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Clinical Development

FTY720/Fingolimod

CFTY720DUS40 / NCT03257358

A 12-Month, prospective, multicenter, two-cohort, nonrandomized, open-label study in adult patients with Relapsing Multiple Sclerosis (RMS), to investigate changes in immune phenotype biomarkers after treatment with 0.5 mg fingolimod (FTY720) [FLUENT]

Statistical Analysis Plan (SAP)

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SIGNATURE PAGE

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List of abbreviations

AE	Adverse event
ANCOVA	analysis of covariance
CSR	Clinical Study report
FAS	Full Analysis Set
eCRF	Electronic Case Report Form
MedDRA	Medical Dictionary for Drug Regulatory Affairs
NCI	National Cancer Institute
o.d.	Once Daily
qd	Qua'que di'e / once a day
SAP	Statistical Analysis Plan
SOC	System Organ Class
TFLs	Tables, Figures, Listings
WHO	World Health Organization

1 Introduction

This Statistical Analysis Plan (SAP) outlines the planned methods and reports to be used in the statistical analysis of study "CFTY720US40". The purpose of the SAP is to document in details the statistical methods and analysis conventions to be used in the completion of the Clinical Study Report (CSR). Specifically, this document will:

1. Briefly review the objectives, design and endpoints of the study
2. Describe the targeted overall patient enrollment
3. Define the analysis populations
4. Define the rules for determining the outcome of the endpoints
5. Provide an overview of the planned primary, secondary and exploratory statistical analyses.

The reader of this SAP is encouraged to also read the clinical protocol for details on conduct of this study.

1.1 Study design

This study uses a two-cohort, nonrandomized, open-label multicenter design. Patients will be enrolled from up to 125 sites in the United States. The study consists of two periods: screening (up to 4 Weeks) and fingolimod treatment period (Baseline to 12 Months). The total study duration is up to 13 Months. Follow-up Visits will be conducted at 3, 6 and 12 Months. Fingolimod treatment includes two cohorts. In the first cohort approximately 200 patients with RMS, who are newly prescribed commercially available fingolimod 0.5mg/day will participate in the study. In the second cohort approximately 200 RMS patients who have been on commercially available fingolimod 0.5mg/day continuously for at least ≥ 2 years will participate in the study. Cohort 2 will only serve as a referent cohort for Cohort 1 and is not intended to be a direct Cohort 1 comparator as no a priori hypotheses are to be examined.

1.2 Study objectives and endpoints

The primary objective of this study aims to characterize phenotypic changes in the cellular components (biomarkers) of the innate (monocytes, neutrophils and NK cells) and adaptive (peripheral blood T cells, B cells) immune system including their subtypes, in patients with RMS, initiating fingolimod therapy of 0.5mg/day up to 12 Months of treatment (Cohort 1) and those patients who have been on fingolimod 0.5mg/day continuously for ≥ 2 years (Cohort 2). Both cohorts will continue treatment for the next 12 Months.

Secondary objectives are to investigate the association between anti-John Cunningham virus (JCV) antibody status/index and phenotypic changes in innate and T and B cell subsets in patients on fingolimod therapy.

[redacted]

2 Statistical methods

2.1 Data analysis general information

Interim analysis/final analysis will be performed [redacted]

SAS® Software version 9.4 or later will be used in this study as primary statistical analysis software.

Statistical methods used in reporting for both efficacy and safety are described below.

In general, categorical measurements will be summarized by frequency counts and percentages. Continuous variables will summarized by numbers of patients, means, standard deviations, standard error of mean, first quartile, medians, third quartile, interquartile range, minimums, and maximums.

In summary statistics of means and medians, one more decimal place beyond the reported data will be displayed, and 2 more decimal places will be displayed for the standard deviation. The minimum and maximum will keep the same decimal places as the reported data.

