PASS INFORMATION

PROTOCOL TITLE:	An observational study utilising data from the US Tysabri TOUCH programme and select EU MS Registries to estimate the risk of progressive multifocal leukoencephalopathy (PML) and other serious opportunistic infections among patients who were exposed to an MS disease modifying treatment prior to treatment with Tysabri
VERSION:	2
DATE:	26 January 2022
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MEDICINAL PRODUCT:	Tysabri
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PROCEDURE NUMBER:	N/A

MARKETING AUTHORISATION HOLDER:	Biogen Idec Limited Innovation House 70 Norden Road Maidenhead Berkshire SL6 4AY United Kingdom
STUDY SPONSOR:	Biogen Idec Limited Innovation House 70 Norden Road Maidenhead Berkshire SL6 4AY United Kingdom
JOINT PASS:	No
RESEARCH QUESTION AND OBJECTIVES:	• estimate the incidence of PML among patients who switched to Tysabri from DMTs, including newer DMTs (including fingolimod, dimethyl fumarate, and teriflunomide) and the established DMTs (interferon beta and glatiramer acetate) • estimate the incidence of SAEs of other serious opportunistic infections among patients who switch to Tysabri from newer DMTs (including fingolimod, dimethyl fumarate, and teriflunomide) and the established DMTs (interferon beta and glatiramer acetate)
COUNTRIES OF THE STUDY:	TOUCH (TYSABRI Outreach: Unified Commitment to Health) programme – US European Union (EU) multiple sclerosis (MS) Registry data
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2. LIST OF ABBREVIATIONS

CHMP	Committee for Medicinal Products for Human Use
CI	confidence interval
CNS	central nervous system
CSF	cerebrospinal fluid
CSR	clinical study report
DMF	dimethyl fumarate
DMT	disease-modifying therapy
DNA	deoxyribonucleic acid
DWI	diffusion weighted imaging
EDSS	Expanded Disability Status Scale
EFNS	European Federation of Neurological Societies
EMEA	European Medicines Agency
EU	European Union
FLAIR	fluid-attenuated inversion recovery
GA	glatiramer acetate
HIV	human immunodeficiency virus
IFN-β	interferon beta
IS	immunosuppressant/immunosuppressive
JCV	John Cunningham Virus
LoD	limit of detection
MAH	Marketing Authorisation Holder
MRI	magnetic resonance imaging
MS	multiple sclerosis
OI	opportunistic infection
PASS	post-authorisation safety study
PCR	polymerase chain reaction
PML	progressive multifocal leukoencephalopathy
PRAC	Pharmacovigilance Risk Assessment Committee
PSUR	periodic safety update report
RMP	risk management plan
RRMS	relapsing remitting multiple sclerosis
SAE	serious adverse event
TOUCH	TYSABRI Outreach: Unified Commitment to Health
US	United States

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4. ABSTRACT

Table 1: Abstract for Protocol 101MS411

Protocol Title:	An observational study utilising data from the US Tysabri TOUCH programme and select EU MS disease registries to estimate the risk of progressive multifocal leukoencephalopathy (PML) and other serious opportunistic infections among patients who were exposed to an MS disease modifying treatment prior to treatment with Tysabri
Version Number:	2
Date of Protocol:	26 January 2022
Name and Affiliation of Main Author:	[redacted], MD [redacted] [redacted] Biogen 225 Binney Street Cambridge, MA 02142 Email: [redacted] Direct: [redacted]
Rationale and Background:	The safety profile of Tysabri in patients switching from the established disease modifying therapies (DMTs) is well established. However, the risk of progressive multifocal leukoencephalopathy (PML) and other serious opportunistic infections (OIs) among Tysabri-treated patients who have switched from the newer generation of DMTs is unclear. This observational study is being conducted to assess the risk of PML and other serious OIs among patients who switched to Tysabri from newer DMTs (including fingolimod, dimethyl fumarate [DMF] and teriflunomide) and the established DMTs (interferon beta and glatiramer acetate [GA]).

