a wheelchair or standing frame) is often needed after six to seven years, while in the RRMS, the average time until such equipment is needed is twenty years (32). However, this means that many individuals with MS will never need a wheelchair (32).

The apparent role of exacerbations early in the disease is more a reflection of the active state of the illness than having a direct causal relation to subsequent disability (14). Once the disease begins to develop a progressive phase, perhaps at a time long before clinical symptoms arise,

the course of deterioration seems remarkably the same irrespective of the presence of prior or subsequent exacerbations or their frequency (14). This has implications for the likelihood that relapse suppression therapies will be effective when progression has begun (14).

The earlier in life MS occurs, the slower disability progresses (32). Individuals who are older than fifty when diagnosed are more likely to experience a chronic progressive course, with a more rapid progression of disability (32). Those diagnosed before the age of 35 years old have the best prognosis (32). Females generally have a better prognosis than males, although women fall in depression and feel fatigue more often (32).

QoL is a multi-dimensional construct which consists of at least three broad domains: physical, mental and social (32). Health-related QoL is a concept which specifically focuses on the impact of an illness and/or treatment on patients perception of their status of health and on subjective wellbeing or satisfaction with life (32). Patients with MS rate their health related QoL lower than general population, and also lower than patients with other chronic diseases (33). Measurement of QoL of patients with MS is of interest for their medical care, rehabilitation and nursing (33).

Cognitive dysfunctions are observed in 40-65% of patients with MS, and there is also a more cognitive impairment in the progressive forms than in the RRMS (32,34). Cognitive dysfunction may subsequently result in reduced fulfilment in work life and social life as well as in a reduction in QoL (34). The prevalence of depression in patients with MS is estimated in 15 to 60% of patients, being more often in women (32). Depressive mood is the main factor influencing QoL (32). Fatigue is one of the most common symptoms of MS and it is associated with reduced QoL, being also more common in women (32). The disability status, fatigue and reduced sleep quality have an impact mainly on physical domains of life quality (32). Depression, fatigue and disability level are significant and independent predictors of QoL (32,34).

Patients with higher disability revealed significantly worse QoL related to activities of daily living than those with less pronounced disabilities (34). Mobility loss was negatively correlated with patients ability to complete instrumental activities of daily living, such as the most complex daily tasks including communication and transportation (30). Patients have more difficulty adjusting to early changes in mobility and consequently have to reduce the level of activity and work in which they engage (30). Further changes in mobility later in the course of the disease appear to have less impact, suggesting patients have already adjusted to their disease state and developed compensatory strategies to overcome or attenuate limitations (30). Wheelchair use is not an inevitable outcome in MS; after 30 years of disease, only about one in five patients report the need for a wheelchair use or worse, and about the same proportion of patients record no or

minimal mobility problems (20). Changes in rate of disease accumulation are due, at least in part, to improvements in prophylactic and symptomatic management of MS, including, importantly, greater emphasis on physical therapy and exercise to maintain ambulatory function (20).

Currently, MS does not have a cure, though several treatments that may slow the appearance of new symptoms are available (32). Treatment with IFN  $\beta$  reduces the progression of impairment in patients with MS but brings about adverse effects, that may have a deleterious effect on QoL (33). Because of slower deterioration caused by these treatments, QoL of patients given DMT is better in spite of the side effects (33). Treatment is also associated with a slower deterioration of health-related QoL (33). There are benefits such as an expected reduction of disease activity and constant access to health care in conjunction with treatment and follow up (33).

The treatment of patients with MS has changed over the past 10 years, with several new potent treatments introduced in an area where treatment options had been limited (35). Compared with the old and inexpensive symptomatic treatments, the new DMT seem costly, and it must be expected that healthcare costs for patients with MS have increased (35). Also, the new treatments are likely to lead to more intensive patient management, thus potentially increasing costs further (35). Finally, as our knowledge of MS has improved, pathological and therapeutic criteria have been modified and a diagnosis is often made earlier, increasing the patient population that is eligible for treatment and thus potentially increasing treatment costs (35). As a consequence, the interest in economic evaluation of multiple sclerosis has intensified (35). On the other hand, treatment for MS aims at avoiding temporary disability due to relapses and, more importantly, delaying the progression to more permanent disability (35). Thus, the major economic benefit of treatment lies in the future; savings will come from delaying or preventing patients' progression to more severe disease, which is associated with high costs and low QoL (35).

MS is associated with a large burden to society, mainly due to substantial increases in indirect costs and decreased health related QoL that occur in conjunction with mobility impairment (30). Costs for patients with MS in Europe increase more than threefold to fourfold in patients with severe disease (EDSS 7.0) compared with patients with an earlier disease state (EDSS 4.0); the effect of advancing disease is detrimental on QoL (35). Drugs that slow disease progression early on, thus avoiding or delaying the severe disease states in which patients are unable to work and become dependent on help from their family, will provide large benefits to society (35).

All types of costs increase with worsening disease (35). Productivity losses still represent the single highest contributor to societal costs (35). Costs of lost productivity (indirect costs) and pain and suffering (intangible costs) increase with worsening disease severity (30). The largest relative increases in indirect costs and utility decrements were seen at earlier mobility impairment stages (30). Informal care use was highly correlated with disease severity, but was further influenced by healthcare systems and family structure (35).

The total mean annual costs per patient were estimated at 18000€ for mild disease (EDSS 4.0), 36500€ for moderate disease (EDSS 4.0–6.5) and 62000€ for severe disease (EDSS 7.0) (35). Utility was similar across countries at around 0.70 for a patient with an EDSS of 2.0 and around 0.45 for a patient with an EDSS of 6.5 (35). Intangible costs were estimated at around 13000€ per patient (35). The cost of a relapse of MS was similar across the countries, ranging between 2800€ and 4000€ (35). Studies have shown that, when controlling for relapses, costs were not different for patients with different courses of MS at the same level of EDSS (35).

The proportion of patients who were working ranged between 25% and 40%, depending on the proportion of patients aged >65 years in the samples (35). An average of 35% of patients were in early retirement because of MS (35). The effect of the disease on employment is very pronounced (35). Although at EDSS 0.0–1.0, about 70–80% of patients <65 years are employed, this proportion is <10% for patients with EDSS 8.0–9.0 (35). Approximately 50% of patients indicated that they had to reduce the number of hours worked or change their type of work, and subsequently, this was associated with a loss of income (35).

In conclusion, if this research study demonstrates that treatment of RIS patients with IFN  $\beta$ -1b has a true effectiveness in reducing the proportion of those experiencing a MS relapse, and subsequently, avoiding the development of this disease, the impact on the clinical management of these patients could be huge. The treatment of these patients would avoid not only the progression towards a disease which almost invariably leads to some degree of disability and reduced QoL, but also save a huge cost to the health system aimed at care of these patients, as well as avoiding losses in productivity.