Statistical tests will be performed at a 2-sided significance level of 0.05 unless otherwise stated. All p-values will be rounded up to 4 decimal places. For example, any p-value strictly greater than 0.0049 and less than or equal to 0.0050 will get displayed as 0.0050. This guarantees that on any printed statistical output, the unrounded p-value will always be less than or equal to the displayed p-value. A displayed p-value of 0.0001 will always be understood to mean ≤ 0.0001. Likewise, any p-value displayed as 1.0000 will be understood to mean >0.9999 and ≤ 1.

All collected data from the eCRFs (including electronic data) and derived analytical data will be included in the listing.

All summaries will be presented by Cohort.

2.1.1 General definitions

As described in the study protocol, fingolimod treatment refers to Fingolimod 0.5 mg/per day.

The last available assessment on or prior to the reference start date is taken as baseline assessment.

For quantitative measurements,

Change from Baseline will be calculated as:

Change from baseline = Test Value at Visit X – Baseline Value

The date of first administration of fingolimod treatment (Day 1) is defined as the first date when a non-zero dose of fingolimod treatment was administrated. For the sake of simplification, the date of first administration of fingolimod treatment will be also referred to start date of fingolimod treatment.

The date of last administration of fingolimod treatment is derived as the last date on or prior to cut-off date when a non-zero dose of fingolimod treatment was administrated.

The study day describes the day of the event or assessment date, relative to the reference start date (start date of fingolimod treatment or randomization date depending on the type of assessment). The study day will be displayed in data listings. For summaries of vital signs, physical examinations, laboratory data, ECG, etc., assessments will be assigned to a visit based on windowing. Observations from Day 2 through Day 135 will be assigned to the 3-month visit. Days 136 through Day 270 will be assigned to the 6-month visit, and Days 271 and beyond will be assigned to the 12-month visit. If more than one observation occurs within a given window, the one closest to the target day will be used.

2.2 Analysis sets

Enrolled Set: All patients enrolled in one of the two cohorts will be included in the Enrolled Set.

Safety Set: The Safety Set will include all patients who enrolled in one of the two cohorts and received at least one dose of fingolimod during the study. Patients will be analyzed according to the cohort enrolled.

Full Analysis Set: For this study, the Full Analysis Set is the same as the safety set defined above.

2.3 Patient disposition, demographics and other baseline characteristics

2.3.1 Patient disposition

The number of patients enrolled, number of screen failure, patients treated, reasons for not receiving fingolimod treatment, patients who discontinued the fingolimod treatment and reasons for fingolimod treatment discontinuation, number and percentage of patients by end of study status will be summarized.

In addition, the number of patients in each analysis population and reasons for exclusion from a population will also be summarized.

A listing of patient disposition will be provided for all enrolled patients, with the extent of their participation in the study and the reason for discontinuation.

2.3.2 Protocol Deviations

Major protocol deviations will be summarized by cohort. All protocol deviations will be listed, with a flag indicating major or minor.

2.3.3 Demographics

Demographics (age, gender, race, and ethnicity) will be summarized by cohort for the Safety Set.

2.3.4 MS History/Baseline disease characteristics

Baseline disease characteristics will be summarized by cohort for the Safety Set.

- Time (in years) from MS diagnosis to first treatment date, calculated as: (Date of MS diagnosis – date of Day 1 + 1)/ 365.25.
- Time (in years) from first MS symptoms to first treatment date, calculated as: (Date of first MS diagnosis – date of Day 1 + 1)/ 365.25.
- Number of relapses in the last year/ Number of required steroids
- Number of relapses in the last 2 years/ Number of required steroids
- Time (in years) from most recent relapse to Day 1, calculated as: (Date of MS diagnosis – date of Day 1 + 1)/ 365.25.

Listings will be provided for demographic and baseline disease characteristics.

2.4 Past or present MS symptoms with Location

Number of each MS symptom/location/persistency/severity by prior and post fingolimod treatment will be provided for the Safety Set.

All past and present MS symptoms will be listed for the Safety Set.

2.5 MS previous therapies

All MS previous therapies will be coded by medication name and will be summarized by preferred term.

