NOVARTIS

Clinical Development

[FTY720/Fingolimod]

Clinical Trial Protocol [CFTY720DTR04] / NCT02373098

Effects of Fingolimod (Gilenya®) on Cytokine and Chemokine Levels in Relapsing Remitting Multiple Sclerosis Patients

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List of abbreviations

AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
b.i.d.	twice a day
CRF	Case Report/Record Form (paper or electronic)
CPO	Country Pharma Organization
CRO	Contract Research Organization
DS&E	Drug Safety & Epidemiology
ECG	Electrocardiogram
EDC	Electronic Data Capture
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
o.d.	once a day
p.o.	oral
SAE	serious adverse event

Glossary of terms

Assessment	A procedure used to generate data required by the study
Control drug	Drugs(s) used as a comparator to reduce assessment bias, preserve blinding of investigational drug, assess internal study validity, and/or evaluate comparative effects of the investigational drug
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
Medication number	A unique identifier on the label of each investigational/study drug package in studies that dispense medication using an IRT system
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)
Randomization number	A unique identifier assigned to each randomized patient, corresponding to a specific treatment arm assignment
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	*CFTY720DTR04*
Title	*Effects of Fingolimod (Gilenya®) on Cytokine and Chemokine Levels in Relapsing Remitting Multiple Sclerosis Patients*
Brief title	*Effects of Fingolimod (Gilenya®) on Cytokine and Chemokine Levels in Relapsing Remitting Multiple Sclerosis Patients*
Sponsor and Clinical Phase	Novartis Phase IV
Investigation type	Drug
Study type	Interventional
Purpose and rationale	The main purpose of this study is to measure the serum levels of major Th1, Th2, Th17 and Treg cytokines as well as cytokines and chemokines that are known to be upregulated during multiple sclerosis relapses. Measurements will be performed at 3 time points; i.e. before treatment, 12±1 weeks and 24±2 weeks after fingolimod treatment. By this way, we will be able to test whether fingolimod treatment shifts the cytokine balance from Th1/Th17 to Th2/Treg and reduces the levels of inflammation related cytokines and chemokines. The basal levels of the cytokines will be compared with the cytokine levels of healthy controls. The second purpose of the study is to test the predictive value of cytokine/chemokine changes measured in the 3rd and 6th months on the EDSS, PASAT, SDMT, 9-hole peg test, timed 25-foot walk test and relapse rate observed after 6 months of treatment. This study design is not randomized or double blind, but it has no bias since the patients themselves will be their own controls if they fulfill the enrollment criteria. This study was designed since there is accumulating evidence that Th1 and Th17-type immunities play major roles in multiple sclerosis pathogenesis in the last decade. By contrast, Th2 and Treg cytokines appear to counterbalance the production of Th1 and Th17 cytokines and thus play an ameliorating role in the natural course of multiple sclerosis. Not surprisingly, many multiple sclerosis medications exert their beneficial effects through modulation of these cytokines. The effects of fingolimod on cytokine production are largely unknown. So this study was designed to see to evaluate the effects of fingolimod on cytokine production.
Primary Objective(s) and Key Secondary Objective	Primary objective of this study is: -To evaluate the differences between serum cytokine and chemokine levels of healthy controls and multiple sclerosis patients.

