PROTOCOL TITLE:	A Retrospective, Multi-Center, Observational Study to Assess the Effect of Tecfidera® Delayed-Release Capsules on Lymphocyte Subsets in Subjects with Relapsing Forms of Multiple Sclerosis (REALIZE)
VERSION:	2.0
DATE:	03 December 2015
ACTIVE SUBSTANCE:	N07XX09 Dimethyl fumarate (DMF)
MEDICINAL PRODUCT:	Tecfidera
STUDY SPONSOR:	Biogen MA Inc. 250 Binney Street Cambridge, MA 02142, USA
RESEARCH QUESTION AND OBJECTIVES:	The primary objective of the study is to retrospectively investigate changes in lymphocyte counts and lymphocyte subtypes, with a focus on $CD4^+$ and $CD8^+$ T cells, in patients on Tecfidera therapy for at least 6 months. The secondary objective is to investigate changes in lymphocyte subtypes other than $CD4^+$ and $CD8^+$ T cells. [redacted]
COUNTRIES OF THE STUDY:	United States

SIGNATURE PAGE

Protocol 109MS419 was approved by:

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[redacted], MD, MSc, DIC Date

[redacted]

Biogen Idec Research Limited

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MD

Biogen MA Inc.

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Date

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[redacted], PhD Date

Biogen MA Inc.

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2. LIST OF ABBREVIATIONS

AE	adverse event
AIDS	acquired immunodeficiency syndrome
ALC	absolute lymphocyte count
CD	cluster of differentiation
CI	confidence interval
CRA	clinical research associate
CRF	case report form
CTCAE	Common Terminology Criteria for Adverse Events
DMF	dimethyl fumarate
eCRF	electronic case report form
EDC	electronic data capture
FAE	fumaric acid ester
ICF	informed consent form
IRB	Institutional Review Board
HIPAA	Health Insurance Portability and Accountability Act of 1996
HIV	human immunodeficiency virus
LLN	lower limit of normal
MMF	monomethyl fumarate
MS	multiple sclerosis
NK	natural killer
PHI	protected health information
PML	progressive multifocal leukoencephalopathy
QPPV	qualified person for pharmacovigilance
RMMM	repeated measures mixed-effects model
SAE	serious adverse event
SAP	statistical analysis plan
TBNK	T, B, and natural killer [NK]
US	United States
WBC	white blood cell

3. RESPONSIBLE PARTIES

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Biogen MA Inc.

[redacted]

Primary Contact for Urgent Medical Issues:

All urgent medical queries should be referred directly to Biogen according to standard commercial product reporting lines. Medical issues directly related to the study should be referred to the relevant [redacted] Clinical Research Associate.

Please refer to the Study Reference Manual for complete contact information.

4. ABSTRACT

Table 1: Abstract for Protocol 109MS419

Protocol Title:	A Retrospective, Multi-Center, Observational Study to Assess the Effect of Tecfidera® Delayed-Release Capsules on Lymphocyte Subsets in Subjects with Relapsing Forms of Multiple Sclerosis (REALIZE)
Version Number:	2.0
Date of Protocol:	03 December 2015
Rationale and Background:	The Phase 2 and 3 clinical development program of dimethyl fumarate (DMF) established the risk-benefit profile, and led to the market authorization of Tecfidera. During this development it was found that mean lymphocyte counts decreased by approximately 30% during the first year of treatment with DMF and then plateaued with mean counts remaining within normal limits; however, changes to specific immune cell subtypes, such as $CD4^+$ and $CD8^+$ T cells, had not been characterized. Small independent observational studies in related compounds and Tecfidera indicate an effect on $CD8^+$ T cells in particular. Immune cells such as $CD4^+$ and $CD8^+$ are known to play a role in multiple sclerosis (MS) pathophysiology, but reductions in specific immune cells can potentially put a patient at risk for opportunistic infections. This study will evaluate the effect of Tecfidera on lymphocytes and lymphocyte subsets, [redacted]

Research Question and Objectives:	The primary objective of the study is to retrospectively investigate changes in lymphocyte counts and lymphocyte subtypes, with a focus on $CD4^+$ and $CD8^+$ T cells, in patients on Tecfidera therapy for at least 6 months. The secondary objective is to investigate changes in lymphocyte subtypes other than $CD4^+$ and $CD8^+$ T cells. [redacted]
Study Design:	This study will be conducted as a retrospective, observational study of patients with relapsing forms of MS who are receiving or received Tecfidera for at least 6 months in routine clinical practice. The study will consist of retrospective medical chart abstraction, conducted at a single time point per patient, with no required study visits or procedures.
Population:	This study will be conducted in patients with relapsing forms of MS who initiated Tecfidera treatment for the first time (treatment naïve) under routine clinical care. For inclusion in the study, patients' charts must have a baseline measurement for absolute lymphocyte count (ALC) and absolute $CD4^+$ or $CD8^+$ count within 6 months prior to Tecfidera initiation, and at least 1 measurement for ALC and absolute $CD4^+$ or $CD8^+$ count while on Tecfidera treatment for at least 6 months. For patients who discontinue Tecfidera, data will be collected for up to 6 months

