NOVARTIS

Clinical Development

[FTY720/Fingolimod]

Clinical Trial Protocol [CFTY720DTR05] / NCT02575365

Effect of Fingolimod (Gilenya®) on neurodegeneration, brain atrophy and cognitive impairment in relapsing remitting multiple sclerosis patients

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Document type:	Clinical Trial Protocol
EUDRACT number:	Not Applicable
Version number:	V1.0
Development phase:	IV
Release date:	07.07.2015

NCDS Template Version 03-Feb-2012

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List of abbreviations

AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
CRF	Case Report/Record Form (paper or electronic)
CRO	Contract Research Organization
ECG	Electrocardiogram
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
IEC	Independent Ethics Committee
i.v.	intravenous
IRB	Institutional Review Board
p.o.	oral
RRMS	relapsing remitting multiple sclerosis
SAE	serious adverse event

Glossary of terms

Assessment	A procedure used to generate data required by the study
Enrollment	Point/time of patient entry into the study; the point at which informed consent must be obtained (i.e. prior to starting any of the procedures described in the protocol)
Epoch	The planned stage of the subjects' participation in the study. Each epoch serves a purpose in the study as a whole. Typical epochs are: determination of subject eligibility, wash-out of previous treatments, exposure of subject to treatment or to follow-up on subjects after treatment has ended.
Investigational drug	The drug whose properties are being tested in the study; this definition is consistent with US CFR 21 Section 312.3 and is synonymous with "investigational new drug" or "investigational medicinal product."
Investigational treatment	All investigational drug(s) whose properties are being tested in the study as well as their associated treatment controls. This *includes* any placebos, any active controls, as well as approved drugs used outside of their indication/approved dosage or tested in a fixed combination. Investigational treatment generally *does not include* other treatments administered as concomitant background therapy required or allowed by the protocol when used within approved indication/dosage
Subject Number	A number assigned to each patient who enrolls into the study
Part	A subdivision of a single protocol into major design components. These parts often are independent of each other and have different populations or objectives. For example, a single dose design, a multiple dose design that are combined into one protocol, or the same design with different patient populations in each part.
Period	A subdivision of a cross-over study
Premature patient withdrawal	Point/time when the patient exits from the study prior to the planned completion of all investigational/study treatment administration and all assessments (including follow-up)
Stop study participation	Point/time at which the patient came in for a final evaluation visit or when study/investigational treatment was discontinued whichever is later
Study drug/ treatment	Any single drug or combination of drugs administered to the patient as part of the required study procedures; includes investigational drug (s), active drug run-ins or background therapy
Study/investigational treatment discontinuation	Point/time when patient permanently stops taking study/investigational treatment for any reason; may or may not also be the point/time of premature patient withdrawal
Variable	Information used in the data analysis; derived directly or indirectly from data collected using specified assessments at specified time points

Protocol synopsis

Protocol number	*CFTY720DTR05*
Title	*Effect of Fingolimod on neurodegeneration, brain atrophy and cognitive impairment in relapsing remitting multiple sclerosis patients*
Brief title	*Effect of Fingolimod on neurodegeneration, brain atrophy and cognitive impairment in relapsing remitting multiple sclerosis patients*
Sponsor and Clinical Phase	Novartis Phase IV
Investigation type	Drug
Study type	Interventional
Purpose and rationale	Multiple sclerosis (MS) is a neurodegenerative disease in which both demyelination and axonal loss occur. Cognitive impairment is a major problem in MS and adversely affects patients' quality of life. Cognitive decline may appear early in the disease process and has been reported even at disease onset. It has previously been shown that a cognitive impairment is present in 53.7% of RRMS patients evaluated at the early disease stage during the onset of neurological symptomatology. (7,8) Our aim in this study is to investigate the effect of fingolimod on cognitive performance. Neuronal loss has been historically detected through MRI quantification of brain volume. Gray matter atrophy occurs in patients with relapsing forms of MS early in the disease course, has a major relationship to physical disability and cognitive impairment, and is a putative measure of neuroprotective therapeutic effects. (9) The paired thalamic nuclei are gray matter structures on both sides of the third ventricle and are involved in a wide range of neurological functions including motor, sensory, integrative, and higher cortical functions.(10) Thalamic location, unique neurologic functions, widespread cortical and subcortical connections and vulnerability to MS pathology from the earliest clinical disease stages make it a critical structure for examining neurodegeneration in MS (11,12) Our other aim in this study is to investigate the effect of fingolimod on brain gray matter atrophy and thalamic atrophy. Cognitive dysfunctions are mainly associated with brain atrophy especially cortical grey matter. (13,14) In this study we will investigate the correlations between the effects of fingolimod on cognitive performances and MRI data. For diagnostic, prognostic and treatment monitoring purposes, biomarkers which reflect the various neurodegenerative processes, would be helpful. The biomarker has to be central nervous system (CNS) specific, and available in concentrations that could be measured in cerebrospinal fluid (CSF) or blood. Serum and CSF oxysterols and cholesterol precursors have been linked to pathological processes in MS in previous studies, and seem to be promising candidate biomarkers. (15-17)

	In this study we will evaluate the effect of fingolimod treatment on the serum levels of a brain specific cholesterol metabolite 24S-hydroxycholesterol (24OHC) which is a biomarker reflecting neurodegeneration. (22) We will also evaluate the effect of fingolimod on plasma levels of osteopontin which is a pro-inflammatory cytokine and matrix metalloproteinase which have a role in the pathology of multiple sclerosis.(23,24)
Primary Objective(s) and Key Secondary Objective	Primary objective of this study is: -To investigate the effects of Fingolimod on cognitive performance in highly active relapsing remitting multiple sclerosis patients
Secondary Objectives	Secondary objectives of this study are: -To investigate the correlation between the effect of fingolimod on cognitive performances and MRI data. -To evaluate the effect of fingolimod on biomarkers (24 hydroxy cholesterol, osteopontin and matrix metalloproteinases) related to neurodegeneration -To investigate the effect of fingolimod on brain gray matter atrophy and thalamic atrophy.
Study design	This is a 24-month, open-label, multicenter study with a single treatment arm design.
Population	We will recruit a minimum of 80 relapsing remitting MS (RRMS) patients according to the McDonald criteria. All subjects will sign an informed consent that will be approved by the Ethics Committee.
Inclusion criteria	1. Diagnosed with RRMS as described in 2010 McDonald criteria 2. Provided written informed consent prior to any intervention 3. Female or male patients aged 18-65 years 4. Unresponsive to treatment with a beta interferon or glatiramer acetate for a minimum of one year at and at adequate dose and with high disease activity (Unresponsive patients: patients with no changes in relapses, increased relapses, severer relapses with one-year treatment or those who had had at least one relapse during the past one year under previous treatments and one or multiple contrast enhancing lesions in cranial MRI or increased T2 lesions in successive MRIs) 5. EDSS score below 5.5 at screening
Exclusion criteria	1. Patients with primary or secondary progressive or progressive relapsing MS 2. Patients with known contraindications for fingolimod treatment. 3. Other coexistent autoimmune diseases including Hashimoto thyroiditis, systemic lupus erythematosus, rheumatoid anthiritis, psoriasis etc. 4. Patients with any of the following cardiovascular conditions: • Resting heart rate < 45 bpm/min

	• Cardiac failure at any time during the first study visit (Class III as per NYHA classification) or significant heart disease as judged by the physician • Myocardial infarction during the last 6 months • History of Mobitz Type II grade 2 AV block • Past or current grade 3 AV block • Confirmed history of sick sinus syndrome or sino-atrial heart block • arrhythmia requiring current treatment with Class Ia drugs (ajmaline, disopyramid, procainamide, quinidine) • hypertension uncontrolled with medication 5. History of malignancy of any organ system (other than localized basal cell carcinoma of the skin), treated or untreated, within the past 5 years, regardless of whether there is evidence of local recurrence or metastases. 6. Pregnant or nursing (lactating) women, where pregnancy is defined as the state of a female after conception and until the termination of gestation, confirmed by a positive hCG laboratory test. 7. Negative for varicella-zoster virus IgG antibodies at screening. Patients who have negative results for varicella-zoster virus IgG antibodies can be included in the study after vaccination for varicella-zoster virus. 8. Active systemic bacterial, viral or fungal infections, or diagnosis of AIDS, Hepatitis B, HepatitisC infection defined as a positive HIV antibody, Hepatitis B surface antigen or Hepatitis C antibody tests, respectively 9. History of previous fingolimod therapy 10. Patient who received any of the treatments below: a. Corticosteroids or adrenocorticotropic hormone (ACTH) during the last 1 month b. Immunosuppressive medications such as azathioprine or methotrexate etc. c. Immunoglobulin treatment during the last 3 months d. Cladribine, cyclophosphamide, mitoxantrone, natalizumab at any time
Investigational and reference therapy	Fingolimod
Efficacy assessments	Cognitive tests, MRI ,biomarkers, EDSS
Safety assessments	This study does not include any planned safety analysis
Other	Not Applicable

assessments	
Data analysis	Data analysis will be performed after all of the data is collected from the enrolled patients. All RRMS patients will be accepted as the analysis sets.
Key words	Fingolimod, neurodegeneration, brain, atrophy, cognitive, impairment, relapsing, remitting, multiple, sclerosis