Secondary Objectives	Secondary objective of this study is: -To test the predictive value of cytokine/chemokine changes measured in the 3rd and 6th months on the PASAT, SDMT, 9-Hole peg test, timed 25-foot walk, EDSS and relapse rate observed after 6 months of treatment.
Study design	This study is a multi-center, open label study.
Population	80 relapsing remitting MS (RRMS) patients will be recruited according to the Mc Donald's criteria and 60 healthy controls will be recruited as the control group. All subjects will sign an informed consent that will be approved by the local Ethics Committee and the applicable health authorities prior to venipuncture. Basal EDSS, relapses during the last 2 years, paced auditory serial addition task (PASAT), 9-Hole peg test, timed 25-foot walk and single digit modalities test (SDMT) scores will be recorded before study entry. Serum and blood samples will be collected from each participant after evaluation for inclusion and exclusion criteria. Additional serum and blood samples at 2 different time points will be collected from MS patients at months 3 (±1 week) and 6 (±2 weeks) after starting fingolimod 0.5 mg/day po. treatment.
Inclusion criteria	1. Written informed consent must be obtained before any assessment is performed. 2. RRMS patients aged between 18-65 years that fulfill following criteria: a) In non-treated/newly-diagnosed patients with two or more serious attacks within one year or three or more serious attacks within two years and one or multiple gadolinium-enhancing lesions in cranial MRI or with significantly increased T_2 lesions compared to the previous MRI. b) In non-responders despite treatment with a beta-interferon or glatiramer acetate with adequate duration and dose, where: a. adequate treatment duration is at least 1 year and, b. non-responders are patients with no changes in attacks or increased number of attacks after one year of treatment, or with more severe attacks, or those with one or multiple contrast enhancing lesions in cranial MRI or where there is an increase in T_2 lesions identified with successive MRI's. c) In RRMS patients who are unable to tolerate first-line treatments due to adverse effects associated with parenteral administration. 3. Last relapse of the patient should be at least 2 months before study entry. 4. Last steroid dose of the patient should be at least 1 month before study entry. 5. Last interferon or glatiramer acetate dose of the patient should be at least 1 month before study entry.

	6. Last cyclophosphamide, mitoxantrone, teriflunamide, natalizumab and azathioprine dose of the patient should be at least 3 months before study entry. **Inclusion Criteria for Healthy Control Group** 1. Healthy subjects who don't have any autoimmune disease and aged >18. 2. Written informed consent must be obtained before any assessment is performed.
Exclusion criteria	Patients fulfilling **any** of the following criteria are not eligible for inclusion in this study. No additional exclusions may be applied by the investigator, in order to ensure that the study population will be representative of all eligible patients. 1. Patients with secondary progressive MS. 2. Patients with known contraindications for fingolimod treatment. 3. Other coexistent autoimmune diseases including Hashimoto thyroiditis, systemic lupus erythematosus, rheumatoid arthritis, 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 (disopyramid, procainamide, quinidine) • hypertension uncontrolled with medication 5. History of malignancy of any organ system other than localized basal cell carcinoma of the skin. 6. Pregnant or nursing (lactating) women 7. Negative for varicella-zoster virus IgG antibodies at screening 8. History of previous fingolimod therapy 9. Presence of any clinically active infections
Investigational and reference therapy	Fingolimod

Efficacy assessments	EDSS, PASAT, SDMT, ,9-hole peg test, timed 25-foot walk and relapse rate
Safety assessments	This study does not include any planned safety analysis
Other assessments	Not Applicable
Data analysis	Data analysis will be performed after all of the data is collected from the enrolled patients. All MS patients and control group will be accepted as the analysis sets. Cytokine and chemokine levels will be analyzed cumulatively at the end of the study. The results will be recorded to the blood sampling logs and laboratory logs cumulatively.
Key words	RRMS, Fingolimod, Cytokine, Chemokine

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.

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.

These effects on lymphocytes are believed to be linked to the efficacy of FTY720 in reducing inflammatory activity as seen in animal models of MS as well as in patients with relapsing MS. However, these effects may also lead to potential immunosuppression and an increased risk for infections. This risk may be further enhanced with the concomitant use of other inmmunosuppressants including corticosteroids.

Fingolimod has been shown to be highly active in a number of animal models for autoimmunity. In particular, fingolimod was highly effective both in a prophylactic and a therapeutic setting in several mouse and rat models of experimental autoimmune encephalomyelitis (EAE), an animal model of MS.

The clinical experience with fingolimod is already extensive. To date, over 12000 subjects (healthy volunteers, transplant patients and 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 MS 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.

There is accumulating evidence that Th1 and Th17-type immunities play major roles in multiple sclerosis pathogenesis in the last decade. By contrast, Th2 and Treg cytokines appear to counterbalance the production of Th1 and Th17 cytokines and thus play an ameliorating role in the natural course of multiple sclerosis. Not surprisingly, many multiple sclerosis medications exert their beneficial effects through modulation of these cytokines. The effects of fingolimod on cytokine production are largely unknown.

