**Official Title:** An Open-label, Multicenter, Biomarker Study to Explore the Mechanism of Action of Ocrelizumab and B-Cell Biology in Patients with Relapsing Multiple Sclerosis or Primary Progressive Multiple Sclerosis

**NCT Number:** NCT02688985

**Document Date:** SAP Version 3: 18-Jul-2023

# Data Analysis Plan (DAP) Module 1

## Sign-Off Sheet

| | |
|---|---|
| **Study title:** | **AN OPEN-LABEL, MULTICENTER, BIOMARKER STUDY TO EXPLORE THE MECHANISM OF ACTION OF OCRELIZUMAB AND B-CELL BIOLOGY IN PATIENTS WITH RELAPSING MULTIPLE SCLEROSIS** |
| **Protocol #:** | ML29966 |

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|---|
| **Study Statistician:** [redacted] |
| |
| **Approver:** |
| [redacted] **Signature** **Date** |

# STATISTICAL ANALYSIS PLAN

**TITLE:** **AN OPEN-LABEL, MULTICENTER, BIOMARKER STUDY TO EXPLORE THE MECHANISM OF ACTION OF OCRELIZUMAB AND B-CELL BIOLOGY IN PATIENTS WITH RELAPSING MULTIPLE SCLEROSIS**

| | |
|---|---|
| **PROTOCOL NUMBER:** | ML29966 |
| **STUDY DRUG:** | Ocrelizumab (RO4964913) |
| **VERSION NUMBER:** | 3 |
| **IND NUMBER:** | 100,593 |
| **EUDRACT NUMBER:** | 2015-004616-37 |
| **SPONSOR:** | Genentech, Inc. |
| **PLAN PREPARED BY:** | [redacted] |
| **DATE FINAL:** | 7/18/2023 |

**STATISTICAL ANALYSIS PLAN AMENDMENT**
**RATIONALE**

## June 2017

Several minor updates have been made following the approval of version 1 of the Statistical Analysis Plan (SAP):

- Biomarker test names have been updated from "CD4+CD3+T cell number" to "CD4+T cell number", and from "CD8+CD3+T cell number" to "CD8+ T cell number".
- Preferred terms of the AESIs (AE of Special Interest) have been updated per MedDRA coding latest version.

## January 2018

In this version of SAP, updates are made to specify analyses for blood NfL data. Blood NfL data up to the same data cut off date as the Interim Analysis 1 (i.e., June 1st 2017) will be summarized as an post-hoc analysis of the Interim Analysis 1 when data become available. All analyses that have been completed during the Interim Analysis 1 (including other biomarker/efficacy analyses, safety analyses etc.) will remain unchanged.

The Statistical Analysis Plan was also amended to incorporate the protocol version 5 updates:

- A Long-Term Extension period has been added to the study to provide ocrelizumab and to collect long-term safety and efficacy information. Updates have been made to align with the protocol on the study design with long term extension period.
- Updates have been made to align with the protocol on exploratory objectives and endpoints incorporating the long-term extension period.
- Updates have been made in the sample size section about additional patients been added to the PPMS cohort.

In addition, scopes of the planned interim and/or primary analyses are described in this version of the SAP.
Additional minor changes have been made to improve clarity and consistency.

## July 2023

Updates for this version of the SAP include expanding the visit windows to include LTE visits due to the impact of the COVID-19 pandemic. In addition, imputation rules for biomarkers measured below the limit of quantification have been defined.

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## 1. BACKGROUND

Multiple sclerosis (MS) is a chronic, inflammatory, demyelinating, and degenerative disease of the central nervous system (CNS) that affects approximately 400,000 people in the United States (U.S.) and 2.5 million worldwide.

There remains an unmet medical need to develop more effective and well tolerated therapies for the treatment of MS. To meet this need, the identification of MS disease biomarkers represents a critical opportunity to potentially understand disease pathogenesis, disease progression, and the mechanism of action of a therapeutic treatment.

Ocrelizumab is a recombinant humanized anti-human monoclonal antibody that selectively targets CD20-expressing B cells. To better understand the mechanism of action of ocrelizumab in MS, this study will assess known biomarkers (OCBs, NfL, B cells, MRI as a surrogate marker, etc.) and explore new potential biomarkers associated with response to ocrelizumab in CSF and peripheral blood.

Refer to the protocol regarding more details of background on biomarkers in MS and background on Ocrelizumab.

## 2. STUDY DESIGN

This is an open-label, multicenter, biomarker study conducted at centers in multiple countries in which all eligible patients will be enrolled to a single treatment arm of ocrelizumab. The study will include an RMS cohort and a PPMS cohort. The RMS cohort is the main cohort and will include the majority of study participants. The RMS cohort will be comprised of four arms, three of which will have patients randomized into one of three arms (Arms 1□3) depending on the time of their second lumbar puncture (LP). In Arm 4, treatment with ocrelizumab will be delayed for 12 weeks after the first LP. The PPMS cohort will comprise a hypothesis-generating portion of the trial, as less is known about PPMS biomarkers.

The study treatment period is comprised of a Main Treatment Period and a Long-term Extension Period. For patients who complete the Main Treatment Period and continue to receive ocrelizumab, a Long-term Extension Period of the study will begin at Week 72 and administer ocrelizumab every 6 months for up to 4 years. The main treatment period will end on the earlier of the dates in which that patient received the Week 72 dose and the date in which Week 72 lab data were collected , or to data cutoff date or end of study date if patient hasn't received the week 72 dose. The long term extension period for each patient will start from the earliest day that patient takes the Week 72 dose or Week 72 lab data is collected till data cutoff date or end of study.

During the Main Treatment Period of the study, two LPs will be drawn from each patient. The two LPs will allow for assessment of biomarkers in CSF that are indicative of response to drug, pharmacokinetic/pharmacodynamic relationships, and/or disease pathogenesis in the CNS. Optional LPs may be collected upon patient's additional consent. Specifically:

Patients in the RMS cohort (Arms 1□3) will receive an LP before the start of dosing with ocrelizumab. Subsequently, these patients will be randomized into one of three arms for timing of the second LP, either Week 12, 24, or 52 following the first dose of ocrelizumab.

Patients in Arm 4 will receive two LPs, separated by a 12-week interval, before administration of the first dose of ocrelizumab. Patients in this arm will be asked if they are willing to give optional CSF samples via LP 12 weeks and 52 weeks after the ocrelizumab dose.

Patients in the PPMS cohort will receive an LP at the start of the study before dosing with ocrelizumab. Subsequently, they will receive a second LP at Week 52 following the first dose of ocrelizumab.

During the Long-term Extension Period, patients will be asked to consent to LPs performed every 2 years. Refer to the protocol regarding more details of study design including the long term extension period.

## 2.1 PROTOCOL SYNOPSIS

The Protocol Synopsis is in Appendix 1.

## 2.2 OUTCOME MEASURES

### 2.2.1 Primary Endpoints

The primary endpoints for the RMS cohort (Arms 1-3) include:

- Change in levels of NfL (pg/mL) in CSF from treatment baseline to post-treatment
- Change in number of CD19 + B cells in CSF (cell number/microliter) from treatment baseline to post-treatment
- Change in number of CD3+T cells in CSF (cell number/microliter) from treatment baseline to post-treatment

### 2.2.2 Exploratory Endpoints

The exploratory endpoints for RMS Arm 4 include:

Change from first LP to second LP comparing Arm 4 to Arm 1 in:

- Levels of NfL in CSF
- Number of CD19 + B cells in CSF

- Number of CD3 + T cells in CSF
- Levels of NfL in blood
- Number of CD19 + B cells in blood
- Number of CD3 + T cells in blood

The exploratory endpoints for the PPMS cohort are:

- Change in levels of NfL in CSF from treatment baseline to post-treatment
- Change in number of CD19 + B cells in CSF from treatment baseline to post-treatment
- Change in number of CD3+T cells in CSF from treatment baseline to post-treatment

For both RMS and PPMS cohorts, other exploratory endpoints include the impact of ocrelizumab treatment on other CSF biomarkers, blood biomarkers and MRI biomarkers listed in this section.