1 Introduction

1.1 Background

Multiple sclerosis (MS) is the leading cause of neurological disability in young adults and the most common demyelinating disorder of the central nervous system. It is widely regarded as a chronic autoimmune disorder encompassing both inflammatory (myelin destruction) and degenerative (axonal loss) features. Disease onset is thought to be mediated by T lymphocytes that recognize myelin antigens, cross the blood brain barrier and drive the immune response against oligodendrocytes and myelin sheaths. MS typically presents in relapsing forms, either with a relapsing-remitting (RRMS) or secondary progressive (SPMS) course. Patients suffer acute self-limiting attacks (relapses) of neurological dysfunction followed by complete or incomplete remission. Between relapses, patients are either neurologically and symptomatically stable (RRMS) or continue to deteriorate in function unrelated to relapses (SPMS). Currently available therapies for patients with RRMS (interferon β and glatiramer acetate) have partial efficacy (about 30-40% reduction in relapse rate and at best a modest effect on disability progression). These agents are biologic parenteral treatments requiring injections associated with side effects (injection site reactions, flu-like symptoms). Therefore, there is a strong medical need for a safe and effective oral treatment of MS. (1)

Fingolimod is a novel, orally active, synthetic small molecule in clinical development for MS. Fingolimod is rapidly phosphorylated in vivo, and fingolimod-phosphate (fingolimod-P) acts as agonist of G protein-coupled receptors for sphingosine-1 phosphate (S1P). More particularly, fingolimod-P acts as 'super agonist' of the S1P1 receptor on thymocytes and lymphocytes, inducing internalization of that receptor. This renders these cells unresponsive to S1P1 signaling, thus depriving them of a signal necessary for egress from lymph nodes and secondary lymphoid tissues. The downstream result of this fingolimod induced interdiction of S1P1 signaling is a marked reduction in the number of both B and T lymphocytes in the intravascular compartment and a decrease in recirculation of these cells to extravascular compartments, including the CNS. In animal immunization models, fingolimod neither impaired T-cell activation, expansion, and memory, nor antibody production by B-cells. However, fingolimod will reduce the ability of an expanded number of activated effector lymphocytes to return to a site of inflammation or infection.

Emerging preclinical data suggest that the mechanism of action of fingolimod may involve CNS effects in addition to the effect on reducing lymphocyte infiltration into the CNS. Fingolimod penetrates into the brain, accumulating predominantly in the white matter. S1P receptors are widely expressed in the CNS, in particular on neurons and glial cells and there is evidence for S1P signaling on CNS cells with involvement in e.g. survival and modulation of process formation in both neurons and oligodendrocytes, neurogenesis, and neuroexcitability.(2) In vitro, fingolimod was shown to activate S1P receptors on astrocytes, stimulating intracellular pathways that regulate a variety of functions such as cell proliferation and migration.(3) Other studies, also in cell culture, demonstrated that fingolimod improved the survival and increased the number of progenitor and mature oligodendrocytes. (4,5) Furthermore,fingolimod, when directly administered in the brain of rats, in doses which did not induce any effect in circulating lymphocytes, significantly suppressed the severity of

clinical Experimental Autoimmune Encephalomyelitis (EAE). This clinical improvement was associated with enhanced myelization and axonal protection (6)

The clinical experience with fingolimod is already extensive. In clinical trials, to date over 15000 MS patients have been exposed to single or multiple doses of fingolimod. Based on this experience, pharmacodynamic effects ascribed to fingolimod are: 1) a rapid and persistent reduction of the peripheral lymphocyte count that is reversible after treatment discontinuation, 2) a predictable reduction in heart rate that is maximal upon treatment initiation and attenuates over time under continued treatment, 3) a mild- to moderate increase in airway resistance early after treatment initiation. The molecular basis of these effects is well understood and compatible with the known mode of action of fingolimod via engagement of S1P receptors.

An increased incidence of macular edema has been observed with fingolimod (in combination with cyclosporine A) in the renal transplantation studies. Regular ophthalmic monitoring in the ongoing clinical development program in RRMS patients has revealed cases of macular edema both prior to and after study drug initiation. The risk of macular edema in multiple sclerosis studies is currently under evaluation in the ongoing program, in which all patients are undergoing regular ophthalmology evaluations.

Multiple sclerosis (MS) is a neurodegenerative disease in which both demyelination and axonal loss occur.

Cognitive impairment is a major problem in MS and adversely affects patients' quality of life. Cognitive decline may appear early in the disease process and has been reported even at disease onset. It has previously been shown that a cognitive impairment is present in 53.7% of RRMS patients evaluated at the early disease stage during the onset of neurological symptomatology. (7,8)

Neuronal loss has been historically detected through MRI quantification of brain volume. Gray matter atrophy occurs in patients with relapsing forms of MS early in the disease course, has a major relationship to physical disability and cognitive impairment, and is a putative measure of neuroprotective therapeutic effects. (9)

The paired thalamic nuclei are gray matter structures on both sides of the third ventricle and are involved in a wide range of neurological functions including motor, sensory, integrative, and higher cortical functions.(10) Thalamic location, unique neurologic functions, widespread cortical and subcortical connections and vulnerability to MS pathology from the earliest clinical disease stages make it a critical structure for examining neurodegeneration in MS (11,12)

Cognitive dysfunctions are mainly associated with brain atrophy especially cortical grey matter. (13,14) In this study we will investigate the correlations between the effects of fingolimod on cognitive performances and MRI data.

For diagnostic, prognostic and treatment monitoring purposes, biomarkers which reflect the various neurodegenerative processes, would be helpful. The biomarker has to be central nervous system (CNS) specific, and available in concentrations that could be measured in cerebrospinal fluid (CSF) or blood. Serum and CSF oxysterols and cholesterol precursors have been linked to pathological processes in MS in previous studies, and seem to be promising candidate biomarkers. (15-17)

1.2 Purpose

Our main aim in this study is to investigate the effect of fingolimod on cognitive performance.

Our other aim in this study is to investigate the effect of fingolimod on brain gray matter atrophy and thalamic atrophy.

In this study we will evaluate the effect of fingolimod treatment on the serum levels of a brain specific cholesterol metabolite 24S-hydroxycholesterol (24OHC) which is a biomarker reflecting neurodegeneration. (22) We will also evaluate the effect of fingolimod on plasma levels of osteopontin which is a pro-inflammatory cytokine and matrix metalloproteinase which have a role in the pathology of multiple sclerosis.(23,24)

2 Study objectives and endpoints

2.1 Primary objective

Primary objective of this study is:
-To investigate the effects of Fingolimod on cognitive performance in highly active relapsing remitting multiple sclerosis patients

2.2 Secondary objectives

Secondary objectives of this study are:

-To evaluate the effect of fingolimod on biomarkers (24 hydroxy cholesterol ,osteopontin and matrix metalloproteinases) related to neurodegeneration

-To investigate the effect of fingolimod on brain gray matter atrophy and thalamic atrophy.

-To investigate the correlation between the effect of fingolimod on cognitive performances and MRI data.

2.3 Primary and secondary endpoints:

2.3.1 Primary endpoint:

- To evaluate the effects of Fingolimod on cognitive performance in highly active relapsing remitting multiple sclerosis patients comparing baseline, month 12 and month 24, as assessed by BICAMS battery test.

2.3.2 Secondary endpoints:

-To measure the difference in serum levels of 24S-hydroxycholesterol (24OHC) , osteopontin and matrix metalloproteinases (and also MMPI's) between baseline and after fingolimod treatment at month 12 and month 24.

-To evaluate the effect of fingolimod on cognitive performance in highly active relapsing remitting multiple sclerosis patients comparing baseline, month 12 and month 24, as assessed by PASAT and Stroop test.

-To evaluate the effect of fingolimod on brain gray matter atrophy and thalamic atrophy by comparing MRI data between baseline and month 24.

-To evaluate the correlation between effect of fingolimod on cognitive performances and brain atrophy (gray matter atrophy and thalamic atrophy) by comparing baseline and month 24.

3 Investigational plan

3.1 Study design

This is a 24-month, open-label, multicenter study with a single treatment arm design.

80 relapsing-remitting MS (RRMS) patients according to the McDonald criteria will be recruited in the study. All subjects will sign an informed consent that will be approved by the Ethics Committee.

Upon signing the informed consent, patients will enter a screening phase to be evaluated for eligibility. Screening visit will take place during month -1 to 0. Eligible patients will be given the study drug at baseline visit (Visit 1) and these patients will enter the 24-month open-label treatment phase.

At screening period, patient's eligibility criteria will be confirmed. Patients, who will be monitored at the site, will receive their first doses at the clinic, be observed during the 6-hour monitoring period.

Serum samples will be collected from each participant after evaluation for inclusion and exclusion criteria at baseline and at months 6, 12 and 24 for measurement of 24 hydroxy cholesterol, osteopontin and matrix metalloproteinases (including MMPI's).