1.2 Purpose

The main purpose of this study is to measure the serum levels of major Th1, Th2, Th17 and Treg cytokines as well as cytokines and chemokines that are known to be upregulated during multiple sclerosis relapses. Measurements will be performed at 3 time points; i.e. before treatment, 12±1 weeks and 24±2 weeks after fingolimod treatment. By this way, we will be able to test whether fingolimod treatment shifts the cytokine balance from Th1/Th17 to Th2/Treg and reduces the levels of inflammation related cytokines and chemokines. The basal levels of the cytokines will be compared with the cytokine levels of healthy controls.

The second purpose of the study is to test the predictive value of cytokine/chemokine changes measured in the 3rd and 6th months on the EDSS, PASAT, SDMT, 9-hole peg test,timed 25-foot walk test and relapse rate observed after 6 months of treatment.

2 Study objectives

2.1 Primary and key secondary objectives

Primary objective of this study is:
-To evaluate the differences between serum cytokine and chemokine levels of healthy controls and multiple sclerosis patients

2.2 Secondary objectives

Secondary objective of this study is:

-To test the predictive value of cytokine/chemokine changes measured in the 3rd and 6th months on the PASAT, SDMT, 9-Hole peg test, timed 25-foot walk, EDSS and relapse rate observed after 6 months of treatment.

[redacted]

3 Investigational plan

3.1 Study design

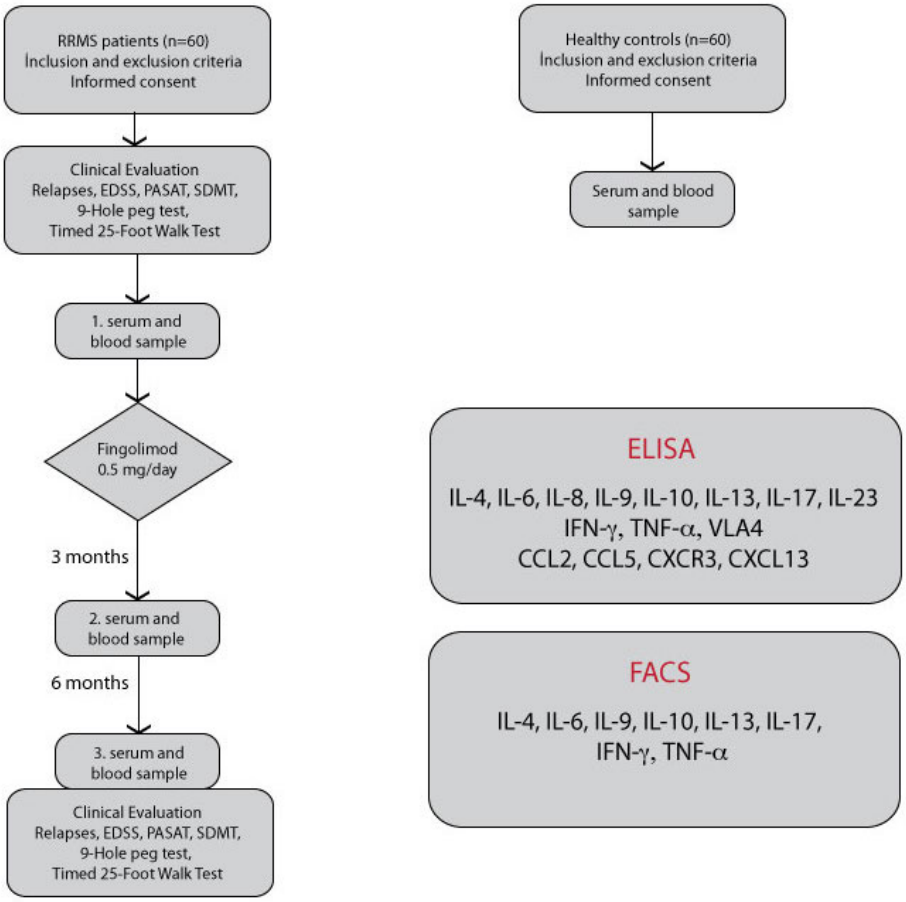
This study is a multi center, open label study.