## 12. ANNEXES

### 12.1 ANNEX 1: CLINICAL COURSES OF MS

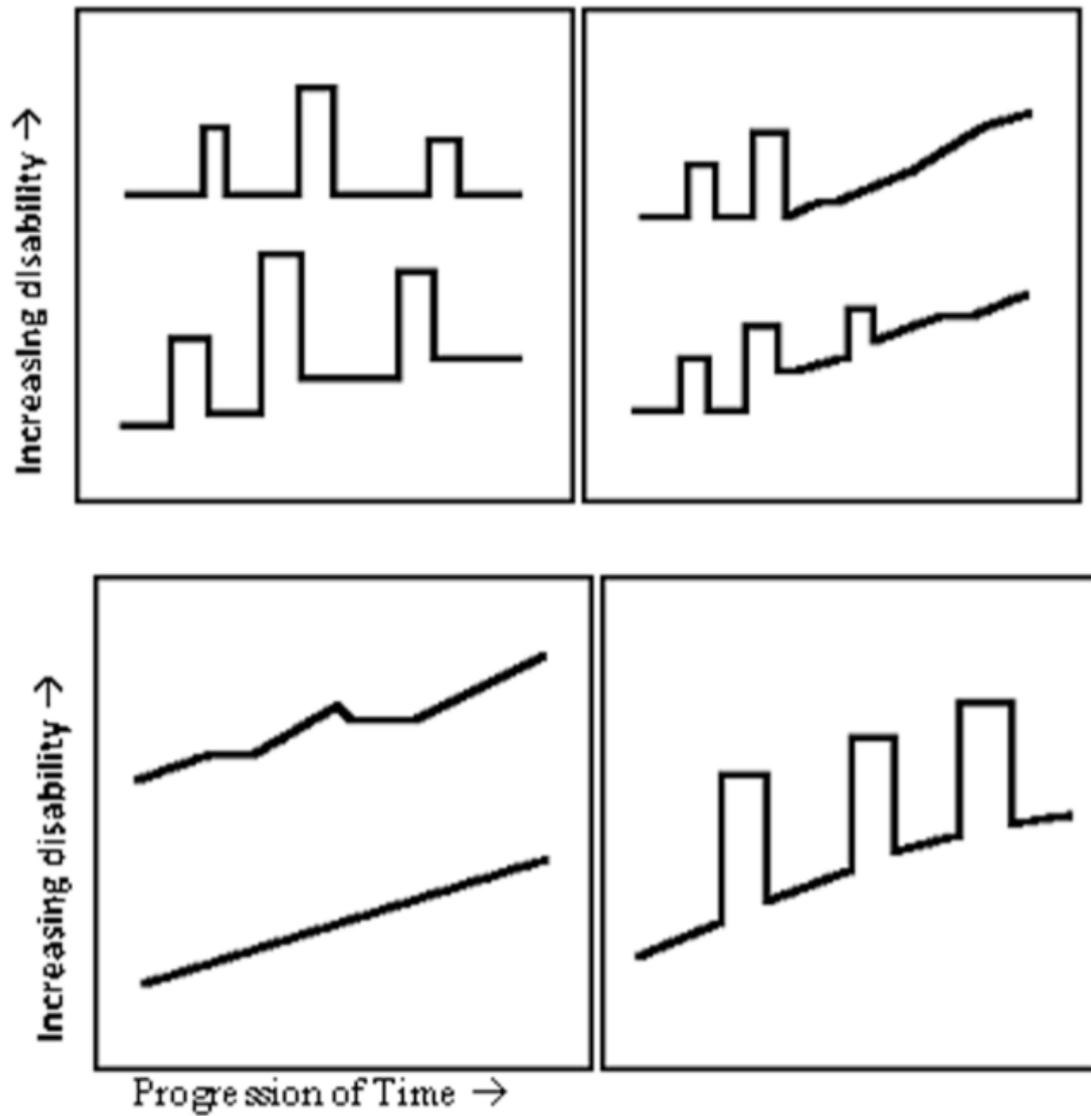


Figure 1 Types of MS (left to right; top to bottom): RRMS, SPMS, PPMS, PRMS (8).

12.2 ANNEX 2: MCDONALD 2010 DIAGNOSTIC CRITERIA FOR MS

TABLE 4: The 2010 McDonald Criteria for Diagnosis of MS	
Clinical Presentation	Additional Data Needed for MS Diagnosis
≥2 attacks <sup>a</sup> ; objective clinical evidence of ≥2 lesions or objective clinical evidence of 1 lesion with reasonable historical evidence of a prior attack <sup>b</sup>	None <sup>c</sup>
≥2 attacks <sup>a</sup> ; objective clinical evidence of 1 lesion	Dissemination in space, demonstrated by: ≥1 T2 lesion in at least 2 of 4 MS-typical regions of the CNS (periventricular, juxtacortical, infratentorial, or spinal cord) <sup>d</sup> ; or Await a further clinical attack <sup>a</sup> implicating a different CNS site
1 attack <sup>a</sup> ; objective clinical evidence of ≥2 lesions	Dissemination in time, demonstrated by: Simultaneous presence of asymptomatic gadolinium-enhancing and nonenhancing lesions at any time; or A new T2 and/or gadolinium-enhancing lesion(s) on follow-up MRI, irrespective of its timing with reference to a baseline scan; or Await a second clinical attack <sup>a</sup>
1 attack <sup>a</sup> ; objective clinical evidence of 1 lesion (clinically isolated syndrome)	Dissemination in space and time, demonstrated by: For DIS: ≥1 T2 lesion in at least 2 of 4 MS-typical regions of the CNS (periventricular, juxtacortical, infratentorial, or spinal cord) <sup>d</sup> ; or Await a second clinical attack <sup>a</sup> implicating a different CNS site; and For DIT: Simultaneous presence of asymptomatic gadolinium-enhancing and nonenhancing lesions at any time; or A new T2 and/or gadolinium-enhancing lesion(s) on follow-up MRI, irrespective of its timing with reference to a baseline scan; or Await a second clinical attack <sup>a</sup>
Insidious neurological progression suggestive of MS (PPMS)	1 year of disease progression (retrospectively or prospectively determined) plus 2 of 3 of the following criteria <sup>d</sup> : 1. Evidence for DIS in the brain based on ≥1 T2 lesions in the MS-characteristic (periventricular, juxtacortical, or infratentorial) regions 2. Evidence for DIS in the spinal cord based on ≥2 T2 lesions in the cord 3. Positive CSF (isoelectric focusing evidence of oligoclonal bands and/or elevated IgG index)

If the Criteria are fulfilled and there is no better explanation for the clinical presentation, the diagnosis is "MS"; if suspicious, but the Criteria are not completely met, the diagnosis is "possible MS"; if another diagnosis arises during the evaluation that better explains the clinical presentation, then the diagnosis is "not MS."

<sup>a</sup>An attack (relapse; exacerbation) is defined as patient-reported or objectively observed events typical of an acute inflammatory demyelinating event in the CNS, current or historical, with duration of at least 24 hours, in the absence of fever or infection. It should be documented by contemporaneous neurological examination, but some historical events with symptoms and evolution characteristic for MS, but for which no objective neurological findings are documented, can provide reasonable evidence of a prior demyelinating event. Reports of paroxysmal symptoms (historical or current) should, however, consist of multiple episodes occurring over not less than 24 hours. Before a definite diagnosis of MS can be made, at least 1 attack must be corroborated by findings on neurological examination, visual evoked potential response in patients reporting prior visual disturbance, or MRI consistent with demyelination in the area of the CNS implicated in the historical report of neurological symptoms.

<sup>b</sup>Clinical diagnosis based on objective clinical findings for 2 attacks is most secure. Reasonable historical evidence for 1 past attack, in the absence of documented objective neurological findings, can include historical events with symptoms and evolution characteristics for a prior inflammatory demyelinating event; at least 1 attack, however, must be supported by objective findings.

<sup>c</sup>No additional tests are required. However, it is desirable that any diagnosis of MS be made with access to imaging based on these Criteria. If imaging or other tests (for instance, CSF) are undertaken and are negative, extreme caution needs to be taken before making a diagnosis of MS, and alternative diagnoses must be considered. There must be no better explanation for the clinical presentation, and objective evidence must be present to support a diagnosis of MS.

<sup>d</sup>Gadolinium-enhancing lesions are not required; symptomatic lesions are excluded from consideration in subjects with brainstem or spinal cord syndromes.

MS = multiple sclerosis; CNS = central nervous system; MRI = magnetic resonance imaging; DIS = dissemination in space; DIT = dissemination in time; PPMS = primary progressive multiple sclerosis; CSF = cerebrospinal fluid; IgG = immunoglobulin G.

Figure 2 The 2010 McDonald Criteria for Diagnosis of MS (17).

**12.3 ANNEX 3: PROPOSED DIAGNOSTIC CRITERIA FOR THE RADIOLOGICALLY ISOLATED SYNDROME (2009)** (Adapted from (24))

- A. The presence of incidentally identified CNS white matter anomalies meeting the following MRI criteria:
  - 1. Ovoid, well-circumscribed, and homogeneous foci with or without involvement of the corpus callosum.
  - 2. T2 hyperintensities measuring >3mm and fulfilling Barkhof criteria (at least 3 out of 4) for dissemination in space\*.
  - 3. CNS white matter anomalies not consistent with a vascular pattern.
- B. No historical accounts of remitting clinical symptoms consistent with neurologic dysfunction.
- C. The MRI anomalies do not account for clinically apparent impairments in social, occupational, or generalized areas of functioning.
- D. The MRI anomalies are not due to the direct physiologic effects of substances (recreational drug abuse, toxic exposure) or a medical condition.
- E. Exclusion of individuals with MRI phenotypes suggestive of leukoaraiosis or extensive white matter pathology lacking involvement of the corpus callosum.
- F. The CNS anomalies are not better accounted for by another disease process.