BICAMS Battery will be used in this study. The Brief International Cognitive Assessment for MS (BICAMS) includes 3 cognitive tests, Symbol Digit Modalities Test (SDMT, the second edition of the California Verbal Learning Test (CVLT2) and the revised Brief Visuospatial Memory Test (BVMTR). BICAMS Battery will be performed to RRMS patients at baseline and at months 6, 12 and 24.

PASAT will be performed at baseline and at months 6, 12 and 24.

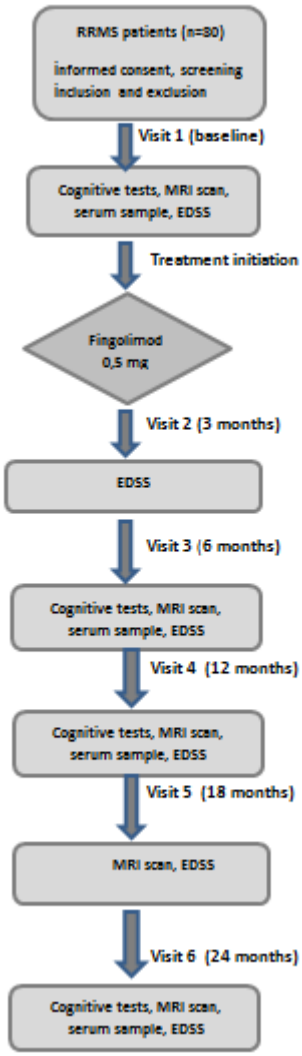
Stroop test will be performed at baseline and at months 6, 12 and 24.

Beck Depression Inventory-II will be performed to distinguish depression and cognitive impairment.

MRI scans will be obtained at baseline and at months 6, 12, 18 and 24 by using 1,5T MRI for measuring gray matter atrophy and thalamic atrophy (a standard scanning protocol will be used for MRI in all centers).

EDSS will be recorded at baseline and at months 3, 6, 12, 18 and 24.

Figure 1: Study protocol flow-chart



3.2 Rationale of dose/regimen, route of administration and duration of treatment

The dose, dose regimen, titration scheme and tapering-off scheme, etc. are in accordance with product labeling.

3.3 Rationale for choice of comparator

A comparator arm is not applicable for this study

3.4 Purpose and timing of interim analyses/design adaptations

Interim analysis might be conducted yearly.

3.5 Risks and benefits

The risk to subjects in this trial will be minimized by compliance with the inclusion/exclusion criteria, close clinical monitoring, and minimal study duration which will be planned as 1 year enrollment time and 2 years follow up.

4 Population

The study will include 80 relapsing remitting MS (RRMS) patients according to the McDonald criteria. All subjects will sign an informed consent that will be approved by the Ethics Committee.

4.1 Inclusion criteria

1. Diagnosed with RRMS as described in 2010 McDonald criteria (36)
2. Provided written informed consent prior to any intervention
3. Female or male patients aged 18-65 years
4. Unresponsive to treatment with a beta interferon or glatiramer acetate for a minimum of one year at and at adequate dose and with high disease activity

   (Unresponsive patients: patients with no changes in relapses, increased relapses, severer relapses with one-year treatment or those who had had at least one relapse during the past one year under previous treatments and one or multiple contrast enhancing lesions in cranial MRI or increased T2 lesions in successive MRIs)
5. EDSS score below 5.5 at screening

4.2 Exclusion criteria

1. Patients with primary or secondary progressive or progressive relapsing MS.
2. Patients with known contraindications for fingolimod treatment.
3. Other coexistent autoimmune diseases including Hashimoto thyroiditis, systemic lupus erythematosus, rheumatoid anthiritis, psoriasis etc.
4. Patients with any of the following cardiovascular conditions:
   - Resting heart rate < 45 bpm/min
   - Cardiac failure at any time during the first study visit (Class III as per NYHA classification) or significant heart disease as judged by the physician
   - Myocardial infarction during the last 6 months
   - History of Mobitz Type II grade 2 AV block
   - Past or current grade 3 AV block

- Confirmed history of sick sinus syndrome or sino-atrial heart block
- arrhythmia requiring current treatment with Class Ia drugs (ajmaline, disopyramid, procainamide, quinidine)
- hypertension uncontrolled with medication

5. History of malignancy of any organ system (other than localized basal cell carcinoma of the skin), treated or untreated, within the past 5 years, regardless of whether there is evidence of local recurrence or metastases.
6. Pregnant or nursing (lactating) women, where pregnancy is defined as the state of a female after conception and until the termination of gestation, detected by urinalysis and confirmed by a positive hCG laboratory test.
7. Negative for varicella-zoster virus IgG antibodies at screening. Patients who have negative results for varicella-zoster virus IgG antibodies can be included in the study after vaccination for varicella-zoster virus.
8. Active systemic bacterial, viral or fungal infections, or diagnosis of AIDS, Hepatitis B, Hepatitis C infection defined as a positive HIV antibody, Hepatitis B surface antigen or Hepatitis C antibody tests, respectively
9. History of previous fingolimod therapy
10. Patient who received any of the treatments below:
    a. Corticosteroids or adrenocorticotropic hormone (ACTH) during the last 1 month
    b. Immunosuppressive medications such as azathioprine or methotrexate etc.
    c. Immunoglobulin treatment during the last 3 months
    d. Cladribine, cyclophosphamide, mitoxantrone, natalizumab at any time

5 Treatment

5.1 Protocol requested treatment

5.1.1 Investigational treatment

Treatment will be 0.5 mg p.o fingolimod daily. The study drug will be provided by Novartis.

5.1.2 Additional study treatment

No additional treatment beyond investigational treatment is requested for this trial.

5.2 Treatment arms

There is only one treatment arm in this study which will include RRMS patients.

5.3 Treatment assignment, randomization

Not Applicable.

5.4 Treating the patient

5.4.1 Patient numbering

Each patient is uniquely identified by a Subject Number which is composed by the site number assigned by Novartis and a sequential number assigned by the investigator. Once assigned to a patient, the Subject Number will not be reused.

Upon signing the informed consent form, the patient is assigned the next sequential number as given by the investigator using the next blank CRF book.

5.4.2 Dispensing the investigational treatment

Each study site will be supplied the investigational treatment in packaging of identical appearance by Novartis.

The investigational treatment packaging has a 2-part label. A unique medication number is printed on each part of this label which corresponds to the treatment. Investigator staff will select the investigational treatment to dispense to the patient using the *visit number* on the label. Immediately before dispensing investigational treatment to the patient, investigator staff will detach the outer part of the label from the packaging and affix it to the source document (Drug Label Form) for that patient's unique patient number. Study treatments will be dispensed to the patients with intervals of 3 months.

5.4.3 Handling of study treatment

5.4.3.1 Handling of investigational treatment

Investigational treatment must be received by a designated person at the study site, handled and stored safely and properly, and kept in a secured location to which only the investigator and designees have access. Upon receipt, all investigational treatment should be stored according to the instructions specified on the labels. Clinical supplies are to be dispensed only in accordance with the protocol.

Medication labels will be in the local language and comply with the legal requirements of each country. They will include storage conditions for the investigational treatment but no information about the patient except for the visit number.

The investigator must maintain an accurate record of the shipment and dispensing of investigational treatment in a drug accountability log. Monitoring of drug accountability will be performed by the field monitor during site visits and at the completion of the trial. Patients will be asked to return all unused investigational treatment and packaging at the end of the study or at the time of discontinuation of investigational treatment.

At the end of the study, and as appropriate during the course of the study, the investigator will return all unused investigational treatment, packaging, drug labels, and a copy of the completed drug accountability log to the Novartis monitor or to the Novartis address provided in the investigator folder at each site.

5.4.3.2 Handling of other study treatment

Not applicable.

5.4.4 Instructions for prescribing and taking study treatment

Prior to administration of the first dose of the study drug the investigator should reconfirm a list of concomitant medications taken by the patient. It is recommended not to initiate treatment with beta-blockers, calcium-channel blockers or digoxin within one week before or after the first dose of the study drug due to a possible additive effect on heart rate reduction.

The first dose of the study drug must be administered under the supervision of the First Dose Administrator or designee. The patient will stay at the study center for a minimum of 6 hours. Heart rate and blood pressure will be monitored hourly during the six hour stay and discharged if specific discharge criteria are met. Hourly monitoring will be extended if the discharge criteria are not met. Some patients may be required to return to the study center for 6 hours following the 2nd dose.

The investigator and/or study personnel should promote compliance by instructing the patient to take one capsule of the study drug orally once daily with or without a meal, preferably at the same time each day and by stating that compliance is necessary for the patient's safety and validity of the study. The patient should be instructed to contact the site if he/she is unable for any reason to take the study drug.

5.4.5 Permitted dose adjustments and interruptions of study treatment

Dose adjustments will not be allowed, however drug interruptions will be allowed based on the judgment of the Investigator.

Conditions/events that may lead to the study drug interruptions based on investigator judgment and overall clinical assessment:

- reported serious adverse event;
- emergency medical condition, unplanned hospitalization, involving use of excluded concomitant medications;
- abnormal laboratory value(s) or abnormal test or examination result(s) (e.g. PFT, liver function tests, ophthalmic findings etc.).
- patient's non-compliance.