Research Question and Objectives:	The primary objectives of the study are as follows: • estimate the incidence of PML among patients who switched to Tysabri from DMTs, including newer DMTs (including fingolimod, dimethyl fumarate and teriflunomide) and the established DMTs (interferon beta and glatiramer acetate) • estimate the incidence of serious adverse events (SAEs) of other serious opportunistic infections among patients who switch to Tysabri from newer DMTs (including fingolimod, dimethyl fumarate and teriflunomide) and the established DMTs (interferon beta and glatiramer acetate)
Study Design:	This is an observational cohort study utilising all available data (retrospective and prospective) from the Tysabri TOUCH (TYSABRI Outreach: Unified Commitment to Health) prescribing programme (US) supplemented with data from European Union (EU) multiple sclerosis (MS) registries to estimate the risk of PML and other serious OIs among patients on Tysabri who have switched from newer DMTs (including fingolimod, dimethyl fumarate and teriflunomide) and the established DMTs (interferon beta and glatiramer acetate). This investigation has two data sources that contribute to the assessment of the PML risk and other serious OI incidences: **TOUCH (US only)** A cohort study utilising all available data from the US Tysabri TOUCH prescribing programme. Annex 3, Table 5 outlines the parameters assessed to support the study objectives.

Study Design (cont.):	**EU MS Registries** Through a feasibility assessment (EMEA/H/C/000603/MEA/064), EU MS registries were identified and assessed to determine whether it was possible to utilise them as a data source for this post-authorisation safety study (PASS). The assessment concluded that it is feasible to supplement the evidence derived from the TOUCH programme provided that the minimum data elements are systematically captured (see Annex 3, Table 6). These EU registries will provide additional data on variables of interest (i.e., PML and other serious OI). Over time, additional EU MS registries will be assessed and potentially used as additional data sources as part of the safety assessment for PML and other serious OIs. **TOUCH and EU MS Registries** This observational study will provide cumulative data from its two components: a retrospective component (data captured prior to 1 January 2016) and a prospective component (data captured, and patients followed up, from 1 January 2016 through 31 December 2023; total prospective study duration of 8 years). All patients who switched to Tysabri from another DMT through 31 December 2020 will be included. The study will continue to follow-up patients until 31 December 2023 to allow for a minimal follow-up of 3 years. Timelines may be extended for some MS registries that may not start prospectively collecting SAE data until later in the study period. A report on the patient numbers for each data source and updates on the risk estimate of PML and the incidence of other serious OIs will be submitted according to the schedule outlined in Section 6, Table 2.

Study Design (cont.):	Although the TOUCH dataset comprises only US patients, the data generated on the risk estimates of PML is expected to be applicable to the patients in the EU as potential differences between geographic regions in terms of the distribution of known risk factors for PML (treatment duration, the presence of anti-JCV [John Cunningham virus] antibodies, and prior immunosuppressant use) will be addressed by the estimates stratified by them. The EU MS registries will be able to provide baseline demographic and treatment history data for a cohort of EU MS patients switching from the newer and established DMTs to Tysabri, together with disease characteristics, exposure and safety information over time. These data would be used to supplement the evidence derived from the analysis of the TOUCH dataset. Patient data from EU MS registries will reflect the use of Tysabri in the post approval setting in the EU and can, therefore, provide information about safety in an expedited time frame than could be obtained by initiating a prospective observational study at the current time.
Population:	All TOUCH and available EU MS registry patients who have switched from DMTs (including fingolimod, dimethyl fumarate, teriflunomide, interferon-beta and glatiramer acetate) and have one or more infusion(s) of Tysabri.
Variables:	Information collected for analysis will include information as outlined in Annex 3, Table 5 and Table 6.
Data Sources:	TOUCH and selected EU MS registries.