**\*BARKHOF/TINTORÉ MRI CRITERIA FOR DISSEMINATION IN SPACE** (Adapted from (36))

Three positive criteria of the following:

- 1 or more enhancing lesions or 9 or more T2 lesions.
- 1 or more juxtacortical lesions.
- 1 or more infratentorial lesions.
- 3 or more periventricular lesions.

**12.4 ANNEX 4: KAPLAN-MEYER SURVIVAL ANALYSIS WITH THE ENDPOINT OF TIME TO FIRST ACUTE OR PROGRESSIVE EVENT AT 5-YEARS FOR THE ENTIRE RIS COHORT**

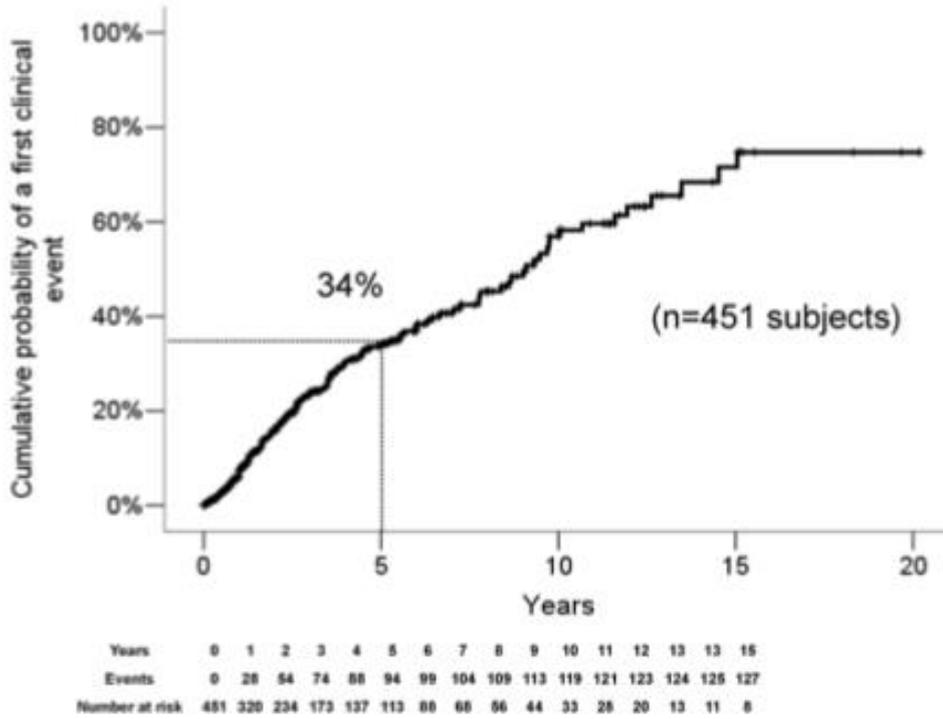


Figure 3 Kaplan-Meier survival analysis with the endpoint of time to the first acute or progressive event at 5-years for the entire RIS cohort (25).

12.5 ANNEX 5: MECHANISM OF ACTION OF INTERFERON BETA-1B

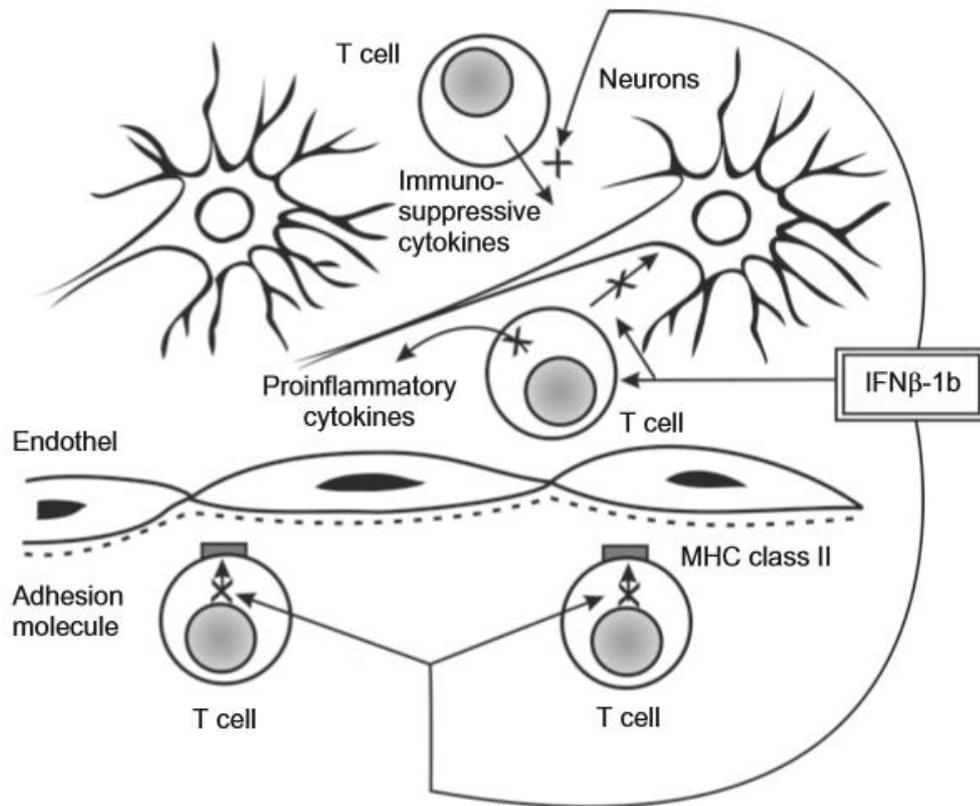


Figure 4 Mechanism of action of interferon beta-1b (11).

## 12.6 ANNEX 6: PARTICIPATING CENTERS WITHIN RISC RESEARCH NETWORK

**Table 2. Participating Centers within the Radiologically Isolated Syndrome Consortium (RISC) Research Network and corresponding contribution of new and previously published RIS cases by region.**

	New Cases (n=264)	Previously Reported (n=187)
<b>United States of America (n=205)</b>		
University of California, San Francisco, San Francisco, California (n=92)	n=21	n=71
Mayo Clinic Medical Center, Rochester, Minnesota (n=30)	n=21	n=9
Mt. Sinai Medical Center, New York, New York (n=34)	n=34	-
Barrow Neurological Institute, Phoenix, Arizona (n=49)	n=49	-
<b>France (n=149)</b>		
<b>Club Francophone de la Sclérose en Plaques</b>		
Centre Hospitalo Universitaire Pasteur, Nice, France (n=51)	n=23	n=28
Centre Hospitalo Universitaire Pontchaillou, Rennes, France (n=4)	n=2	n=2
Centre Hospitalo Universitaire Purpan, Toulouse, France (n=9)	n=6	n=3
Centre Hospitalo Universitaire Salengro, Lille, France (n=25)	n=13	n=12
Centre Hospitalo Universitaire Clermont, Clermont-Ferrand, France (n=2)	n=1	n=1
Centre Hospitalo Universitaire Central, Nancy, France (n=9)	n=3	n=6
Centre Hospitalo Universitaire Besançon, Besançon, France (n=4)	n=2	n=2
Centre Hospitalo Universitaire Strasbourg, Strasbourg, France (n=8)	n=5	n=3
Centre Hospitalo Universitaire tripode, Bordeaux, France (n=10)	n=7	n=3
Centre Hospitalo Universitaire Nantes, Nantes, France (n=7)	n=3	n=4
Centre Hospitalo Universitaire Timone, Marseille, France (n=3)	n=3	-
Fondation Rothschild, Paris, France (n=11)	n=5	n=6
Centre Hospitalo Universitaire Reims, France (n=3)	n=3	-
Centre Hospitalo Universitaire Montpellier Nimes, France (n=3)	n=3	-
<b>Italy (n=24)</b>		
University of Siena, Siena, Italy (n=12)	-	n=12
University of Florence, Florence, Italy (n=12)	-	n=12
<b>Turkey (n=44)</b>		
University of Istanbul, Istanbul, Turkey	n=31	n=13
<b>Spain (n=29)</b>		
MS Center of Catalunya Cemcat, Vall d'Hebron Hospital, Barcelona, Spain	n=29	-

doi:10.1371/journal.pone.0090509.t002

Figure 5 Participating centres within the Radiologically Isolated Syndrome Consortium (RISC) Research Network and corresponding contribution of new and previously published RIS cases by region (25).