80 relapsing remitting MS (RRMS) patients will be recruited according to the Mc Donald's criteria and 60 healthy controls will be recruited as the control group. All subjects will sign an informed consent that will be approved by the local Ethics Committee and the applicable health authorities prior to venipuncture. Basal EDSS, relapses during the last 2 years, paced auditory serial addition task (PASAT), 9-Hole peg test, timed 25-foot walk and single digit modalities test (SDMT) scores will be recorded before study entry. Serum and blood samples will be collected from each participant after evaluation for inclusion and exclusion criteria. Additional serum and blood samples at 2 different time points will be collected from MS patients at months 3 (±1 week) and 6 (±2 weeks) after starting fingolimod 0.5 mg/day po. treatment (Figure 1). Serum samples will be stored at -20ºC at local centers for a longest duration of two weeks and kept frozen at -80ºC [redacted] until assayed. The levels of IL-4, IL-6, IL-8, IL-9, IL-10, IL-13, IL-17, IL-23, IFN-γ, TNF-α, VLA4, CCL2, CCL5, CXCR3, CXCL13 will be measured by ELISA according to the manufacturer′s protocols. Optical density (OD) will be measured at 450 nm and cytokine/chemokine concentrations (pg/ml) will be calculated by referring to a standard curve.

Whole blood samples will be dispatched [redacted] In order to perform flow cytometry assay for intracellular cytokine measurement PBMC will be isolated freshly.

EDSS and relapses will be recorded after 6 months of treatment. PASAT, 9-hole peg test, timed 25-foot walk and SDMT's will be performed only at study entry and termination after 6 months (Figure 1).

Figure 1: Study protocol (PASAT: Paced auditory serial addition task, SDMT: single digit modalities test, FACS: Flow assisted cell sorting, flow cytometry)



3.2 Rationale of study design

This study design is not randomized or double blind, but it has no bias since the patients themselves will be their own controls if they fulfill the enrollment criteria.

This study was designed since there is accumulating evidence that Th1 and Th17-type immunities play major roles in multiple sclerosis pathogenesis in the last decade. By contrast, Th2 and Treg cytokines appear to counterbalance the production of Th1 and Th17 cytokines and thus play an ameliorating role in the natural course of multiple sclerosis. Not surprisingly, many multiple sclerosis medications exert their beneficial effects through modulation of these cytokines. The effects of fingolimod on cytokine production are largely unknown. So this study was designed to see to evaluate the effects of fingolimod on cytokine production.

3.3 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.

3.4 Rationale for choice of comparator

A comparator arm is not applicable for this study

3.5 Purpose and timing of interim analyses/design adaptations

Not applicable

3.6 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 1,5 years enrollment time and 6 months follow up.

4 Population

The study will include only 80 relapsing remitting MS patients and 60 healthy controls without any known autoimmune disease. RRMS patients will be followed for 6 months.

4.1 Inclusion criteria

1. Written informed consent must be obtained before any assessment is performed.
2. RRMS patients aged between 18-65 years that fulfill following criteria:

   d) In non-treated/newly-diagnosed patients with two or more serious attacks within one year or three or more serious attacks within two years and one or multiple gadolinium-enhancing lesions in cranial MRI or with significantly increased T_2 lesions compared to the previous MRI.

   e) In non-responders despite treatment with a beta-interferon or glatiramer acetate with adequate duration and dose, where:
   a. adequate treatment duration is at least 1 year and,
   b. non-responders are patients with no changes in attacks or increased number of attacks after one year of treatment, or with more severe attacks, or those with one or multiple contrast enhancing lesions in cranial MRI or where there is an increase in T_2 lesions identified with successive MRI's.
   f) In RRMS patients who are unable to tolerate first-line treatments due to adverse effects associated with parenteral administration.

3. Last relapse of the patient should be at least 2 months before study entry.
4. Last steroid dose of the patient should be at least 1 month before study entry.

5. Last interferon or glatiramer acetate dose of the patient should be at least 1 month before study entry.

6. Last cyclophosphamide, mitoxantrone, teriflunamide, natalizumab and azathioprine dose of the patient should be at least 3 months before study entry.

Inclusion Criteria for Healthy Control Group

3. Healthy people who don't have MS and aged >18.
4. Written informed consent must be obtained before any assessment is performed.

4.2 Exclusion criteria

Patients fulfilling **any** of the following criteria are not eligible for inclusion in this study. No additional exclusions may be applied by the investigator, in order to ensure that the study population will be representative of all eligible patients.

1.Patients with secondary progressive MS.

2.Patients with known contraindications for fingolimod treatment.