<u>Change from treatment baseline in CSF biomarkers:</u>

- %CD19+ B cell
- %CD3+ T cell
- %CD4+ (CD3+)
- CD4+CD3+ number
- %CD4- (CD3+)
- CD4-CD3+ number
- %IgD+CD27+ (CD19+)
- IgD+CD27+CD19+ number
- %IgD+CD27- (CD19+)
- IgD+CD27-CD19+ number
- %IgD-CD27- (CD19+)
- IgD-CD27-CD19+ number
- %IgD-CD27+ (CD19+)
- IgD-CD27+CD19+ number
- %CD138+CD38+ (IgD-CD27+)
- CD138+CD38+IgD-CD27+CD19+ number
- %CD138-CD38- (IgD-CD27+)
- CD138-CD38-IgD CD27+CD19+ number
- %CD138-CD38+ (IgD-CD27+)
- CD138-CD38+IgD-CD27+CD19+ number
- %CD38+ (IgD+CD27-)
- CD38+IgD+CD27-CD19+ number
- IgG
- IgM
- CXCL13
- CCL19
- Strem2

- YKL40
- IL-6

Change from treatment baseline in Blood biomarkers:

- %CD19+ B cell
- %CD3+ T cell
- CD3+ T cell number
- CD4+T cell number
- CD8+T cell number
- CD19+ B cell number
- CD56+CD16+ NK cell number
- CD4/CD8 Ratio
- NfL
- CXCL13
- IL-6

MRI biomarkers:

- Mean number of T1 Gd-enhanced lesions
- Mean number of new and/or enlarging T2 lesions
- Mean T2 lesion volume
- Mean Regional or total brain volume
- Mean number of leptomeningeal-enhancing regions

### 2.2.3 Pharmacokinetic Outcome Measures

Pharmacokinetics and immunogenicity may be explored in relation to the pharmacodynamics of blood and/or CSF biomarkers in both cohorts. Serum concentration of ocrelizumab may be measured at Weeks 12, 24, 48, and 52; in the case of early termination; and at safety follow-up. The level of ocrelizumab may be measured in the CSF at Weeks 12, 24, and/or 52 for the RMS cohort; and at Week 52 for the PPMS cohort; and/or in a CSF sample taken at an unscheduled visit or from an optional sample.

Assessment may include, but may not be limited to, the relationship of pharmacokinetics in CSF to levels of cells in CSF, or to NfL levels in CSF, and/or compared with serum concentration.

### 2.2.4 Immunogenicity Outcome Measures

- Incidence of anti-drug antibodies (ADAs) to ocrelizumab.

### 2.2.5 Safety Outcome Measures

- Exposure to ocrelizumab, including information such as duration and dosage
- Incidence of
- Treatment emergent adverse events
- Serious treatment emergent adverse events

- Treatment emergent adverse events of special interest
- Treatment emergent adverse events leading to withdrawal of treatment
- Fatal adverse events
- Infusion-related reactions (IRR)
- Listing of lab abnormalities as needed

## 2.3 DETERMINATION OF SAMPLE SIZE

The sample size calculations for this study are based on the assumed reduction from baseline in NfL at Weeks 24 and 52 combined (Arms 2 and 3) in the RMS cohort. Other biomarker measures are not considered for power determination.

Assuming (a) a 30% reduction from pre-treatment NfL (mean [SD] = 860 pg/mL [780 pg/mL]) to posttreatment NfL (mean [SD] = 602 pg/mL [310 pg/mL]) and a correlation coefficient of 0.6, resulting in an SD of 643 pg/mL for the change from pre- to post-treatment, and (b) a desired power of 80% with a Type 1 error of 5%, a sample size of 51 patients is required, with approximately 25 patients in Arm 2 and 26 patients in Arm 3.

This is the first study to explore the impact of ocrelizumab on CSF B-cell count. In a study by Piccio et al. (2010), the rate of undetectable B cells in CSF was 74% (21/26) after 24□30 weeks of rituximab as an add-on to immunomodulatory therapy. Based on this information and a sample size of 51 patients (Arm 2 and 3 combined), the expected 95% CI for the CSF Bcell undetectable rate will be: (0.61, 0.85).

In the RMS cohort, 15 patients will be included in Arm 1 to explore the possible reduction in NfL at Week 12 post-treatment and 15 patients will be assigned to the delayed start RMS control arm (Arm 4). At least 15 patients will be recruited to the PPMS cohort. The above sample sizes are not based on statistical considerations and are meant to help explore the changes in biomarkers and generate hypotheses for future studies.

Based on the above, the total sample size for this study will be at least 96 patients. The sample size will be increased by approximately 10% in order to account for potential missing post-treatment CSF information; therefore, the number of patients to be enrolled in the RMS cohort will be N = 88 (Arm 1: 16 patients, Arm 2: 28 patients, Arm 3: 28 patients, and Arm 4: 16 patients).

The PPMS cohort will enroll at least 16 patients, and additional PPMS patients were allowed to enroll to collect more information on biomarkers. The total sample size of the PPMS cohort may approximate 30 patients. Therefore, the total number of patients to be enrolled is at least 104, and may reach approximately 130 patients.

## 2.4 ANALYSIS TIMING

For publication purpose, an interim analysis (Interim Analysis 1, details specified in SAP version 1) has been conducted based on a clinical data cutoff date (CCOD) of June 1st, 2017. Biomarker data collected from RRMS Arm 1 and Arm 2 patients were summarized based on available data. Blood NfL analyte data was not available at the time of Interim Analysis 1, a post-hoc analysis may be conducted for blood NfL data with CCOD of June 1st 2017 upon its availability.

Table 1 shows the timing of planned interim and final analyses for the main study and LTE period. Additional interim analyses may also be performed for internal exploration and/or publication purposes. No formal adjusting of alpha level is necessary for the multiple interim analyses, as no decisions will be made regarding study conduct based on the interim analyses results.

**Table 1 Outline of Analysis Timings**

| Analysis | Timing of Analysis |
|---|---|
| Interim Analysis 1 | Approximately 50% of patients have had their second LP (CCOD June 1st 2017) Refer to SAP for IA1 for analysis details |
| Interim Analysis 2 | Addition IA planned for publication needs. E.g., when RMS Arm1-4 patients complete 2nd LP. |
| Interim Analysis 3 (Internal review) | Approximately 50% of PPMS patients had their 2nd LP |
| Final Analysis | RMS and PPMS patients complete Main Treatment Period |
| LTE Final Analysis | All patients complete the LTE period (4-Year summary) & Safety Follow-up assessments |

# 3. STUDY CONDUCT

## 3.1 RANDOMIZATION ISSUES

Patients in Arms 1-3 of the RMS cohort will be randomized into each of the three arms at 1:1:1 ratio where the timing of the second LP will be at Week 12, 24, or 52, respectively, following the first dose of Ocrelizumab. An independent interactive voice/web response system (IXRS) provider will conduct randomization and maintain the treatment assignment code. Randomization will be stratified by the previous disease-modifying therapy (DMT) treatment status (DMT-naïve vs. DMT-experienced). Therefore, the proportion of DMT-naïve patients will be approximately equal in each of three arms.

In the event that one of the randomized arms (e.g., Arm 1) reached the planned target patient enrollment (e.g., ~16 patients in Arm 1), subsequent new patients will be randomized into the remaining arms (e.g., Arm 2 and 3) at a 1:1 ratio.

Arm 4 of the RMS cohort with delayed treatment will not be part of the randomization and will be recruited separately.

# 4. STATISTICAL METHODS

This study is designed to assess levels of NfL, levels of lymphocyte populations in CSF, and to generate hypotheses about biomarkers of ocrelizumab treatment in patients with RMS and PPMS. Levels of NfL in CSF and levels of lymphocyte populations in CSF will be compared to baseline levels. In addition, Arm 1 of the RMS cohort (first LP at first dose and second LP at 12 weeks) will be compared to Arm 4 (first LP at 12 weeks before and second LP at the first dose of study drug).

In this exploratory biomarker study, the analyses will be mostly descriptive and hypothesis-generating. Unless otherwise specified, statistical tests will be two-sided and the statistical significance level will be 5%. Corresponding 95% confidence intervals will be presented as appropriate. No corrections for multiple testing will be applied to the primary endpoint analyses, exploratory endpoint analyses, or interim analyses.

The statistical summaries will be descriptive if not otherwise specified. For continuous variables, the mean, median, standard deviation, 25th and 75th percentiles, minimum and maximum will be calculated. For categorical variables, number and percentage in each category will be displayed.