**12.7 ANNEX 7: FUNCTIONAL SYSTEMS IN MS AND KURTZKE EXPANDED DISABILITY STATUS SCALE (EDSS) (37)**

# neurostatus

Standardised Neurological Examination and Assessment of Kurtzke's *Functional Systems*<sup>1</sup> and *Expanded Disability Status Scale*<sup>1</sup>

## SOURCE DOCUMENT

**SUBJECT NO/SUBJECT INITIALS**


**COUNTRY/CENTRE NO**


**NAME OF EDSS RATER**


**DATE OF EXAMINATION**


<sup>1</sup> slightly modified from J. F. Kurtzke, *Neurology* 1983;33,1444-52  
© L. Kappos, Department of Neurology, University Hospitals,  
CH-4031 Basel, Version 06/98

*To ensure unbiased EDSS assessment, the EDSS rater should not inquire about the patients' condition except as necessary to perform the EDSS assessment.*

*Patients must be observed to walk the required distance.*

**NEUROSTATUS (NS)**

In the Neurostatus «signs only» is noted when the examination reveals signs of which the patient is unaware.

**FUNCTIONAL SYSTEMS (FS)**

A score of 1 in the Functional Systems implies that the patient is not aware of the deficit and that the deficit or sign does not interfere with normal daily activities (with the exceptions of optic, vegetative and cerebral functions).

**EXPANDED DISABILITY STATUS SCALE (EDSS)**

EDSS should not be lower than the highest score of the FS. Symptoms which are not MS-related will not be taken into consideration for assessments, but should be noted.

In the definitions of EDSS grades 6.0 and 6.5 both a description of assistance required and of the walking range are included. In general, the distinction of bilateral versus unilateral assistance required to walk overrules the walking range. However, the following exceptions are suggested, if a patient is able to walk considerably longer than 100 m with two sticks, crutches or braces he is in grade 6.0. If a patient is able to walk more than 10 m and less than 100 m with two sticks, crutches or braces he is in grade 6.5. If a patient needs assistance by another person (as opposed to one stick, crutch or brace) and/or is not able to walk more than 50 m with one stick, crutch or brace he is in grade 6.5.

**Definitions****Visual acuity**

The visual acuity score is based upon the line on the Snellen chart at 20 feet (5 m) for which the patient makes no more than one error (use best available correction).

**Fields**

0 – normal

1 – signs only, deficits present only on formal testing

2 – moderate, patient aware of deficit, but incomplete hemianopsia on examination

3 – marked, complete homonymous hemianopsia or equivalent

**Scotoma**

0 – none

1 – small, detectable only on formal (confrontational) testing

2 – large, spontaneously reported by patient

**Disc pallor**

0 – not present

1 – present

**VISUAL (OPTIC) FUNCTIONS**

<b>OPTIC FUNCTIONS</b>	<b>OD</b>	<b>OS</b>
Visual acuity (corrected)		
Visual fields		
Scotoma		
Disc pallor		

**FUNCTIONAL SYSTEM SCORE**

0 – normal
1 – disc pallor and/or mild scotoma and/or visual acuity of worse eye (corrected) less than 30/30 (1.0) but better than 20/30 (0.67)
2 – worse eye with large scotoma and/or maximal visual acuity (corrected) of 20/30 to 20/59 (0.67–0.34)
3 – worse eye with large scotoma or moderate decrease in fields and/or maximal visual acuity (corrected) of 20/60 to 20/99 (0.33–0.2)
4 – worse eye with marked decrease of fields and/or maximal visual acuity (corrected) of 20/100 to 20/200 (0.1–0.2); grade 3 plus maximal acuity of better eye of 20/60 (0.3) or less
5 – worse eye with maximal visual acuity (corrected) less than 20/200 (0.1); grade 4 plus maximal acuity of better eye of 20/60 (0.3) or less
6 – grade 5 plus maximal visual acuity of better eye of 20/60 (0.3) or less

## Definitions

**Assessment of impairment/disability**

0 = normal

1 = signs only

2 = mild, clinically detectable numbness, facial weakness, dysarthria or cranial nerve deficits of which patient is aware

3 = moderate, diplopia with incomplete paralysis of any eye movement, impaired discrimination of sharp/dull in 1 or 2 trigeminal branches, trigeminal neuralgia, weakness of eye closure, cannot hear finger rub and/or misses several whispered numbers, obvious dysarthria during ordinary conversation impairing comprehensibility

4 = severe, complete loss of movement of either eye in one direction, impaired discrimination of sharp/dull or complete loss of sensation in the entire distribution of one or both trigeminal nerves, unilateral or bilateral facial palsy with lagophthalmus or difficulty with liquids, sustained difficulty with swallowing, incomprehensible voice

**Nystagmus**

0 = normal

1 = signs only

2 = mild, patient feels disturbed

3 = moderate, sustained nystagmus on 30° horizontal or vertical gaze, but not in primary position

4 = severe, sustained nystagmus in primary position or coarse persistent nystagmus in any direction interfering with visual acuity, complete internuclear ophthalmoplegia with sustained nystagmus of abducting eye, oscillopsia

**CRANIAL NERVE EXAMINATION**

EOM (extra ocular movements) impaired

Nystagmus

Trigeminal damage

Facial weakness

Hearing loss

Dysarthria

Dysphagia

Other bulbar signs

**FUNCTIONAL SYSTEM SCORE**

0 = normal

1 = signs only

2a = moderate nystagmus

2b = other mild disability

3a = severe nystagmus

3b = marked extraocular weakness

3c = moderate disability of other cranial nerves

4a = marked dysarthria

4b = other marked disability

5 = inability to swallow or speak

## Definitions

\* = optional

**REFLEXES**

0 = absent, 1 = weak, 2 = normal, 3 = exaggerated, 4 = cloniform, 5 = inexhaustible (indicate difference between R &amp; L by &lt; or &gt;)

**Plantar response**

0 = flexor, 1 = neutral, 2 = extensor

**Cutaneous reflexes**

0 = normal, 1 = weak, 2 = absent

**\*Palmomental reflex**

0 = absent, 1 = present

**LIMB STRENGTH**

The weakest muscle in each group defines the score for that group. Each movement should be tested, but only pathological findings should be noted using the BMRC grades. Use of functional tests like jumping with one foot, walking on toes or heels are recommended in order to assess grades 3-5 BMRC.

**BMRC Rating scale**

0 = no activity, 1 = visible contraction without visible joint movement, 2 = visible movements with elimination of gravity, 3 = movements against gravity possible but impaired, 4 = movements against resistance possible but impaired, 5 = normal strength

**FUNCTIONAL TESTS****\*Position test UE (upper extremities)**

Sinking, 0 = none, 1 = mild, 2 = evident

**\*Position test LE (lower extremities)**

Sinking, 0 = none, 1 = mild, 2 = evident

1 = only separate lifting possible (grades from horizontal position in hip joints...\*)

2 = even separate lifting not possible

**\*Walking on heels/tiptoes**

0 = normal, 1 = impaired, 2 = not possible

**\*Monopodal hopping**

0 = normal, 1 = 6-10 times, 2 = 1-5 times, 3 = not possible

**LIMB SPASTICITY**

0 = normal, 1 = mild, barely increased muscular tone after rapid flexion of an extremity, 2 = moderate, 3 = severe, barely surmountable increased spastic tonus after rapid flexion of an extremity, 4 = contracted

**Gait spasticity**

0 = normal, 1 = barely perceptible, 2 = evident, minor interference with function, 3 = permanent shuffling, major interference with function

## PYRAMIDAL FUNCTIONS

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

**LIMB STRENGTH**

Shoulder			
Elbow flexors			
Elbow extensors			
Hand/finger flexors			
Hand/finger extensors			
Hip flexion			
Knee flexors			
Knee extensors			
Foot/toe flexors			
Foot/toe extensors			
*Position test UE, pronation			
*Position test UE, sinking			
*Position test LE, sinking			
only lifting of single leg possible		°	°
*Walking on heels			
*Walking on tiptoes			
*Hopping on one foot			