3.Other coexistent autoimmune diseases including Hashimoto thyroiditis, systemic lupus erythematosus, rheumatoid arthritis, 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

6.Pregnant or nursing (lactating) women7.Negative for varicella-zoster virus IgG antibodies at screening

8.History of previous fingolimod therapy

9.Presence of any clinically active infections

5 Treatment

5.1 Protocol requested treatment

5.1.1 Investigational treatment

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

Control group will not receive any treatment.

5.1.2 Additional study treatment

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

5.2 Treatment arms

Not Applicable.

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 by Novartis with investigational treatment in packaging of identical appearance.

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.

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.

Handling of other study treatment

Not applicable.

5.4.4 Administering 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. It is recommended that the first dose be administered in the morning time, before 12:00 noon. 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 less than 8 days and was interrupted for more than 1 day
- The treatment lasted for 8 days or more and was interrupted for 8 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.

Steroid treatment should consist of 3-5 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.

If the patient is currently under treatment with corticosteroids, the blood sample should be obtained at least 1 month after the end of the corticosteroid treatment.

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.

Patients who are prematurely withdrawn from the study will be replaced by an equal number of newly enrolled patients.

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 and Table 6-2 lists all of the assessments and indicates with an "x" when the visits are performed.

Table 6-1 Assessment schedule for MS Patients

Visit	**1**	**2**	**3**
Day	**0**	**90±7**	**180±14**
Obtain Informed Consent	X		
Evaluation of Inclusion/Exclusion Criteria	X		
Demgraphic and Previous Data	X		
Other Concomitant Diseases	X		
MS and Treatment History	X		
Assessment of the MS Relapses Within Last 2 Years	X		
Recording of Relapses			X
Pregnancy Test (Urine)	X		
Varicella-Zoster Ab	X		
Hematology *	X**	X	X
Clinical Chemistry *	X**	X	X
ECG	X		
Optical Cohorence Tomography	X	X	
EDSS Measurements	X		X
PASAT Measurements	X		X
Symbol Digit Modalities Test (SDMT) Measurements	X		X
9-Hole Peg Test	X		X
25 Foot Walk Test	X		X
Collecting Serum and Blood Samples for Biomarker Research	X	X	X
Dispense Study Medication	X	X	
First Dose Administration	X		
End of the Study Visit			X

.

* Hematology: Leukocytes, Hemoglobin, Absolute Neutrophilis, Absolut, Lymphocyte, Platelet
Clinical Chemistry: AST, ALT, BUN, Creatinine, Total Cholesterol, Tryglicerides, LDL-K, HDL-K, TSH,ST4, Glucose, HbA1c

**** If the patient has hematology and clinical chemistry results within the last 1month, the tests do not have to be repeated.**

Table 6-2 Assessment schedule for Healthy Control Group

Visit	**1**	**2**	**3**
Day	**0**	**90±7**	**180±14**
Obtain İnformed Consent	X		
Evaluation of Inclusion/Exclusion Criteria	X		
Collecting Serum and Blood Samples	X		

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 , or as close to it as possible, or as shortly after it as possible, or as shortly before it as possible, etc.

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

Since the study is aiming to collect serum samples from the MS patients and healthy volunteers, screening visit is not applicable. The investigators will assess the inclusion/exclusion criteria in visit 1 and the patients who are not eligible for the study will not give any serum samples. There is no information to be collected for screen failures.

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 dieases will be recorded. In addition, MS history and MS treatment will be recorded.

At Visit 1, an ECG will be performed to ensure that the patients continue to meet inclusion criteria prior to taking the first dose of fingolimod. After 6 hours of the first dose, the ECG will be repeated. Should any of the cardiac inclusion 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 for 1 month totally will be considered withdrawn.

6.4 Efficacy

General definition of relapse: Appearance of a new neurological abnormality or worsening of previously stable or improving pre-existing neurological abnormality, separated by at least 30 days from onset of a preceding clinical demyelinating event. The abnormality must be present for at least 24 hours and occur in the absence of fever (<37.5C) or infection.