	following Tecfidera discontinuation. Detailed eligibility criteria are described in the protocol.
Variables:	The following will be collected for all patients: • Assessment of eligibility • Documentation of written informed consent, if required • Demographic characteristics • Relevant medical history and comorbidities • MS disease history • Prior and current MS treatment • Concomitant medications and therapies, including immunomodulatory and immunosuppressive treatment • Tecfidera prescription information • [redacted] • Laboratory values (ALC, lymphocyte subset count [i.e., $CD4^+$ and $CD8^+$, as well as any additional lymphocyte subsets if available], absolute leukocyte count) • [redacted] • [redacted]
Data Sources:	Data will be collected retrospectively directly from the patient's medical record and other source documents available at the clinical sites. All data will be entered into an electronic case report form (eCRF).

Study Size:	The study will enroll approximately 400 to 1500 patients from approximately 5 to 8 sites in the United States (US).
Data Analysis:	Descriptive analyses corresponding to the specific research objectives will be performed to characterize the study population. The primary analysis will estimate the mean change from baseline in ALC, $CD4^+$, and $CD8^+$ counts at month 6 and month 12. Longitudinal plots of lymphocyte counts will be produced. The absolute value, change from baseline, and percent change from baseline for leukocyte, lymphocyte, lymphocyte subset counts, and the $CD4^+/CD8^+$ ratio will be summarized cross-sectionally at 6 and 12 months among patients with available data. Kaplan-Meier curves will display the time to pre-determined lymphocyte counts following the start of Tecfidera therapy. Logistic regression will be conducted to determine potential predictors of low lymphocyte counts. Similar analyses as described for the primary endpoints will be performed for the additional lymphocyte subsets.  Summary statistics will be presented for lymphocyte counts among patients who discontinued Tecfidera. For patients with low white blood cell (WBC), ALC, $CD4^+$,

	or $CD8^+$ counts during Tecfidera treatment, the time to recovery to baseline and normal values will be plotted and mean and median of the time to recovery will be calculated. [redacted]

5. AMENDMENTS AND UPDATES

Table 2: Substantial Amendments and Updates to Protocol 109MS419 After the Start of Data Collection

Number	Date	Section of Study Protocol	Amendment or Update	Reason
1	03 December 2015	• 9.1.1, 9.1.2, 9.1.3, 9.5, 9.7.4, 9.7.5, 9.7.6 • 9.2.1, 9.2.1.1 • 9.2.3	Amendment	• Align endpoints and analyses with statistical analysis plan (SAP) and interim data • Remove end date for Tecfidera initiation • Extension of data collection duration

6. MILESTONES

Milestone dates for this protocol are provided in a separate communication.

7. RATIONALE AND BACKGROUND

7.1. Profile of Previous Experience

7.1.1. General Experience with Fumaric Acid Esters

While the mechanism of action of dimethyl fumarate (DMF) is not fully understood, DMF and its metabolite monomethyl fumarate (MMF) have been shown to activate the nuclear factor (erythroid-derived 2)-related factor 2 (Nrf2) *in vitro* and *in vivo* in animals and humans. The Nrf2 pathway is involved in the cellular response to oxidative stress [Linker 2011]. In addition, MMF has been identified as a nicotinic acid receptor agonist in *in vitro* and in *in vivo* animal models [Scannevin 2012]. Initial studies of fumaric acid esters (FAE) for psoriasis showed a decrease in T cells among nearly all treated patients [Altmeyer 1996], and subsequent in vitro studies showed that DMF can induce apoptosis in human T cells [Treumer 2003]. Various studies have shown immunomodulatory effects on T cell subsets. Hoxtermann et al. observed a 49% reduction in overall lymphocyte count among patients receiving FAE for 3 months and continuous reductions up to 60% throughout 12 months. Within the lymphocyte subsets, $CD4^+$ and $CD8^+$ cells were shown to consistently decrease after 2 to 3 months on treatment [Höxtermann 1998]. Treumer et al. showed a 90% reduction in $CD4^+$ cells and 53% reduction in $CD8^+$ cells in patients receiving DMF [Treumer 2003]. An exploratory study of FAE in patients with multiple sclerosis (MS) found that the rate of apoptosis increased in both total lymphocytes and $CD4^+$ cells after 6 weeks on therapy, however the increases in apoptotic rates were not sustained and the rate of apoptosis returned to baseline values by week 12 [Schimrigk 2006].

