PASS PROTOCOL

TITLE:	A retrospective analysis in real world on lymphocyte reconstitution after lymphopenia in patients treated by Tecfidera and description of management strategies in France.
PROTOCOL VERSION IDENTIFIER:	1.0
DATE OF LAST VERSION OF PROTOCOL:	Not Applicable
EU PAS REGISTER NUMBER:	TBD
ACTIVE SUBSTANCE:	L04AX07: Dimethyl fumarate
MEDICINAL PRODUCT:	TECFIDERA
PRODUCT REFERENCE:	EU/1/13/837/001 EU/1/13/837/002 EU/1/13/837/003
PROCEDURE NUMBER:	FR-BGT-11758
MARKETING AUTHORISATION HOLDER:	Biogen Netherlands B.V. Prins Mauritslaan 13 1171 LP Badhoevedorp The Netherlands
JOINT PASS:	No
RESEARCH QUESTION AND OBJECTIVES:	This study's research question is to describe management strategies of DMF-associated lymphopenia in France and to identify clinical risk factors predisposing for lymphopenia. The main objective of this study is to describe absolute lymphocyte count (ALC) reconstitution after DMF discontinuation because of lymphopenia. Secondary objectives include characterization of lymphopenia, evolution of ALC during and after

	DMF treatment, exploration of clinical outcomes and safety.
COUNTRIES OF THE STUDY:	France
AUTHOR:	[redacted]

MARKETING AUTHORISATION HOLDER

MARKETING AUTHORISATION HOLDER (MAH):	Biogen Netherlands B.V. Prins Mauritslaan 13 1171 LP Badhoevedorp The Netherlands
MAH CONTACT PERSON:	EU QPPV Biogen

SIGNATURE PAGE

Protocol FR-BGT-11758 was approved by:

[redacted] 12-Feb-2021

[redacted] Date (*DD MMM YYYY*)

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Protocol FR-BGT-11758 was approved by:

[redacted] 12-Feb-2021

[redacted] Date (*DD MMM YYYY*)

[redacted]

Biogen

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1. LIST OF ABBREVIATIONS

AE	Adverse Event
ALC	Absolute Lymphocyte count
CRF	Case Report Form
CRO	Contract Research Organization
CTCAE	Common Terminology Criteria for Adverse Events
DMF	Dimethyl fumarate
DMT	Disease Modifying Therapy
EDC	Electronic Data Capture
EDSS	Expanded Disability Status Scale
GCP	Good Clinical Practice
GVP	Good Pharmacovigilance Practice
ICF	Informed Consent Form
ICH	International Council for Harmonisation
MAH	Marketing Authorisation Holder
MS	Multiple Sclerosis
NSAE	Nonserious Adverse Event
OFSEP	Observatoire Français de la Sclérose en Plaques
RRMS	Relapsing-Remitting Multiple Sclerosis
SAE	Serious Adverse Event

2. RESPONSIBLE PARTIES

A list of all Investigators and other key sites (e.g., central laboratories), with their contact information, is available upon request (see Section 13).

Qualified Person for Pharmacovigilance:

[redacted]
Biogen

Global Safety Officer:

[redacted]
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Biogen

Main Author:

[redacted]
[redacted] France
Biogen

Biogen may transfer any or all of its study-related responsibilities to a contract research organization (CRO) and other third parties; however, Biogen retains overall accountability for these activities.

3. ABSTRACT

Protocol Title:	A retrospective analysis in real world on lymphocyte reconstitution after lymphopenia in patients treated by Tecfidera and description of management strategies in France.
Version Number:	0.2
Date of Protocol:	29-DEC-2020
Name and Affiliation of Main Author:	[redacted] Biogen
Rationale and Background:	Dimethyl fumarate (DMF) has demonstrated a favorable benefit-risk profile in patients with relapsing-remitting multiple sclerosis (RRMS). However, as shown in clinical studies, 2.2% patients developed severe and prolonged lymphopenia. Few data existed on lymphocyte reconstitution after dis-continuation. The main goal of this retrospective study is to describe lymphocyte reconstitution after DMF discontinuation because of lymphopenia, in RRMS patients. This study could potentially contribute to describe management strategies in France and to identify clinical risk factors predisposing for lymphopenia in patients treated by DMF.
Research Question and Objectives:	This study's research question is to describe management strategies of DMF-associated lymphopenia in France. The main objective of this study is to describe absolute lymphocyte count (ALC) reconstitution after DMF discontinuation because of lymphopenia. Secondary objectives include characterization of lymphopenia, evolution of ALC during and after DMF treatment, exploration of clinical outcomes and safety and identification of clinical risk factors predisposing for lymphopenia.
Study Design:	Retrospective, multicentric, medical chart review, French metropolitan study.
Population:	RRMS patients treated with DMF for at least 3 months
Variables:	

Data Sources:	• OFSEP database (EDMUS) • Patient's medical file
Study Size:	2,000 patients
Data Analysis:	Data will be summarized classically. Kaplan-Meier curves will display the time to lymphopenia defined as first ALC $<0.91x10^9/L$ following the start of DMF therapy. This outcome will be assessed for any lymphopenia and for each grade (CTCAE) grading).
Milestones:	Post of study information on the OFSEP website: 15 January 2021 Start of data collection for the study: 15 February 2021 End of data collection (data base lock): 15 June 2021 Interim analysis report: 29 October 2021 Registration in EU PAS register: TBD Final report of study results: 31 January 2022

4. AMENDMENTS AND UPDATES

None

5. MILESTONES

Table 1: Milestones for Protocol FR-BGT-11758

Milestone	Planned Date
Post of study information on the OFSEP website	15 January 2021
Start of data collection for the study	15 February 2021
End of data collection (data base lock)	15 June 2021
Interim analysis report	29 October 2021
Registration in EU PAS register	DD Month YYYY
Final report of study results	31 January 2022