Severity of MS relapse

Mild relapse	Moderate relapse	Severe relapse
EDSS increase of 0.5 point	EDSS increase of 1 or 2 points	Exceeding Moderate criteria
Or	or	or
1 point FS change in one to three systems	2-point FS change in one or two systems	Exceeding Moderate criteria
	or	or
	1-point change in four or more systems	Exceeding Moderate criteria
Reference: Panitch, H (2002)		

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 baseline and at the end of the 6 monthly treatments on Visit 3.

6.4.1 Efficacy assessment

MS Relapses and EDSS is the key efficacy assessment criteria. Efficacy assessment will be performed at the end of the study.

6.4.2 Appropriateness of efficacy assessments

Measurements are the standard key efficacy criteria for MS.

6.5 Safety

This study does not include any planned safety analysis.

6.5.1 Laboratory evaluations

Blood samples will be collected from each patient and healthy control after evaluation for inclusion and exclusion criteria. Additional blood samples at 2 different time points will be collected from MS patients at months 3 (±1 week) and 6 (±2 weeks) after starting fingolimod 0.5 mg/day po. treatment. Serum samples will be sent to the central laboratory (DETAE) for flow cytometry assay on the same day they are collected. Serum samples will be stored at -20ºC at local centers for a longest duration of two week and kept frozen at -80ºC [redacted]

[redacted] until assayed. The levels of IL-4, IL-6, IL-8, IL-9, IL-10, IL-13, IL-17, IL-23, IFN-γ, TNF-α, VLA4, CCL2, CCL5, CXCR3, CXCL13 will be measured by ELISA according to the manufacturer′s protocols. Optical density (OD) will be measured at 450 nm and cytokine/chemokine concentrations (pg/ml) will be calculated by referring to a standard curve.

In order to perform the flow cytometry assay freshly purified peripheral blood mononuclear cells (PBMC) from venous blood will be stimulated for 24 h by a combination of phorbol ester (PMA, 50 ng/ml) and Ca2+ Ionophore (Ionomycin, 250 ng/ml). The combination of these two stimuli will be used to achieve the strongest stimulus for intracytoplasmic cytokine secretion. Monensin will be added at a final concentration of 1 mM. After stimulation, the cells will be washed with PBS and will be stained with anti-CD4, anti-CD8, anti-CD25, and then fixed and permeabilized. After washing with PBS, the cells will be stained with PE-conjugated anti-IL-4, anti-IL-6, anti-IL-9, anti-IL-10, anti-IL-13, anti-IL-17, anti-IFN-γ, anti-TNF-α for 30 min at room temperature and analyzed by flow cytometry.
Cytokine and chemokine levels will be analyzed cumulatively at the end of the study. The results will be recorded to the blood sampling logs and laboratory logs cumulatively.

6.5.1.1 Hematology

Hematology parameters will be collected at screening and each scheduled visit 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 (Visit 1) 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, Tryglicerides, LDL-K, HDL-K, TSH,ST4, Glucose, HbA1c

6.5.2 Electrocardiogram (ECG)

A standard 12 lead ECG will be performed at baseline. The ECG will be performed 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 the baseline 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.

6.5.5 Appropriateness of safety measurements

The safety assessments selected are standard for this indication/patient population.

6.6 Other assessments

For clinical measures; EDSS and relapses will be recorded after 6 months of treatment. EDSS scoring will also be performed at study entry. PASAT, 9-Hole Peg test, timed 25-foot walk and SDMT tests will be performed only at study entry and termination after 6 months.

6.6.1 Resource utilization

Not Applicable

6.6.2 Health-related Quality of Life

Not Applicable

6.6.3 Pharmacokinetics

Not Applicable

6.6.4 Pharmacogenetics/pharmacogenomics

Not Applicable

6.6.5 Other biomarkers

Not Applicable

7 Safety monitoring

7.1.1 Serious Adverse Events

A Serious Adverse Event (SAE) is defined as an event which:

- 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 the start of study drug
• 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

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

To ensure patient safety, every SAE, regardless of suspected causality, occurring after the patient has provided informed consent and until 30 days after the patient has stopped study participation must be reported to Novartis within 24 hours of learning of its occurrence.
Any SAEs experienced after this 4-week period should only be reported to Novartis if the investigator suspects a causal relationship to the study drug.
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 is collected and recorded on the Serious Adverse Event Report Form. The investigator must assess the relationship to study drug, complete the SAE Report Form in English, and send the completed, signed form by fax within 24 hours to the local Novartis Novartis Drug Safety and Epidemiology Department. The telephone and telefax number of the contact persons in the local department of Novartis Integrated Medical Safety are listed in the investigator folder provided to each site and is also listed below. 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 is sent to the same person to whom the original SAE Report Form was sent, using a new SAE Report Form stating that this is a follow-up to the previously reported SAE and giving the date of the original report. The follow-up information should describe whether the event has resolved or continues, if and how it was treated, and whether the patient continued or withdrew from study participation.
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 Novartis study drug, a Novartis 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 drug that this SAE has been reported.