**SPASTICITY**

Arm			
Leg			
Gait			

**FUNCTIONAL SYSTEM SCORE**

0 = normal
1 = abnormal signs without disability
2 = minimal disability, patient complains about fatiguability in motor tasks and/or BMRC grade 4 in one or two muscle groups
3a = mild to moderate paraparesis or hemiparesis, full range of movement against gravity
3b = severe monoparesis, refers to BMRC grade 2 or less in one muscle group
4a = marked paraparesis or hemiparesis
4b = moderate tetraparesis (refers to BMRC grade 3)
4c = Monoplegia
5a = Paraplegia, grade 0 or 1 in all muscle groups of the lower limbs
5b = Hemiplegia
5c = Marked tetraparesis (BMRC grade 2 or less)
6 = Tetraplegia (grade 0 or 1 in all muscle groups of upper and lower limbs)

**Definitions**

UE = upper extremities

LE = lower extremities

EO = eyes open

EC = eyes closed

**Head tremor, rebound**

0 = normal

1 = mild abnormality

2 = moderate abnormality

3 = severe abnormality

**Truncal ataxia**

0 = none

1 = signs only

2 = mild, swaying with EC

3 = moderate, swaying with EO

4 = severe, unable to sit without assistance

**Limb ataxia**

0 = none

1 = signs only

2 = mild, tremor or clumsy movements seen easily, minor interference with function

3 = moderate, tremor or clumsy movements interfere with function in all spheres

4 = severe, most functions are very difficult

**Gait ataxia**

0 = none

1 = signs only

2 = mild, abnormal balance only on heel or toe walking, or walking along a line

3 = moderate, abnormal balance on ordinary walking or while seated

4 = severe, unable to walk more than a few steps or requires support by another person or walking aid because of ataxia

**Romberg test**

0 = normal

1 = mild, mild insecurity with EC

2 = moderate, not stable with EC

3 = severe, not stable with EO

**Straight line walking**

0 = without problems

1 = impaired

2 = not possible

**Note**

The presence of severe gait ataxia alone results in a grade of 3 in the cerebellar FS. If weakness interferes with the testing of ataxia, score the patient's actual performance, but also indicate the possible role of weakness by marking the box marked 'X'.

## CEREBELLAR FUNCTIONS

CEREBELLAR EXAMINATION		
Head tremor		
Truncal ataxia, EO		
Truncal ataxia, EC		
	<b>R</b>	<b>L</b>
Tremor/dysmetria UE		
Tremor/dysmetria LE		
Rapid alternate movements impaired UE		
Rapid alternate movements impaired LE		
Gait ataxia, EO		
Straight line walking, EO		
Other, e.g. rebound		
Romberg test		

FUNCTIONAL SYSTEM SCORE	
	0 = normal
	1 = abnormal signs without disability
	2 = mild ataxia
	3a = moderate truncal ataxia
	3b = moderate limb ataxia
	4 = severe ataxia in all limbs or trunk
	5 = unable to perform coordinated movements due to ataxia
X	weakness (grade 3 or more on pyramidal) interferes with testing

## Definitions

UE = upper extremities

LE = lower extremities

\* = optional

**Superficial sensation – Touch/pain**

0 = normal

1 = mild, patient is aware of impaired light touch or pain, but able to discriminate sharp/dull

2 = moderate, impaired discrimination of sharp/dull

3 = severe, no discrimination of sharp/dull and/or unable to feel light touch

4 = complete loss, anaesthesia

**Vibration sense**

0 = normal

1 = mild, graded tuning fork 5–7 of 8 (alternatively) detects more than 10 sec. but less than examiner

2 = moderate, graded tuning fork 1–4 of 8 (alternatively) detects more than 2 sec. but less than 11 sec.

3 = marked, complete loss of vibration sense

**Position sense / Romberg test**

0 = normal

1 = mild, 1–2 incorrect responses on testing, only distal joints affected/slight stagger during Romberg testing

2 = moderate, misses many movements of fingers or toes, proximal joints affected/unable to stand during Romberg testing without assistance

3 = marked, no perception of movement/astasia

**\*Lhermitte**

0 = negative

1 = positive

**\*Paraesthesia (tingling)**

0 = none

1 = present

SENSORY EXAMINATION	R	L
Superficial sensation (touch/pain) UE		
Superficial sensation trunk		
Superficial sensation LE		
Vibration sense UE		
Vibration sense LE		
Position sense UE		
Position sense LE		
*Lhermitte		
*Paraesthesiae UE		
*Paraesthesiae trunk		
*Paraesthesiae LE		

**FUNCTIONAL SYSTEM SCORE**

0 = normal
1 = mild vibration or figure-writing decrease only in 1 or 2 limbs
2a = mild decrease in touch or pain or position sense and/or moderate decrease in vibration in 1 or 2 limbs
2b = vibration or figure-writing decrease, alone or in 3 or 4 limbs
3a = moderate decrease in touch or pain or position sense and/or essentially lost vibration in 1 or 2 limbs
3b = mild decrease in touch or pain and/or moderated decrease in all proprioceptive tests in 3 or 4 limbs
4a = marked decrease in touch or pain or loss of proprioception, alone or combined in 1 or 2 limbs
4b = moderate decrease in touch or pain and/or severe proprioceptive decrease in more than 2 limbs
5a = loss (essentially) of sensation in 1 or 2 limbs
5b = moderate decrease in touch or pain and/or loss of proprioception for most of the body below the head
6 = sensation essentially lost below the head

## Definitions

\* = optional

**BLADDER****Hesitancy/retention**

- 0 = none
- 1 = mild, no major impact on lifestyle
- 2 = moderate, urine retention, frequent UTI
- 3 = severe, requires catheterisation
- 4 = loss of function, overflow incontinence

**Urgency/incontinence**

- 0 = none
- 1 = mild, no major impact on lifestyle
- 2 = moderate, rare incontinence, no more than once a week, must wear pads
- 3 = severe, frequent incontinence, several times a week up to once daily, must wear urinal
- 4 = loss of function, loss of bladder control

**Catheterisation**

- 0 = none
- 1 = intermittent, up to twice daily
- 2 = intermittent, > twice daily
- 3 = constant

**Bowel**

- 0 = none
- 1 = mild, no incontinence, no major impact on lifestyle, constipation
- 2 = moderate, must wear pads or alter lifestyle to be near lavatory
- 3 = severe, in need of intermittent enemas
- 4 = complete loss of function

**\*Sexual dysfunction**

- 0 = none
- 1 = mild
- 2 = moderate
- 3 = severe
- 4 = loss

**BOWEL/BLADDER FUNCTIONS**

<b>BLADDER AND BOWEL FUNCTIONS</b>	
Hesitancy/retention	
Urgency/incontinence	
Catheterisation	
Bowel dysfunction	
*Sexual dysfunction	

**FUNCTIONAL SYSTEM SCORE**

0 = normal
1 = mild urinary hesitancy, urgency and/or constipation
2 = moderate urinary hesitancy and/or urgency and/or rare incontinence and/or severe constipation
3 = frequent urinary incontinence or intermittent self catheterisation once or twice a day, needs constantly enemas or manual measures to evacuate bowel
4 = in need of almost constant catheterisation, intermittent self catheterisation more than twice a day
5 = loss of bladder function, external or indwelling catheter
6 = loss of bowel and bladder function

**Definitions**

The presence of depression and/or euphoria alone results in a score of 1 on the cerebral FS, but does not affect the EDSS score.

**Depression/euphoria**

0 – none

1 – present

Patient complains of depression or is considered depressed or euphoric by the investigator or «significant other».

**Decrease in mentation**

0 – none

1 – mild, difficulties apparent to patient and «significant other» such as impaired ability to follow a rapid course of association and of surveying complex matters, impaired judgement in certain demanding situations, able to handle the daily routine, but no tolerance for additional stressors, intermittently symptomatic to even normal levels of stress, reduced performance, tendency toward negligence due to obliviousness or fatigue.

However, not apparent while taking the history or performing the routine neurological examination.

2 – moderate, definite abnormalities on formal mental status testing, but still oriented to time, place and person

3 – marked, not oriented in 1 or 2 spheres of time, place or person, marked effect on lifestyle

4 – dementia, confusion and/or complete disorientation

**Fatigue**

0 – none

1 – present

Fatigue that interferes with daily activity will be scored with a maximum of 2 in the FS.

