STATISTICAL ANALYSIS PLAN

Version 2.0

EFFECTS OF FINGOLIMOD (GILENYA®) ON CYTOKINE AND CHEMOKINE LEVELS IN RELAPSING REMITTING MULTIPLE SCLEROSIS PATIENTS

STUDY NUMBER: FTY720/Fingolimod

STUDY NAME: BIYOMARKER

Statistical Analysis Plan Signature Page

Version 2.0

Sponsor	:	NOVARTIS
Protocol	:	[CFTY720DTR04] / NCT02373098
Study title	:	Effects of Fingolimod (Gilenya®) on Cytokine and Chemokine Levels in Relapsing Remitting Multiple Sclerosis Patients

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1 ABBREVIATIONS

AE	Adverse Event
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
CCL2	Chemokine (C-C motif) ligand 2
CXCL13	Chemokine (C-X-C motif) ligand 13
CXCR3	Chemokine (C-X-C motif) receptor 3
ELISA	Enzyme-linked Immunosorbent Assay
EDSS	Expanded Disability Status Scale
GCP	Good Clinical Practice
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
MS	Multiple Sclerosis
PASAT	Paced Auditory Serial Addition Test
RRMS	Relapsing-remitting Multiple Sclerosis
SAE	Serious Adverse Event
SDMT	Symbol Digit Modalities Test
TNF	Tumor Necrosis Factor
VLA	Very Late Antigen
WBC	White Blood Cell

2 BACKGROUND

The purpose of this statistical plan is to define the tables, lists, data summaries and graphs to be prepared and to determine the statistical methods to be used for the analysis of the results of the study titled, "Effects of Fingolimod (Gilenya®) on Cytokine and Chemokine Levels in Relapsing Remitting Multiple Sclerosis Patients".
This statistical analysis plan has been prepared in pursuant to and in line with ICH-GCP principles and protocol no. CFTY720DTR04.

3 OBJECTIVES OF THE STUDY

3.1. PRIMARY OBJECTIVE

The primary objective of this study is to evaluate the differences between serum cytokine and chemokine levels of healthy controls and multiple sclerosis patients.

3.2. SECONDARY OBJECTIVE

The 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.

4 STUDY DESIGN

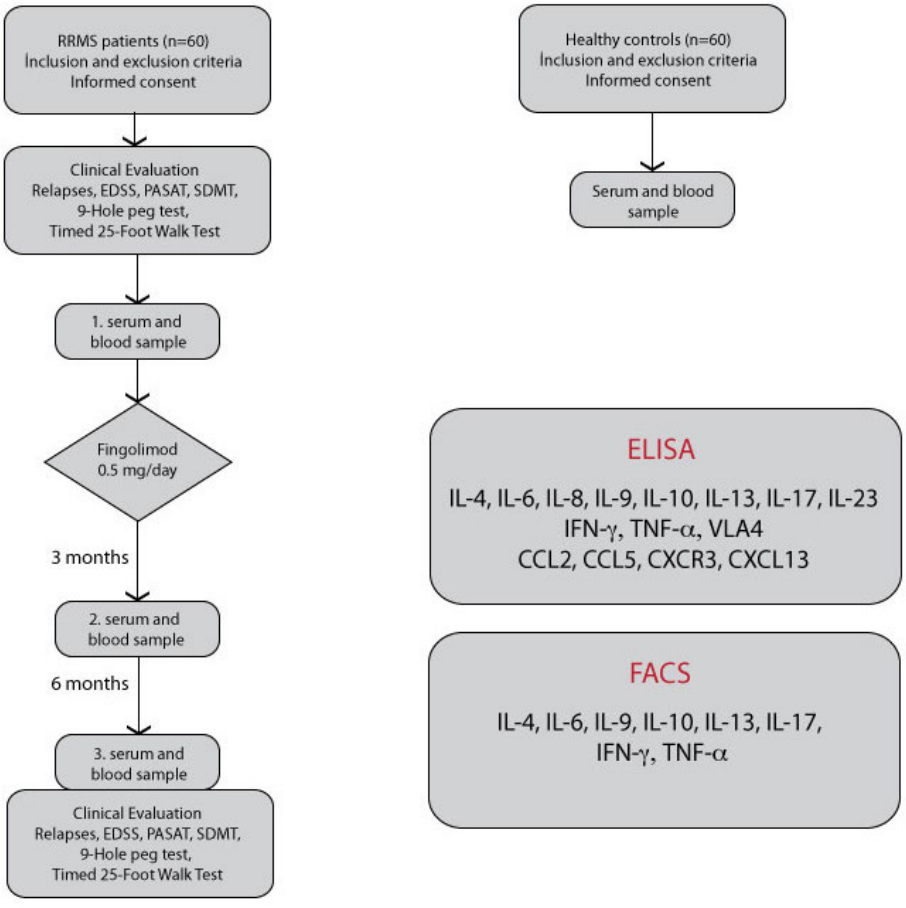
This study is a multi center, open label study.
80 relapsing remitting MS (RRMS) patients will be recruited according to the Mc Donald's criteria and 60 healthy controls will be recruited as the control group. All subjects will sign an informed consent that will be approved by the local Ethics Committee 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]

[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)



5 ANALYZED POPULATIONS

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.