2.6 Fingolimod treatment / compliance / Dose interruption

The duration of fingolimod treatment exposure (days) for patients who took at least one dose of fingolimod treatment will be summarized with descriptive statistics. Duration will be calculated as:

Duration of exposure (days) = (Date of last administration of fingolimod treatment – Date of first administration of fingolimod treatment) +1 .

The actual number of days of fingolimod treatment exposure will be calculated as:

Actual days of fingolimod treatment exposure = (Date of last administration of fingolimod treatment – Date of first administration of fingolimod treatment) +1 – days not on treatment.

Compliance(%) will be calculated as:

Compliance (%) = actual days of fingolimod treatment exposure / duration of exposure *100

2.7 Analysis of the primary objective

2.7.1 Primary endpoint

All analyses will be conducted by cohort for the Safety Set. There are 22 primary endpoints for this study, and Change from Baseline to Month 6 will be calculated as:

Change from baseline to Month 6 = Observed Value at Month 6 (Days 136 to 270) – Baseline Value

1. Change from Baseline to Month 6 in CD4+ naïve T cells (CCR7+CD45RA+)
2. Change from Baseline to Month 6 in CD4+ central memory T cells (CCR7+CD45RACD45RO+)
3. Change from Baseline to Month 6 in CD4+ effector memory T cells (CCR7-CD45RACD45RO+)
4. Change from Baseline to Month 6 in CD4+ Th1 cells (CXCR3+)
5. Change from Baseline to Month 6 in CD4+ Th2 cells (CCR4+)
6. Change from Baseline to Month 6 in CD4+ Th17 cells (CCR6+)
7. Change from Baseline to Month 6 in CD8+ naïve T cells (CCR7+CD45RA+)
8. Change from Baseline to Month 6 in CD8+ central memory T cells (CCR7+CD45RACD45RO+)
9. Change from Baseline to Month 6 in CD8+ effector memory T cells (CCR7-CD45RACD45RO+)
10. Change from Baseline to Month 6 in naïve B Lymphocytes (CD19+CD27-)
11. Change from Baseline to Month 6 in memory B Lymphocytes (CD19+CD27+)
12. Change from Baseline to Month 6 in regulatory B Lymphocytes (CD19+CD24+CD38+)
13. Change from Baseline to Month 6 in monocytes (CD14+)
14. Change from Baseline to Month 6 in neutrophils (CD16+)
15. Change from Baseline to Month 6 in NK cells (CD56+)
16. Change from Baseline to Month 6 in Total CD4+ Absolute Cell Counts
17. Change from Baseline to Month 6 in Total CD4+ Differential Cell Counts
18. Change from Baseline to Month 6 in Total CD8+ Absolute Cell Counts
19. Change from Baseline to Month 6 in Total CD8+ Differential Cell Counts
20. Change from Baseline to Month 6 in Total CD19+ Absolute Cell Counts
21. Change from Baseline to Month 6 in Total CD19+ Differential Cell Counts

[redacted]

Descriptive statistics (sample size, mean, standard deviation, standard error of mean, minimum, first quartile, median, third quartile, interquartile range, and maximum) will be provided. The 95% confidence interval for the mean will be calculated.

Analyses will be performed with respect to the actual value of primary variables by employing an analysis of covariance (ANCOVA) model with gender as a factor and duration of disease at Baseline (either time since first symptom or time since diagnosis) and corresponding baseline as covariates. The unadjusted as well as the adjusted least squares means will be provided together with a p-value.

Percent change from baseline mean will also be reported.

2.7.2 Statistical hypothesis, model, and method of analysis

The majority of analyses will be descriptive in nature. The 95% confidence intervals will be provided for the parameters of interest.

This is an open-label, single arm study, and has no active control. And there is no confirmatory hypothesis to be tested. Thus, the multiplicity adjustment of the level of significance for the primary analysis is not relevant in this study.

2.7.3 Handling of missing values/censoring/discontinuations

No imputation will be performed for efficacy endpoints.