6. RATIONALE AND BACKGROUND

6.1. Background

Multiple sclerosis (MS) is a chronic, inflammatory, demyelinating and neurodegenerating disease, affecting 2.3 million people worldwide (Giovannoni et al., 2020). MS is the most common cause of non-traumatic disability in young adults. The vast majority of patients diagnosed with MS have a relapsing-remitting type (RRMS). Dimethyl fumarate (DMF), a disease-modifying therapy (DMT), has demonstrated a favorable benefit-risk profile in patients with RRMS (Fox et al., 2012, 2014; Gold et al., 2012, 2017; Kappos et al., 2008) but is associated with decreases in absolute lymphocyte counts (ALC) (Mehta et al., 2019). In clinical trials, ALC decreased by approximately 30% during the first year of treatment with DMF and then plateaued (Buckle et al., 2020; Fox et al., 2016). DMF-associated ALC decrease within the first year of treatment is correlated to the decline in CD4+ and CD8+ T cells (more important than CD4+) and an increase in the CD4/CD8 ratio (Buckle et al., 2020). Rare cases of progressive multifocal leukoencephalopathy (PML) have occurred in patients treated with DMF in the setting of lymphopenia, predominantly in the context of moderate ($\geq 0.5 \times 10^9$/L to $< 0.8 \times 10^9$/L) to severe ($< 0.5 \times 10^9$/L) lymphopenia persisting for 6 months or more (Buckle et al., 2020).

To minimize potential risks of lymphopenia, DMF labels recommend monitoring lymphocyte counts before initiation and then every 3 months after DMF initiation. DMF should not be initiated in patients with severe lymphopenia (lymphocyte counts $< 0.5 \times 10^9$/L). DMF label recommends also: enhanced vigilance due to an increased risk for PML is recommended in patients with lymphopenia as follows (Tecfidera, SmPC, November, 2020):

- Tecfidera should be discontinued in patients with prolonged severe lymphopenia (lymphocyte counts $< 0.5 \times 10^9$/L) persisting for more than 6 months.
- In patients with sustained moderate reductions of absolute lymphocyte counts $\geq 0.5 \times 10^9$/L and $< 0.8 \times 10^9$/L for more than 6 months, the benefit/risk of DMF treatment should be re-assessed.
- In patients with lymphocyte counts below lower limit of normal (LLN) as defined by local laboratory reference range, regular monitoring of absolute lymphocyte counts is recommended.

Meta-analysis of DMF study data showed among patients treated for ≥6 months (n = 2,099), 2.2% patients developed lymphopenia that persisted for ≥6 months (Fox et al., 2016). This impact on ALC has been confirmed in numerous real-world study (Briner et al., 2019; Buckle et al., 2020; Longbrake et al., 2018). Nevertheless, few data are available about lymphocytes reconstitution after DMF withdrawal. Chan et al suggested most patients who discontinued DMF due to lymphopenia experienced ALC reconstitution within 2–4 months following DMF discontinuation. Prolonged lymphopenia on DMF treatment is associated with slow lymphocyte reconstitution after DMF discontinuation. ~~Based on available data, ALCs generally increase following discontinuation of DMF, however lymphocyte reconstitution time varied by individual patient, and little data exists~~

~~to inform on how lymphocyte reconstitution post DMF is influence by the choice of next DMT (Chan et al., 2020).~~ In an interim analysis of ENDORSE at 9 years, of 138 patients with lymphopenia at any time while on DMF, and with an ALC < LLN at DMF discontinuation, 62% (86/138) patients reached an ALC threshold of at least LLN after DMF discontinuation. The median time to reach an ALC threshold of ⩾LLN was 7 weeks after discontinuation. Patients with severe prolonged lymphopenia, defined as $<0.5\times10^9$/L for ⩾6 months, were excluded from the post-DMF lymphocyte reconstitution analyses due to the prolonged duration of on-treatment lymphopenia (n = 38) (Gold et al., 2020). In controlled and uncontrolled clinical studies (including patients in DEFINE/CONFIRM/ENDORSE, the mean time for lymphocyte counts to return to normal after discontinuing dimethyl fumarate treatment was 4.7 weeks in patients without prolonged, severe lymphopenia and 29 weeks in patients with prolonged, severe lymphopenia (Biogen data on file). In summary, there is no available data on the dynamic of ALC for subjects who may have had shorter duration of therapy with DMF prior to discontinuation, shorter duration of on-treatment lymphopenia prior to discontinuation, or less severe on-treatment lymphopenia prior to discontinuation in real world.

6.2. Study Rationale

In this study, we will retrospectively review the medical records of DMF-treated patients in academic hospitals, general hospitals and by office-based neurologists. MS centers that will participate in this study are already involved in the OFSEP registry, the largest French national MS cohort (EDMUS database). Upon review, we will report ALC before DMF initiation, during DMF treatment and after DMF discontinuation. ALC before initiation of DMF and ALC monitoring during and after end of DMF treatment have been recommended by ANSM since November 2015 (ANSM, 2015; DHCP ANSM, november 2020). Therefore, the study will be performed on patients having started DMF between 01/01/2016 and 15/12/2020 with an end of follow-up in the study on 15/06/2021. This project will investigate evolution of ALC and frequency, dynamics and duration of lymphopenia in patients on DMF or after DMF discontinuation for the first time in a real word setting in France. It will also describe management strategies and try to identify clinical risk factors predisposing for lymphopenia, as described recently (Goldman et al., 2020; Morales et al., 2020). The results could potentially contribute to the development of new recommendations on how to manage the risk of lymphopenia during treatment with DMF.

7. RESEARCH QUESTION AND OBJECTIVES

7.1. Research Question

This study's research question is to describe lymphocyte reconstitution after DMF discontinuation because of lymphopenia, in RRMS patients. ~~to describe management strategies of DMF-associated lymphopenia in France and to identify clinical risk factors predisposing for lymphopenia.~~

7.2. Primary Objective

The primary objective(s) of the study is to describe absolute lymphocyte count (ALC) reconstitution after DMF discontinuation because of lymphopenia, in RRMS patients.