7.1.2. Clinical Experience With Tecfidera

Tecfidera (DMF) is approved in the US for the treatment of relapsing forms of MS. In 2 Phase 3 studies, Tecfidera demonstrated significant clinical and neuroradiological benefits at 2 years, including a reduction in annualized relapse rate, proportion of patients relapsed, time to confirmed disability progression, and various brain lesions with an acceptable safety profile [Fox 2012; Gold 2012]. While a direct role for DMF on immune cells is not well understood, in Phase 3 and extension studies, mean lymphocyte counts decreased by approximately 30% during the first year of treatment with DMF and then plateaued with mean counts remaining within normal limits. However, 28% of subjects had at least 1 minimum post-baseline lymphocyte count of less than 800 cells/µl, and 6% percent of subjects had a minimum post-baseline lymphocyte counts less than 500 cells/µl. In controlled and uncontrolled clinical trials, 2% of patients experienced prolonged lymphopenia with lymphocyte counts less than 500 cells/µl for at least six months. In these patients, the majority of lymphocyte counts remained less than 500 cells/µl with continued therapy. There was no increased incidence of serious infections observed in patients with lymphocyte counts less than 800 cells/µl or 500 cells/µl in controlled trials or in the post-marketing setting, although 1 patient in an extension study

developed progressive multifocal leukoencephalopathy (PML) in the setting of prolonged lymphopenia (lymphocyte counts predominantly <500 cells/µl for 3.5 years).

7.2. Study Rationale

Two large Phase 3 studies of Tecfidera demonstrated reduced white-cell counts and lymphocyte counts, however specific changes to immune cell subtypes were not characterized in patients taking Tecfidera. While small studies of FAE for psoriasis and in vitro experiments have shown the immunomodulatory effects of DMF on T cell subsets, few studies have investigated the influence of Tecfidera treatment for MS on lymphocyte subsets in clinical practice. Spencer et al. conducted an observational study in patients with relapsing forms of MS receiving DMF therapy to investigate the effect of DMF on leukocyte and lymphocyte subsets through 12 months of treatment. Blood cell counts were obtained at baseline (n=34) and at month 3 (n=21), month 6 (n=15), and month 12 (n=17) of treatment. Lymphocyte counts were below the lower limit of normal (LLN) in 50% of patients at month 12, and lymphocyte subsets also decreased at month 12. $CD4^+$ T cell counts decreased by 39.2% and $CD8^+$ T cell counts decreased by 54.6%. The authors also reported reductions in $CD3^+$ T cells and $CD19^+$ B cells [Spencer 2015].

Immune cells such as $CD4^+$ and $CD8^+$ cells are known to play a role in MS pathophysiology, but reductions in specific immune cells can potentially put a patient at risk for infections or malignancies. To date, no increased risk of serious or opportunistic infections, nor for malignancies has been reported for FAE used in the treatment of MS or psoriasis. However, rare cases of PML have been reported in psoriasis patients receiving FAE, and recently a case of PML was reported in a MS patient receiving Tecfidera who had experienced prolonged lymphopenia [TECFIDERA™ USPI 2014].

This retrospective chart review study aims to investigate the effect of Tecfidera on lymphocytes and lymphocyte subsets, [redacted]

8. RESEARCH QUESTION AND OBJECTIVES

8.1. Primary Objective

The primary objective of the study is to retrospectively investigate changes in lymphocyte counts and lymphocyte subtypes, with a focus on $CD4^+$ and $CD8^+$ T cells, in patients on Tecfidera therapy for at least 6 months.

8.2. Secondary Objectives

The secondary objective of this study in this study population is to investigate changes in lymphocyte subtypes other than $CD4^+$ and $CD8^+$ T cells.

9. RESEARCH METHODS

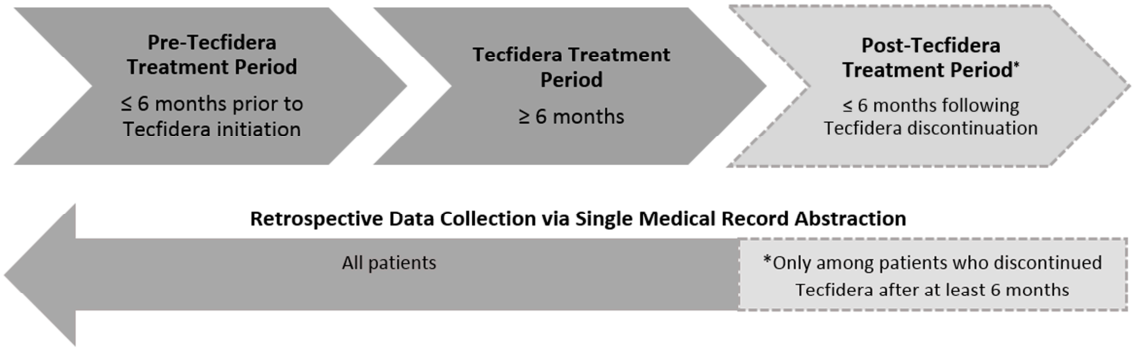
9.1. Study Design

The study will be conducted as a retrospective, observational study of patients with relapsing forms of MS who are receiving or received Tecfidera for at least 6 months in routine clinical practice. Data will be collected retrospectively directly from the patient's medical record and other source documents available at the clinical sites and entered into an electronic case report form (eCRF). The data will be collected at a single medical record review per patient. The medical record review and data collection will be performed either by designated investigator site staff or a person external to the site.