7.1.2 SAE Reporting to Ministry of Health and Ethical Committee

Local Novartis Turkey Drug Safety Department will report all SAEs to Ministry of Health within 7 days of receipt by Novartis. SAEs are also reported to relevant Ethics Committee simultaneously in the same frame.

Suspected Unexpected Serious Adverse Events will be collected and reported to Ministry of Health and to relevant Ethics Committee every 6 months as a line listing.

Annual Safety Report will be submitted to Ministry of Health and to relevant Ethics Committee on an annual basis.

7.1.3 Pregnancies

To ensure patient safety, each pregnancy in a patient on study drug 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 Novartis study drug of any pregnancy outcome. Any SAE experienced during pregnancy must be reported on the SAE Report Form.

7.1.4 Contact information for Novartis Drug Safety department:

[redacted]

Tel: [redacted]
Novartis Drug Safety Fax: [redacted]

Novartis Drug Safety Mailbox: [redacted]

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. *[Data not requiring a separate written record will be defined before study start and will be recorded directly on the CRFs.]* 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 MS patients and control group will be accepted as the analysis sets.

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 the primary variable(s)

Serum IL-4, IL-6, IL-8, IL-9, IL-10, IL-13, IL-17, IL-23, IFN-γ, TNF-α, VLA4, CCL2, CCL5, CXCR3, CXCL13 levels, PBMC intracellular IL-4, IL-6, IL-9, IL-10, IL-13, IL-17, IFN-γ, TNF-α levels, EDSS, PASAT, 9-Hole peg test, timed 25-foot walk, SDMT scores and relapse rates will be analyzed.

9.5 Statistical model, hypothesis, and method of analysis

There is no special hypothesis planned for this study.

9.6 Supportive analyses

Not Applicable

9.7 Safety variables .

Adverse and serious advers events and pregnancies will be summarized.

9.8 Sample size calculation

Due to the lack of previous studies using all of the cytokines and chemokines and multitudinous analyses, it is difficult to estimate a precise sample size for our study. Despite this, our previous publications indicate that the basal IL-17 concentration is normally distributed with a mean of 32 pg/ml (±stdev of 15 pg/ml) in RRMS patients. If the true difference in the mean concentration of matched pairs is 7 pg/ml, we will need to study 50 pairs of subjects to be able to reject the null hypothesis with a power of 90%. The Type I error probability associated with this test of this null hypothesis is 0,05.
With assumption of drop out rate of 20% minimum 60 RRMS patients and 60 healthy control subjects should enroll to the study. Due to expected high rate of screen failure patients, we decided to screen 80 RRMS patients in order to reach 60 eligible RRMS subjects for the study.

9.9 Interim analyses

Not Applicable

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: 2005 Revisions to the McDonald diagnosis criteria for MS Guidelines from International Panel on the diagnosis of MS

(McDonald 2001,Polman 2005,)

Clinical Presentation	Additional Data Needed for MS Diagnosis
Two or more attacks; objective clinical evidence of 2 or more lesions	None[a]
Two or more attacks; objective clinical evidence of 1 lesion	**Dissemination in space**, demonstrated by: MRI[b] *or* Two or more MRI-detected lesions consistent with MS plus positive CSF[c] *or* Await further clinical attack implicating a different site
One attack; objective clinical evidence of 2 or more lesions	**Dissemination in time**, demonstrated by: MRI[d] *or* Second clinical attack
One attack; objective clinical evidence of 1 lesion (monosymptomatic presentation; clinically isolated syndrome)	**Dissemination in space**, demonstrated by: MRI[b] *or* Two or more MRI-detected lesions consistent with MS plus positive CSF[c] *and* **Dissemination in time**, demonstrated by: MRI[d] *or* Second clinical attack

If the criteria indicated are fulfilled, the diagnosis is multiple sclerosis (MS)

[a]Not additional tests are required; however, if tests [magnetic resonance imaging (MRI), cerebral spinal fluid (CSF)] are undertaken and are negative, extreme caution should be taken before making a diagnosis of MS. Alternative diagnoses must be considered. There must be not better explanation for the clinical picture.