When establishing statistical significance, the non-parametric method will be applied as appropriate, i.e., the Mann-Whitney test will be used between two groups and the Kruskal-Wallis test will be used for three or more groups. For the analysis of continuous data, the paired/unpaired t-test and non-parametric test (i.e., the Wilcoxon matched pairs test) will be performed as appropriate. Some variables may be appropriately transformed as needed (e.g., log transformation of CSF NfL level, CD19+ B cell and CD3+ T cell numbers) before performing statistical analysis. The Spearman's rank-correlation coefficient will be used to evaluate the relationship among the levels of biomarkers or between levels of biomarkers and clinical parameters.

## 4.1 ANALYSIS POPULATIONS

One patient population will be defined for the purpose of the safety analysis, and two patient populations will be defined for the efficacy analysis. All primary efficacy analyses will be performed using the intent-to-treat (ITT) population. The biomarker population was used in the *Interim Analysis 1* on summaries of all biomarker parameters. It may not be used in the other planned analyses if, based on the timing of planned analyses, the biomarker population will be approximately the same as the ITT population. Demographics, baseline MRI, and disease history will be summarized using the enrolled population, defined as all

patients who were enrolled in the study, regardless of whether they received any Ocrelizumab.

### 4.1.1 Intent-to-treat (ITT) Population

The ITT population is defined as all patients enrolled in the study who received at least one dose of Ocrevus. Summaries such as patient disposition and baseline biomarkers will be performed on the ITT population. Summaries of all primary and exploratory endpoints will also be performed using the ITT population.

Patients in this study were randomized/assigned into arms to take the 2nd LP at different timings, while receiving the same treatment. In order to summarize the effect of ocrelizumab on biomarkers over time more accurately, patients will be summarized by the actual "arm" depending on when the patient actually received the 2nd LP.

### 4.1.2 Biomarker Population

A Biomarker population is defined as all enrolled patients who (a) received at least one infusion of Ocrelizumab, even if the infusion was incomplete and (b) received two LPs. Summaries of biomarkers in this population were performed in *Interim Analysis 1*.

### 4.1.3 Pharmacokinetic-Evaluable Population

The definition of pharmacokinetic evaluable population will be specified in a separate analysis plan together with the pharmacokinetic endpoints, if applicable.

### 4.1.4 Safety Population

Safety population is defined as all enrolled patients who received at least one infusion of ocrelizumab, even if the infusion was incomplete. Summary of safety outcome measures will be performed using the safety population. Patients will be summarized by the actual "arm" depending on when the patient actually received the 2nd LP.

## 4.2 ANALYSIS OF STUDY CONDUCT

The following information on patient disposition will be summarized using the ITT population by the actual arm:

- Number and percentage of patients who received ocrelizumab treatment during the Main Treatment Period and the Long-term Extension Period.

- Number and percentage of patients who received LP at each scheduled visit.

- Number and percentage of patients who completed the Main Treatment Period and the Long-term Extension Period of the study.

- Reason for study discontinuation. At interim analysis, patients who haven't filled out the Study Completion CRF by the CCOD of analyses will be considered as "ongoing" in study.

- Reason for discontinuation from ocrelizumab during MainTreatment Period and the Long-term Extension Period. At interim analysis, patients who haven't filled out the Ocrelizumab Completion/Discontinuation CRF by the time CCOD will be considered as "ongoing" on treatment.

- Summaries or listing of major protocol violations.

## 4.3 ANALYSIS OF TREATMENT GROUP COMPARABILITY

For continuous variables, the mean, median, SD, minimum, and maximum will be calculated. For categorical variables, number and percentage in each category will be displayed. The units/categories to be used are indicated within the brackets and separated by commas.

Except where stated, all assessments of treatment group comparability will utilize the date of the baseline visit (not the date of the screening visit) as the reference point in time, unless otherwise stated. For Arm 4, the treatment baseline will be used when comparing to other arms.

Biomarker values for Arm 4 at pre-treatment baseline will be summarized separately and compared to Arm 1 baseline values.

### 4.3.1 Demographics and Baseline Characteristics

The following patient demographics and baseline characteristics will be summarized:

Age (years)

Age (years) group: (age categories < 40, ≥ 40, and age categories < 18, >=18−<40, >= 40−=<55, > 55)

Gender

Self reported race and ethnicity

Female and male reproductive statuses

Region (USA, Canada, EU)

Baseline Height, weight, and BMI

### 4.3.2 Multiple Sclerosis Disease History

Patient disease history summaries will include the following variables:

Duration since MS first symptoms (in years), categorical (=<3, >3 - =<5, >5 - =<10 and >10).

MS diagnosis categories (RRMS, SPMS, PPMS)

Duration since MS diagnosis (in years), categorical (=<3, > 3 - =<6, >6 - =< 9 and >9; =<2, > 2 - =<5, >5 - =< 10 and >10).

Previous disease-modifying therapy (DMT) treatment status (DMT-naïve vs. DMT-experienced)

Number of previous unique DMTs

Number of switches of previous DMTs

Last DMT before study enrollment

Duration of last DMT

Reasons for enrollment eligibility

Baseline EDSS (rounded) and categorical (0-<3, 3- <4, 4-< 6, >=6)

### 4.3.3 Baseline biomarker data

Patient baseline CSF and blood biomarker data summaries will include the variables described in Sections 2.2.1 and 2.2.2. The summaries may also be done by:

Previous disease-modifying therapy (DMT) treatment status (DMT-naïve vs. DMT-experienced).

### 4.3.4 Baseline MRI Data

Patient baseline MRI data summaries will be done as follows:

Number of Gd-enhancing T1 lesions at baseline and categorical (1, 2, 3, 4-8, 9-20, >20)

Number of T2 lesions at baseline and categorical (0-3, 4-5, 6-9, >9)

Volume of T2 lesions at baseline

Brain volume at baseline

## 4.4 EFFICACY ANALYSIS

All biomarker assessments (scheduled or unscheduled) will be summarized after applying visit windows. Summary of the visit windowing rules can be found in Appendix 2. See Module 3 for full visit window specifications. If multiple values of the same parameter occur within the same time window for blood biomarkers

or regular lab values, the worst value for that parameter will be presented in the summary table. If multiple MRI scans occur within the same time window, the scan closest to the scheduled visit date will be included in the analysis.

All summaries will be done based on the ITT population and summaries will be provided by the actual arm, as determined by the actual timing of 2nd LP:

For patients randomized to Arm 1-3,

If the second LP date is missing then patient is included in the arm where originally randomized to;

Else:

| Study Day of second LP (= date of the 2nd LP – 1st infusion start date +1) | Treatment Arm |
|---|---|
| ≤126 | Arm 1 |
| 127-266 | Arm 2 |
| ≥267 | Arm 3 |

Patients randomized to Arm 4 will be summarized as Arm 4 regardless of the timing of 2nd LP.

Unless otherwise specified and where appropriate, statistical tests, if any, will be performed at the two-sided 5% significance level. Corresponding 95% confidence intervals will be presented as appropriate. No corrections for multiple testing will be applied.

For biomarkers measured below the lower limit of quantification (LLOQ), numeric values will be imputed using half of the LLOQ.

### 4.4.1 Primary Endpoints

For RMS cohort arm 1-3, the following values at treatment baseline and post-baseline visits, and changes and percent changes from treatment baseline to post-baseline visits will be summarized by arm:

- Levels of NfL in CSF
- Number of CD19 + B cells in CSF
- Number of CD3+T cells in CSF

The median (IQR) value at pre-treatment baseline, treatment baseline and post-baseline visits will be plotted over time and color coded by each arm. Scatter points of data will be laid over the median (IQR) plot.

### 4.4.2 Exploratory Endpoints

#### 4.4.2.1 Summaries of Exploratory Endpoints Over Time

For RMS patients in Arm 4, values at pre-treatment baseline and treatment baseline, and change and percent change from treatment baseline to post-baseline visits will be summarized.

For RMS patients in arms 1-3 and PPMS patient arm, CSF biomarker endpoints, blood biomarker endpoints and MRI biomarker endpoints stated in Section 2.2.2 will be summarized over time following the same rule as the primary efficacy endpoint, as stated in Section 4.4.1. The median (IQR) value of CSF and blood biomarkers at pre-treatment baseline, treatment baseline and post-baseline visits will be plotted over time and color coded by each arm. Scatter points of data will be laid over the median (IQR) plot for blood NfL level, CD19+ B cell and CD3+ T cell numbers.