Study Size:	It is expected that at least 16,095 patients switching from DMTs to Tysabri are projected to be captured over the study period (based on patients included as of 31 December 2020). To obtain the targeted threshold of 0.5 per 1000 person-years, a required sample size of 9449 patients is required to switch from newer DMTs to Tysabri, with 1754 from prior fingolimod, 4077 from prior DMF treatment, and 3618 from prior teriflunomide treatment. When the three newer DMTs are combined into one group, 3004 patients are required to switch from newer DMTs to Tysabri. To provide a contemporaneous reference group, the patients who switch to Tysabri from interferon beta and glatiramer acetate (approximately 64,000 patients, as projected) will also be included in the study.

Data Analysis:	**General:** It is not planned to pool patient-level data from TOUCH and EU MS registries. Established DMTs (IFN-β and GA) are pooled into one group, while newer DMTs (fingolimod, DMF, and teriflunomide) are both pooled into one group and analysed separately. Furthermore, pooling of patient-level data from different EU registries is currently not planned (due to different minimal data elements/data capturing between the EU MS registries) but may be considered for later analysis time points (closer to the end of this observational study period). The analysis of the EU MS registries follows as much as possible (if data capturing and actual patient and event numbers allow) the analysis of TOUCH. **Analysis of TOUCH:** Descriptive analyses of TOUCH patients will be performed to characterise the study population. The distribution of age, gender, prior IS (yes/no), anti-JCV antibody status, and duration of Tysabri treatment will be summarised for each prior DMT group. The PML risk estimate will be determined by the yearly exposure interval for each prior DMT, with and without prior IS exposure, among anti-JCV antibody positive patients, anti-JCV antibody positive and unknown anti-JCV antibody status, and across all patients (anti-JCV antibody positive, negative, and unknown status) using the life table method. The number of patients with use of multiple newer DMTs will be determined. Due to the likely small sample size, no separate estimates of PML risk are currently planned for this group of patients. The time to PML following initiation of Tysabri therapy will also be assessed using a Kaplan-Meier approach for each prior DMT among anti-JCV antibody positive patients (with and without prior IS exposure). The incidence and incidence rate (per 1000 person-years) of other OIs will be determined for each prior DMT. Kaplan-Meier curves may also be used to

	explore exposure and event relationship if the number of cases allows such analyses. A sensitivity analysis may be performed for TOUCH, limited to patients who have switched to Tysabri from another DMT on or after 31 January 2012, when risk stratification was added to the PML estimated incidence table. An additional sensitivity analysis may also be performed for TOUCH, limited to patients who have switched to Tysabri from another DMT on or after 01 January 2016 to align with the EU registries.

Data Analysis (cont.):	**<u>Analysis of EU MS registries:</u>** Descriptive analyses of patients captured within EU MS registries will be performed to characterise the study population. Patient and disease characteristics like country, age, gender, disease duration, prior IS usage (yes/no), disease activity (relapse and Expanded Disability Status Scale [EDSS]), if available and anti- JCV antibody status will be summarised at Tysabri initiation for each prior DMT group. During Tysabri exposure, the anti-JCV antibody status and any concomitant medication (related or unrelated to MS) will be summarised if available. Follow-up time (including frequency/ density of follow-up) and Tysabri exposure (including reasons for Tysabri discontinuation other than PML) will be analysed if available. The PML risk estimate will be determined by yearly Tysabri exposure intervals for each prior DMT, with and without prior IS exposure, among anti-JCV antibody positive patients, anti-JCV antibody positive and unknown, and across all patients (anti-JCV antibody positive, negative, and unknown status), using the life table method. The prior DMT refers to the DMT most recently used prior to switching to Tysabri. The time to PML following initiation of Tysabri therapy will also be assessed using a Kaplan-Meier approach for each prior DMT among anti-JCV antibody positive patients (with and without prior IS exposure). The incidence and incidence rate (per 1000 person-years) of other serious OIs will be determined for any prior DMT. A Kaplan-Meier approach may also be used to explore exposure and event relationship (if event numbers allow). For a subset of registries that can confirm that data capture of SAEs and DMT history was systematic prior to the date of the prospective component of this study, a

	sensitivity analysis of the retrospective component (data captured prior to 01 January 2016) may be included.
Milestones:	The first interim report will be submitted along with the PSUR in 2017. Interim reports will be submitted on annual basis thereafter. The final clinical study report (CSR) will be delivered by 30 June 2024.