7.3. Secondary Objectives

The secondary objectives of this study in this study population are as follows:

1. Characterization of lymphopenia;
2. Characterization of lymphopenia having led to DMF discontinuation treatment;
3. Description of the evolution of ALC during DMF treatment;
4. Description of discontinuation of DMF treatment;
5. Exploration of clinical outcomes in patients with lymphopenia on DMF and/or after DMF discontinuation due to lymphopenia;
6. Description of vigilance and patient management overall and in patients with lymphopenia who continued DMF treatment;
7. Incidence assessment of opportunistic or serious infections;
8. Investigation of the potential impact of baseline demographic and disease characteristics in risk of developing lymphopenia while on DMF;
9. Investigation of the potential impact of baseline demographics on the kinetics of ALC reconstitution upon discontinuation of DMF;
10. Investigation of the changes of lymphocyte subtypes in patients on DMF if available.

8. RESEARCH METHODS

8.1. Study Design

This is a retrospective, multicentric, medical chart review, French metropolitan study.

8.1.1. Primary Endpoint

The primary endpoint is the time to ALC reconstitution after DMF discontinuation.

Lymphopenia will be defined and graded according to Common Terminology Criteria for Adverse Events (CTCAE) grade (version 5.0).

8.1.2. Secondary Endpoints

Secondary endpoints are:

1. Time from DMF initiation to lymphopenia;
    - Severity of lymphopenia assessed by CTCAE grade: maximum CTCAE grade in patients with and without DMF discontinuation;
    - Longitudinal evolution of ALC after the first occurrence of lymphopenia;
2. Time from DMF initiation to lymphopenia;
    - CTCAE grade at time of DMF discontinuation;
    - (Subgroup of patients who discontinued DMF because of lymphopenia)
3. Longitudinal evolution of ALC over time from DMF initiation to DMF discontinuation or end of study;
4. Time from DMF initiation to DMF discontinuation;
    - Percentage of patients with discontinuation of DMF treatment;
    - (Overall and per cause of DMF discontinuation)
5. Percentage of patients showing relapses and/or an EDSS progression in patients with lymphopenia on DMF and/or after DMF discontinuation due to lymphopenia;
6. Serious Adverse events;
    - Serious Adverse Events related to DMF treatment;
    - Frequency of monitoring of ALC;
    - (Overall and, in patients with continuation of DMF treatment, after onset of lymphopenia)
7. Opportunistic or serious infections;
    - (Overall and in patients with lymphopenia (according to DMF discontinuation or not))

8. Association between baseline demographic and clinical characteristics (age, gender, diabetes, smoking habits, baseline ALC, time since MS diagnosis, number of relapses in prior year, baseline EDSS, duration of treatment, any prior DMT, duration of lymphopenia, and grade of lymphopenia) on the occurrence of lymphopenia during DMF treatment;
9. Association between baseline demographic and clinical characteristics (age, gender, diabetes, smoking habits, baseline ALC, time since MS diagnosis, number of relapses in prior year, baseline EDSS, duration of treatment, any prior DMT, prior DMT, duration of lymphopenia, and grade of lymphopenia) on lymphocyte reconstitution after DMF discontinuation;
10. Absolute CD4 count, absolute CD8 count and ratio.

8.2. Setting

8.2.1. Selection Criteria

To be eligible to participate in this study, candidates must meet the following criteria:

1. Clinical diagnosis of relapsing remitting forms of MS (RRMS) at DMF initiation;
2. Minimum of 3 months of continuous treatment with DMF*;
3. Initiation of DMF between January 1st, 2016 and December 15th, 2020;
4. Minimum of 2 ALC assessments:
   - 1 ALC at DMF initiation (or within 6 months before DMF initiation); or under DMF treatment before lymphopenia;
   - and 1 ALC before the database extraction (15/06/2021).

*to avoid the early DMF discontinuations for reasons other than lymphopenia.

Patients will be excluded from the study entry if they express their opposition to collect the data upon the information.

Discontinuation of DMF for reasons other than lymphopenia is not an exclusion criterion.

8.2.2. Study Location

The study will be conducted in metropolitan France. The study will be proposed mainly to sites involved in the OFSEP registry but also to other sites out of the registry for all practice representativeness.

8.2.3. Overall Study Duration and Follow-Up

This is a retrospective study, based on the review of medical charts from patients. Schedule of activities is presented in Table 2.

Identification and enrollment of patients fulfilling the inclusion criteria will occur in February 2021. As a retrospective study, no patient visit is planned.

Table 2: Schedule of activities

		Day 0	Day 30
Global Information Sheet publicly posted in the center and on the OFSEP website		X	
Review of eligibility criteria for all patients		X	
For each living patient	Data collection on the database (at least 30 days after individual information)		X
For each deceased patient	Individual Information Letter provided	*Not applicable*	
	Data collection on the database	X	

8.2.3.1. Enrollment

8.2.3.1.1. OFSEP patients

RRMS patients having initiated DMF (see 8.2.1 for inclusion criteria) will be identified in the EDMUS database for OFSEP sites. OFSEP will extract data from EDMUS database in order to identify the potentially eligible patients. Extracted data will include the EDMUS patient's number.

The collective study information to inform the patients on the purpose of the study, the planned treatment of their personal data and their rights to opposition, will be posted on the OFSEP website, as stated in the OFSEP consent. When the one-month delay is over, sites will be able to collect the data required for the study.

8.2.3.2. Follow-Up Period

Not applicable as it is a retrospective study.

8.2.3.3. Discontinuation of dimethyl fumarate

Patients who have discontinued DMF are eligible in the study (see section 8.2.1). Information will be collected as described in Section 8.3.2.

8.2.3.4. Withdrawal of Participants from the Study

All participating sites will document and collect patient's opposition. Even after a period

of 1 month with no opposition, all patients can still express their opposition to the use of their data at any moment of the study progression and the corresponding data will be erased from the study database. However, suppression of the collected data will no more be possible if the patient's opposition is received after the database lock.

8.2.3.5. Lost to Follow-Up

Not applicable for a retrospective study. All data available for an eligible patient will be collected. For all patients, the investigator will be asked whether the patient is lost to follow-up at the end of the follow-up in the study and if yes, the date of last contact.

8.2.3.6. Participant Transfers

Not applicable.