Should the patient interrupt the study drug, and should the investigator decide in agreement with the sponsor to re-initiate treatment with the study drug, depending on the duration of the interruption (see below), the first dose at re-start may need to take place under supervision of the First Dose Administrator in a similar manner as the first intake of the study medication. It is recommended not to initiate beta-blockers, calcium-channel blockers or digoxin treatment within one week before or after the day of re-initiation of the study drug administration due to a possible additive effect on heart rate reduction.

When re-starting of study drug monitoring (as for first dose) is mandatory in the following cases:

- The treatment lasted for 14 days or less and was interrupted for more than 1 day

- The treatment lasted for more than 14 days and less than 29 days and was interrupted for 7 days or more

- The treatment lasted for 29 days or more and was interrupted for 14 days or more.

Re-start decision should be made on a case by case basis and should be discussed with the Medical Advisor at Novartis. A reason for the interruption of treatment and time of interruption should be appropriately documented in the source documents as well as in the Dosage Administration Record CRF. All required guidelines for the above maters are given as an appendix to this protocol

5.4.6 Rescue medication

A standard course of corticosteroids (methylprednisolone) on an inpatient or outpatient basis is allowed for treatment of relapses as clinically warranted.

Duration of steroid treatment will be under discretion of the investigator (in general it consists of 5-10 days and up to 1,000 mg methylprednisolone/day). Standard of care procedures will be followed during treatment.

Use of any oral tapering is not permitted.

The use of steroid therapy should be recorded in the related section of CRF.

Investigators should consider the added immunosuppressive effects of corticosteroid therapy and increase vigilance regarding infections during such treatment and in the weeks following administration.

Patients should be reminded of the importance of reporting any signs or symptoms of an infection during treatment. Special consideration should be given to symptoms or signs of herpes simplex or zoster reactivation (e.g. lancinating pain, skin lesions) and appropriate antiviral therapy (e.g acyclovir, valcyclovir) should be promptly initiated and continued for up to 30 days after stopping high dose steroid treatment. An infectious disease specialist may be consulted to guide such therapy if needed.

Should a patient develop any neurological symptoms or signs, unexpected for MS in the opinion of the investigator or accelerated neurological deterioration, the investigator should immediately schedule an MRI and follow the “Guidance on monitoring of patients with symptoms or signs of neurological deterioration inconsistent with MS”. Steroids should not be taken prior to conducting the unscheduled MRI.

5.4.7 Concomitant treatment

The investigator should instruct the patient to notify the study site about any new medications he/she takes after the patient was enrolled into the study.

5.4.8 Prohibited Treatment

Use of the following treatments is NOT allowed concomitantly with the study drug during the course of the study: (use can only be considered if the study drug is permanently discontinued):

- Immunosuppressive medication (e.g. cyclosporine, azathioprine, methotrexate, cyclophosphamide, mitoxantrone, cladribine);
- Other concomitant medications: immunoglobulins, monoclonal antibodies (including natalizumab), INF-ß, glatiramer acetate, adrenocorticotropic hormone (ACTH). The administration of any live or live attenuated vaccines (including for measles) is prohibited while patients are receiving study drug and for 3 months after study drug discontinuation. They may be administered, thereafter, once there is confirmation that lymphocyte counts are in the laboratory normal range. Use of the following treatments is allowed to manage potential adverse reactions associated with the study drug:
    i. anticholinergics (atropine s.c. or i.v.) for treatment of symptomatic bradycardia as the first line treatment, up to 3 mg/day;
    ii. beta-agonists/sympathomimetics (dopamine drip 5-20µg/kg/min or epinephrine drip 2- 10 µg/min) for treatment of non-responsive bradycardia. Standard short course of corticosteroids (methylprednisolone i.v.) is allowed for treatment of relapses.

The medications allowed for treatment of adverse reactions and relapses are not considered study supplies, and therefore, need to be supplied by the study site. The investigator should instruct the patient to notify the study site about any new medications he/she takes after the start of the study drug.

5.4.9 Discontinuation of study treatment and premature patient withdrawal

Patients may voluntarily withdraw from the study for any reason at any time. They may be considered withdrawn if they state an intention to withdraw, fail to return for visits, or become lost to follow-up for any other reason.

If premature withdrawal occurs for any reason, the investigator must make every effort to determine the primary reason for a patient's premature withdrawal from the study and record this information on the Study Completion CRF

The investigator should discontinue study treatment for a given patient or withdraw the patient from study if, on balance, he/she believes that continuation would be detrimental to the patient's well-being.

Study drug must be discontinued for a given patient if the investigator determines that continuing it would result in a significant risk for that patient. The following conditions/events may be considered sufficient to support a decision about the study drug discontinuation in individual cases:

- serious adverse event (e.g. cardiac failure, diagnosed malignancy)
- abnormal laboratory value(s) including liver function tests or abnormal test result(s) (e.g. PFTs, MRI and ophthalmic findings). See below and Appendix 7 and 8 for guidance for safety monitoring
- withdrawal of consent (eg., in case the patient does not consent to continue in the study after a confirmed relapse or disability progression)
- pregnancy

- use of prohibited medications, listed in Section 5.1 and 6.6.5
- adverse events
- protocol violation
- unsatisfactory therapeutic effect
- patient's condition no longer requires study treatment
- administrative problems (e.g. patient's non-compliance)

In addition, the following conditions based on the Guidance on safety and ophthalmic monitoring should result in study drug discontinuation:

- Hepatic Increase in ALT > 5 x ULN Increase in AST > 5 x ULN
- New neurological symptoms accompanied by MRI findings unexpected for MS
- Diagnosis of macular edema
- Decrease in visual acuity with abnormal OCT (increase in central foveal thickness of > 20% compared to screening or cystic changes in the macula).

Patients who discontinue study drug should not be considered withdrawn from the study, unless one of the conditions listed above have been met. The sponsor should be notified about any decision regarding discontinuation of the study medication. Patients who discontinue the study drug should be treated according to the best standard of care. In addition to scheduled visits, patients who discontinue study drug due to adverse events or abnormalities on safety monitoring tests must be followed up with additional visits as needed in order to confirm the resolution of abnormalities. A Study Drug Discontinuation CRF should be completed, giving the date and primary reason for stopping the study drug.

For patients who are lost to follow-up (i.e. those patients whose status is unclear because they fail to appear for study visits without stating an intention to withdraw), the investigator should show "due diligence" by documenting in the source documents steps taken to contact the patient, e.g. dates of telephone calls, registered letters, etc.

5.4.10 Study completion and post-study treatment

The investigator must provide follow-up medical care for all patients who are prematurely withdrawn from the study, or must refer them for appropriate ongoing care.

5.4.11 Early study termination.

The study can be terminated at any time for any reason by Novartis. Should this be necessary, the patient should be seen as soon as possible and treated for a prematurely withdrawn patient. The investigator may be informed of additional procedures to be followed in order to ensure that adequate consideration is given to the protection of the patient's interests. The investigator will be responsible for informing IRBs and/or ECs of the early termination of the trial.

6 Visit schedule and assessments

Table 6-1 lists all of the assessments and indicates with an "x" when the visits are performed.

Table 6-1 Assessment schedule

Visit	Screening Period	1(Baseline) [2]	2	3	4	5	6
Month	**-1 to 0**	**0**	**3**	**6**	**12**	**18**	**24**
Obtain informed consent [1]	X						
Background / Demography	X						
Inclusion / exclusion criteria	X						
Medical history	X						
MS history / MS treatment	X						
Concomitant medication	X		X	X	X	X	X
Physical examination	X		X	X	X	X	X
Vital findings	X	X	X	X	X	X	X
Hematology	X		X	X	X	X	X
Serology	X						
Blood biochemistry	X		X	X	X	X	X
ECG	X	X					
Optical Cohorence Tomography (OCT)	X		X				
Pregnancy test	X						
EDSS	X		X	X	X	X	X
6-hour first dose monitoring		X					
Beck Depression Inventory (BDI-II)		X		X	X		X
PASAT		X		X	X		X
Stroop test		X		X	X		X
BICAMS Battery		X		X	X		X
MRI		X		X	X	X	X
Collecting serum samples for biomarkers		X		X	X		X
Advers events / SAE	X	X	X	X	X	X	X

[1] All assessments must be performed after obtaining informed consents.

[2] At visit 1, all assessments must be performed before initiating the study drug.

Patients should be seen for all visits on the designated day with an allowed "visit window" of "±7" days at visit 2 and "±14" days at visit 3, 4, 5 or as close to it as possible, or as shortly after it as possible, or as shortly before it as possible and "±30" days for the last visit

At a minimum, patients will be contacted for safety evaluations during the 30 days following the last study visit or following the last administration of study treatment if there are post-treatment follow-up visits (whichever is later), including a final contact at the 30-day point. Documentation of attempts to contact the patient should be recorded in the source documentation.

6.1 Information to be collected on screening failures

Patients who sign the informed consent form but fail to meet eligibility criteria during the Screening Phase will be considered screening failure and the reason will be documented on the Screening Record CRF.

6.2 Patient demographics/other baseline characteristics

Data of patient demographics and baseline characteristics will be collected from all patients that include: date of birth and sex. Relevant medical history/current medical condition data includes data until the start of study drug. If there is, concomitant diseases will be recorded. In addition, MS history and MS treatment will be recorded.