Mean (SD) of MRI biomarker endpoints will be summarized over time.

For blood biomarkers, patient arms will also be pooled to look at the effect of ocrelizumab over time.

#### 4.4.2.2 Comparison between Arm 1 and Arm 4

For patients in Arm 4, changes from pre-treatment baseline to treatment baseline will be compared to Arm 1 change from treatment baseline to Week 12 through an ANCOVA model adjusting for baseline DMT status (DMT naïve vs. DMT experienced) and baseline biomarker level for the following endpoints:

- CSF CD19+ B cell, CD3+ T cell and NfL
- Blood CD19+ B cell, CD3+ T cell and NfL

If data are highly skewed, ANCOVA model will be based on percent changes.

#### 4.4.2.3 Associations Among Biomarker Data

The following associations will be assessed separately for each arm at each scheduled visit:

- CSF with blood NfL, CD19+ B cells, and $CD3_+$ T cells.
- change in CSF NfL levels, CD19+ B cells, $CD3_+$ T cells with changes in T1 Gd-enhanced lesions from treatment baseline to post-baseline scheduled visits for arms 1-3 patients, and from pre-treatment baseline to treatment baseline visit for arm 4 patients.

- brain volume with CSF NfL levels.
- change in CSF NfL levels with new/enlargingT2 Gd-enhanced lesions.
- blood NfL with CSF CD19+B cells, CD3+T cells.
- blood NfL with T1 Gd-enhanced lesions.
- change in blood NfL with new and/or enlarging T2 lesions.

The Spearman's rank correlation coefficient will be used to evaluate the relationship among the levels of biomarkers or between levels of biomarkers and clinical parameters. Baseline values across patient arms may be pooled when appropriate. Scatterplots of the two-by-two associations may also be constructed to aid in the interpretation of the coefficients.

The following associations will be assessed by Mann-Whitney test:

- Baseline CSF NfL in DMT-experienced patients versus DMT-naïve patients.
- Baseline CSF NfL levels in patients with 0, 1 and >1 baseline T1 Gd-enhanced lesions.
- Percent change in CSF NfL in patients having at least one relapse event versus patients without relapse during the study.
- Percent change in CSF NfL in DMT-experienced patients versus DMT-naïve patients.

### 4.4.3 Sensitivity Analyses

Due to potentially skewed distribution of the biomarker endpoints, a sensitivity analysis using the non-parametric Mann-Whitney test may be performed as appropriate.

For each of the primary endpoints and exploratory endpoints listed below, an ANCOVA model adjusting for baseline DMT status, baseline biomarker level and other covariates, such as baseline Gd+ T1 lesions category (0, 1, >1) and age, may be conducted, providing sufficient sample size in each covariate category:

- CSF NfL, CD19+ B cell and CD3+T cell
- Blood NfL, CD19+ B cell and CD3+T cell

### 4.4.4 Subgroup Analyses

The primary efficacy endpoints (change in levels of NfL in CSF, change in number of CD19 + B cells and change in number of CD3 + T cells in CSF) will be further summarized for DMT-naïve and DMT-experienced patients separately, and for patients who experienced at least one relapse during the study and patients experienced no relapse separately.

## 4.5 PHARMACOKINETIC AND PHARMACODYNAMIC ANALYSES

Details of pharmacokinetic and pharmacodynamics analyses will be described in a Modeling and Simulation Analysis Plan and results will be reported separately.

## 4.6 SAFETY ANALYSES

Safety analyses will be performed on the safety population. Summaries of adverse events will be generated by summarizing the incidence of treatment-emergent adverse events only. Treatment-emergent events are defined as those adverse events with observed or imputed onset date on or after the start date of study treatment. Only where the most extreme intensity is greater than the initial intensity will events with an onset date prior to the start of study treatment (and with an end date on or after the start of study treatment) be considered treatment emergent. An adverse event with a completely missing non-imputed start date will be assumed to be treatment emergent unless the adverse event has a complete non-imputed end date that is prior to start of study treatment. Protocol-defined relapses over the study period will also be summarized for the safety population.

### 4.6.1 Exposure of Study Medication

Ocrelizumab exposure during the main treatment period will be summarized, including duration and dosage. The main treatment period is defined in Section 2. Accordingly, the duration is calculated as follows:

For patients who received Week 72 dose during the long term extension period, the duration of ocrelizumab exposure during the main treatment period = (Date prior to Week 72 dose – date of first dose) + 1

For patients who did not receive Week 72 dose, the duration = (the earlier of CCOD/completing/discontinuing treatment − date of first dose) +1

Ocrelizumab exposure during the long term extension treatment period will also be summarized for patients who received Week 72 dose, including duration and dosage. The long term extension treatment period is defined in Section 2. Accordingly, the duration is calculated as (the earlier of CCOD/date of completing/discontinuing long term extension treatment − date of Week 72 dose) +1.

Ocrelizumab exposure for the entire study will be summarized and calculated as the (date of last dose - date of first dose) + 1.

### 4.6.2 Adverse Events

Treatment emergent adverse events and serious treatment emergent adverse events will be coded, summarized overall and by NCI CTCAE v4.0 grade, and tabulated by body system and Preferred Term for individual events within each body system.

The number and percentage of patients with at least one IRR, and the intensity (highest grade for each patient) will be summarized in total and by dose cycle (patients with multiple events within an infusion will count only once). In addition, the total number of IRRs will be summarized (multiple events will be counted) in total and by dose cycle.

### 4.6.3 Laboratory Data

Subject listing of abnormalities in selected safety labs will be provided if needed.

### 4.6.4 Anti-drug Antibodies (ADAs)

The number and percentage of patients that developed ADAs will be summarized.

### 4.6.5 Adverse Event of Special Interest (AESI)

All reported AESI will be summarized overall and by NCI CTCAE v4.0 grade, and tabulated by specific MedDRA bucket terms and preferred terms. The following types of events are of special interest:

- Drug-induced liver injury
- Suspected transmission of an infectious agent via product

### 4.6.6 Vital Signs

Vital signs and physical examination results will be presented in patient listings if deemed necessary.

## 4.7 MISSING DATA

Missing data will not be imputed for efficacy analyses. For partial dates used for summaries such as treatment emergent adverse events, refer to the DAP module 3 for imputation of partial dates.

## 4.8 INTERIM ANALYSES

Given the hypothesis-generating nature and publication intent of this study, several interim analyses are planned as listed in Table 1, Section 2.4 and no formal multiplicity adjustment is necessary. The interim analyses will be performed and interpreted by members of the Sponsor study team and appropriate senior management personnel. No decision will be made on study conduct based on the interim analyses.

## 4.9 SCOPE OF PLANNED ANALYSES

*Interim Analysis 1* was limited to patients in the RMS cohorts Arms 1-3 who were enrolled by the CCOD. Biomarker analyses were limited to the Biomarker Population.

*Interim Analysis 2* will be limited to patients in the RMS cohort Arms 1□4. Only data during the Main Treatment Period (defined in Section 2, before receiving Week 72 treatment) will be summarized at this interim analysis, which applies to patient disposition information, efficacy endpoints and safety endpoints.

The Final Analysis will include all patients (RMS cohort Arms 1-4 and PPMS cohort). Only data during the Main Treatment Period will be summarized.

The LTE Final Study Analysis will include all patients and all data collected during the Long Term Extension Period and the Safety Follow Up period.

The endpoints that will be included in each of the analyses and the analysis populations are specified in Table 2. Specific list of planned outputs will be available in SAP Module 2.