**MENTAL STATUS EXAMINATION**

Depression	
Euphoria	
Decrease in mentation	
Fatigue	

**FUNCTIONAL SYSTEM SCORE**

0 – normal
1 – mood alteration only (does not affect EDSS score)
2 – mild decrease in mentation/fatigue
3 – moderate decrease in mentation
4 – marked decrease in mentation
5 – dementia

**Definitions**

Actual walking distance without assistance obligatory up to 500 m (if possible). Actual walking distance with assistance obligatory up to 150 m (if possible).

In the definitions of EDSS grades 6.0 and 6.5 both a description of assistance required and of the walking range are included. In general, the distinction of bilateral versus unilateral assistance required to walk overrules the walking range.

However, the following exceptions are suggested,

If a patient is able to walk considerably longer than 100 m (> 120) with two sticks, crutches or braces he is in grade 6.0.

If a patient is able to walk more than 10 m and less than 100 m with two sticks, crutches or braces he is in grade 6.5.

If a patient needs assistance by another person (as opposed to one stick, crutch or brace) and/or is not able to walk more than 50 m with one stick, crutch or brace he is in grade 6.5.

**AMBULATION****Walking range as reported (without help or sticks)**

	meters
--	--------

In	min
----	-----

**Able to walk without rest or assistance**

> 100 meters, but < 200 meters
> 200 meters, but < 300 meters
> 300 meters, but < 500 meters
> 500 meters
Unrestricted

**Actual distance (obligatory up to 500 m if possible)**

	meters
--	--------

**Unable to walk 100 m without constant assistance**

Unilateral assistance		meters
	Cane/crutch	
	Other	
Bilateral assistance		meters
	Cane/crutch	
	Other	
Other person		

**SYNOPSIS OF FS SCORES**

Visual <sup>1</sup>
Brainstem
Pyramidal
Cerebellar
Sensory
Bladder/Bowel <sup>2</sup>
Mental

<sup>1</sup> For calculation of the EDSS the score of the visual FS is to be converted as follows, 6-4; 5-3; 4-3; 3-2; 2-2; 1-1.

<sup>2</sup> Scores 5 and 6 in the bowel/bladder FS are converted to 4 and 5 respectively.

EDSS steps below 4 refer to patients who are fully ambulatory, and the precise step is defined by the functional systems (FS) score(s). EDSS steps between 4.0 and 5.0 are defined by both FS-scores and walking range. In general, the worst of both should determine the score. Steps 5.5-8.0 are exclusively defined by ability to ambulate or use wheelchair.

EDSS should not change by 1.0 step unless there is a change in same direction of at least one step in at least one FS.  
EDSS should not be lower than each of FS (excepted visual and bowel/bladder FS).

0	normal neurological exam (all grade 0 in FS)	5.0	ambulatory without aid or rest for > 200 m (usual FS equivalents are one grade 5 alone, others 0 or 1; or combinations of lesser grades usually exceeding specifications for step 4.5)
1.0	no disability, minimal signs in one FS1 (i.e. grade 1)	5.5	ambulatory without aid or rest > 100 m
1.5	no disability, minimal signs in more than one FS 1 (more than one grade 1)	6.0	unilateral assistance (cane or crutch) required to walk at least 100 m with or without resting
2.0	minimal disability in one FS (one FS grade 2, others 0 or 1)	6.5	constant bilateral assistance (canes or crutches) required to walk at least 20 m without resting
2.5	minimal disability in two FS (two FS grade 2, others 0 or 1)	7.0	unable to walk 20 m even with aid, essentially restricted to wheelchair; wheels self and transfers alone; up and about in wheelchair some 12 h a day
3.0	moderate disability in one FS (one FS grade 3, others 0 or 1) or mild disability in three or four FS (three/four FS grade 2, others 0 or 1) though fully ambulatory	7.5	unable to take more than a few steps; restricted to wheelchair; may need some help in transfer and in wheeling self
3.5	fully ambulatory but with moderate disability in one FS (one grade 3) and one or two FS grade 2; or two FS grade 3; or five FS grade 2 (others 0 or 1)	8.0	essentially restricted to bed or chair or perambulated in wheelchair, but out of bed most of day; retains many self-care functions; generally has effective use of arms
4.0	ambulatory without aid or rest for > 500 m; up and about some 12 hours a day despite relatively severe disability consisting of one FS grade 4 (others 0 or 1), or combinations of lesser grades exceeding limits of previous steps	8.5	essentially restricted to bed much of the day; has some effective use of arm(s); retains some self-care functions
4.5	ambulatory without aid or rest for > 300 m; up and about much of the day; characterised by relatively severe disability usually consisting of one FS grade 4 or combinations of lesser grades exceeding limits of previous steps	9.0	helpless bed patient; can communicate and eat
		9.5	totally helpless bed patient; unable to communicate effectively or eat/swallow
		10.0	death due to MS

<sup>1</sup> Mental function's grade 1 does not contribute to EDSS-step definitions

Actual EDSS

Signature

## **12.8 ANNEX 8: MRI SPECIFICATIONS (Adapted from (38))**

### **Patient positioning**

Patients must be placed in the magnet in the most comfortable way possible, trying to fix his head firmly using pads. Patients will be informed about the study procedure and its approximate duration. It is advisable not to communicate orally with the patient during this exploration since this favours the movements of the head.

Patients must be aligned as symmetrically as possible with the help of laser positioning. The isocenter of the coil must be placed next to the naso-frontal union.

To all patients participating in the study contrast will be administered. Thus, a series of measures have to be taken:

- Ensure that patient consent the administration of contrast and there are no contraindications to the use of paramagnetic contrast.
- Ensure that the patient has normal kidney function.
- Place an intravenous via (antecubital via) before placing the patient into the magnet, in order to administrate the contrast without removing the patient from the tube. Patients must not be removed from the magnet once the study to administer contrast has begun. Keep this via with serum.
- Inject serum after administration of contrast to purge the line.
- In case that we need to repeat the T1 sequences after contrast, there is a room for 20 minutes to do so. In case that this time has passed, the exploration must be repeated.
- Write down the exact time from the injection of contrast and the start of the acquisition sequence.

### **Analysis of images**

The analysis of images will be done in the reference centre (Hospital Josep Trueta).

The quantification of each of the 3 explorations performed in patients will be done by the following parameters, which measure lesion activity:

- Number of total and apparent active lesions (measured in T1 sequence by the enhancement with gadolinium).
- Number of new active lesions.
- Number of new lesions/augmented lesions in DP/T2.
- Volume of active lesions (T1Gd).

- Percentage of lesions with enhancement in the basal study that are hypointense (black holes) in the final study.
- Volume of T1Gd lesions and black holes.

#### **Guidelines for the identification of contrast enhancing lesions**

- a. A lesion that is enhanced with contrast is defined as an area of evident increased signal on T1 sequences in relation to the same area on a T1 sequence of the same characteristics obtained before contrast administration, or in case not to have it, in relation to the adjacent normal tissue (with normal signal on T2 sequences). Hyperintense foci of small size (1 pixel or with a diameter less than 3mm) should not be considered as enhancing lesions because most of these lesions correspond to vascular structures.
- b. Lesions that enhance with contrast are associated in virtually all cases with hyperintense foci on T2 sequences. This condition is obligatory when lesions in the posterior fossa are considered and highly recommended in supratentorial lesions. This condition is not essential in supratentorial lesions with cortico-yuxtacortical location, where the sensibility of T2 sequences is not as high. However, in the latter situation, it is important not to confuse an enhancing lesion with a vascular leptomenigeal structure.
- c. In some lesions that show themselves hypointense on T1 it can be identified a peripheral hyperintense signal not due to enhancement but a false visual perception (“mach” effect). In these cases it is necessary to compare the level of hypersignal with a T1 sequence without contrast and in case they show similar, it must not be considered enhancement.
- d. Flow artefacts can difficult the interpretation of enhancing lesions especially in the posterior fossa. It is therefore necessary that enhancing lesions located in the posterior fossa are associated with an area of hypersignal in T2, and sequences in T1 after contrast are obtained with compensation gradients flow (they minimize flow artefacts).
- e. The valuations of contrast enhancing lesions can be made from the visual analysis of the total number (given that a lesion identified in consecutive cuts only counts as one) or quantifying the number of areas in which enhancement is seen (apparent number). This latter form of quantization is closer to the volumetric analysis of enhancing lesions.

#### **General recommendations on the identification of new T2 lesions.**

It is generally advisable to adopt a conservative attitude in identifying active T2 lesions to reduce the rate of false positives.