At screening, an ECG will be performed to ensure that the patients continue to meet inclusion criteria. Also an ECG will be performed prior to taking the first dose of fingolimod and after 6 hours of the first dose, the ECG will be repeated. Should any of the cardiac exclusion criteria is met based on the ECG results, the patient will not be allowed to participate in the study. ECGs will be printed out and will be kept at the study site as a source document.

6.3 Treatment exposure and compliance

In order to collect accurate information about the study drug exposure, the following records should be maintained for each patient: records of study medication dispensed and returned, dosages administered and intervals between visits.

Compliance will be assessed by the investigator and/or study personnel at each visit using capsule counts and information provided by the patient. A monitor will perform and document drug accountability during site visits and at the end of the study.

Patients who discontinue study medication consecutively for 1 month will be considered withdrawn.

6.4 Efficacy

6.4.1 Cognitive Tests

The Brief International Cognitive Assessment for Multiple Sclerosis (BICAMS) Battery test is the primary efficacy assessment criteria. Cognitive tests will be performed to RRMS patients at baseline and at months 6, 12 and 24 at the study center.

The Brief International Cognitive Assessment for MS (BICAMS)
The Brief International Cognitive Assessment for MS (BICAMS Battery) includes 3 cognitive tests, 1-Symbol Digit Modalities Test (SDMT, 2-the second edition of the California Verbal Learning Test (CVLT2) and 3-the revised Brief Visuospatial Memory Test (BVMTR).

Symbol Digit Modalities Test (SDMT) will be used as the test of information processing speed. It can be completed in 5 minutes for each patient including instructions, practice and testing. It's a highly efficient tool in determination of cognitive impairment in MS patients with a sensitivity rate of 82% and specificity rate of 60%. During the SDMT a series of nine symbols, each paired with a single digit in a key at the top of a standard sheet of paper similar to the one presented below is shown to the patients and patients are asked to voice the digit associated with each symbol as rapidly as possible for 90 seconds for the rest of the figures.

≥	±	«	Π	Ж	ψ	Δ	o	↑
1	2	3	4	5	6	7	8	9

ψ	±	«	Π	ψ	Δ	↑	Ж	±

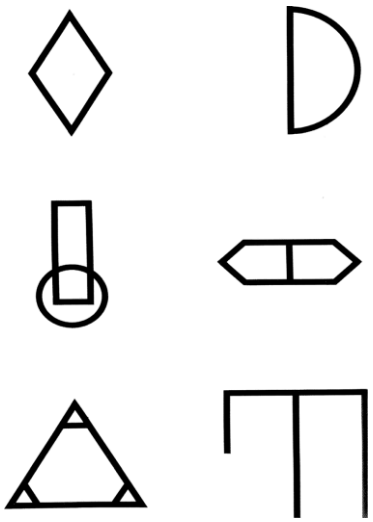
ψ	«	Ж	±	Δ	↑	«	ψ	Π

California Verbal Learning Test 2 (CVLT2) is an auditory/verbal learning test ant it begins with the examiner reading a list of 16 words. Patients listen to the list and report as many of the items as possible. There is no instruction as to the order in which items are recalled. After recall is recorded, the entire list is read again followed by a second attempt at recall. Altogether, there are five learning trials. After 25 min there is a delayed recall trial as well as a yes/no recognition memory task. The 16-item list (an example is given below) will have words that conform to four semantic categories, in this case sports, vegetables, clothes, and tools. Some subjects will recall items in a grouped fashion, and others may recall the list in serial order. There are many variables of recall available in the CVLT2, as a second list is presented, and after 25 min there is a delayed recall trial as well as a yes/no recognition memory task. The list will be read aloud five times in the same order to the patient, at a slightly slower rate than one item per second. Patients will be required to recall as many items as possible, in any order, after each reading of the list. The CVLT-II can be completed in 5–10 minutes, including instructions, testing and responses. The CVLT-II (T1-5 version) has been

validated with brain MR total lesion area and right superior frontal atrophy, 44 MR T1 and FLAIR lesion volume, BPF and third ventricular width32 and MR diffusion measures.

Football	Paper	Folder	Candy
Notebook	River	Boxing	Envelope
Island	Tennis	Mountain	Valley
Billiards	Cake	Pie	Ice cream

Brief Visuospatial Memory Test (BVMTR) will assess visual/spatial memory in BICAMS. In this test, six abstract designs such as the figure shown below are presented for 10 seconds. The display is removed from view and patients render the stimuli via pencil on paper manual responses. Each design receives from 0 to 2 points representing accuracy and location. Thus, scores range from 0 to 12. There will be three learning trials, and the primary outcome measure is the total number of points earned over the three learning trials. Because there is little evidence that the delayed recall trial adds to discriminant validity in MS, as in the MATRICS consensus battery, only the initial learning trials will be included for BICAMS.



Paced Auditory Serial Addition Test (PASAT)

The Paced Auditory Serial Addition Test (PASAT) has been widely used in MS trials and is viewed as one of the most adequate measures of cognitive dysfunction in multiple sclerosis. PASAT was introduced by Gronwall in the 1970s as a clinical tool for measuring the severity of brain injury and its recovery. This test is considered to be a measure of sustained attention, divided attention, concentration, and information processing speed.

During the test the participant is asked to listen to a recorded series of single digits (from 1 to 9) and add each number to the one presented previously under a considerably time pressure. MS patients have been found to be impaired compared with healthy controls at the rapid rates (1.2 and 1.6 sec), at the slow rates (2.0 and 2.4 sec), or at all of the rates of presentation. In the Multiple Sclerosis Functional Composite (MSFC) version, the presentation rate is 3.0 seconds.

The 3-minute version of the PASAT will be used in this study and the number of correct answers will be the outcome variable. PASAT will be performed to RRMS patients at baseline and at months 6, 12 and 24.

Stroop Test:

The Stroop Color and Word Test assesses cognitive processing and provides valuable diagnostic information on brain dysfunction, cognition, and psychopathology. It is based on the observation that individuals can read words much faster than they can identify and name colors. It is a five minute test is applicable for those between the ages of 15 and 90.

This test was adapted from the paper-based Stroop Color-Word Interference Test (Golden, 1978) and consists of three 60-s trials, each of which was preceded by a set of eight practice items to insure the participant understood the task to follow. In the first trial (word reading, W), participants will read color words (RED, BLUE, YELLOW, GREEN) printed in black letters; in the second trial (color naming, C), they will name the colors used to print a row of four Xs; and in the third trial (color-word naming, CW), they will name the color of the print for a set of incongruent Stroop stimuli (e.g., BLUE printed with red letters). In each trial, the stimulus item appeared on the paper, the participant will respond verbally to the item, and either the experimenter (E-press condition) immediately will show the next item. The number of items completed in the allotted time will be recorded. In addition to the W, C, and CW scores, the combined score for word-reading and color-naming (W + C) might be examined as a particularly good measure of processing speed. Also, a measure of relative interference (RI) will be computed by dividing the difference between the color-naming and the color-word naming scores by the color-naming score (RI ¼ [C 2 CW]/C).

Stroop test will be performed to RRMS patients at baseline and at months 6, 12 and 24.

Beck Depression Inventory (BDI-II)

BDI-II is the 1996 revision of BDI. It is a multiple-choice inventory including 21 items and it takes ~ 10 minutes to complete.

Beck Depression Inventory will be assessed to distinguish depression and cognitive impairment. The maximum total score is 63 and scores 0-13 indicates minimal depression, 14-19 indicates mild depression, 20-28 indicates moderate depression and 29-63 indicates severe depression. Beck Depression Inventory will be also performed to distinguish depression and cognitive impairment.

6.4.2 Magnetic Resonance Imaging (MRI) Assessments

MRI scans will be obtained at baseline and at months 6, 12, 18 and 24 by using 1,5T MRI for measuring gray matter atrophy and thalamic atrophy.

A standard scanning protocol will be used for MRI in all centers.

MRI scanning protocol:
MR imaging studies will be performed using a 1.5 Tesla scanner with a dedicated head coil. Patients will be placed in supine position.

For volumetric measurements, 3D T1 FFE sequence will be obtained. Parameters will be as TE: 2.3 ms, TR: 5.1ms, Flip angle: 8°, 1 mm slice thickness, TFE factor: 232, TFE shots: 46

MRI scans will be sent to the Kocaeli University Faculty of Medicine which is the center of the study coordinator investigator for atrophy analysis.

6.4.3 Biomarkers

Serum samples will be collected at baseline and at months 6, 12 and 24 from each participant after evaluation for inclusion and exclusion criteria for measurement of 24 hydroxy cholesterol, osteopontin and matrix metalloproteinases (including MMPI's).

6.4.4 Expanded Disability Status Scale (EDSS)

EDSS is a scale for assessing neurologic impairment in MS (Kurtzke 1983) including (1) a series of scores in each of eight functional systems, and (2) the EDSS steps (ranging from 0 (normal) to 10 (death due to MS). The functional systems are Visual, Brain Stem, Pyramidal, Cerebellar, Sensory, Bowel & Bladder, Cerebral and Other functions. It is recommended that fatigue is not included in the Cerebral score of the EDSS.