**Table 2 Scope of Planned Analyses**

| Endpoint | Interim Analysis 1 | Interim Analysis 2 | Final Analysis | LTE Final Analysis |
|---|---|---|---|---|
| Disposition & Baseline Demographics, characteristics | X<br><br>(RMS Enrolled Population, Biomarker Population) | X<br><br>(RMS Enrolled Population) | X<br><br>(Enrolled Population) | X<br><br>(Enrolled Population) |
| Efficacy Endpoints (depend on data availability) | X<br><br>(RMS Biomarker Population) | X<br><br>Selected biomarkers as specified in SAP Module 2.<br><br>(RMS Enrolled Population) | X<br><br>(Enrolled Population, Per-Protocol Population) | X<br><br>(Enrolled Population, Per-Protocol Population) |
| Pharmacokinetic (depend on data availability) | Not Planned | Depend on data availability | X<br>(Pharmacokinetic Evaluable Population) | X<br>(Pharmacokinetic Evaluable Population) |
| Exposure, AE, IRR | X<br><br>(RMS Safety Population) | X<br><br>(RMS Safety Population) | X<br><br>(Safety Population) | X<br><br>(Safety Population) |
| ADA | Not Planned | Not Planned | X<br><br>(Safety Population) | X<br><br>(Safety Population) |
| Vital Signs and General Laboratory Data | Not Planned | Not Planned | X<br><br>(Safety Population) | X<br><br>(Safety Population) |

# APPENDIX 1
# PROTOCOL SYNOPSIS

| | |
|---|---|
| **TITLE:** | **AN OPEN-LABEL, MULTICENTER, BIOMARKER STUDY TO EXPLORE THE MECHANISM OF ACTION OF OCRELIZUMAB AND B-CELL BIOLOGY IN PATIENTS WITH RELAPSING MULTIPLE SCLEROSIS OR PRIMARY PROGRESSIVE MULTIPLE SCLEROSIS** |
| **PROTOCOL NUMBER:** | ML29966 |
| **VERSION NUMBER:** | 5 (U.S.) |
| **EUDRACT NUMBER:** | 2015-004616-37 |
| **IND NUMBER:** | 100,593 |
| **TEST PRODUCT:** | Ocrelizumab (RO4964913) |
| **PHASE:** | IIIb |
| **INDICATION:** | Relapsing multiple sclerosis and primary progressive multiple sclerosis |
| **SPONSOR:** | Genentech, Inc. |

## Objectives and Endpoints

This is an exploratory biomarker study designed to be hypothesis-generating in order to better understand the mechanism of action of ocrelizumab and B-cell biology in relapsing multiple sclerosis (RMS) and primary progressive multiple sclerosis (PPMS).

### Primary Objectives

The primary objectives for the RMS cohort in this study are:

- To understand the impact of ocrelizumab treatment on neurofilament light (NfL) as a biomarker of neuronal damage in cerebrospinal fluid (CSF)
- To assess the number of CD19+ B cells in CSF (cell number/microliter) before and after treatment with ocrelizumab
- To assess the number of CD3+ T cells in CSF (cell number/microliter) before and after treatment with ocrelizumab

### Exploratory Objectives

The exploratory objectives for the PPMS cohort in this study are:

- To understand the impact of ocrelizumab treatment on NfL as a biomarker of neuronal damage in CSF
- To assess the number of CD19+ B cells in CSF (cell number/microliter) before and after treatment with ocrelizumab
- To assess the number of CD3+ T cells in CSF (cell number/microliter) before and after treatment with ocrelizumab

The exploratory objectives for both cohorts in this study are:

- To understand the impact of ocrelizumab treatment on NfL and other biomarkers of neurodegeneration in CSF as a reflection of activity in the central nervous system (CNS) and compared with peripheral blood

- To measure the impact of ocrelizumab treatment on B cells/B-cell subsets, T cells/T-cell subsets, and other cell types (e.g., natural killer cells, monocytes, etc.), functional parameters of B/T/other cell types (activation, cell products), and other biomarkers of inflammation in CSF as a reflection of activity in the CNS and compared with peripheral blood
- To assess potential correlation between change in blood or CSF biomarkers of neurodegeneration or inflammation and change in magnetic resonance imaging (MRI) or efficacy outcome measures, such as reduction in gadolinium (Gd)-positive lesions or Expanded Disability Status Scale (EDSS) score

*The exploratory objectives for the Long-Term Extension are:*

- *To understand the impact of ocrelizumab on indices of neurodegeneration*
- *To monitor the clinical condition of the patient over an extended period of time*

Exploratory objectives for the RMS delayed time to start control arm (Arm 4) will include comparisons between Arm 1 of the RMS cohort (second lumbar puncture [LP] at 12 weeks) and Arm 4 (*first* LP at 12 weeks before the first dose of study drug), and may include but not be limited to changes in CSF, blood, and MRI biomarkers or efficacy, as outlined above.

**Pharmacokinetic Objective**

The pharmacokinetic objective for this study is:

- To assess pharmacokinetics of ocrelizumab in CSF compared with serum concentration

**Immunogenicity Objective**

The immunogenicity objective for this study is:

- To assess the incidence of anti-drug antibodies to ocrelizumab

**Safety Objectives**

The safety objective for this study, *including for the Long-Term Extension,* is to evaluate the safety of ocrelizumab on the basis of the following endpoints:

- Nature, frequency, severity, and timing of adverse events
- Changes in vital signs, physical findings, and clinical laboratory results during and following ocrelizumab administration
- Adverse events related to biomarker sample collection

## Study Design

**Description of Study**

This is an open-label, multicenter, biomarker study conducted at centers in multiple countries in which all eligible patients will be enrolled to a single treatment arm of ocrelizumab. The study will include an RMS cohort and a PPMS cohort (see Table 1). The RMS cohort is the main cohort and will include the majority of study participants. The RMS cohort will be comprised of four arms, three of which will have patients randomized into one of three arms (Arms 1–3) depending on the time of their second LP. In Arm 4, treatment with ocrelizumab will be delayed for 12 weeks after the first LP. The PPMS cohort will comprise a smaller, hypothesis-generating portion of the trial, as less is known about PPMS biomarkers.

Arm 4 will provide a control for Arm 1 of the RMS cohort. Due to the relapsing and remitting nature of RMS, some biomarkers may change due to disease course rather than based on influence of a drug treatment. Therefore, some biomarkers may "regress to the mean" across the disease course over time, and this change could then inaccurately be reported as a change due to drug treatment over time. Arm 4 will allow for an estimate of the natural variability of the disease when analyzing the other treated arms.

Male and female patients age 18–55 years with a diagnosis of RMS or PPMS in accordance with the 2010 revised McDonald criteria (Polman et al. 2011; Appendix 7) and an EDSS score of 0–5.5 points for RMS patients, or an EDSS score of 3.0–6.5 for PPMS patients, at screening will be eligible. Screening will occur over a 4-week period, after which eligible patients may begin treatment with ocrelizumab.

For patients in the RMS cohort (Arms 1–3; *see Appendix 1*), ocrelizumab will be administered at Week 1, Week 3, Week 24, and Week 48 *(and every 24 weeks beginning at Week 72 per the*

*Long-Term Extension; see Appendix 9)*. The first dose will be administered as two infusions of 300 mg given on Day 1 and Day 15. The subsequent doses will be given as single 600-mg infusions.

For patients in the RMS cohort (Arm 4; *see Appendix 2*), after receiving two LPs at Week –12 and Week 1, the first dose of ocrelizumab will be administered as two 300-mg infusions on Week 1 (Day 1) and Week 3 (Day 15), with subsequent doses given as single 600-mg infusions at Weeks 24 and 48 *(and every 24 weeks beginning at Week 72 per the Long-Term Extension; see Appendix 9)*. The timeline for Arm 4 will continue for an extra 12 weeks to accommodate the same one-year dosing regimen.

For patients in the PPMS cohort *(see Appendix 3)*, ocrelizumab 600 mg will be administered as two 300-mg IV infusions separated by 14 days at a scheduled interval of every 24 weeks *(and every 24 weeks as a single dose beginning at Week 72 per the Long-Term Extension; see Appendix 9)*.

Treatment with ocrelizumab will continue for approximately 4.5 years after the first infusion; however, treatment may be stopped at any time due to lack of clinical benefit, unacceptable toxicity, withdrawal of consent, patient or physician decision to discontinue treatment, death, or if the Sponsor decides to close the trial, whichever occurs first.

Patients who complete the study and choose not to continue on ocrelizumab treatment, or who discontinue from treatment early, should enter the Safety Follow-up Period and be assessed every 24 weeks for 48 weeks counting from the date of the last infusion of ocrelizumab. After 48 weeks, if the peripheral blood B-cell count remains depleted, monitoring of the patient should continue at 24-week intervals until the B-cell count has returned to the baseline value or to the lower limit of the normal range, whichever is lower (see Appendix 8).