5.1. 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.

6 ANALYSIS PLAN

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.

6.1 DEMOGRAPHICS

- **Gender**: Subjects, male and female, in both groups (Control & MS patients) will be expressed separately as n and %.
- **Age**: Age data for subjects in the “Control” and in the “MS patients” groups will be tabulated by calculating n, mean, standard deviation, minimum, maximum and median.

6.2 CLINICAL DATA

- **Previous MS treatment** of the patients will be expressed as n and %.
- **Age of onset of MS** of the patients will be tabulated by calculating n, mean, standard deviation, minimum, maximum and median.
- **Duration of MS (years)** of the patients will be tabulated by calculating n, mean, standard deviation, minimum, maximum and median.
- **MS relapses in the previous 2 years** of the patients will be tabulated by calculating n, mean, standard deviation, minimum, maximum and median.
- **MS relapses requiring corticosteroids in the previous 2 years** of the patients will be tabulated by calculating n, mean, standard deviation, minimum, maximum and median.
- **MS relapses in the previous 1 year** of the patients will be tabulated by calculating n, mean, standard deviation, minimum, maximum and median.
- **MS relapses requiring corticosteroids in the previous 1 year** of the patients will be tabulated by calculating n, mean, standard deviation, minimum, maximum and median.
- **Duration of treatments (months)** of the patients will be tabulated by calculating n, mean, standard deviation, minimum, maximum and median.
- **Relapse rates** of the patients will be evaluated.

6.3 LABORATORY TESTS

6.3.1 Clinical Biochemistry

Patients' **AST**, **ALT**, **BUN**, **Creatinine**, **Total Cholesterol**, **Tryglicerides**, **LDL-C**, **HDL-C**, **TSH**, **ST4**, **Glucose** and **HbA1c** values will be tabulated by calculating mean and standart deviation and compared as Visit 1 vs. Visit 2 and Visit 1 vs. Visit 3 by using appropriate statistical test. Wilcoxon test will be used in the dependent variables with non-normal distributions whereas Paired sample t test will be used in the dependent variables with normal distributions.

6.3.2 Hematology

Patients' **WBC**, **Absolute Lymphocyte Count**, **Platelet**, **Absolute Neutrophil Count** and **Hemoglobin** values will be tabulated by calculating mean and standart deviation and compared as Visit 1 vs. Visit 2 and Visit 1 vs. Visit 3 by using appropriate statistical test. Wilcoxon test will be used in the dependent variables with non-normal distributions whereas Paired sample t test will be used in the dependent variables with normal distributions.

6.4 ASSESSMENTS OF SCALE

Patients' **EDSS**, **PASAT**, **SDMT**, **9-hole peg test** and **timed 25-foot walk test** values will be tabulated by calculating mean and standart deviation and compared as Visit 1 vs. Visit 3 by using appropriate statistical test. Wilcoxon test will be used in the dependent variables with non-normal distributions whereas Paired sample t test will be used in the dependent variables with normal distributions.

6.5 ASSESSMENT OF CYTOKINE AND CHEMOKINE LEVELS

6.5.1 ELISA

Subjects' **IL-4**, **IL-6**, **IL-8**, **IL-9**, **IL-10**, **IL-13**, **IL-17**, **IL-23**, **IFN-γ**, **TNF-α**, **VLA4**, **CCL2**, **CCL5**, **CXCR3** and **CXCL13** values will be tabulated by calculating mean and standart error and compared as Control group vs. Visit 1 (MS patients), Visit 1 (MS patients) vs. Visit 2 (MS patients), Visit 1 (MS patients) vs. Visit 3 (MS patients) and Control group vs. Visit 3 (MS patients) by using appropriate statistical test. Mann Whitney U test with significance level of 0.05 was used for the independent variables since non-normal distributions had been observed. Wilcoxon test was used for the dependent variables with non-normal distributions. For the independent variables with normal distributions Independent Sample t test was used whereas Paired sample t test was used for the dependent variables with normal distributions.

6.5.2 Flow Cytometry

Subjects' **IL-4**, **IL-6**, **IL-9**, **IL-10**, **IL-13**, **IL-17**, **IFN-γ** and **TNF-α** values will be tabulated by calculating mean and standart error and compared as Control group vs. Visit 1 (MS patients), Visit 1 (MS patients) vs. Visit 2 (MS patients), Visit 1 (MS patients) vs. Visit 3 (MS patients) and Control group vs. Visit 3 (MS patients) by using appropriate statistical test. Mann Whitney U test with significance level of 0.05 was used for the independent variables since non-normal distributions had been observed. Wilcoxon test was used for the dependent variables with non-normal distributions. For the independent variables with normal distributions Independent Sample t test was used whereas Paired sample t test was used for the dependent variables with normal distributions.

6.6 SAFETY ASSESSMENT

Incidence of AEs, incidence of SAEs and number of patients who died or experienced other serious or clinically significant events will be tabulated.