2.8 Analysis of the key secondary objective

Secondary variables consist of both efficacy and safety variables.

2.8.1 Efficacy variables

MS relapses that occur while on study will be summarized by cohort for the FAS. The number of patients with at least one relapse, along with the total number of episodes across all patients, will be presented. The proportion of episodes requiring steroid use, proportion requiring hospitalization, and proportion at each severity level will be summarized. All steroid treatment of MS relapses will be coded by World Health Organization (WHO) drug anatomical therapeutic chemical (ATC) classification. All steroid treatment of MS relapses will be summarized by ATC level 2 and preferred term.

All past and present MS symptoms will be listed for the FAS.

The following additional efficacy variables will be analyzed by cohort using data from patients in FAS.

1. Change from Baseline in Patient Determined Disease Steps (PDDS);
2. Change from Baseline in T2 lesion burden;
3. New Gd-enhancing T1 lesion count

Descriptive statistics (sample size, mean, standard deviation, standard error of mean, minimum, first quartile, median, third quartile, interquartile range, and maximum) will be provided. The 95% confidence interval for the mean will be calculated.

2.8.2 Safety variables

The Safety Set will be used for the following analyses.

1. The assessment of safety will be based mainly on the frequency of adverse events.
2. Change from Baseline to Months 3, and 12 in CD4+ naïve T cells (CCR7+CD45RA+);
3. Change from Baseline to Months 3, and 12 in CD4+ central memory T cells (CCR7+CD45RA-CD45RO+);
4. Change from Baseline to Months 3, and 12 in CD4+ effector memory T cells (CCR7-CD45RA-CD45RO+);
5. Change from Baseline to Months 3, and 12 in CD4+ Th1 cells (CXCR3+);
6. Change from Baseline to Months 3, and 12 in CD4+ Th2 cells (CCR4+);
7. Change from Baseline to Months 3, and 12 in CD4+ Th17 cells (CCR6+);
8. Change from Baseline to Months 3, and 12 in CD8+ naïve T cells (CCR7+CD45RA+);
9. Change from Baseline to Months 3, and 12 in CD8+ central memory T cells(CCR7+CD45RA-CD45RO+);

10. Change from Baseline to Months 3, and 12 in CD8+ effector memory T cells (CCR7-CD45RA-CD45RO+);
11. Change from Baseline to Months 3, and 12 in naïve B cells (CD19+CD27-);
12. Change from Baseline to Months 3, and 12 in memory B cells (CD19+CD27+);
13. Change from Baseline to Months 3, and 12 in regulatory B cells (CD19+CD24+CD38+);
14. Change from Baseline to Months 3, and 12 in monocytes (CD14+);
15. Change from Baseline to Months 3, and 12 in neutrophils (CD16+);
16. Change from Baseline to Months 3, and 12 in NK cells (CD56+);
17. Change from Baseline to Months 3, 6, and 12 in the anti-JCV antibody index;
18. Change from Baseline to Months 3, 6, and 12 in Hematology measurements;
19. Anti-JCV antibody status (+ or -) at Months 3, 6, and 12.
20. Change from Baseline to Months 3, and 12 in Total CD4+ Absolute Cell Counts
21. Change from Baseline to Months 3, and 12 in Total CD4+ Differential Cell Counts
22. Change from Baseline to Months 3, and 12 in Total CD8+ Absolute Cell Counts
23. Change from Baseline to Months 3, and 12 in Total CD8+ Differential Cell Counts
24. Change from Baseline to Months 3, and 12 in Total CD19+ Absolute Cell Counts
25. Change from Baseline to Months 3, and 12 in Total CD19+ Differential Cell Counts

[redacted]

For safety Variable 1, please refer to section 2.11.4 for more details.

For safety Variable 19, the frequency count and percentage together with the 95% confidence interval for the proportion of JCV antibody positive patients will be presented. The shift from baseline to Months 3, 6, and 12 in anti-JCV screening index will also be summarized.