*For patients who complete the study and continue to receive ocrelizumab, a Long-Term Extension of the study beginning at Week 72 will be conducted every 6 months for up to 4 years at the time of treatment infusion for as long as they continue to receive ocrelizumab (Appendix 9). A Long-Term Extension of the study is needed to understand the correlation between long-term changes in biomarker assessments with long-term safety and long-term efficacy of ocrelizumab. The Long-Term Extension extends the study to a total of 5 years, which is the necessary timeframe to investigate whether baseline CSF NfL levels may be prognostic for long-term brain atrophy as assessed by 5-year declines in whole brain volume in MS patients (Arrambide et al. 2016). CSF NfL and serum NfL levels are highly correlated in the disease (Disanto et al. 2017), and therefore measurement of blood NfL levels may allow for predictions of brain atrophy in MS patients. Treatment of MS patients with disease-modifying therapies has been demonstrated to cause short-term (6 months to 1 year) accelerations in whole brain-volume loss (pseudoatrophy) but slows long-term (3 –5 years) brain atrophy (Zivadinov et al. 2008). Therefore, a Long-Term Extension is needed in order to accurately establish an association between serum NfL levels and brain atrophy in patients in the study.*

Biomarkers will be monitored in a longitudinal fashion before and after treatment with ocrelizumab in order to assess dynamic changes in biomarkers as they relate to drug exposure, length of time on drug, response to drug, and MS disease pathogenesis. Biomarkers will be assessed via blood draws throughout the study and via LP in CSF at baseline before the first dose of ocrelizumab and at one other timepoint.

Two LPs will be drawn from each patient during the study *(except in the case of consent for optional LPs)*. The two LPs will allow for assessment of biomarkers in CSF that are indicative of response to drug, pharmacokinetic/pharmacodynamic relationships, and/or disease pathogenesis in the CNS.

Patients in the RMS cohort (Arms 1–3) will receive an LP before the start of dosing with ocrelizumab. Subsequently, these patients will be randomized into one of three arms for timing of the second LP, either Week 12, 24, or 52 following the first dose of ocrelizumab.

Patients in Arm 4 will receive two LPs, separated by a 12-week interval, before administration of the first dose of ocrelizumab. Patients in this arm will be asked if they are willing to give an optional CSF sample via LP 12 weeks after the ocrelizumab dose.

Patients in the PPMS cohort will receive an LP at the start of the study before dosing with ocrelizumab. Subsequently, they will receive a second LP at Week 52 following the first dose of ocrelizumab.

In the case of MS relapse or worsening (for PPMS patients) during the study, the patient should have an unscheduled visit and will be asked to receive an optional LP at the time of relapse or worsening (for PPMS patients). The patient will have another LP at the originally scheduled time based on their randomization, or if a relapse occurs after they have already given the second LP, the relapse LP will remain optional. *Patients in Arms 1, 2, and 4 will also be asked to have an additional, optional LP at Week 52.*

*Patients will be asked to consent to LPs during the Long-Term Extension.*

All patients will be evaluated for safety throughout the study.

In the case of early termination, the patient will be asked to have an early termination visit.

**Number of Patients**

Approximately *120* patients will be enrolled in this study; 88 patients with RMS and *up to* 32 patients with PPMS.

**Target Population**

Inclusion Criteria

General Inclusion Criteria

Patients must meet the following criteria for study entry:

- Signed Informed Consent Form
- Ability to comply with the protocol, in the investigator's judgment
- Age 18–55 years, inclusive
- For women of childbearing potential: agreement to remain abstinent (refrain from heterosexual intercourse) or use contraceptive methods that result in a failure rate of <1% per year during the treatment period and for at least 24 weeks after the last dose of study treatment or until their B cells have repleted, whichever is longer
  - A woman is considered to be of childbearing potential if she is postmenarcheal, has not reached a postmenopausal state (≥ 12 continuous months of amenorrhea with no identified cause other than menopause), and has not undergone surgical sterilization (removal of ovaries and/or uterus).
  - Examples of contraceptive methods with a failure rate of <1% per year include bilateral tubal ligation, male sterilization, established, proper use of hormonal contraceptives that inhibit ovulation, hormone-releasing intrauterine devices, and copper intrauterine devices.
  - The reliability of sexual abstinence should be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, or postovulation methods) and withdrawal are not acceptable methods of contraception.

Inclusion Criteria Specific to RMS Patients

- Diagnosis of RMS in accordance with the 2010 revised McDonald criteria (Polman et al. 2011; Appendix 7)
- EDSS score of 0–5.5 points, inclusive, at screening
- Disease duration from the onset of MS symptoms:
  - Less than 15 years in patients with an EDSS >5.0 at screening
- Either treatment-naïve or receiving treatment with disease-modifying therapies, including prior use of interferon (IFN)-β-1a (Avonex®, Rebif®), IFN-β-1b (Betaseron®/Betaferon), or glatiramer acetate (Copaxone®)
- At least one clinically documented relapse in the past year and/or at least one T1-weighted Gd-enhancing lesion in the past year and/or at least one new T2 lesion in the past year at the time of enrollment

Inclusion Criteria Specific to RMS Cohort Arm 4

- Must meet inclusion criteria for the RMS cohort
- Separate signed Informed Consent Form for the RMS Delayed Time to Start Control Arm (Arm 4)
- Must be willing to remain on the same dose and regimen of current standard of care, or no treatment if treatment-naïve, for 12 weeks after study enrollment
  - The treating and/or study physician must agree that the patient is eligible to remain on the same dose and regimen of their current standard of care at screening, or to receive no treatment if the patient is treatment-naïve, for 12 weeks after study enrollment.

Inclusion Criteria Specific to PPMS Patients

- Diagnosis of PPMS in accordance with the 2010 revised McDonald criteria (Polman et al. 2011; Appendix 7)
- EDSS score of 3.0–6.5 points, inclusive, at screening
- Disease duration from the onset of MS symptoms:
  - Less than 10 years in patients with an EDSS at screening ≤ 5.0
- Documented history of at least one of the following laboratory findings in CSF:
  - Elevated IgG Index
  - One or more IgG oligoclonal bands detected by isoelectric focusing

Exclusion Criteria

General Inclusion Criteria

Patients who meet any of the following criteria will be excluded from study entry:

- Diagnosis of secondary progressive MS without relapses for at least 1 year
- History or known presence of recurrent or chronic infection (e.g., HIV, syphilis, tuberculosis)
- History of recurrent aspiration pneumonia requiring antibiotic therapy
- History of cancer, including solid tumors and hematological malignancies (except basal cell, in situ squamous cell carcinomas of the skin, and in situ carcinoma of the cervix or the uterus that have been excised and resolved with documented clean margins on pathology)
- History of or currently active primary or secondary immunodeficiency
- History of coagulation disorders
- History of severe allergic or anaphylactic reactions to humanized or murine monoclonal antibodies
- History of alcohol or other drug abuse within 24 weeks prior to enrollment
- Known presence or history of other neurologic disorders, including but not limited to, the following:
  - History or known presence of progressive multifocal leukoencephalopathy
  - History or known presence of CNS or spinal cord tumor (e.g., meningioma, glioma)
  - History or known presence of potential metabolic causes of myelopathy (e.g., untreated vitamin B12 deficiency)
  - History or known presence of infectious causes of myelopathy (e.g., syphilis, Lyme disease, HTLV-1, herpes zoster myelopathy)
  - History of genetically inherited progressive CNS degenerative disorder (e.g., hereditary paraparesis; mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes [MELAS])
  - History or known presence of systemic autoimmune disorders, potentially causing progressive neurologic disease (e.g., lupus, anti-phospholipid antibody syndrome, Sjögren's syndrome, Behçet's disease)
  - History or known presence of sarcoidosis
  - Neuromyelitis optica