- a. Small foci of hypersignal (<3mm) should not be considered relevant.
- b. Areas where only a small hypersignal relative to normal parenchyma is identified should not be taken into account.
- c. The signal intensity of a potentially active lesion must be greater than the adjacent gray matter in the T2 sequence obtained with short echo.
- d. If a potentially active lesion isointense relative to the adjacent gray matter in the T2 short echo sequence, it may still be considered a lesion if its signal is clearly hyperintense in the long echo or if it is identified in two consecutive cuts.
- e. The correct positioning between the studies is critical when assessing active T2 lesions. If the positioning is suboptimal, the adjacent cuts should be analysed with special detail before assigning a lesion as active, as rotational and parallel displacements can cause apparent shifts in the size and position of lesions.
- f. In patients with elevated lesion volumes it is particularly difficult to detect active lesions on T2 especially if repositioning is not optimal. In this situation you should take a particularly conservative attitude.

**Definition of active T2 lesions "new lesions" and "enlarged lesions."**

- a. "New" lesion: It is defined as an area that appears hyperintense on T2 in an area of tissue that was normal in a previous study in T2 sequences with short echo. In general, it is recommended that this hypersignal is confirmed both on T2 sequences with short echo and long echo. This condition is required in those anatomical regions most susceptible to flow artefacts, such as the poles of the temporal lobes and the posterior fossa. A lesion should also be considered "new" if it is contiguous with a pre-existing lesion but connected to it by an area of relative low signal. In situations of suboptimal repositioning, a "new" lesion can only be considered in an area with pre-existing injury if it is confirmed in at least two consecutive cuts. In the posterior fossa any "new" lesion must be identified both in the T2 sequences obtained with long echo in the short echo. The latter are the most affected by flow artefacts so that the detection of new lesions should be based on long echo sequences.
- b. "Enlarged" lesions: It is sometimes extremely difficult to determine whether an injury is enlarged or has simply changed its size or shape as a result of suboptimal repositioning. Therefore never a lesion must be considered as "increased" if it only has changed its form. Lesions >5mm in diameter should be considered "increased" only if they have increased their diameter by at least 100%, or when an increasing in size is detected in at least two consecutive sections. In lesions <5 mm both criteria must be met for

classifying a lesion as "increased". Given the difficulty in enforcing these criteria in potentially "increased" lesions on the posterior fossa, you should not see this possibility in lesions of this location.

**Definition of T1-hypointense lesions (black holes)**

They are T1 hypointense lesions in relation to the normal gray matter, always associated with an area of hypersignal on T2.

## **12.9 ANNEX 9: TECHNICAL SLUG OF INTERFERON BETA-1B (Adapted from (39))**

### **Mechanism of action**

Antiviral and immunoregulatory activity mediated by interaction with specific cell receptors on the surface of human cells.

### **Therapeutic indications**

RRMS (and 2 or more relapses within the last two years) and SPMS who have active disease. Patients with a single demyelinating event with an active inflammatory process, if it is severe enough to warrant treatment with intravenous corticosteroids, if alternative diagnoses have been excluded, and if high risk of developing CDMS is determined.

### **Posology**

Subcutaneous, adults and children 12-17 years: 0.25 mg every 2 days. Should not be used in children <12 years.

### **Contraindications**

Hypersensitivity to IFN  $\beta$  natural or recombinant, initiation of treatment in pregnancy, severe depression and/or suicidal ideation, decompensated liver disease.

### **Warnings and Precautions**

Previous or current depressive disorder, history of heart disease or seizures, epilepsy not adequately controlled by treatment, renal failure, anaemia, thrombocytopenia or leukopenia, pre-existing monoclonal gammopathy. Perform thyroid function tests, complete blood counts, biochemical parameters including PFH. Discontinue if severe hypersensitivity reaction occurs. Development of serum neutralizing activity. Take contraceptive measures. The use of IFN  $\beta$  can be associated with the occurrence of thrombotic microangiopathy (TMA) and nephrotic syndrome (NS), and can be present from several weeks to years after starting treatment. Monitor for signs and symptoms of TMA and NS, if they appeared initiate appropriate treatment and discontinuation of IFN  $\beta$ .

### **Liver failure**

Contraindicated in decompensated liver disease. Precaution, should monitor patients for signs of liver damage.

### **Renal insufficiency**

Caution. Monitor renal function.

**Interactions**

Caution with: antiepileptics.

It is not recommended with: other immunomodulators except corticosteroids or ACTH.

**Pregnancy**

Contraindicated.

**Lactation**

It is unknown whether IFN  $\beta$ -1b is excreted in breast milk. Because of the potential for serious adverse reactions in nursing infants a decision should be made whether to discontinue lactation or treatment with IFN  $\beta$ -1b.

**Effects on ability to drive**

There have been no studies on the effects on the ability to drive and use machines. Adverse effects on the CNS associated with the use of IFN  $\beta$ -1b may impair the ability to drive and use machines in susceptible patients.

**Adverse reactions**

Infection, abscess; decrease of lymphocytes, neutrophils or leukocytes, lymphadenopathy; lowering blood glucose; depression, anxiety; headache, dizziness, insomnia, migraine, paresthesia; conjunctivitis, abnormal vision; earache; palpitation; vasodilatation, hypertension; upper respiratory tract infection, sinusitis, increased cough, dyspnoea; diarrhoea, constipation, nausea, vomiting, abdominal pain; SGOT and SGPT increased; skin disorder, rash; hypertonia, myalgia, myasthenia, back pain, pain in limb; urinary retention, proteinuria +, incontinence or urinary frequency or urgency; dysmenorrhoea, menstrual disorders, vaginal bleeding, impotence; reaction or necrosis at injection site, flu-like symptom complex, fever, pain, chest pain, peripheral oedema, fatigue, chills, sweating, malaise.

## INFORMATION SHEET FOR PATIENTS

### “Interferon $\beta$ -1b as a first-line treatment in patients with Radiologically Isolated Syndrome”

*Multicenter study, double-blind, randomized in two groups: one group will be treated with Interferon  $\beta$ -1b and the other one with placebo in patients with Radiologically Isolated Syndrome.*

Dear Patient,

You are at risk for developing a disease called Multiple Sclerosis (MS), a chronic neurological disorder that affects the central nervous system. That’s why you have been invited to participate in a research study for assessing the efficacy of interferon  $\beta$ -1b as a first-line treatment in patients with Radiologically Isolated Syndrome (RIS) in order to prevent the occurrence of a first clinical event of Multiple Sclerosis. Before you decide whether to participate in the study or not, it is important that you read this Patient Information Sheet and ask the study doctor to explain anything you do not understand. Your participation in the study is voluntary. This study has been approved by an ethics committee.

#### **Purpose of the study**

The main purpose of the study is to study the effect of treatment with interferon  $\beta$ -1b in patients with RIS on delaying or avoiding the occurrence of a first clinical event of MS. This study also attempts to determine whether this treatment avoids the appearance of new lesions and reduces the size of pre-existing lesions or avoids their enlargement in the MRI, as well as the effect on disability progression. Interferon  $\beta$ -1b is a drug used in first-line treatment of MS, and that’s why the evidence suggests that could be useful in patients suffering from RIS.

#### **Treatment groups**

It is expected that about 522 patients are included in this study, approximately in 23 study centers.

To study the efficacy of interferon  $\beta$ -1b these drug must be compared with placebo. There will be 2 treatment groups in the study, and the results of each treatment group will be compared

to find out if the group treated with interferon  $\beta$ -1b has less proportion of cases developing a first clinical event of MS. To ensure that the treatment groups are similar, at baseline you will be assigned randomly to a group, which means that neither you nor the study doctor can choose your treatment group. You have the same probability of being in each of the treatment groups. To avoid that you know what treatment you are receiving, both treatments will have the same presentation. The placebo has the same appearance as the study drug but contains no active drug.

The treatment groups of this study are:

- Group 1: Nearly 261 patients. Recombinant IFN  $\beta$ -1b (Betaferon) 0.25 mg subcutaneous every-other day. It contains 0.25 mg per ml of solution.
- Group 2: Nearly 261 patients. Placebo (physiological saline serum) 1ml subcutaneous infusion every other day.

You will receive the study drug for up to 72 weeks (about 18 months). Neither you nor the study doctor or study staff will know if you are receiving interferon  $\beta$ -1b or placebo. However, in an emergency, we can figure out what treatment you are receiving.