For safety Variables 2–18, descriptive statistics (sample size, mean, standard deviation, standard error of mean, minimum, first quartile, median, third quartile, interquartile range, and maximum) will be provided. The 95% confidence interval for the mean will be calculated.

In addition, correlation coefficients between changes in cellular (T, B and monocyte, neutrophil, NK) subsets and efficacy (MRI based variables and PDDS) and safety outcomes (anti-JCVantibody status and index) will be computed. Analyses of safety data will be by cohort based on the Safety Set.

2.8.3 Handling of missing values/censoring/discontinuations

No impuatition will be performed for safety endpoints.

2.8.4 Adverse events (AEs)

All AE verbatim descriptions will be coded using the Medical Dictionary for Regulatory Activities (MedDRA), Version 19.1 or the most updated version.

Treatment-emergent adverse events (TEAEs) are defined as AEs that started or worsened in severity on or after the first dose of fingolimod treatment and no later than the last treatment date + 14.

All TEAEs will be summarized by presenting the number and percentage of patients having at least one TEAE, and the number of TEAEs that occurred in each System Organ Class (SOC) and Preferred Term (PT). A patient with multiple occurrences of an AE will be counted only once in the corresponding AE category.

In the TEAE summary outputs, adverse events will be sorted by descending percent of patients.

The AE summaries below will be performed:

- An overall summary of the number and percentage of patients

- TEAEs, regardless of fingolimod relationship by SOC and PT
- TEAEs suspected to be fingolimod-related by SOC and PT
- TEAEs, regardless of fingolimod treatment relationship by SOC, PT and maximum severity

TEAEs starting after the first dose of fingolimod treatment with a missing severity will be classified as severe. If a patient reports a TEAE more than once within that System Organ Class (SOC)/ Preferred Term (PT), the AE with the highest severity will be used in the corresponding severity summaries.

2.8.4.1 Deaths and Serious Adverse Events

TEAEs leading to Death are those events with "Outcome" response recorded as "Fatal" on the Adverse Events page of the eCRF. Number of patients with TEAEs leading to death will be summarized by SOC and PT. A listing of all TEAEs leading to death will be provided.

All reported deaths will be listed. Death will also be summarized for overall and for on treatment period (from first dose to 14 days after last dose). The number of deaths and the primary cause of death will be presented by treatment group in the summary table.

Serious adverse events are those events with a response of "Yes" for the item "Was AE Serious" on the AE form. Summaries of incidence rates (frequencies and percentages) of serious AEs by SOC and PT will be prepared. A patient data listing of all SAEs will be provided.

In summary, following summaries of TEAEs will be provided:

- TEAEs leading to death, by SOC and PT
- Serious TEAEs, regardless of fingolimod treatment relationship, by SOC and PT
- TEAEs leading to fingolimod treatment discontinuation, regardless of fingolimod treatment relationship, by SOC and PT

Listings to be provided are :

- AEs leading to Deaths (CRF Adverse Event page: AE outcome is Fatal.)
- Serious AEs
- AEs leading to dose discontinuation

2.9 Interim analysis

Two interim analyses of data will be performed prior to the final analysis at Month 12.

1. The first interim analysis is an early assessment to evaluate safety in Cohort 2 patients. This will be conducted when 50% of patients in Cohort 2 have completed the Month 6 follow up. For this analysis, primary variables, demographics, baseline characteristics, adverse events, and serious adverse events will be summarized.

2. The second interim analysis is the primary analysis for this study. This analysis will be conducted when all patients in Cohorts 1 and 2 complete Month 6 (primary time point of interest) of study. All data collected on patients from both Cohorts 1 and 2 will be included in this analysis. These data will be analyzed as mentioned in protocol Sections 9.4, 9.5, and 9.6.

This is an open-label, single arm study, and has no active control. The first interim analysis is a summarization of data from Cohort 2 only and no confirmatory hypothesis will be tested. Thus, the multiplicity adjustment of the level of significance for the primary analysis due to the first interim analysis is not relevant in this study. Results obtained from these interim analyses may be reported and published externally for communication to health care professions, as appropriate.