8.3. Variables

Data will be collected at treatment initiation (baseline= first day of DMF or if not available day of first prescription), during the time of DMF treatment, and up to 15/12/2020 using the database extraction (see Table 3 for data collection period). However, for patients who discontinued treatment for reasons other than lymphopenia, data will be collected up to DMF discontinuation. For patients who discontinued DMF treatment due to lymphopenia, data will be collected until lymphocyte reconstitution to normal values (as determined by first determination ≥ LLN=0.91×10^9/L) and prescription of a new DMT or end of study, which event occurs first. For ALC prior to DMF initiation, if none is available at baseline, then the earliest available ALC will be collected within the last 6 months prior to DMF initiation

Information collected for analysis is outlined in Section 15.

Table 3: Study data collection

Initiation of DMF	01/01/2016 to 15/12/2020
Data collection/ Follow-up	01/03/2016 to 15/06/2021
Database extraction	15/06/2021

8.3.1. Information Collected at Enrollment

Once the one-month delay after informing the patients has expired, for the patients not having expressed their opposition, data collection can start.

For all patients, will be collected:

- Demographic data
  - Birth date (MM/YYYY)
  - Gender (M/F)
  - Smoking habits (never, current, former)
  - Diabetes type
- MS history:
  - RRMS diagnosis date (MM/YYYY)
  - Number of relapses in the year preceding DMF initiation
  - Relapse treatment (corticoids)
  - Previous DMT prior to DMF:
    - DCI of prior DMT
    - Start date
    - End date
    - Cause of permanent DMT discontinuation
  - EDSS
    - Date of examination
    - EDSS score
- DMF treatment
  - Start date
  - If applicable, permanent discontinuation date
    - Cause of discontinuation (lymphopenia only, other reason only, both)
  - Temporary Interruption, if any, with end date and date of DMF resume
    - Cause of temporary DMF discontinuation (lymphopenia only, other reason only, both)
- Laboratory Data
  - Date of sampling,
  - Absolute Lymphocyte Count (ALC in x109/L)
  - If available, CD4 and CD8 immunophenotyping
  - Laboratory data will be collected at the following time-points:
    - Lab results before DMF initiation: Lab results closest to DMF initiation will be collected if done within the 6 months preceding treatment initiation,

        - All blood samples will be collected from treatment initiation and up to the end of the patient's defined study collection period.

- Safety data
    - Opportunistic infections
        - Start Date,
        - Type (Tuberculosis, Systemic mycosis, Opportunistic bacteria or parasitosis, Opportunistic virus, Other)
        - Serious or not serious,
        - Relationship with a medical treatment, if yes which one (DMF/other DMT/other),
        - End date and Outcome
    - Serious infections (bacterial, viral or fungal)
        - Start Date,
        - Type (PML: progressive multifocal leukoencephalopathy, septicemia, Meningitis, Encephalitis, other)
        - Serious or not serious,
        - Relationship with a medical treatment, if yes which one (DMF/other DMT/other),
        - End date and Outcome
    - Other serious adverse events
        - Start Date,
        - Type
        - Serious or not serious,
        - Relationship with a medical treatment, if yes which one (DMF/other DMT/other),
        - End date and Outcome

8.3.2. Information Collected During the Follow-Up Period

Not applicable as it is a retrospective study. All the data will be collected at enrollment.

8.4. Data Sources

Data will be collected by two different ways:

- Data extracted from the sites follow-up database (EDMUS): Pseudo-anonymized data will be up-loaded in the database. These data can be patient's demographic data, patient's medical or MS history, etc. These data are present within the "OFSEP minimal form"
- Data from the patient's medical file: Sites will enter the study data, missing from the data base extraction but present in the patient's medical file. These data will mainly be patient's laboratory results.

For sites requiring support either for patient's selection or for database completion, a Study Technician support will be available on request. This Study Technician will be provided by the OFSEP but will work on site under the responsibility of the investigator of the site.

OFSEP database and specific study database will be hosted by the Hospices Civils of Lyon which is a health data storage.

8.5. Study Size

It is expected that a minimum of 150-200 subjects who discontinued DMF treatment because of lymphopenia will allow to follow evolution of ALC after treatment discontinuation and to evaluate time to ALC reconstitution. The percentage of patients presenting lymphopenia on DMF discontinuation is not established but according to several publications (Condé et al., 2019; Miclea et al., 2016), this percentage could be estimated around 10%. Because of the retrospective design of the study, missing or incomplete data are expected. Furthermore, no accurate estimation of median time to DMF discontinuation because of lymphopenia is available. With those estimations, a minimum of 2000 subjects must be included.

8.6. Data Management

Data collection will be performed using local EDMUS database. Data will be entered into a central database managed by the OFSEP.

OFSEP will extract from the EDMUS database, data of the patients selected for the study. Extracted data will include the EDMUS patient number and data required for the study.

At the end of the data collection process, after the data review meeting, database will be frozen and pseudo-anonymized data under SAS format will be extracted and transmitted to study statistician to perform the analysis as planned in the Statistical Analysis Plan (SAP).

8.7. Data Analysis

Data will be summarized as described in the SAP. Normality of distribution of continuous parameters will be verified by at least 2 statistical tests and visually checked.

All statistical tests will be 2-sided and with a statistically significant threshold set at 5%.

Missing data will be handled for the main criterion only. At least 2 different methods will be used and detailed in the SAP.

8.7.1. Analysis Population

All patients fulfilling all eligibility criteria and with at least one ALC determination after initiation of DMF, will be included in the population of analysis, the full analysis set (FAS).

Two other populations of analyses are defined:

- Lymphopenic population: all patients from the FAS with lymphopenia under DMF treatment
- Reconstitution population: all patients from the lymphopenic subpopulation who discontinued DMF treatment.

8.7.2. Demography and Baseline Disease Characteristics

Demography and Baseline Disease Characteristics will be summarized, on the FAS, in patients with no lymphopenia, in patients with lymphopenia at least once under DMF treatment and overall.

8.7.3. Safety

8.7.3.1. Absolute Lymphocyte count

The baseline lab value will be the most recent lab value occurring prior to DMF initiation or, if not available, the first determination after initiation and before lymphopenia.

Mean (or median if normality of distribution at baseline is rejected) ALC and lymphocyte subset counts will be summarized at baseline and quarterly until end of study or DMF discontinuation on the FAS. Percent changes from baseline will be displayed with their 95% confidence interval (CI) and modelled on a log scale. Absolute values of CD4+, CD8+ and their ratio will be summarized bi-annually on the FAS. If more than one determination is available on a given period, the last available value will be used for the analysis.