5. AMENDMENTS AND UPDATES

No.	Date	Section of Study Protocol	Amendment or Update	Reason
1	26 Jan 2022	Section 4, 9.5	Version 2	The number of patients to be included in the overall study was reduced. This change was the primary reason for the amendment.
2	26 Jan 2022	Sections 4, 6, 9.1 and 9.2.3	Version 2	Table 2 was updated to dissociate study reporting milestones from the submission of future PSURs.
3	26 Jan 2022	Section 4, 9.7	Version 2	Newer DMTs were pooled into one group.
4	26 Jan 2022	Section 4, 9.7	Version 2	Text was clarified as to how PML will be determined by anti-JCV antibody status.
5	26 Jan 2022	Section 4, 9.7	Version 2	Additional sensitivity analyses were added to the protocol.

Additional information on changes to the protocol can be found in the associated amendment summary document.

6. MILESTONES

Table 2: Milestones for Protocol 101MS411

Milestone	Planned Date
Start of prospective study data collection	01 January 2016
End of data collection	31 December 2023
Interim report 1	2017
Interim report 2	2018
Interim report 3	2019
Interim report 4	2020
Interim report 5	2021
Interim report 6	by 31 October 2022
Interim report 7	by 31 October 2023
Final report of study results	30 June 2024

7. RATIONALE AND BACKGROUND

Scientific rationale

Prior immunosuppressants (IS) use in patients with anti-JCV (John Cunningham virus) antibody positive status is identified as a risk factor for progressive multifocal leukoencephalopathy (PML). This is because these natalizumab-treated patients with prior IS have a greater risk of PML than patients with no prior IS use [Bloomgren 2012] except for those without prior IS who have a high anti-JCV antibody index. There is a relative paucity of data for those natalizumab patients exposed previously to newer disease-modifying therapies (DMTs) (e.g., teriflunomide, fingolimod, dimethyl fumarate [DMF]). For the newer DMTs, it is currently unknown how the risk of PML in patients that switch to natalizumab compares to the increased risk observed in patients previously treated with traditional IS (e.g., azathioprine, methotrexate, cyclophosphamide, mitoxantrone, mycophenolate moxetil). Due to the unknown risk of PML and other serious opportunistic infections (OIs) among Tysabri-treated patients who have switched from the newer generation of DMTs, this study is being conducted to assess those risks among patients who switched to Tysabri from newer DMTs (including fingolimod, DMF and teriflunomide) and the established DMTs (interferon beta and glatiramer acetate [GA]).

Scientific background

Natalizumab is a humanised monoclonal antibody which, *in vivo*, reduces inflammation by preventing the transmigration of mononuclear leukocytes from the bloodstream across the endothelium into inflamed parenchymal tissue, including in the central nervous system (CNS) [Wiendl 2008].

Tysabri (natalizumab) significantly reduced the rate of clinical relapse and the risk of progression of disability in pre-marketing efficacy trials including AFFIRM (monotherapy in patients naïve to previous DMTs).