Throughout the study, all subcutaneous injections (administered every other day) will be administered by the patient at home. However, your first subcutaneous injection will be administered in the centre. At hospital a nurse will explain you how to do it.

### **Study procedures**

During the study, you will come at hospital for follow-up visits 8 times for a period of about 24 months. The first visit will be a screening visit to see if you meet the conditions for participation in the study. Then you will have to return to the centre for a baseline visit in the following 2 months. The doctor will collect your clinical history and will perform a neurological exploration, and he will ask you questions about your process. A magnetic resonance imaging will be performed too. After additional tests you will be assigned to a treatment group and you will receive your first dose of the drug.

You will have visits every 3 months. If you have symptoms of an MS relapse or another significant change in your health that may be related to use of the study drug, additional visits may be necessary to perform additional blood tests.

During the study the following tests shall be performed in some or all visits: blood test, urine strip, measurement of blood pressure, magnetic resonance imaging and clinical evaluation. The

doctor will also ask you how you tolerate the medication, if you had any side effect of the drug and in affirmative case, what other medications have you taken to get better.

During the study you will be asked if you had new symptoms of MS or if you are experiencing worsening symptoms. Any side effects will be registered and if some of the laboratory tests show significantly abnormal results or other changes in your health, such as infection, your study treatment will be temporarily interrupted or permanently discontinued.

### **Interruption and withdrawal**

You have taken the decision to participate in the study. Your signature indicates that you have read the above information and you have decided to participate in the study. You can leave at any time without explanation and without this decision affecting your future medical care. Before doing it, you should discuss your decision with the study doctor.

The treatment with the study drug will be interrupted if:

- You become pregnant
- You have symptoms of an allergic reaction to the study drug
- You show signs of impaired liver function
- You have a medical reason that needs stopping the study drug
- You do not follow the indications of the study

The study doctor can decide to discontinue participation in this study at any time.

If you need to interrupt the study drug permanently because of liver tests are abnormal, a blood and urine test will be done to you for safety analysis. Your doctor will tell you the result of abnormal tests.

If you are removed from the study, you will be asked to return to the study centre for follow-up visits.

### **Benefits**

We hope that your participation in the study will be benefit. However, there is no guarantee that in all cases the occurrence of a clinical event of MS will be avoided by participating in this study, and we cannot ensure that all patients participating in the study will have developed a clinical event in all cases if they had not been treated. The results of the study can help in the future for people with the same disorder.

## **Risks**

If you think you are having an allergic reaction to medication you should contact the study doctor or seek medical attention immediately.

- **Risks of MRI**

Serial MRI will be performed to you, which allows the obtainment of images of your central nervous system without being exposed to X-rays or ionizing radiation. The disadvantage of this test is the irritating noise that produces and the fact that the patient should be placed inside a device for an extended period of time trying to stay still, which can upset people with claustrophobia. It should not be performed on people who carry metal devices such as prostheses or pacemakers.

MRI with contrast will be performed. Rarely, it may cause a severe allergic reaction due to the use of radiological agent (gadolinium). The radiological agent may cause you headaches, dizziness or fainting, nausea, vomiting, sweating, changes in the flavour of food and symptoms at the injection site. If you have experienced any of these symptoms prior to a radiological agent, report it to the study doctor.

It has recently been linked such contrast with the appearance of nephrogenic systemic fibrosis in patients suffering from moderate to severe renal insufficiency. It is a rare but serious complication that causes a skin thickening that can cause inability to move the joints and get to invalidate patients. This only occurs in patients with renal failure and patients with renal impairment will not be included in this study, however it cannot be excluded absolutely that this could not happen.

- **Risks of interferon  $\beta$ -1b**

The most common adverse reactions (incidence  $\geq 5\%$ ) are: injection-site reaction, lymphopenia, flu-like symptoms, myalgia, leukopenia, neutropenia, increased liver enzymes, headache, hypertonia, pain, rash, insomnia, abdominal pain and asthenia.

## **Serious side effects**

### **Anaphylaxis**

Anaphylaxis has been reported as a rare complication on interferon  $\beta$ -1b use but if it occurs, the treatment will be discontinued.

### **Flu-like symptom complex**

It is one of the most common side effect among patients using Betaferon but the incidence decreases over treatment period. Normally the median duration is 7 days. Analgesics and antipyretics on treatment days may help flu-like symptoms.

### **Injection-site necrosis**

It typically occurs within the first 4 months of therapy. The necrotic lesions are usually 3 cm and rarely larger areas are affected. The treatment will be discontinued if multiple lesions occurs.

### **Depression and suicide**

Depression and suicide have been reported to occur with increased frequency in patients receiving interferon beta products. If a patient develops depression, discontinuation of the therapy should be considered.

### **Compensation for damages**

It has taken out an insurance, covering all patients participating in this study according to the Spanish Royal Decree 223/2004 of 6th February. This insurance will cover you if you suffer damages related to the study.

You must tell the study doctor immediately if you believe you have suffered damages for participating in this study. Insurance does not cover the normal progression of their disease or any damage, injury or complication due to a medical condition pre-existing. If you have damages related to the study, the doctor will decide what medical care you need.

### **Confidentiality notice**

You will not be identified by name but by an identification code. In all written reports and publications it will appear only your reference codes. Your medical information and any information obtained about you during this study will be kept confidential in accordance with the Organic Law 15/1999 on Protection of Personal Data and the corresponding Royal Decree 1720/2007 and will not be made public.

## INFORMED CONSENT FORM FOR PATIENTS

### “Interferon $\beta$ -1b as a first-line treatment in patients with Radiologically Isolated Syndrome”

*Multicenter study, double-blind, randomized in two groups: one group will be treated with Interferon  $\beta$ -1b and the other one with placebo in patients with Radiologically Isolated Syndrome.*

Name of the participant: \_\_\_\_\_

Date of birth: \_\_\_\_\_

- I have read this Informed Consent Form or someone has read to me. It is written in a language that I understand.
- I understand what I was asked to do during this study and I have had time to think about what the study means for me.
- I have discussed this with the doctor/study personnel; I asked questions about the study and they have answered satisfactorily.
- I have received enough information about the study.
- I told the person obtaining consent if I am involved in other medical research studies.
- I have talked to ..... (Name of the investigator/person obtaining informed consent).
- I understand my participation is voluntary.
- I understand I have to decide if I want to participate in the study and can later change my mind, and I can withdraw from the study at any time, without giving explanations, and without affecting my health care.
- I understand that whatever I decide, my care and my legal rights are not affected.
- I understand that I can save a copy of the Patient Information Sheet and Informed Consent Form.

- I willingly pay my agreement to participate in this research study.

Signed and dated by the patient or the patient's legal representative:

**Participant's signature:** \_\_\_\_\_ **Date:** \_\_\_\_\_

**Investigator's signature:** \_\_\_\_\_ **Date:** \_\_\_\_\_

### 12.12 ANNEX 12: STUDY CHRONOGRAM

ACTIVITIES	PERSONNEL	2015				2016			2017	2018							2019			
		Sept	Oct	Nov	Dec	Jan-Apr	May-Jun	Jul-Dec	Jan-Dec	Jan-Jun	Jul	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr
<b>PREPARATION AND COORDINATION</b>																				
Bibliographic research, literature review, protocol elaboration and evaluation	PI, N																			
Selection of participating centres	PI, M																			
Meeting 2: Study research proposal and evaluation by clinical committees	PI, M, N, CEIC																			
Pilot data extraction	PI, M, N																			
Meeting 2	PI, M, N																			
<b>FIELD WORK AND DATA COLLECTION</b>																				
Subjects recruitment, randomization and data collection	N, NR, SS																			
- Screening visit	N																			
- Basal visit	N																			
Data collection	N, NR, Nu																			
Evaluation of correct data collection	PI, M																			
Teleconference meetings	PI, M, N																			
Follow-up period	N, NR																			
<b>DATA ANALYSIS AND FINAL EVALUATION</b>																				
Statistical analysis	SS																			
Analysis and interpretation of the results	PI, SS																			
Discussion of the results	PI, N																			
<b>ARTICLE PUBLICATION AND SCIENTIFIC DIFFUSION</b>																				
Final report elaboration	PI, N																			
Final report evaluation	PI, N																			
Scientific publications	PI																			
Attendance to conferences	PI																			

Figure 6 Study Chronogram.