A patient will be enrolled into the study after the Study Physician determines that the patient meets all eligibility criteria. As no identifying data for the patient will be collected, a waiver of informed consent will be requested based on non-personally identifiable retrospective data collection and minimal risk.

Enrollment of approximately 400 to 1500 patients from approximately 5 to 8 US sites is planned.

Figure 1: Study Schematic



9.1.1. Primary Endpoints

The primary endpoints are as follows:

- Estimated absolute lymphocyte count (ALC) change from baseline at 6 month intervals within the first 12 months following Tecfidera initiation
- Estimated $CD4^+$ count change from baseline at 6 month intervals within the first 12 months following Tecfidera initiation
- Estimated $CD8^+$ count change from baseline at 6 month intervals within the first 12 months following Tecfidera initiation

9.1.2. Secondary Endpoints

The secondary endpoints are as follows:

- Raw absolute counts, change from baseline, and percentage change from baseline for leukocyte, lymphocyte, $CD4^+/CD8^+$ ratio, and for additional lymphocyte subsets (T, B, and natural killer [NK] [TBNK] cells, e.g., $CD19^+$, $CD45RA^+$, $CD45RO^+$, $CD26^+$, $CD3^+$, $CD56^+$, and $CD16^+$) at 6 and 12 months following Tecfidera initiation
- Time to pre-determined lymphocyte counts following Tecfidera initiation
- Potential predictors of low lymphocyte counts at 6 and 12 months following Tecfidera initiation

9.2. Setting

9.2.1. Selection Criteria

Approximately 5 to 8 sites will be selected to participate based on the available pool of Tecfidera patients who meet the selection criteria for this study.

[redacted]

9.2.1.1. Inclusion Criteria

To be eligible to participate in this study, patients must meet the following eligibility criteria at the time of enrollment:

- Written informed consent, if required

  Note: Waivers of informed consent will be pursued as permitted by local legislation. In cases where informed consent waivers are not granted, evidence of a personally signed and dated consent form indicating that the patient (or legally acceptable representative) has been informed of all pertinent aspects of the study will be required for inclusion in the study.
- Aged ≥ 18 years
- Initiated Tecfidera treatment for the first time on or after 27 March 2013, and received at least 6 months of continuous treatment with Tecfidera
- Clinical diagnosis of a relapsing form of MS
- A baseline measurement for ALC and absolute $CD4^+$ or $CD8^+$ count within 6 months prior to Tecfidera initiation
- At least 1 measurement for ALC and absolute $CD4^+$ or $CD8^+$ count while on Tecfidera therapy for at least 6 months
- Patient has sufficient available medical records for data abstraction to meet the objectives of the study, i.e., the patient was either under the medical care of the participating site within 6 months of Tecfidera treatment initiation, during the entire period of Tecfidera treatment, and up to 6 months following Tecfidera treatment, or the patient's complete MS disease and treatment history is otherwise available at the participating site

9.2.1.2. Exclusion Criteria

Patients will be excluded from study entry if any of the following exclusion criteria exist at enrollment:

- _msocom_36Clinical diagnosis of human immunodeficiency virus (HIV) or acquired immunodeficiency syndrome (AIDS) prior to Tecfidera initiation
- Participation in DEFINE 109MS301 or CONFIRM 109MS302
- Concurrent enrollment in any clinical trial of an investigational product during time evaluated for chart abstraction

9.2.2. Study Location

The study will be conducted at approximately 5 to 8 sites located in the US.

9.2.3. Overall Study Duration and Follow-Up

The study will consist of retrospective medical chart abstraction, conducted at a single time point per patient, with no required study visits or procedures. Following appropriate approvals, data collection is expected to last up to approximately 3 to 8 months.

9.2.3.1. Enrollment

Sites will be expected to access relevant local databases and records to identify eligible patients by applicable disease codes for MS and treatment with Tecfidera.

Patients must be consented (or a waiver of consent must be obtained, if applicable) before any data collection is performed. If required by the Institutional Review Board (IRB), a signed and dated informed consent must be obtained from all patients prior to their entering the study. At the time of consent, the patient will be enrolled into the study. Participating study sites are required to document all eligible candidates initially considered for inclusion in this study. If an eligible patient is excluded from the study, the reason(s) for exclusion will be documented in the screening log.