Tysabri was authorised in the EU in June 2006. In June 2016, the European Commission authorised the extension of indication for Tysabri. Tysabri is now indicated as a single DMT in adults with highly active relapsing remitting Multiple Sclerosis (RRMS) for the following patient groups:

1. Patients with highly active disease activity despite a full and adequate course of treatment with at least one DMT (with some exceptions and required washout periods)

Or

2. Patients with rapidly evolving severe RRMS defined by 2 or more disabling relapses in one year, and with 1 or more Gadolinium enhancing lesions on brain Magnetic Resonance Imaging (MRI) or a significant increase in T2 lesion load as compared to a previous recent MRI

Reports of the viral brain disease, PML, caused by JCV were initially reported during the premarketing efficacy trials and PML is an important identified risk in the Tysabri Risk Management Plan (RMP).

Progressive Multifocal Leukoencephalopathy (PML)

PML is a sub-acute, evolving infectious disease of the central nervous system (CNS) caused by the JCV. The disease affects the sub-cortical white matter [Safak and Khalili 2003] and is caused by the reactivation of JCV, which is a human polyomavirus [Berger 1998]. The triggers for JCV replication are unknown but may result from confluence of risk factors, one of which is a compromised cellular immune system.

The European Federation of Neurological Societies (EFNS) published guidelines for the diagnosis and management of neurological complications of human immunodeficiency virus (HIV) infection including PML [Portegies 2004]. The diagnostic criteria are reproduced here.

Slowly progressive focal neurological deficits with asymmetrical white matter abnormalities on MRI suggest PML. The lesions are generally subcortical in location with finger like projections toward the cortex and have no mass effect. The lesions are hypointense on T1W MRI sequences, hyperintense on T2W and FLAIR (fluid-attenuated inversion recovery), hyperintense on DWI (diffusion weighted imaging) and generally do not enhance with contrast.

Detection of JCV deoxyribonucleic acid (DNA) in the cerebrospinal fluid (CSF) by polymerase chain reaction (PCR) strongly supports the diagnosis because it has a sensitivity of 72–100% and a specificity of 92–100% [Cinque 1997]. If the CSF-PCR is negative, it is recommended to repeat CSF-PCR. Use of an ultrasensitive PCR JCV DNA test is important (e.g., with a Limit of Detection (LoD) of 10 copies/mL) as many confirmed PML cases have demonstrated a low copy count. Brain biopsy remains the final confirmatory test, but a positive CSF-PCR offers acceptable evidence. MRI is the sensitive paraclinical tool for detection of symptomatic and asymptomatic PML in Tysabri treated patients [Wattjes and Barkhof 2014]. A previous baseline brain MRI scan should be available for use as a reference to help in differentiating between PML and other neurological diseases, e.g., MS lesions.

Regulatory background

In 2015, Biogen, the Marketing Authorisation Holder (MAH) sought to extend the approved indication for Tysabri. At the time the indication was restricted to non-responders to IFN-β or GA, to include patients who are non-responders to other first-line DMTs. During the assessment of variation EMEA/H/C/000603/II/0077 for Tysabri (natalizumab), the Pharmacovigilance Risk Assessment Committee (PRAC) required the MAH to make a proposal of a non-interventional Post-Authorisation Safety Study (PASS) aimed at determining the risk of PML and other opportunistic infections in patients who were previously treated with at least one DMT. The MAH proposed to run an observational cohort study using data from all patients included in the TYSABRI Outreach: Unified Commitment to Health (TOUCH) programme, supplemented with European Union (EU) patient data from existing registries.

On 26 May 2016, the Committee for Medicinal Products for Human Use (CHMP) opinion on the Type II variation concluded with the request to update the RMP with the inclusion of this Category 3 study.

8. RESEARCH QUESTION AND OBJECTIVES

8.1. Primary Objectives

The primary objectives of the study are as follows:

- estimate the incidence of PML among patients who switched to Tysabri from DMTs, including newer DMTs (including fingolimod, dimethyl fumarate and teriflunomide) and the established DMTs (interferon beta and glatiramer acetate)
- estimate the incidence of serious adverse events (SAEs) of other serious opportunistic infections among patients who switch to Tysabri from newer DMTs (including fingolimod, dimethyl fumarate and teriflunomide) and the established DMTs (interferon beta and glatiramer acetate)

8.2. Secondary Objectives

Not applicable.