The same quarterly data will be displayed but after DMF discontinuation in the Reconstitution population. Absolute values of CD4+, CD8+ and their ratio will be summarized bi-annually after DMF discontinuation.

Kaplan-Meier curves will display the time to lymphopenia defined as first ALC $<0.91 \times 10^9$/L (lower limit of normal (LLN), Gold et al., 2020) following the start of DMF therapy. This time will be assessed for any lymphopenia and for each grade (CTCAE grading). A Cox regression model will be used to estimate potential predictors of lymphopenia under DMF treatment. Time to censorship will be determined by the end of follow-up or discontinuation of treatment for those who discontinued DMF. Potential

predictors may include demographic characteristics (e.g., age, sex), relevant disease history or past and current DMT.

Time to discontinuation of DMF will be estimated overall using a Kaplan-Meier method and by cause of DMF discontinuation using a competitive risk model (with other causes as competitive risk) and percentages at end of study estimated with their 2-sided 95%CI.

Median time to ALC reconstitution will be estimated by Kaplan-Meier method with its 2-sided 95% CI in the Reconstitution population. The percentage of patients with ALC reconstitution at end of study will be estimated with its 2-sided CI.

In the Reconstitution population, in the subgroup of patients with CD4+ below LLN at time of DMF discontinuation, the time to reconstitution to normal values (as determined by first determination ≥ LLN) will be estimated using Kaplan Meier method. The same analysis will be performed on the subgroup of patients with CD8+ below LLN at time of DMF discontinuation.

Sub-group analyses may be conducted among important subgroups of interest and will be described in the statistical analysis plan (SAP).

8.7.3.2. Opportunistic and Serious Infections

The frequency and proportion of opportunistic and serious infections will be summarized during DMF treatment and after DMF discontinuation, if applicable. Chi-square tests or Fisher exact tests if appropriate will be used to assess the association between the incidence of these events by low or high values of ALC (as determined by <LLN or ≥LLN and by CTCAE).

8.7.4. Effectiveness

Relapses requiring hospitalization or requiring steroid use will be summarized during DMF treatment and after DMF discontinuation.

8.7.5. Interim Analyses

An interim analysis on primary criteria will be performed after extraction of the database on 15/06/2021. Demographics and baseline characteristics will be displayed. The interim analysis will be detailed on the SAP and before database lock.

8.8. Quality Control

8.8.1. Site Initiation

The Investigator must not enroll any participants in this study prior to completion of a study initiation visit. This initiation visit will include a detailed review of the protocol.

8.8.2. Quality Assurance

Quality control procedures will be implemented at each stage of data handling (as per OFSEP procedures) to ensure that all data are reliable and have been processed correctly.

Data anomalies will be communicated to the sites for clarification and resolution, as appropriate. The Investigators are responsible for demonstrating timely oversight of all clinical trial data from their site. Investigators must approve all their data on completed EDMUS database, at the subject, visit, or casebook level, at any time prior to an interim lock or database lock, as well as before any subsequent re-lock.

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

8.8.3. Monitoring of the Study

As the study is retrospective, no onsite monitoring visits by a CRA are planned.

8.8.4. 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, national, or regional laws or regulations. Prior to proceeding with destruction of records, the Investigator must notify Biogen in writing and receive written authorization from Biogen to destroy study records. In addition, the Investigator must notify Biogen of any changes in the archival arrangements (e.g., archival at an off-site facility or transfer of ownership if the Investigator leaves the site).

8.9. Limitations of the Research Methods

As a retrospective study, there are limitations inherent to the study design; the quality of a study based on existing data relies on the accuracy of the recorded data. Important data may not be available. Also, in the absence of randomization and blinding, bias and confounder controls can be challenging. The absence of randomization could create a selection bias.

To limit the risk of errors in the results, a quality control of the SAP and the statistical analysis may be implemented by appointing an independent reviewer.

8.10. Other Aspects

8.10.1. Study Funding

Biogen is the MAH of the study and is funding the study. All financial details are provided in the separate contracts between the institution, Investigator, and Biogen.

8.10.2. Steering committee

A Steering Committee has been formed to provide scientific and medical direction for the study. This committee will also rule on any protocol amendments or suspension of the study, if necessary.

The scientific committee consists of:

- [redacted] as study coordinator,
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]
- [redacted]

8.10.3. Publications

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

9. PROTECTION OF HUMAN PARTICIPANTS

Biogen and participating Investigators must comply with this protocol and applicable International Council for Harmonisation (ICH), Good Clinical Practice (GCP), and Good Pharmacovigilance Practice (GVP) guidelines, and conduct the study according to local regulations.

The Investigator may delegate responsibilities for study-related tasks where appropriate to individuals sufficiently qualified by education, training, and experience, in accordance with applicable ICH, GCP, and GVP guidelines. The Investigator should maintain a list of the appropriately qualified persons to whom significant study-related duties have been delegated. The Investigator is responsible for supervising those individuals and for implementing procedures to ensure the integrity of the tasks performed and any data generated.

This study will be performed in alignment with the ethical principles outlined in the Declaration of Helsinki

According to French legislation ("Loi Informatique et Libertés" No. 78-17 on January 6, 1978 modified by Law No. 2016-41 on January 26, 2016), the processing of personal data for the purpose of research field fit into the scope of Chapter IX of Law No. 78-17 of 6 January 1978 relating to data processing, files and liberties. Researches relating to the re-use of data already acquired (historical data) and which do not involve the human person within the meaning of Law No. 2012-300 on March 5, 2012 (known as the "Loi Jardé"), do not require ethics committee approval, but just a registration of the study on the Health Data Hub website, and a compliance with the “French Methodology of reference MR-004.”

9.1. Ethics Committee

As stated above, retrospective studies do not require ethics committee approval, but a registration of the study on Health Data Hub website, and a compliance with the French Methodology of reference MR-004.

9.2. Participant Information and Consent

In order to comply with the French Methodology of Reference MR-004, at least 1 month prior to data collection, information will be given to all eligible patients, except for deceased patients (in compliance with French reference methodology MR-004).