As this is a retrospective study, the index date will be the date a patient initiated Tecfidera therapy for the first time. Data will be collected as described in Section 9.2.3.2.

9.2.3.2. Follow-Up Period

As this is a retrospective study, there are no required study visits or procedures. Data collection will be performed through medical chart abstraction conducted at a single time point per patient. Data will be collected for each patient for up to 6 months prior to Tecfidera initiation, and for at least 6 months of continuous Tecfidera treatment. If a patient discontinues Tecfidera, up to 6 months of data after Tecfidera discontinuation will be collected. Refer to Figure 1 Study Schematic for more information.

9.2.3.3. Discontinuation of Tecfidera

Patients who discontinue treatment with Tecfidera will be eligible for enrollment into the study, provided they have at least 6 months of treatment with Tecfidera and meet all other eligibility criteria. Data on patients who discontinue Tecfidera will continue to be collected as described in Section 9.3. The Study Physician will record the primary reason for Tecfidera discontinuation, including diagnosis of adverse events (AEs) leading to Tecfidera discontinuation.

9.2.3.4. Withdrawal of Patients From the Study

As this is a retrospective study, there are no other formal withdrawal criteria for this study other than patient withdrawal of consent (if consent is required by the IRB). If a patient is withdrawn prior to study completion, all information already collected as part of the study will be retained for analysis; however, no further efforts will be made to obtain or record additional information regarding the patient. Patients may withdraw consent to participate in the study at any time without penalty.

9.3. Variables

Data elements will be collected from information routinely recorded in the medical record. Data pertaining to, but not limited to, patient demographics, medical history, MS disease history, MS treatment including Tecfidera, [redacted] will be abstracted.

9.3.1. Demographics and Baseline Disease Characteristics

The following will be collected for all patients from their medical charts:

- Assessment of eligibility
- Date of written informed consent for participating in the study, if required
- Demographic characteristics at the time of Tecfidera initiation such as age, sex, weight, race/ethnicity, etc.
- Medical history at the time of Tecfidera initiation (e.g., autoimmune disorders, malignancy, history of leukopenia and lymphopenia) and comorbidities
- MS disease history including type of MS at the time of Tecfidera initiation
- Malignancies (e.g., cancer type, date of diagnosis, stage)
    - NOTE: Malignancy information will also be collected prior to, during, and after Tecfidera treatment.

Refer to Section 14 for timing of assessments.

9.3.2. Treatment

The following will be collected for all patients from their medical charts:

- MS treatment in the 6 months prior to Tecfidera and during Tecfidera treatment (e.g., type of treatment, start and stop dates [at minimum MM/YYYY, preferably DD/MM/YYYY], dose, prescribed dosing frequency, and reason for discontinuation [if available])
- Tecfidera treatment from initiation to discontinuation, if applicable (e.g., initial dose, dose reductions or escalations, prescribing frequency, start date [at minimum MM/YYYY, preferably DD/MM/YYYY], and if applicable, date of Tecfidera discontinuation, and reason(s) for treatment discontinuation [including diagnosis of AE, lost to follow up, death])
- Immunomodulatory and immunosuppressive treatment (including corticosteroids) (e.g., type of treatment, start and stop dates [at minimum MM/YYYY, preferably DD/MM/YYYY], dose, prescribed dosing frequency, reason for discontinuation)
- All other concomitant medications (e.g., type of treatment, start and stop dates, dose, prescribed dosing frequency)

- MS treatment following Tecfidera discontinuation, if applicable (e.g., type of treatment, start and stop dates [at minimum MM/YYYY, preferably DD/MM/YYYY], dose, prescribed dosing frequency, and reason for discontinuation [if available])

Refer to Section 14 for timing of assessments.

[redacted]

9.3.4. Laboratory Assessments

The following information will be collected for all patients from their medical charts:

- Absolute leukocyte count (e.g., test date [DD/MM/YYYY], reference range)
- Absolute lymphocyte count (e.g., test date [DD/MM/YYYY], reference range)
- Absolute $CD4^+$ count [or $CD4^+$ percentage if absolute count is unavailable] (e.g., test date [DD/MM/YYYY], reference range)
- Absolute $CD8^+$ count [or $CD8^+$ percentage if absolute count is unavailable] (e.g., test date [DD/MM/YYYY], reference range)
- Absolute count of other lymphocyte subsets (TBNK) (e.g., test date [DD/MM/YYYY], reference range)

Refer to Section 14 for timing of assessments.

[redacted]

9.4. Data Sources

All data elements will be collected from information routinely recorded in the medical record.

9.5. Study Size

The study will enroll approximately 400 to 1500 patients across participating US sites. Based on the sample size range, the precision of the month 6 change from baseline in lymphocytes based on a 2-sided 95% confidence interval (CI) will be at least ± 0.06 ($\times 10^9$ cells/L). This is based on data from the DEFINE (109MS301) and CONFIRM (109MS302) studies which had an observed change in lymphocytes of approximately -0.5 ($\times 10^9$ cells/L) from baseline to week 24 among patients with Tecfidera twice daily with a standard deviation of approximately 0.61.