EDSS will be assessed by the investigator at each site, who has successfully completed his/her certification requirements. EDSS assessments are scheduled at screening and at month 3, 6, 12, 18 and 24.

6.5 Safety

6.5.1 Laboratory evaluations

6.5.1.1 Hematology

Hematology parameters will be collected at screening and other visits as shown in the assessment schedule and will include: WBC count, absolute lymphocytes, platelet, absolute neutrophil count, and hemoglobin.

6.5.1.2 Clinical chemistry

Blood samples will be collected at screening and at each scheduled visit to verify a patient's eligibility and will include but not limited to the following parameters:
AST, ALT, BUN, Creatinine, Total Cholesterol, Triglycerides, LDL-K, HDL-K, TSH, ST4, Glucose, HbA1c

6.5.2 Electrocardiogram (ECG)

A standard 12 lead ECG will be performed at screening, right before the intake of the first dose of the study drug and after 6 hours of the intake of the study drug. Interpretation of the tracing must be made by a qualified physician and documented on the ECG section of the CRF. Each ECG tracing should be labeled with the study and subject number, date, and kept in the source documents at the study site. Clinically significant abnormalities should also be recorded on the Medical History/Adverse event CRF page. Clinically significant findings must be discussed with the Novartis Medical Monitor prior to enrolling the patient in the study.

6.5.3 Optical Cohorence Tomography (OCT)

An optical coherence tomography for the eyes will be performed in screening and in Visit 2. The tracing of the OCT must be made by a qualified physician and documented on the OCT section of the CRF. Each OCT tracing should be labeled with the study and subject number, date, and kept in the source documents at the study site. Clinically significant abnormalities should also be recorded on the Medical History/Adverse event CRF page. Clinically significant findings must be discussed with the Novartis Medical Monitor prior to enrolling the patient in the study.

6.5.4 Pregnancy and assessments of fertility

All pre-menopausal women who are not surgically sterile will have a urine pregnancy test. A positive urine pregnancy test requires immediate interruption of study drug until serum β-hCG is performed and found to be negative. If positive, the patient must be discontinued from the trial.

7 Safety monitoring

7.1 Adverse events

An adverse event (AE) is any untoward medical occurrence (i.e., any unfavorable and unintended sign [including abnormal laboratory findings], symptom or disease) in a subject or clinical investigation subject *after providing written informed consent* for participation in the study. Therefore, an AE may or may not be temporally or causally associated with the use of a medicinal (investigational) product.

The occurrence of adverse events should be sought by non-directive questioning of the patient at each visit during the study. Adverse events also may be detected when they are volunteered by the patient during or between visits or through physical examination, laboratory test, or other assessments.

Abnormal laboratory values or test results constitute adverse events only if they fulfill at least one of the following criteria:

- they induce clinical signs or symptoms,
- they are considered clinically significant,
- they require therapy.

Clinically significant abnormal laboratory values or test results should be identified through a review of values outside of normal ranges/clinically notable ranges, significant changes from baseline or the previous visit, or values which are considered to be non-typical in patient with underlying disease. Investigators have the responsibility for managing the safety of individual patient and identifying adverse events. Alert ranges for labs and other test abnormalities are included in Appendix 1.

Adverse events should be recorded in the Adverse Events CRF under the signs, symptoms or diagnosis associated with them accompanied by the following information.

- the severity grade
    - mild: usually transient in nature and generally not interfering with normal activities

  - moderate: sufficiently discomforting to interfere with normal activities
  - severe: prevents normal activities
- its relationship to the *(select what is applicable)*
  study treatment (no/yes), or
  investigational treatment (no/yes), or
  the other study treatment(non-investigational) (no/yes), or
  both or indistinguishable,
- its duration (start and end dates) or if the event is ongoing an outcome of not recovered/not resolved should be reported.
- whether it constitutes a serious adverse event (SAE)
- action taken regarding study treatment
- whether other medication or therapies have been taken (concomitant medication/non-drug therapy)
- its outcome (not recovered/not resolved; recovered/resolved; recovering/resolving, recovered/resolved with sequelae; fatal; or unknown)

An SAE is any adverse event (appearance of (or worsening of any pre-existing) undesirable sign(s), symptom(s) or medical conditions(s) which meets any one of the following criteria

- is fatal or life-threatening
- results in persistent or significant disability/incapacity
- constitutes a congenital anomaly/birth defect
- requires inpatient hospitalization or prolongation of existing hospitalization, unless hospitalization is for*:*
  - routine treatment or monitoring of the studied indication, not associated with any deterioration in condition
  - elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since signing the informed consent
  - treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of a SAE given above and not resulting in hospital admission
  - social reasons and respite care in the absence of any deterioration in the patient's general condition
- is medically significant, i.e. defined as an event that jeopardizes the patient or may require medical or surgical intervention to prevent one of the outcomes listed above.

All malignant neoplasms will be assessed as serious under "medically significant" if other seriousness criteria are not met.

Unlike routine safety assessments, SAEs are monitored continuously and have special reporting requirements; see Section 7.2.

All adverse events should be treated appropriately. Treatment may include one or more of the following: no action taken (i.e. further observation only); [study/investigational] *(select)* treatment dosage adjusted/temporarily interrupted; study drug(s) permanently discontinued;

concomitant medication given; non-drug therapy given. The action taken to treat the adverse event should be recorded on the Adverse Event CRF.

Once an adverse event is detected, it should be followed until its resolution or until it is judged to be permanent, and assessment should be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the study treatment, the interventions required to treat it, and the outcome.

Information about common side effects already known about the investigational drug can be found in the Investigator Brochure (IB) or will be communicated between IB updates in the form of Investigator Notifications. This information will be included in the patient informed consent and should be discussed with the patient during the study as needed.

The investigator should also instruct each patient to report any new adverse event (beyond the protocol observation period) that the patient, or the patient's personal physician, believes might reasonably be related to study treatment. This information should be recorded in the investigator's source documents, however, if the AE meets the criteria of an SAE, it must be reported to Novartis.

7.2 Serious adverse event reporting

To ensure patient safety, every SAE, regardless of causality, occurring after the patient has provided informed consent and until 30 days after the last study visit must be reported to Novartis within 24 hours of learning of its occurrence. Any SAEs experienced after the 4 weeks period should only be reported to Novartis if the investigator suspects a causal relationship to study treatment.

Recurrent episodes, complications, or progression of the initial SAE must be reported as follow-up to the original episode, regardless of when the event occurs. This report must be submitted within 24 hours of the investigator receiving the follow-up information. An SAE that is considered completely unrelated to a previously reported one should be reported separately as a new event.

Information about all SAEs (*either initial or follow up information)* is collected and recorded on the paper Serious Adverse Event Report Form. The investigator must assess the relationship to *each specific component of study treatment (if study treatment consists of several drugs)* complete the SAE Report Form in English, and send the completed, signed form by fax within 24 hours after awareness of the SAE to the local Novartis Drug Safety and Epidemiology Department. The telephone and fax number of the contact persons in the local department of Drug Safety and Epidemiology, specific to the site, are listed in the investigator folder provided to each site. The original copy of the SAE Report Form and the fax confirmation sheet must be kept with the case report form documentation at the study site. Follow-up information should be provided using a new paper SAE Report Form stating that this is a follow-up to a previously reported SAE

Follow- up information provided should describe whether the event has resolved or continues, if and how it was treated, whether the treatment code was broken or not and whether the patient continued or withdrew from study participation. Each re-occurrence, complication, or progression of the original event should be reported as a follow-up to that event regardless of when it occurs.

If the SAE is not previously documented in the Investigator's Brochure or Package Insert (new occurrence) and is thought to be related to the investigational treatment a Drug Safety and Epidemiology Department associate may urgently require further information from the investigator for Health Authority reporting. Novartis may need to issue an Investigator Notification (IN) to inform all investigators involved in any study with the same investigational treatment that this SAE has been reported.
Local Novartis Turkey Drug Safety Department will report all fatal or life-threatening suspected unexpected serious adverse reactions (SUSARs) to Ministry of Health as per The Regulation on Clinical Trials and Guidance on the collection, verification and submission of Adverse Event/Reaction Reports Occurring During Clinical Trials **of Medicinal Products and Biological Products.**

7.3 Pregnancy reporting

To ensure patient safety, each pregnancy occurring while the patient is on study treatment must be reported to Novartis within 24 hours of learning of its occurrence. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications.

Pregnancy should be recorded on a Clinical Trial Pregnancy Form and reported by the investigator to the local Novartis Drug Safety and Epidemiology Department. Pregnancy follow-up should be recorded on the same form and should include an assessment of the possible relationship to the study treatment.

Any SAE experienced during pregnancy must be reported on the SAE Report Form.

8 Data review and database management

8.1 Site monitoring

Before study initiation, at a site initiation visit or at an investigator's meeting, a Novartis representative will review the protocol and CRFs with the investigators and their staff. During the study, the field monitor will visit the site regularly to check the completeness of patient records, the accuracy of entries on the CRFs, the adherence to the protocol and to Good Clinical Practice, the progress of enrollment, and to ensure that study treatment is being stored, dispensed, and accounted for according to specifications. Key study personnel must be available to assist the field monitor during these visits.