A general information concerning the study will be posted on the OFSEP website,

General information informs patients about:

- The nature of the information used in the research,
- The purpose of data processing,
- The recipients of the processed data,
- The rights of access, rectification and opposition of their data.

A one-month delay after proper information is deemed sufficient for the patients to express their opposition, if any, before data collection. At the end of this delay, the physician will be responsible to not enter on the database, the data of patients having expressed their opposition to the processing of their data.

If ever a patient expresses her/his opposition right after entering the data on the database, the physician will be responsible to inform OFSEP of patient's opposition and her/his identification number in the study (in no way full identity of the patient will be provided). Then, OFSEP will be responsible to delete all data of the corresponding study identification number, from the database.

9.3. Changes to Final Study Protocol

As a retrospective study compliant to the MR-004 methodology, protocol amendments do not need to be approved by local authorities.

However, in the event of a protocol modification, the subject information letter may require similar modifications (see Sections 9.1 and 9.2).

9.4. Participant Data Protection

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

The participant will not be identified by name in the CRFs, study-related forms, study reports, and related publications, and these reports will be used for research purposes only. Biogen, its partners and designee(s), and various government health agencies may inspect the records of this study. Every effort will be made to keep the participant's personal medical data confidential.

The study will comply with the General Data Protection Regulation (GDPR) and CNIL (2019-536 published 2019/05/30), which require data to be de-identified as soon as it is practical to do so. The processing of the personal data of participants will be minimized by making use of a unique participant study number on all study documents and any electronic database. All documents will be stored securely and only accessible by study staff and authorized personnel. The study team will safeguard the privacy of participants' personal data.

Study reports will be used for research purposes only. The participant will not be identified by name in eCRFs, study-related forms, study reports, or any related publications. The Sponsor, its partners and designees, and various government health agencies may inspect the records of this study. Every effort will be made to keep the participant's personal medical data confidential.

9.5. Internal Safety Review

Not applicable.

9.6. Compensation for Injury

Biogen maintains appropriate insurance coverage for clinical trials and will follow applicable local compensation laws.

9.7. Conflict of Interest

The Investigator should address any potential conflicts of interest (e.g., financial interest in the Sponsor) with the participant before the participant makes a decision be in the study.

10. SAFETY DEFINITIONS, RECORDING, REPORTING, AND RESPONSIBILITIES

10.1. Definitions

10.1.1. Adverse Event

An AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

Determination of whether an abnormal laboratory value, vital sign result, and/or ECG result meets the definition of an AE will be made by the Investigator. Abnormal results are not considered AEs unless one or more of the following criteria are met:

- The result meets the criteria for an SAE
- The result requires the participant to receive specific corrective therapy
- The result is considered by the Investigator to be clinically significant

10.1.2. Serious Adverse Event

An SAE is any untoward medical occurrence that at any dose:

- results in death
- in the view of the Investigator, places the participant at immediate risk of death (a life-threatening event); however, this does not include an event that, had it occurred in a more severe form, might have caused death
- requires inpatient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability/incapacity
- results in a congenital anomaly/birth defect
- Is a medically important event

A medically important event is an AE that, in the opinion of the Investigator, may jeopardise the participant or may require intervention to prevent one of the other outcomes listed in the definition above. (Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or convulsions occurring at home that do not require an inpatient hospitalization.)

10.2. Safety Classifications

10.2.1. Investigator Assessment of Events

All events must be assessed to determine the following:

- If the event meets the criteria for an SAE as defined in Section 10.1.2.

- The relationship of the event to study treatment as defined in Section 10.2.2
- The severity of the event as defined in Section 10.2.3

10.2.2. Relationship of Events to dimethyl fumarate

The following definitions should be considered when evaluating the relationship of AEs and SAEs to dimethyl fumarate:

Relationship of Event to Commercial Drug	
Not related	An AE will be considered "not related" to the use of dimethyl fumarate if there is not a possibility that the event has been caused by it. Factors pointing toward this assessment include, but are not limited to: the lack of reasonable temporal relationship between administration of the drug and the event, the presence of a biologically implausible relationship between the product and the AE (e.g., the event occurred before administration of drug), or the presence of a more likely alternative explanation for the AE.
Related	An AE will be considered "related" to the use of dimethyl fumarate if there is a possibility that the event may have been caused by it. Factors that point toward this assessment include, but are not limited to: a positive rechallenge, a reasonable temporal sequence between administration of the drug and the event, a known response pattern of the suspected drug, improvement following discontinuation or dose reduction, a biologically plausible relationship between the drug and the AE, or a lack of an alternative explanation for the AE.

10.2.3. Severity of Events

The following definitions should be considered when evaluating the severity of AEs and SAEs:

Severity of Event	
Mild	Symptom(s) barely noticeable to subject or does not make subject uncomfortable; does not influence performance or functioning; prescription drug not ordinarily needed for relief of symptom(s) but may be given because of personality of subject.
Moderate	Symptom(s) of a sufficient severity to make subject uncomfortable; performance of daily activity is influenced; subject is able to continue in study; treatment for symptom(s) may be needed.
Severe	Symptom(s) causes severe discomfort; symptoms cause incapacitation or significant impact on subject's daily life; severity may cause cessation of treatment with study treatment; treatment for symptom(s) may be given and/or subject hospitalised.

10.2.4. Expectedness of Events

Expectedness of all AEs will be determined by Biogen according to the approved local label.

10.3. Monitoring and Recording Events

This retrospective study collects secondary data within medical record review.

Based on the secondary objectives, the collection of the opportunistic infections, serious infections and overall serious adverse events will be collected.

The submission of suspected adverse reactions in the form of ICSRs is not required. All adverse events/reactions collected for the study should be recorded and summarized in the final study report.

10.3.1. Adverse Events

Any NSAE that is to be collected as part of the study and that occurs between the time of first dose on the study and study completion or premature study withdrawal must be extracted from the database.

10.3.2. Serious Adverse Events

SAEs should be extracted from the database. No SAE Form is required

10.3.2.1. Deaths

Death is an outcome of an event. The event that resulted in death should be recorded and reported as an SAE within 24 hours of the site becoming aware of the event. The Investigator should make every effort to obtain and send death certificates and autopsy reports to Biogen or designee. The term death should be reported as an SAE only if the cause of death is not known and cannot be determined.