[b]MRI demonstrated of space dissemination must fulfill the criteria listed below.

[c]Positive CSF determined by oligoclonal bands detected by established methods (preferably isoelectric focusing) different from any such bands in serum or by a raised IgG index.

[d]MRI demonstration of time dissemination must fulfill the criteria listed below.

14 Appendix 2: Instructions for the Paced Auditory Serial Addition Test (PASAT)

The PASAT is a measure of cognitive function that specifically assesses auditory information processing speed and flexibility, as well as calculation ability. The PASAT is the last measure administered at each visit. It is presented on audio CD to control the rate of stimulus presentation. Single digits are presented every 3 seconds and the patient must add each new digit to the one immediately prior to it. The test result is the number of correct sums given (out of 60 possible). To minimize familiarity with stimulus items in clinical trails and other serial studies, two alternate forms have been developed; the order of these should be counterbalanced across testing sessions.

MATERIALS NEEDED

CD player, audio CD with PASAT stimuli, clipboard, PASAT Record Forms

15 Appendix 3: EDSS Assessment Criteria

EXPANDED DISABILITY STATUS SCALE

0= normal neurological exam (all FS grade 0)

1.0 = no disability, minimal signs in one FS (one FS grade 1)

1.5 = no disability, minimal signs in more than one FS (more than one FS grade 1)

2.0 = minimal disability in one FS (one FS grade 2, others 1 or 0)

2.5 = minimal disability in two FS (two FS grade 2, others 1 or 0)

3.0 = moderate disability in one FS (one FS grade 3, others 1 or 0) though fully ambulatory; or mild disability in three or four FS (three/four FS grade 2, others 1 or 0) though fully ambulatory

3.5 = fully ambulatory but with moderate disability in one FS (one FS grade 3) and mild disability in one or two FS (one/two FS grade 2) and others 0 or 1;or fully ambulatory with two FS grade 3 (others 0 or 1);or fully ambulatory with five FS grade 2 (others 0 or 1)

4.0 = ambulatory without aid or rest for 3500 meters; up and about some 12 hours a day despite relatively severe disability consisting of one FS grade 4 (others 0 or 1)or combinations of lesser grades exceeding limits of previous steps

4.5 = ambulatory without aid or rest for 3300 meters; up and about much of the day, characterised by relatively severe disability usually consisting of one FS grade 4 and combination of lesser grades exceeding limits of previous steps

5.0 = ambulatory without aid or rest for 3200 meters (usual FS equivalents include at least one FS grade 5, or combinations of lesser grades usually exceeding specifications for step 4.5)

5.5 = ambulatory without aid or rest 3100 meters

6.0 = unilateral assistance (cane or crutch) required to walk at least 100 meters with or without resting

6.5 = constant bilateral assistance (canes or crutches) required to walk at least 20 meters without resting

7.0 = unable to walk 5 meters even with aid, essentially restricted to wheelchair; wheels self and transfers alone; up and about in wheelchair some 12 hours a day

7.5 = unable to take more than a few steps; restricted to wheelchair; may need some normal neurological exam (all FS grade 0) no disability, minimal signs in one FS (one FS grade 1)no disability, minimal signs in more than one FS (more than one FS grade 1) minimal disability in one FS (one FS grade 2, others 0 or 1) minimal disability in two FS (two FS grade 2, others 0 or 1)moderate disability in one FS (one FS grade 3, others 0 or 1) though fully help in transferring and in wheeling self

8.0 = essentially restricted to bed or chair or perambulated in wheelchair, but out of bed most of day; retains many self-care functions; generally has effective use of arms

8.5 = essentially restricted to bed much of the day; has some effective use of arm(s); retains some self-care functions

9.0 = helpless bed patient; can communicate and eat

9.5 = totally helpless bed patient; unable to communicate effectively or eat/swallow

10.0 = death due to MS