The investigator must maintain source documents for each patient in the study, consisting of case and visit notes (hospital or clinic medical records) containing demographic and medical information, laboratory data, electrocardiograms, and the results of any other tests or assessments. All information on CRFs must be traceable to these source documents in the patient's file. The investigator must also keep the original informed consent form signed by the patient (a signed copy is given to the patient).

The investigator must give the monitor access to all relevant source documents to confirm their consistency with the CRF entries. Novartis monitoring standards require full verification for the presence of informed consent, adherence to the inclusion/exclusion criteria, documentation of SAEs, and of data that will be used for all primary variables. Additional

checks of the consistency of the source data with the CRFs are performed according to the study-specific monitoring plan. No information in source documents about the identity of the patients will be disclosed.

8.2 Data collection

Designated investigator staff must enter the information required by the protocol onto the Novartis CRFs that are printed on 3-part, non-carbon-required paper. Field monitors will review the CRFs for completeness and accuracy and instruct site personnel to make any required corrections or additions. The CRFs are forwarded to the Medical Documents Reception Center of Novartis *[or CRO working on behalf of Novartis]* by field monitors or by the investigational site, with one copy being retained at the investigational site. Once the CRFs are received by Novartis *[or CRO working on behalf of Novartis]*, their receipt is recorded, the original copy is placed in Central Files, and the non-carbon-required copy is forwarded to the responsible Data Management staff for processing.

8.3 Database management and quality control

Data from the CRFs are entered into the study database by Novartis Data Management staff using single data entry. Verification is performed manually by a separate member of the Data Management staff by comparing the Case Report Form to the data entered into the database.

Data from the CRFs are entered into the study database by Contract Research Organization (CRO) staff following their own internal standard operating procedures that have been reviewed and approved by Novartis.

Subsequently, the entered data are systematically checked by Data Management staff, using error messages printed from validation programs and database listings. Other errors or omissions are entered on Data Query Forms, which are returned to the investigational site for resolution. The signed original and resolved Data Query Forms are kept with the CRFs at the investigator site, and a copy is sent to Novartis so the resolutions can be entered into the database. Quality control audits of all key safety and efficacy data in the database are made prior to locking the database.

Adverse events will be coded using the Medical dictionary for regulatory activities (MedDRA) terminology.

Laboratory samples will be processed centrally and the results will be sent electronically to Novartis (or a designated CRO).

8.4 Data Monitoring Committee

Not required.

8.5 Adjudication Committee

Not required.

9 Data analysis

9.1 Analysis sets

Data analysis will be performed after all of the data is collected from the enrolled patients. All RRMS patients will be accepted as the analysis sets.

The statistical analyses of this study will be performed by a Contract Research Organization

9.2 Patient demographics and other baseline characteristics

Data collected for patient demographics and the baseline characteristics will be summarized by descriptive statistics with summary tables.

9.3 Treatments (study drug, rescue medication, other concomitant therapies, compliance)

Data collected for treatments will be summarized by descriptive statistics with summary tables.

9.4 Analysis of Efficacy Variables

9.4.1 Primary efficacy endpoint:

The primary end-point was planned as evaluating the effects of Fingolimod on cognitive performance in highly active, RRMS patients comparing cognitive performance at baseline and at Month 24, after treatment for two years as assessed by BICAMS battery test.

In this study, it is proposed to observe an increase of 3 points in SDMT testing (which roughly equals to 5 % increase in SDMT overall score) is meaningful and in our study, with treatment we may expect a 5% increase in cognitive functioning during a considerably longer duration of treatment and follow-up.

9.4.2 Secondary efficacy endpoints:

The metric for the PASAT assessment is the number of correct answers.

The Stroop test yields three scores based on the number of items completed on each of the three stimulus sheets.

All MRI analysis will be performed at the Kocaeli University Faculty of Medicine which is the center of the study coordinator investigator.

SIENAX—cross-sectional method will be used to measure atrophy. (25)

Whole brain volume, total gray matter volume, cortical gray matter volume and deep gray matter volume will be measured.

White matter lesions will be segmented by using *k* nearest neighbor classification with tissue-type priors, The resulting lesion volume will be normalized for head size to create normalized lesion volume (26,27) Lesion filling will be applied to minimize the effect of hypointense lesions on atrophy measurements (28). Normalized whole-brain volume, normalized gray

matter volume (NGMV), and normalized white matter volume (NWMV) will be measured with SIENAX (part of FSL [Functional MRI of the Brain, or FMRIB, Software Library] 5.0.2, *http://www.fmrib.ox.ac.uk/fsl*) by using optimized parameters for brain extraction (29,30).

Deep gray matter volumes will be measured by using FSL FIRST software (31). Cortical thickness will be measured by using FreeSurfer 5.1 software *(https://surfer.nmr.mgh.harvard.edu/)* (32,33). FreeSurfer uses the T1-weighted image to locate the white matter and pial surface. The distance between both surfaces gives the cortical thickness at each location.

Thalamic and intracranial volumes will be segmented automatically. Thalamic volumes and thalamic fractions will be calculated as ratios of the left and right thalamic volume to the intracranial volume.

Correlation between effect of fingolimod on cognitive performances and brain atrophy will be explored.

9.4.3 Statistical model, hypothesis, and method of analysis

The basic hypothesis of the study is improvements in cognitive tests in RRMS patients. The sample size calculation for the study has been based on an improvement with treatment in a cognitive test (SDMT).

9.4.3.1 Data analysis

General Approach

All Statistical analyses will be performed with MedCalc Statistical Software version 13.1.2 (MedCalc Software bvba, Ostend, Belgium; http://www.medcalc.org; 2014) at the 5% significance level using 2-sided tests or 2-sided Confidence Intervals.

Descriptive Statistics

The quantitative variables will be summarized using the following parameters:

- Number of non-missing data (n),
- Mean,
- Standard deviation,
- 2-sided 95% CI of the mean,
- Median,
- Minimum,
- Maximum.

The qualitative variables will be summarized using the following parameters:

- Number of non-missing data (n),
- Counts and percentages.

Missing data or unknown responses will not be counted in the percentages.

Comparisons of Two Groups

Student t test will be used for comparisons of two independent normally distributed groups, Mann Whitney u test will be used for comparisons of two independent non-normally distributed groups.

Paired samples t test will be used for comparisons of two dependent normally distributed groups, Wilcoxon Signed Rank test will be used for comparisons of two independent normally distributed groups.

For comparisons of categorical and independent data Chi-Square test or Fisher Exact test (where appropriate) will be used.

To asses categorical and dependent variables Mc Nemar test will be used.

Normality of continuous data will be tested by using Shapiro-Wilk test.

Comparisons of Several Groups

All continuous data (non-normally distributed) with several dependent groups (Baseline, 12th month, 1. Year) will be compared by Friedman's test.

Normally distributed continuous data with several dependent groups (Baseline, 12th month, 1. Year) will be compared by Repeated Measures ANOVA test.

Continuous, normally distributed data with several independent groups will be compared by ANOVA test. For statistically significant results, Post Hoc comparisons will be done with Tukey HSD test.

Continuous, non-normally distributed data with several independent groups will be compared by Kruskal-Wallis test. For statistically significant results, Post Hoc comparisons will be done with Mann Whitney u test.

9.4.3.2 Statistical Methods by Evaluation of Study Objective

Primary Objective:

Primary objective of the study will be evaluated by the statistical methods which are stated below:

- Calculated scores of BICAMS (SDMT, CVLT2 and BVMTR) at baseline, 12th month and 24th month will be compared by using Repeated Measures ANOVA (if variables are non-normally distributed Friedman Test).

  Post-Hoc comparisons will be done with Tukey test. (if Friedman test was used, Wilcoxon Signed Rank test will be used for Post-Hoc comparisons.)

Secondary Objectives:

Secondary objectives of the study will be evaluated by the statistical methods which are stated below:

- The effect of fingolimod on biomarkers (24OHC, Osteopontin and Metalloproteinase) at months 6, 12, 24 will be evaluated by comparing the levels of biomarkers. The evaluation among time points will be made by using Repeated Measures ANOVA.
- The effect of fingolimod on brain gray matter atrophy and thalamic atrophy will be evaluated by comparing MRI parameters at baseline, 6^{th}, 12^{th}, 24^{th} months.

  The comparison among time points will be made by Repeated Measures ANOVA. (if variables are non-normally distributed Friedman Test).

- Investigation the effect of fingolimod on cognitive performance in highly active relapsing remitting multiple sclerosis patients comparing baseline, 12^{th} and 24^{th} month, as assessed by PASAT and Stroop test will be made by Repeated Measures ANOVA. (if variables are non-normally distributed Friedman Test).

- Correlation between cognitive performance and MRI data will be calculated by using Pearson Correlation Coefficient (or Spearman Correlation Coefficient) between PASAT, STROOP and BICAMS tests scores and MRI parameters.

9.4.4 Supportive analyses

Not Applicable

9.5 Safety variables

Adverse and serious advers events and pregnancies will be summarized.