9.6. Data Management

Patient information will be captured and managed by study sites on eCRFs using a secure, web-based electronic data capture (EDC) system. All data will be collected and entered directly into the EDC system. Participating sites will only have access to the data on its own site and enrolled patients. All sites will be fully trained on using the online data capture system, including eCRF completion guidelines and help files. Site staff or external medical chart abstractors will be responsible for entering extracted patient data into the EDC system via the eCRF. All changes or corrections to eCRFs will require an adequate explanation for the update and this change data will be documented in an audit trail within the EDC database.

A data management plan will be created before data collection begins and will describe all functions, processes, and specifications for data collection, cleaning, and validation. The eCRFs will include programmable edits to obtain immediate feedback if the data are missing, out of range, illogical, or potentially erroneous. Concurrent manual data review will be performed based on parameters dictated by the plan. Ad hoc queries will be generated within the EDC system and followed up on for resolution.

High data quality standards will be maintained and processes and procedures utilized to repeatedly ensure that the data are as clean and accurate as possible when presented for analysis. Data quality will be enhanced through a series of programmed data quality checks that automatically detect out-of-range or anomalous data.

9.7. Data Analysis

All computation and generation of tables, listings, and data for figures will be performed using SAS® version 9.2 or higher (SAS Institute, Cary, NC, USA).

The analysis plan will be fully described in a written and approved Statistical Analysis Plan (SAP).

9.7.1. Analysis Population

The full analysis population includes all enrolled patients with an approved waiver of informed consent or written informed consent, if required, who meet all of the inclusion criteria and none of the exclusion criteria for this study.

A secondary analysis population includes patients from the full analysis population who discontinued Tecfidera treatment (after at least 6 months on Tecfidera treatment).

9.7.2. Demographic and Baseline Disease Characteristics

Baseline and disease characteristics will be summarized overall. The demographic profile (e.g., age, sex, and race/ethnicity), relevant medical history and comorbidities, MS disease history, MS treatment prior to start of Tecfidera treatment, concomitant medications and therapies, and laboratory values will be listed and summarized using the appropriate descriptive statistics for categorical and continuous variables as described in Section 9.7.3.

9.7.3. General Methods of Analysis

Descriptive analyses corresponding to the specific research objectives will be performed to characterize the study population. Continuous variables will be reported as number of patients, mean, standard deviation, median, 25th and 75th percentile, minimum, and maximum where appropriate. Categorical variables will be summarized as number of patients, frequency, and percentage. Proportions will be presented with 95% CIs.

Full details on the handling of all missing data, which are common in retrospective chart review studies, will be described in the SAP. The proportion of missing data will be reported for each measured variable in the study.

9.7.4. Primary Endpoint Analysis

Lymphocyte, $CD4^+$, and $CD8^+$ counts will be analyzed separately, using a repeated measures mixed-effects model (RMMM) of the longitudinal data.

The RMMM will be used to estimate an average change from baseline and will be reported for time points month 6 and month 12 for each laboratory parameter. The dependent variable will be the change from baseline, where the baseline value is defined as the most recent lab value occurring prior to Tecfidera initiation which has non-missing lymphocyte, $CD4^+$, or $CD8^+$ values.

The following covariates will be considered: site, baseline lymphocyte value, time from assessment of baseline value to initiation of Tecfidera, and any other demographic or baseline variable found to be predictive. The appropriate functional form to assess the longitudinal changes in the lab values over time (e.g., linear, quadratic, or other) will be determined using Akaike Information Criterion (or a likelihood ratio test, as appropriate). Variables may be transformed as deemed appropriate. Interaction effects may be included in the model as appropriate. Population averages will be reported.

The primary analysis will exclude patients with idiopathic $CD4^+$ lymphopenia.

Sub-analyses may be conducted among important subgroups of interest and will be described in the SAP.

9.7.5. Secondary Endpoint Analysis

The absolute value, change from baseline, and percent change from baseline for leukocyte, lymphocyte, lymphocyte subset counts, and the $CD4^+/CD8^+$ ratio at 6 and 12 months will be summarized cross-sectionally among patients with available data and plotted.

Kaplan-Meier curves will display the time to pre-determined lymphocyte counts (as defined in the SAP) following the start of Tecfidera therapy. ALCs will be categorized for analysis using the Common Terminology Criteria for Adverse Events (CTCAE) grading. Time to censorship will be determined by the end of follow-up or discontinuation of treatment for those who discontinued.