3 Sample size calculation

Cohort 1: A sample size of 200 patients will be able to detect an effect size of 0.30 with 89% power after adjusting for multiplicity of testing changes in 15 primary variables, at an overall significance level of 0.05.

In addition, for both cohorts the primary interest is to estimate mean change from Baseline at various time points (Months 3, 6, and 12) of the primary and secondary variables. A sample size of 200 patients in each cohort will provide us the precision of estimates within 0.14 of the corresponding standard deviation of the variables of interest.

4 Change to protocol specified analyses

Not applicable.

5 Appendix

5.1 Imputation rules

In general, other than for partial dates, missing data will not be imputed and will be treated as missing.

Partial date will be imputed as below:

- If year is missing (or completely missing), do not impute;
- 15th of the month will be used to impute the initial diagnosis date if only the day is missing;
- July of the year will be used to impute as the initial diagnosis date if both the day and the month are missing;

5.1.1 Concomitant Medications Start/End Date

Partial missing start/end date to concomitant medications will be imputed as below:

Start date of concomitant medications

- If only the day of the month is missing, use the first day of the month to replace the missing part.
- If both the day and the month are missing, January 1stwill be used to replace the missing part.
- If Day, Month and Year are all missing, use a date one day before the first date of fingolimod treatment.

End date of concomitant medications

- If only Day is missing, use the last day of the month.
- If Day and Month are both missing, use the last day of the year.
- If Day, Month and Year are all missing, assign ‘continuing’ status to stop date

5.1.2 Adverse Events Start/End Date

Missing or partial missing date to the onset date of AE will be imputed as below:

- If the AE onset date is completely missing, the AE start date will be imputed as the first date of fingolimod treatment;

- If the AE onset date is partial missing, then
  - If both the year and the month are available and the year and the month are the corresponding year and month of the first dosing date, then the AE start date will be imputed as the first dosing date;
  - If both the year and the month are available and the year and the month are not equal to the corresponding year and month of the first dosing date, then the AE start date will be imputed as the 1st date of the month;
  - If only the year is available and the available year is the corresponding year of the first dosing date, then the AE start date will be imputed as the first dosing date;
  - If only the year is available, and the available year is not equal to the corresponding year of the first date of fingolimod treatment, then the AE start date will be imputed as the January1st of the year;

Adverse event end date will be imputed as below for the partial date only.

- If both the year and the month are available, AE end date will be imputed as the last day of the month;
- If only the year is available, AE end date will be imputed as the December 31st of the year.

If the imputed AE end date is after the death date, then the date of the death will be imputed as the AE end date.

For adverse events continuing at the cut-off date, the end date will not be imputed and instead will be reported as "ongoing".

5.2 Statistical models

5.2.1 Primary analysis

The primary statistical analysis will use an analysis of covariance (ANCOVA) model with gender as a factor and the duration of disease and baseline as covariates. The primary analysis population will be the Safety Set. The unadjusted as well as the adjusted least squares means will be provided together with a p-value.

5.2.2 Key secondary analysis

The correlation of changes from baseline in cellular (T, B and monocyte, neutrophil, NK) subsets with efficacy (MRI based variables and PDDS) and safety outcomes (anti-JCV antibody status and index) will be estimated within each cohort for each assessment time separately. Analyses involving the MRI based variables or the PDDS will be conducted on the FAS, while analyses of safety outcomes will use the Safety Set.

A Pearson correlation coefficient will be used to estimate the correlation of changes in cellular subsets with changes in T2 lesion volume, changes in PDDS score, and changes in anti-JCV index.

The correlation between changes in cellular subsets and number of new Gd-enhancing T1 lesions will be estimated by a Spearman coefficient.

The relationship between changes in cellular subsets and anti-JCV antibody status (positive or negative) will be assessed using a t-test to compare the mean change in cellular subset across anti-JCV antibody status.

5.3 Reference

Not applicable.