9.6 Sample size calculation

The primary objective of this study was set as investigating the effects of Fingolimod on cognitive performance in highly active relapsing remitting multiple sclerosis (RRMS) patients. Thus the primary end-point was planned as evaluating the effects of Fingolimod on cognitive performance in highly active, RRMS patients comparing cognitive performance at baseline and at Month 24, after treatment for two years as assessed by BICAMS battery test.

The Symbol Digit Modalities Test (SDMT) is one of the best known tests for detecting the cognitive impairment in patients with organic cerebral dysfunctions. The SDMT has a high sensitivity in detecting the presence of brain damage with providing data on cognitive functioning over time in response to treatment. The scoring of SDMT varies between 0-110 and in a study which evaluated age-related cognitive decline, baseline SDMT values for 50-64 years age group was 52.9 and for the 65+age group was 45.9 (34).

There are currently various clinical work is on-going for detection of decremetal or incremental changes of cognitive function in RRMS patients and in a recent publication by Benedict et al which evaluated cognitive decline in RRMS patients, the mean SDMT score was found to be 53.1 for stable RRMS patients at baseline. In this study, the authors concluded that the increment of decline on SDMT was 3.5 raw score points, or roughly 6%. it was concluded that in overall, there seems to be an emerging consensus that a score change of 3–4 points on SDMT is clinically meaningful (35).
In this study, it is proposed to observe an increase of 3 points in SDMT testing (which roughly equals to 5 % increase in SDMT overall score) is meaningful and in our study, with treatment

we may expect a 5% increase in cognitive functioning during a considerably longer duration of treatment and follow-up.
As per published literature, any cognitive improvement on the basis of SDMT test at a rate of % 5 should be evaluated as a significant improvement for RRMS patient with cognitive dysfunction. Thus, in this study we have postulated to reach a 5 % improvement in SDMT test at 24 months when compared to baseline in the cognitively impaired RRMS patients.
Thus, on the basis of percentage improvement expected and by setting alpha at 0.05 and without considering power (no treatment group comparisons will be made); a minimum of 73 RRMS patients has been calculated to be enrolled. However, due to debilitating nature of RRMS as a disease and considerably longer duration of treatment and/or follow-up, a 10 % LFU should be expected and for providing coverage for such cases, 80 RRMS patients should be enrolled to the study.

9.7 Interim analyses

Interim analysis might be conducted yearly.

10 Ethical considerations

10.1 Regulatory and ethical compliance

This clinical study was designed and shall be implemented and reported in accordance with the ICH Harmonized Tripartite Guidelines for Good Clinical Practice, with applicable local regulations (including European Directive 2001/20/EC, US Code of Federal Regulations Title 21, and Japanese Ministry of Health, Labor, and Welfare), and with the ethical principles laid down in the Declaration of Helsinki.

10.2 Informed consent procedures

Eligible patients may only be included in the study after providing written (witnessed, where required by law or regulation), IRB/IEC-approved informed consent, or, if incapable of doing so, after such consent has been provided by a legally acceptable representative of the patient. In cases where the patient's representative gives consent, the patient should be informed about the study to the extent possible given his/her understanding. If the patient is capable of doing so, he/she should indicate assent by personally signing and dating the written informed consent document or a separate assent form. Informed consent must be obtained before conducting any study-specific procedures (i.e. all of the procedures described in the protocol). The process of obtaining informed consent should be documented in the patient source documents.

Novartis will provide to investigators in a separate document a proposed informed consent form that complies with the ICH GCP guideline and regulatory requirements and is considered appropriate for this study. Any changes to the proposed consent form suggested by the investigator must be agreed to by Novartis before submission to the IRB/IEC, and a copy of the approved version must be provided to the Novartis monitor after IRB/IEC approval.

10.3 Responsibilities of the investigator and IRB/IEC

The protocol and the proposed informed consent form must be reviewed and approved by a properly constituted Institutional Review Board/Independent Ethics Committee/Research Ethics Board (IRB/IEC) before study start. A signed and dated statement that the protocol and informed consent have been approved by the IRB/IEC must be given to Novartis before study initiation. Prior to study start, the investigator is required to sign a protocol signature page confirming his/her agreement to conduct the study in accordance with these documents and all of the instructions and procedures found in this protocol and to give access to all relevant data and records to Novartis monitors, auditors, Novartis Clinical Quality Assurance representatives, designated agents of Novartis, IRBs/IECs, and regulatory authorities as required. If an inspection of the clinical site is requested by a regulatory authority, the investigator must inform Novartis immediately that this request has been made.

10.4 Publication of study protocol and results

Novartis assures that the key design elements of this protocol will be posted in a publicly accessible database such as clinicaltrials.gov. In addition, upon study completion and finalization of the study report the results of this trial will be either submitted for publication and/or posted in a publicly accessible database of clinical trial results.

11 Protocol adherence

Investigators ascertain they will apply due diligence to avoid protocol deviations. Under no circumstances should the investigator contact Novartis or its agents, if any, monitoring the trial to request approval of a protocol deviation, as requests to approve deviations will not be granted.

This protocol defines the study objectives, the study procedures and the data to be collected on study participants. Under no circumstances should an investigator collect additional data or conduct any additional procedures for any research related purpose involving any investigational drugs.

If the investigator feels a protocol deviation would improve the conduct of the study this must be considered a protocol amendment, and unless such an amendment is agreed upon by Novartis and approved by the IRB/IEC it cannot be implemented. All significant protocol deviations will be recorded and reported in the CSR.

11.1 Protocol Amendments

Any change or addition to the protocol can only be made in a written protocol amendment that must be approved by Novartis, Health Authorities where required, and the IRB/IEC. Only amendments that are required for patient safety may be implemented prior to IRB/IEC approval. Notwithstanding the need for approval of formal protocol amendments, the investigator is expected to take any immediate action required for the safety of any patient included in this study, even if this action represents a deviation from the protocol. In such cases, Novartis should be notified of this action and the IRB/IEC at the study site should be informed within 10 working days or less, if required by local regulations.

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13 Appendix 1: 2010 Revisions to the McDonald diagnosis criteria for MS Guidelines from International Panel on the diagnosis of MS

Table 1: 2010 McDonald MRI Criteria for Demonstration of DIS
DIS Can Be Demonstrated by ≥1 T2 Lesion[a] in at Least 2 of 4 Areas of the CNS:
• Periventricular
• Juxtacortical
• Infratentorial
• Spinal cord[b]
Based on Swanton et al 2006, 2007. [a]Gadolinium enhancement of lesions is not required for DIS. [b]If a subject has a brainstem or spinal cord syndrome, the symptomatic lesions are excluded from the Criteria and do not contribute to lesion count. MRI = magnetic resonance imaging; DIS = lesion dissemination in space; CNS = central nervous system.

Table 2: 2010 McDonald MRI Criteria for Demonstration of DIT
DIT Can Be Demonstrated by:
1. A new T2 and/or gadolinium-enhancing lesion(s) on follow-up MRI, with reference to a baseline scan, irrespective of the timing of the baseline MRI 2. Simultaneous presence of asymptomatic gadolinium-enhancing and non-enhancing lesions at any time
Based on Montalban et al 2010. MRI = magnetic resonance imaging; DIT = lesion dissemination in time.

Table 3: 2010 McDonald MRI Criteria for Diagnosis of MS in Disease with Progression from Onset
PPMS May be Diagnosed in Subjects With:
1. One year of disease progression (retrospectively or prospectively determined) 2. Plus 2 of the 3 following criteria[a]: A. Evidence for DIS in the brain based on ≥1 T2[b] lesions in at least 1 area characteristic for MS (periventricular, juxtacortical, or infratentorial) B. Evidence for DIS in the spinal cord based on ≥2 T2[b] lesions in the cord C. Positive CSF (isoelectric focusing evidence of oligoclonal bands and/or elevated IgG index)
[a] If a subject has a brainstem or spinal cord syndrome, all symptomatic lesions are excluded from the Criteria. [b] Gadolinium enhancement of lesions is not required. MS = multiple sclerosis; PPMS = primary progressive MS; DIS = lesion dissemination in space; CSF = cerebrospinal fluid; IgG = immunoglobulin G.

Table 4: 2010 McDonald Criteria for Diagnosis of MS

Clinical Presentation	Additional Data Needed for MS Diagnosis
≥2 attacks [a]; objective clinical evidence of ≥2 lesions or objective clinical evidence of 1 lesion with reasonable historical evidence of a prior attack [b]	None [c]
≥2 attacks [a]; 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)[d]; or Await a further clinical attack [a] implicating a different CNS site
1 attack [a]; objective clinical evidence of ≥2 lesions	Dissemination in time, demonstrated by: Simultaneous presence of asymptomatic gadolinium-enhancing and non-enhancing 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 [a]
1 attack [a]; objective clinical evidence of 1lesion (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)[d]; or Await a second clinical attack [a] implicating a different CNS site; and For DIT: Simultaneous presence of asymptomatic gadolinium-enhancing and non-enhancing 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 [a]
Insidious neurological progression suggestive of MS (PPMS)	1 year of disease progression (retrospectively or prospectively determined) plus 2 of 3 of the following criteria [d]: 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.''

[a] 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 occur- ring 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.

[b] 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.

[c] 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.

[d] 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.