Logistic regression will be conducted to determine potential predictors of low lymphocyte counts (as determined by <LLN) at 6 months and 12 months following the start of Tecfidera therapy. Potential predictors may include demographic characteristics (e.g., age, sex, race/ethnicity), relevant disease history and comorbidities, past and current MS treatment, and other concomitant medications. Similar analyses as described for the primary endpoints will be performed for the additional lymphocyte subsets.

9.7.7. Interim Analyses

No interim analyses will be performed for this study. Data from the EDC will be reviewed on an ongoing basis.

9.8. Quality Control

9.8.1. Study Site Initiation

The Study Physician must not enroll any patients in this study prior to completion of a study initiation visit, conducted by Biogen or designee. This initiation visit will include a detailed review of the protocol and study procedures.

9.8.2. Quality Assurance

During and/or after completion of the study, quality assurance officers named by Biogen or the regulatory authorities may wish to perform onsite audits. The Study Physician will be expected to cooperate with any audit and to provide assistance and documentation (including source data) as requested.

9.8.3. Monitoring of the Study

Biogen or its designee representatives may conduct onsite visits at the study facilities for the purpose of monitoring various aspects of the study. The Study Physician must agree to Sponsor-authorized personnel having direct access to the patient (or associated) files for the purpose of verifying entries made in the eCRF, and assist with their activities, if requested. Adequate time and space for monitoring visits should be made available by the physicians or study staff. The site must complete the eCRFs in a timely manner and on an ongoing basis to allow regular review by the study team.

A study monitoring plan that is appropriate for the study design will be developed and implemented. During the site initiation visit, the monitor will provide training on the conduct of the study to the Study Physician and all site staff involved in the study. In order to ensure the integrity of the data, sites will be monitored. Site monitoring, including remote monitoring, may be performed by [redacted] Clinical Research Associates (CRAs) based on criteria established in the study monitoring plan. The monitor will close out each site after the last patient's data collection is completed, all data have been entered and all outstanding monitoring issues have been resolved or addressed. All monitoring procedures and frequency of monitoring visits will be described in a Monitoring Plan. Monitor contact details for each participating site will be maintained in the Investigator Site File.

9.8.4. Retention of Study Data

The minimum retention time for study records will meet the strictest standard applicable to that site, as dictated by any institutional requirements or local laws or regulations. Prior to proceeding with destruction of records, the physician is requested to notify Biogen in writing. In addition, the physician is requested to notify Biogen of any changes in the archival arrangements (e.g., archival at an off-site facility or transfer of ownership if the physician leaves the site).

9.9. Limitations of the Research Methods

Selection bias may arise if the study sample differs substantively from the underlying target population of patients with MS receiving Tecfidera as part of routine care. To minimize selection bias, the eligibility criteria were selected to be as broad as possible for this patient population. To address enrollment bias, sites will be expected to consecutively enroll eligible subjects. Sites will be asked to maintain a screening log of all eligible subjects, along with reasons for non-enrollment. As a waiver of informed consent is anticipated, non-participation may not be an issue for this study.

The final sample of participating sites may not be representative because they are selected on the basis of performing lymphocyte subset testing among their patients, which may not be routine clinical care across the general practice setting for MS.

Inaccurate assessment of study variables can occur in observational research, particularly in retrospective studies based on medical records. The current study will provide individual or centralized training for the site abstractors and detailed abstraction instructions in the eCRF completion guidelines. Furthermore, clear definitions of variables of interest will be provided to ensure accurate assessment of desired data elements. Missing data is a common occurrence in retrospective chart review studies. The impact of missing data on the analysis will be evaluated, and appropriate measures will be taken to address the missing information if any bias is likely.

9.10. Other Aspects

9.10.1. Study Funding

Biogen is the Sponsor of the study and is funding the study. All financial details are provided in the separate contracts between the institution, Study Physician, and Biogen.

9.10.2. Publications

Details on any restrictions on the publication of study data by Study Physicians are included in the clinical study agreement for this study.

10. PROTECTION OF HUMAN SUBJECTS

Biogen and participating physicians must comply with this protocol and applicable International Conference on Harmonisation and Good Pharmacovigilance Practices guidelines, and conduct the study according to local regulations. The patient's privacy; physical, mental, and social integrity; and the confidentiality of his or her personal information will be strictly respected in accordance with the World Medical Association Declaration of Helsinki and the Health Insurance Portability and Accountability Act of 1996 (HIPAA).

10.1. Ethics Committee

Participating physicians must obtain IRB approval of the protocol, informed consent form (ICF), and other required study documents prior to starting the study.

If the physician makes any changes to the ICF, Biogen must approve the changes before the ICF is submitted to the IRB. A copy of the approved ICF must be provided to Biogen. After approval, the ICF must not be altered without the agreement of the relevant IRB and Biogen.

It is the responsibility of the physicians to ensure that all aspects of institutional review are conducted in accordance with current governmental regulations.