10.3.3. Reporting Events

Not applicable.

10.4. Investigator Responsibilities

Not applicable.

10.5. Biogen Responsibilities

Not applicable.

As this is a study conducted from "secondary use data", EDMUS SERVICES/OFSEP undertakes to communicate to BIOGEN all pharmacovigilance information that it may come to identify during the Study and summarize them in aggregate form in the Report submitted to BIOGEN.

11. PLANS FOR DISSEMINATING AND COMMUNICATING STUDY RESULTS

A final study report will be produced and data disclosed publicly within 12 months of the end of data collection.

11.1. Ethics Committee Notification of Study Completion or Termination

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

11.2. 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.

12. REFERENCES

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13. ANNEX 1: LIST OF STAND-ALONE DOCUMENTS

Table 4: List of Stand-Alone Documents for Protocol FR-BGT-11758

No.	Document Reference Number	Date of Document	Title or Content of Document
1	0.5	30-Sept-2020	OFSEP_Lympho_Tec_0.5_20200930_BIOGEN
2	2.0	06-Apr-2020	OFSEP_Cohorte-Mère_Consentement_Majeurs_v2.0
3	2.0	06-Apr-2020	OFSEP_Cohorte-Mère_Note d'information_Majeurs_&_plus_de_13_ans_v2.0

14. ANNEX 2: ENCEPP CHECKLIST FOR STUDY PROTOCOLS (REVISION 4)

Study title:
A retrospective analysis in real world on lymphocyte reconstitution after lymphopenia in patients treated by Tecfidera and description of management strategies in France.

EU PAS Register® number:
Study reference number (if applicable): FR-BGT-11758

Section 1: Milestones	**Yes**	**No**	**N/A**	**Section Number**
1.1 Does the protocol specify timelines for				
1.1.1 Start of data collection[1]	☒	☐	☐	6
1.1.2 End of data collection[2]	☒	☐	☐	6
1.1.3 Progress report(s)	☒	☐	☐	6
1.1.4 Interim report(s)	☒	☐	☐	6
1.1.5 Registration in the EU PAS Register	☒	☐	☐	6
1.1.6 Final report of study results.	☒	☐	☐	6

Comments:

Section 2: Research question	**Yes**	**No**	**N/A**	**Section Number**
2.1 Does the formulation of the research question and objectives clearly explain:	☒	☐	☐	8
2.1.1 Why the study is conducted? (e.g. to address an important public health concern, a risk identified in the risk management plan, an emerging safety issue)	☒	☐	☐	7
2.1.2 The objective(s) of the study?	☒	☐	☐	8
2.1.3 The target population? (i.e. population or subgroup to whom the study results are intended to be generalised)	☒	☐	☐	9.2.1
2.1.4 Which hypothesis(-es) is (are) to be tested?	☐	☐	☒	
2.1.5 If applicable, that there is no *a priori* hypothesis?	☐	☐	☒	

[1] Date from which information on the first study is first recorded in the study dataset or, in the case of secondary use of data, the date from which data extraction starts.
[2] Date from which the analytical dataset is completely available.

Comments:

Section 3: Study design	**Yes**	**No**	**N/A**	**Section Number**
3.1 Is the study design described? (e.g. cohort, case-control, cross-sectional, other design)	☒	☐	☐	9.1
3.2 Does the protocol specify whether the study is based on primary, secondary or combined data collection?	☒	☐	☐	9.4
3.3 Does the protocol specify measures of occurrence? (e.g., rate, risk, prevalence)	☒	☐	☐	9.7
3.4 Does the protocol specify measure(s) of association? (e.g., risk, odds ratio, excess risk, rate ratio, hazard ratio, risk/rate difference, number needed to harm (NNH))	☒	☐	☐	9.7; 9.5
3.5 Does the protocol describe the approach for the collection and reporting of adverse events/adverse reactions? (e.g. adverse events that will not be collected in case of primary data collection)	☐	☐	☒	

Comments:

Section 4: Source and study populations	**Yes**	**No**	**N/A**	**Section Number**
4.1 Is the source population described?	☒	☐	☐	9.2.2
4.2 Is the planned study population defined in terms of:				
4.2.1 Study time period	☒	☐	☐	9.2.1
4.2.2 Age and sex	☐	☐	☒	
4.2.3 Country of origin	☐	☐	☒	
4.2.4 Disease/indication	☒	☐	☐	9.2.1
4.2.5 Duration of follow-up	☒	☐	☐	9.2.1; 9.2.3
4.3 Does the protocol define how the study population will be sampled from the source population? (e.g. event or inclusion/exclusion criteria)	☒	☐	☐	9.2.1

Comments:

Section 5: Exposure definition and measurement	**Yes**	**No**	**N/A**	**Section Number**
5.1 Does the protocol describe how the study exposure is defined and measured? (e.g. operational details for defining and categorising exposure, measurement of dose and duration of drug exposure)	☐	☐	☒	
5.2 Does the protocol address the validity of the exposure measurement? (e.g. precision, accuracy, use of validation sub-study)	☐	☐	☒	
5.3 Is exposure categorised according to time windows?	☐	☐	☒	
5.4 Is intensity of exposure addressed? (e.g. dose, duration)	☐	☐	☒	
5.5 Is exposure categorised based on biological mechanism of action and taking into account the pharmacokinetics and pharmacodynamics of the drug?	☐	☐	☒	
5.6 Is (are) (an) appropriate comparator(s) identified?	☐	☐	☒	

Comments:

Section 6: Outcome definition and measurement	**Yes**	**No**	**N/A**	**Section Number**
6.1 Does the protocol specify the primary and secondary (if applicable) outcome(s) to be investigated?	☒	☐	☐	9.1.1; 9.1.2
6.2 Does the protocol describe how the outcomes are defined and measured?	☒	☐	☐	9.1.1; 9.1.2; 9.3.1; 9.7
6.3 Does the protocol address the validity of outcome measurement? (e.g. precision, accuracy, sensitivity, specificity, positive predictive value, use of validation sub-study)	☐	☐	☒	
6.4 Does the protocol describe specific outcomes relevant for Health Technology Assessment? (e.g. HRQoL, QALYs, DALYS, health care services utilisation, burden of disease or treatment, compliance, disease management)	☐	☐	☒	