  - Ischemic cerebrovascular disorders (e.g., stroke, transient ischemic attack) or ischemia of the spinal cord
  - Severe, clinically significant brain or spinal cord trauma (e.g., cerebral contusion, spinal cord compression)
- Significant, uncontrolled disease, such as cardiovascular (including cardiac arrhythmia), pulmonary (including chronic obstructive pulmonary disease), renal, hepatic, endocrine, gastrointestinal, or any other significant disease
- Congestive heart failure (according to New York Heart Association III or IV functional severity)
- Known active bacterial, viral, fungal, mycobacterial infection, or other infection (including tuberculosis or atypical mycobacterial disease, but excluding fungal infection of nail beds) or any major episode of infection requiring hospitalization or treatment with IV antibiotics within 4 weeks prior to baseline visit or oral antibiotics within 2 weeks prior to baseline visit
- Any concomitant disease that may require chronic treatment with systemic corticosteroids or immunosuppressants during the course of the study
- Contraindications for, or intolerance to, oral or IV corticosteroids, including IV methylprednisolone, according to the country label, including:
  - Psychosis not yet controlled by a treatment
  - Hypersensitivity to any of the treatment drug constituents
- Contraindication for LP
- Previous treatment with B cell-targeted therapies (e.g., rituximab, ocrelizumab, atacicept, belimumab, or ofatumumab)
- Previous treatment with natalizumab (Tysabri®), alemtuzumab, anti-CD4 agents, cladribine, teriflunomide, cyclophosphamide, mitoxantrone, azathioprine, mycophenolate mofetil, cyclosporine, methotrexate, total body irradiation, or bone marrow transplantation
- Treatment with fingolimod (Gilenya®), dimethyl fumarate (Tecfidera®), or similar treatment within 6 months prior to enrollment
- Receipt of a live vaccine within 6 weeks prior to enrollment
  - Vaccinations before baseline: In rare cases where a live vaccine must be administered by the patient's physician, the screening period may need to be prolonged but cannot exceed 12 weeks.
- Systemic corticosteroid therapy within 4 weeks prior to baseline
  - The screening period may be extended (but cannot exceed 8 weeks) for patients who have used systemic corticosteroids for their MS before screening.
- Previous or concurrent treatment with any investigational agent or treatment with any experimental procedure for MS (e.g., treatment for chronic cerebrospinal venous insufficiency)
- Certain laboratory abnormalities or findings at screening, including the following:
  - Positive serum β-hCG
  - Positive for hepatitis B (hepatitis B surface antigen [HBsAg] positive or hepatitis B core antibody [total HBcAb] confirmed by positive viral DNA polymerase chain reaction [PCR]) or hepatitis C (HepCAb)
  - AST or ALT ≥ 2.0 upper limit of normal
  - Platelet count < 100,000/μL (< 100 × 109/L)
  - ANC < 1.5 × 103/μL
  - Abnormal lymphocyte count (below lower level of normal)

Re-testing before baseline: in rare cases in which the screening laboratory samples are rejected by the laboratory (e.g., hemolyzed sample) or the results are not assessable (e.g., indeterminate) or abnormal, the tests need to be repeated. Any abnormal screening laboratory value that is clinically relevant should be retested in order to rule out any progressive or uncontrolled underlying condition. The last value before randomization must meet study criteria. In such circumstances, the screening period may need to be prolonged but should not exceed 8 weeks.

- Inability to complete an MRI (contraindications for MRI include but are not restricted to weight ≥ 140 kg, pacemaker, cochlear implants, intracranial vascular clips, surgery within 6 weeks of entry into the study, coronary stent implanted within 8 weeks prior to the time of the intended MRI, contraindication to gadolinium, etc.)
- Lack of peripheral venous access
- Pregnant or lactating, or intending to become pregnant during the study
  - Women of childbearing potential must have a negative serum *or urine* pregnancy test result within *14* days prior to initiation of study drug.

Exclusion Criteria Specific to RMS Patients

- Diagnosis of PPMS or secondary progressive multiple sclerosis without relapses

**End of Study**

The end of this study is defined as the date when the last patient, last visit (LPLV) occurs or the date at which the last data point required for statistical analysis or safety follow-up is received from the last patient, whichever occurs later.

**Length of Study**

The total length of the study is expected to be approximately *6.5* years from the first patient enrolled to LPLV.

**Investigational Medicinal Products**

**Test Product (Investigational Drug)**

The investigational medicinal product for this study is ocrelizumab.

**Non-Investigational Medicinal Products**

Premedicate with 100 mg of methylprednisolone (or an equivalent) approximately 30 minutes prior to each ocrelizumab infusion *and with an antihistaminic drug (e.g., diphenhydramine) approximately 30 –60 minutes before each infusion of ocrelizumab* to reduce the frequency and severity of infusion-related reactions (IRRs).

The addition of an antipyretic (e.g., acetaminophen/paracetamol) may also be considered *to further reduce the frequency and severity of IRRs*.

**Statistical Methods**

Statistical analyses will be performed on the analysis population, defined as all enrolled patients who received at least one infusion of ocrelizumab, even if the infusion was incomplete.

**Primary Endpoints**

Primary endpoints for the RMS cohort include:

- Change in levels of NfL in CSF from treatment baseline to post-treatment with ocrelizumab
- Change in number of CD19+ B cells in CSF from treatment baseline to post-treatment with ocrelizumab
- Change in number of CD3+ T cells in CSF from treatment baseline to post-treatment with ocrelizumab

**Determination of Sample Size**

The sample size calculations for this study are based on the assumed reduction from baseline in NfL (Gunnarsson et al. 2011) at Weeks 24 and 52 combined (Arms 2 and 3) in the RMS cohort. Other biomarker measures are not considered for power determination.

Assuming (a) a 30% reduction from pre-treatment NfL (mean [SD] = 860 pg/mL [780 pg/mL]) to post-treatment NfL (mean [SD] = 602 pg/mL [310 pg/mL]) and a correlation coefficient of 0.6, resulting in an SD of 643 pg/mL for the change from pre- to post-treatment, and (b) a desired

power of 80% with a Type 1 error of 5%, a sample size of 51 patients is required, with approximately 25 patients in Arm 2 and 26 patients in Arm 3.

This is the first study to explore the impact of ocrelizumab on CSF B-cell count. In a study by Piccio et al. (2010), the rate of undetectable B cells in CSF was 74% (21/26) after 24–30 weeks of rituximab as an add-on to immunomodulatory therapy.

In the RMS cohort, 15 patients will be included in Arm 1 to explore the possible reduction in NfL at Week 12 post-treatment and 15 patients will be assigned to the delayed start RMS control arm (Arm 4). *At least 15* patients will be recruited to the PPMS cohort. The above sample sizes are not based on statistical considerations and are meant to help explore the changes in biomarkers and generate hypotheses for future studies.

Based on the above, the total sample size for this study will be *at least* 96 patients. The sample size will be increased by approximately 10% in order to account for potential missing post-treatment CSF information; therefore, the number of patients to be enrolled in the RMS cohort will be 88 (Arm 1: 16 patients, Arm 2: 28 patients, Arm 3: 28 patients, and Arm 4: 16 patients), and *at least* 16 in the PPMS cohort. Therefore, the total number of patients to be enrolled is *at least* 104; *however, the addition of patients to the PPMS cohort would allow for even more detailed information on biomarkers.*

**Interim Analyses**

*For the RMS patients,* interim analysis may be performed when approximately 50% of patients have had their second LP. *For the PPMS patients, interim analyses may be performed after approximately 50% of patients have had their baseline LP and/or approximately 50% have had their second LP.*

# APPENDIX 2

# VISIT WINDOWING FOR MAIN TREATMENT AND LTE PERIOD

**Visit window for vital sign and blood biomarkers (Arm 1-4 & PPMS):**

| Report Visit Label | Nominal study days (from baseline) | Visit window ( in terms of study days from baseline) | Notes |
|---|---|---|---|
| Week -12 (Arm 4) | -84 | < -14 | |
| Baseline (Arm 1-3 & PPMS) | 1 | <=1 | ANLFL for the record most close to day 1 if there are multiple records in the window |
| Baseline (Arm 4) | 1 | -14 - 1 | Give two week window for baseline |
| Week 3 | 21 | 2<= and <=53 | Window is from Day 2 until Week 7.5+0.5 day |
| Week 12 | 84 | 54<= and <=126 | Window is from Week 7.5+1.5 day until Week 18 |
| Week 24 | 168 | 127<= and <=210 | Window is from Week 18+1 day until Week 30 |
| Week 36 | 252 | 211<= and <=294 | Window is from Week 30+1 day until Week 42 |
| Week 48 | 336 | 295<= and <=350 | Window is from Week 42+1 day until Week 50 |
| Week 52 | 364 | 351 - < LTE Day 1 (if entered LTE)<br>351 – patient end of main study date (if not entered LTE) | Window is from Week 50+1 day until end of main study date |
| Week 96 | 672 | LTE Day 1 - <=840 | Window is from LTE Day 1 to Week 120 |
| Week 144 | 1008 | >=841 and <=1,176 | Window is from Week 120+1 day until Week 168 |
| Week 192 | 1344 | >=1,177 and <=1,512 | Window is from Week 168+1 day until Week 216 |
| Week 240 | 1680 | >= 1,513 | Window is from Week 216 to end of LTE |