Biogen must receive a letter documenting IRB approval, which specifically identifies the protocol, protocol number, and ICF (or acknowledgement of waiver of consent), prior to the initiation of the study. Protocol amendments will be subject to the same requirements as the original protocol.

A progress report must be submitted to the IRB at required intervals and not less than annually.

At the completion or termination of the study, the study site must submit a close-out letter to the IRB and Biogen.

10.2. Subject Information and Consent

A waiver of informed consent, based on non-personally identifiable retrospective data collection and minimal risk, will be at the discretion of the IRB.

If required by the IRB, prior to any data collection under this protocol, written informed consent with the approved ICF must be obtained from the patient or patient's legally authorized representative, as applicable, in accordance with local practice and regulations. Written informed consent, if required, must be obtained from all patients participating in a clinical study conducted by Biogen.

If written informed consent is required by the IRB, information about the study and the voluntary nature of study participation must be explained to the patient. The patient must be given sufficient time to consider whether to participate in the study. A copy of the ICF, signed and dated by the patient, must be given to the patient. Confirmation of a

patient's informed consent must be documented in the patient's medical record prior to any data collection under this protocol.

If required by the IRB, each ICF should contain an authorization allowing the physician and Biogen to use and disclose protected health information (i.e., patient-identifiable health information) in compliance with local law.

If written informed consent is required by the IRB, the signed ICF will be retained with the study records.

10.3. Changes to Final Study Protocol

All protocol amendments must be submitted to the IRB and regulatory authorities if required by local law. Protocol modifications that affect patient safety, the scope of the investigation, or the scientific quality of the study must be approved by the IRB before implementation of such modifications to the conduct of the study. If required by local law, such modifications must also be approved by the appropriate regulatory agency prior to implementation.

However, Biogen may, at any time, amend this protocol to eliminate an apparent immediate hazard to a patient. In this case, the appropriate regulatory authorities will be notified subsequent to the modification.

In the event of a protocol modification, the patient ICF, if applicable, may require similar modifications (see Sections 10.1 and 10.2).

10.4. Subject Data Protection

Prior to any data collection under this protocol, patients must also provide all authorizations required by local law (e.g., protected health information authorization in North America).

The patient will not be identified by name in the case report form (CRF) or in any study reports, and these reports will be used for research purposes only. Biogen and designee(s), ethics committees, and various government health agencies may inspect the records of this study. Every effort will be made to keep the patient's personal medical data confidential.

10.5. Internal Safety Review

Not applicable as there is no prospective serious adverse event (SAE) collection for this study.

11. MANAGEMENT AND REPORTING OF ADVERSE EVENTS/ADVERSE REACTIONS

Adverse event reporting in the form of individual case safety reports is not required for non-interventional post-authorization studies based on secondary use of data. Adverse events captured as endpoint(s) defined in the study protocol will be summarized in the final study report.

12. PLANS FOR DISSEMINATING AND COMMUNICATING STUDY RESULTS

A final study report will be sent to regulators within 12 months of the end of data collection.

12.1. Ethics Committee Notification of Study Completion or Termination

Where required, the Health Authorities and IRBs must be notified of completion or termination of this study, and sent a copy of the study synopsis in accordance with necessary timelines.

12.2. Registration of Study and Disclosure of Study Results

Biogen will register the study and post study results regardless of outcome on a publicly accessible website in accordance with the applicable laws and regulations.

13. REFERENCES

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14. ANNEX 1: ADDITIONAL INFORMATION

Table 3: Schedule of Assessments

Assessment	Pre-Tecfidera Treatment (Baseline)[a]	Tecfidera Treatment Period[b]	Post-Tecfidera Treatment[c]
Informed Consent Form, if required	X		
Eligibility Criteria	X		
Demographic characteristics	X		
Relevant medical history and comorbidities	X		
MS disease history	X		
Prior and current MS treatment	X	X	X
Concomitant medications and therapies, including immunomodulatory and immunosuppressive treatment	X	X	X
[redacted]			
Tecfidera prescription information: start and stop dates, dose, dosing frequency, reason for discontinuation (if applicable)		X	
Absolute lymphocyte count	X	X	X
Absolute $CD4^+$ count	X	X	X
Absolute $CD8^+$ count	X	X	X
Absolute leukocyte (WBC) count	X	X	X
Absolute count of other lymphocyte subsets, e.g., TBNK (if available)	X	X	X
[redacted]			
Malignancies	X	X	X

[a] Up to 6 months prior to Tecfidera initiation

[b] For a minimum of 6 months

[c] Up to 6 months following Tecfidera discontinuation, among patients who discontinued

Signature Page

Document Name: 109MS419 Protocol V2 Final 03Dec2015

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Signed by	Role	Date / Time (UTC)
[redacted]	Signing as Approver	12/07/2015 21:36:22
[redacted]	Signing as Approver	12/09/2015 13:24:41
[redacted]	Signing as Author	12/17/2015 18:44:54