Comments:

Section 7: Bias	**Yes**	**No**	**N/A**	**Section Number**
7.1 Does the protocol address ways to measure confounding? (e.g. confounding by indication)	☐	☐	☒	

Section 7: Bias	**Yes**	**No**	**N/A**	**Section Number**
7.2 Does the protocol address selection bias? (e.g. healthy user/adherer bias)	☐	☐	☒	
7.3 Does the protocol address information bias (e.g. misclassification of exposure and outcomes, time-related bias)	☐	☐	☒	

Comments:

Section 8: Effect measure modification	**Yes**	**No**	**N/A**	**Section Number**
8.1 Does the protocol address effect modifiers? (e.g. collection of data on known effect modifiers, sub-group analyses, anticipated direction of effect)	☐	☐	☒	

Comments:

Section 9: Data sources	**Yes**	**No**	**N/A**	**Section Number**
9.1 Does the protocol describe the data source(s) used in the study for the ascertainment of:				
9.1.1 Exposure? (e.g. pharmacy dispensing, general practice prescribing, claims data, self-report, face-to-face interview)	☒	☐	☐	9.4
9.1.2 Outcomes? (e.g. clinical records, laboratory markers or values, claims data, self-report, patient interview including scales and questionnaires, vital statistics)	☒	☐	☐	9.4
9.1.3 Covariates and other characteristics?	☒	☐	☐	9.4
9.2 Does the protocol describe the information available from the data source(s) on:				
9.2.1 Exposure? (e.g. date of dispensing, drug quantity, dose, number of days of supply prescription, daily dosage, prescriber)	☒	☐	☐	9.4
9.2.2 Outcomes? (e.g. date of occurrence, multiple event, severity measures related to event)	☒	☐	☐	9.4
9.2.3 Covariates and other characteristics? (e.g. age, sex, clinical and drug use history, co-morbidity, co-medications, lifestyle)	☒	☐	☐	9.4
9.3 Is a coding system described for:				
9.3.1 Exposure? (e.g. WHO Drug Dictionary, Anatomical Therapeutic Chemical (ATC) Classification System)	☐	☒	☐	

Section 9: Data sources	**Yes**	**No**	**N/A**	**Section Number**
9.3.2 Outcomes? (e.g. International Classification of Diseases (ICD), Medical Dictionary for Regulatory Activities (MedDRA))	☐	☒	☐	
9.3.3 Covariates and other characteristics?	☐	☒	☐	
9.4 Is a linkage method between data sources described? (e.g. based on a unique identifier or other)	☐	☐	☒	

Comments:

Coding system will be detailed in the SAP

Section 10: Analysis plan	**Yes**	**No**	**N/A**	**Section Number**
10.1 Are the statistical methods and the reason for their choice described?	☒	☐	☐	9.7
10.2 Is study size and/or statistical precision estimated?	☒	☐	☐	9.5
10.3 Are descriptive analyses included?	☒	☐	☐	9.7
10.4 Are stratified analyses included?	☒	☐	☐	9.7
10.5 Does the plan describe methods for analytic control of confounding?	☐	☐	☒	
10.6 Does the plan describe methods for analytic control of outcome misclassification?	☐	☐	☒	
10.7 Does the plan describe methods for handling missing data?	☐	☒	☐	
10.8 Are relevant sensitivity analyses described?	☐	☒	☐	

Comments:

Section 11: Data management and quality control	**Yes**	**No**	**N/A**	**Section Number**
11.1 Does the protocol provide information on data storage? (e.g. software and IT environment, database maintenance and anti-fraud protection, archiving)	☒	☐	☐	9.4
11.2 Are methods of quality assurance described?	☒	☐	☐	9.8
11.3 Is there a system in place for independent review of study results?	☐	☒	☐	

Comments:

Section 12: Limitations	**Yes**	**No**	**N/A**	**Section Number**
12.1 Does the protocol discuss the impact on the study results of:				
12.1.1 Selection bias?	☐	☐	☒	
12.1.2 Information bias?	☐	☐	☒	
12.1.3 Residual/unmeasured confounding? (e.g. anticipated direction and magnitude of such biases, validation sub-study, use of validation and external data, analytical methods)	☐	☐	☒	
12.2 Does the protocol discuss study feasibility? (e.g. study size, anticipated exposure uptake, duration of follow-up in a cohort study, patient recruitment, precision of the estimates)	☐	☐	☒	

Comments:

Section 13: Ethical/data protection issues	**Yes**	**No**	**N/A**	**Section Number**
13.1 Have requirements of Ethics Committee/ Institutional Review Board been described?	☐	☐	☒	
13.2 Has any outcome of an ethical review procedure been addressed?	☐	☐	☒	
13.3 Have data protection requirements been described?	☒	☐	☐	10.5

Comments:

Section 14: Amendments and deviations	**Yes**	**No**	**N/A**	**Section Number**
14.1 Does the protocol include a section to document amendments and deviations?	☒	☐	☐	5

Comments:

Section 15: Plans for communication of study results	**Yes**	**No**	**N/A**	**Section Number**
15.1 Are plans described for communicating study results (e.g. to regulatory authorities)?	☒	☐	☐	12
15.2 Are plans described for disseminating study results externally, including publication?	☒	☐	☐	12

Comments:

Name of the main author of the protocol: [redacted]

Date: dd/Month/year

Signature: _____

15. ANNEX 3: ADDITIONAL INFORMATION

16. SIGNED AGREEMENT OF THE STUDY PROTOCOL

I have read the foregoing protocol, "A retrospective analysis in real world on lymphocyte reconstitution after lymphopenia in patients treated by Tecfidera and description of management strategies in France." and agree to conduct the study according to the protocol and the applicable ICH, GCP, and GVP guidelines, and to inform all who assist me in the conduct of this study of their responsibilities and obligations.

_____

Investigator's Signature Date

_____

Investigator's Name (Print)

_____

Study Site (Print)