**Visit window for regular lab: hematology, chemistry and urinalysis (Arm 1-4 & PPMS):**

| Report Visit Label | Nominal study days (from baseline) | Visit window ( in terms of study days from baseline) | Notes |
|---|---|---|---|
| Screening (Arm 4) | N/A | <=-92 | |
| Screening (Arms 1-3 & PPMS) | N/A | <=-8 | |
| Week -12 (Arm 4) | -84 | -92< and <=-8 | |
| Baseline (Arm 4) | 1 | -8< and <=1 | |
| Baseline (Arms 1-3 & PPMS) | 1 | -8< and <=1 | |
| Week 24 | 168 | 2<= and <=252 | Window is from day 2 until Week 36 |
| Week 48 | 336 | 253<= and <=350 | Window is from Week 36+1 day until Week 50 |
| Week 52 | 364 | 351 - <LTE Day 1 (if entered LTE)<br>351 – patient end of main study date (if not entered LTE) | Window is from Week 50+1 day until start of LTE |
| Week 72 | 504 | LTE Day 1 - <=623 | Window is from LTE Day 1 to Week 89 |
| Week 96 | 672 | >=624 and <=756 | Window is from Week 89+1 day to Week 108 |
| Week 120 | 840 | >=757 and <=924 | Window is from Week 108+1 day to Week 132 |
| Week 144 | 1008 | >=925 and <=1,092 | Window is from Week 132+1 day to Week 156 |
| Week 168 | 1176 | >=1,093 and <=1,260 | Window is from Week 156+1 day to Week 180 |
| Week 192 | 1344 | >=1,261 and <=1,428 | Window is from Week 180+1 day to Week 204 |
| Week 216 | 1512 | >=1,429 and <=1,596 | Window is from Week 204+1 day to Week 228 |
| Week 240 | 1680 | >=1,597 | Window is Week 228+1 day to end of LTE |

**Visit window for pregnancy (Arm 1-4 & PPMS):**

| Report Visit Label | Nominal study days (from baseline) | Visit window ( in terms of study days from baseline) | Notes |
|---|---|---|---|

| | | | |
|---|---|---|---|
| Screening (Arm 4) | N/A | <-92 | |
| Screening (Arms 1-3 & PPMS) | N/A | <=-8 | |
| Week -12 (Arm 4) | -84 | -91<= and <=-8 | |
| Baseline (Arm 4) | 1 | -8< and <=1 | |
| Baseline (Arms 1-3 & PPMS) | 1 | -8< and <=1 | |
| Week 24 | 168 | 2<= and <=210 | Window is from Week 18+1 day until Week 30 |
| Week 36 | 252 | 211<= and <=294 | Window is from Week 30+1 day until Week 42 |
| Week 48 | 336 | 295<= and <=350 | Window is from Week 42+1 day until Week 50 |
| Week 52 | 364 | 351 - < LTE Day 1 (if entered LTE)<br>351 – patient end of main study date (if not entered LTE) | Window is from Week 50+1 day until start of LTE |
| Week 72 | 504 | LTE Day 1 - <=623 | Window is from LTE Day 1 to Week 89 |
| Week 96 | 672 | >=624 and <=756 | Window is from Week 89+1 day to Week 108 |
| Week 120 | 840 | >=757 and <=924 | Window is from Week 108+1 day to Week 132 |
| Week 144 | 1008 | >=925 and <=1,092 | Window is from Week 132+1 day to Week 156 |
| Week 168 | 1176 | >=1,093 and <=1,260 | Window is from Week 156+1 day to Week 180 |
| Week 192 | 1344 | >=1,261 and <=1,428 | Window is from Week 180+1 day to Week 204 |
| Week 216 | 1512 | >=1,429 and <=1,596 | Window is from Week 204+1 day to Week 228 |
| Week 240 | 1680 | >=1,597 | Window is Week 228+1 day to end of LTE |

**Visit window for MRI biomarkers (Arm 1-4 & PPMS):**

| Report Visit Label | Nominal study days (from baseline) | Visit window ( in terms of study days from baseline) | Notes |
|---|---|---|---|
| Week -12 (Arm 4) | -84 | <=-28 | |
| Baseline (Arm 4) | 1 | >-28 and <=1 | Give a 4-week window for baseline visit |

| | | | |
|---|---|---|---|
| Baseline (Arm 1-3 & PPMS) | 1 | <=1 | |
| Week 12 | 84 | 2<= and <=126 | Window is from Day 2 until Week 18 |
| Week 24 | 168 | 127<= and <=266 | Window is from Week 18+1 day until Week 38 |
| Week 52 | 364 | 267 - < LTE Day 1 (if entered LTE)<br>267 – patient end of main study date (if not entered LTE) | Window is from Week 38 +1 day |
| Week 96 | 672 | LTE Day 1 - <=840 | Window is from LTE Day 1 to Week 120 |
| Week 144 | 1008 | >=841 and <=1,176 | Window is from Week 120+1 day to Week 168 |
| Week 192 | 1344 | >=1,177 and <=1,512 | Window is from Week 168+1 day to Week 216 |
| Week 240 | 1680 | >=1,513 | Window is from Week 216+1 day to end of LTE |

*Early withdrawal, B cell monitoring and safety follow up 1 and 2 excluded from windowing

**Visit window for CSF Biomarkers (Arm 1-4 & PPMS):**

| Report Visit Label | Nominal study days (from baseline) | Visit window ( in terms of study days from baseline) | Notes |
|---|---|---|---|
| Week -12 (Arm 4) | -84 | < -14 | |
| Baseline (Arm 4) | 1 | -14 - 1 | Give a 2-week window for baseline visit |
| Baseline (All patients except Arm 4) | 1 | <=1 | |
| Week 12 (All patients) | 84 | 2 - 126 | |
| Week 24 (All patients) | 168 | 127-266 | |
| Week 52 (All patients) | 364 | 267 - < LTE Day 1 (if entered LTE)<br>267 – patient end of main study date (if not entered LTE) | |
| Week 144 (All patients) | 1,008 | LTE Day 1 - <=1,344 | Window is from LTE Day 1 to Week 192 |

| | | | |
|---|---|---|---|
| Week 240 (All patients) | 1,680 | >=1,345 | Window is from Week 192+1 day to end of LTE |

**Visit window for EDSS:**

| Report Visit Label | Nominal study days (from baseline) | Visit window ( in terms of study days from baseline) | Notes |
|---|---|---|---|
| Screening (Arm 4) | N/A | <=-97 | |
| Screening (Arm 1-3 & PPMS) | N/A | <=-13 | |
| Week -12 Pre-Baseline (Arm 4) | -84 | -97 - <=-14 | Give a 2-week window for baseline visit |
| Baseline (Arm 1-3 & PPMS) | 1 | -13 – <=1 | Give a 2-week window for baseline visit |
| Baseline (Arm 4) | 1 | -14 – <=1 | Give a 2-week window for baseline visit |
| Week 12 (All Patients) | 84 | 2<= and <=126 | Window is from Day 2 until Week 18 |
| Week 24 (All Patients) | 168 | 127<= and <=210 | |
| Week 36 (All Patients) | 252 | 211<= and <=294 | |
| Week 48 (All Patients) | 336 | 295<= and <=350 | |
| Week 52 | 364 | 267 - < LTE Day 1 (if entered LTE)<br>267 – patient end of main study date (if not entered LTE) | Window is from Week 38 +1 day |
| Week 96 | 672 | LTE Day 1 - <=840 | Window is from LTE Day 1 to Week 120 |
| Week 144 | 1008 | >=841 and <=1,176 | Window is from Week 120+1 day to Week 168 |
| Week 192 | 1344 | >=1,177 and <=1,512 | Window is from Week 168+1 day to Week 216 |
| Week 240 | 1680 | >=1,513 | Window is from Week 216+1 day to end of LTE |

*Early withdrawal, B cell monitoring and safety follow up 1 and 2 excluded from windowing