9. RESEARCH METHODS

The MAH continues to work with select MS registries, both in the US (TOUCH) and the EU MS registries, to collect data relevant to the assessment of the occurrence of PML and other serious OIs.

9.1. Study Design

This is an observational cohort study utilising all available data (retrospective and prospective) from the Tysabri TOUCH prescribing programme (US) supplemented with data from EU MS registries to estimate the risk of PML and other serious OIs among patients on Tysabri who have switched from newer DMTs (including fingolimod, dimethyl fumarate and teriflunomide) and the established DMTs (interferon beta and GA).

This investigation has two data sources that contribute to the assessment of the PML risk and other serious OI incidences:

TOUCH (US only)

A cohort study utilising all available data from the US Tysabri TOUCH prescribing programme. Annex 3, Table 5 outlines the parameters assessed to support the study objectives.

EU MS Registries

Through a feasibility assessment (EMEA/H/C/000603/MEA/064), EU MS registries were identified and assessed to determine whether it was possible to utilise them as a data source for this post-authorisation safety study (PASS). The assessment concluded that it is feasible to supplement the evidence derived from the TOUCH programme provided that the minimum data elements are systematically captured (see Annex 3, Table 6). These EU registries will provide additional data on variables of interest (i.e., PML and other serious OI). Over time, additional EU MS registries will be assessed and potentially used as additional data sources as part of the safety assessment for PML and other serious OIs.

TOUCH and EU MS Registries

This observational study will provide cumulative data from its two components: a retrospective component (data captured prior to 01 January 2016) and a prospective component (data captured, and patients followed up, from 01 January 2016 through 31 December 2023; total prospective study duration of 8 years).

All patients who switched to Tysabri from another DMT through 31 December 2020 will be included. The study will continue to follow-up patients until 31 December 2023 to allow for a minimal follow-up of 3 years.

Timelines may be extended for some MS registries that may not start prospectively collecting SAE data until later in the study period. A report on the patient numbers for each data source and updates on the risk estimate of PML and the incidence of other serious OIs will be submitted according to the schedule outlined in Section 6, Table 2.

Although the TOUCH dataset comprises only US patients, the data generated on the risk estimates of PML is expected to be applicable to the patients in the EU as potential differences between geographic regions in terms of the distribution of known risk factors for PML (treatment duration, the presence of anti-JCV [John Cunningham virus] antibodies, and prior immunosuppressant use) will be addressed by the estimates stratified by them.

The EU MS registries will be able to provide baseline demographic and treatment history data for a cohort of EU MS patients switching from the newer and established DMTs to Tysabri, together with disease characteristics, exposure and safety information over time. These data would be used to supplement the evidence derived from the analysis of the TOUCH dataset. Patient data from EU MS registries will reflect the use of Tysabri in the post approval setting in the EU and can, therefore, provide information about safety in an expedited time frame than could be obtained by initiating a prospective observational study at the current time.

9.1.1. Primary Endpoint

- Occurrence of confirmed PML among Tysabri patients who switched from MS DMTs
- Occurrence of SAEs of other serious OIs among Tysabri patients who switched from MS DMTs

9.2. Setting

9.2.1. Selection Criteria

All TOUCH and available EU MS registry patients who have switched from DMTs (including fingolimod, dimethyl fumarate, teriflunomide, interferon beta and glatiramer acetate) and have one or more infusion(s) of Tysabri.

9.2.2. Study Location

The data sources will be the TOUCH registry (US) and select EU MS registries.

9.2.3. Overall Study Duration and Follow-Up

This observational study will provide cumulative data from its two components: a retrospective component (data captured prior to 1 January 2016) and a prospective component (data captured, and patients followed up, from 1 January 2016 through 31 December 2023; total prospective study duration of 8 years).

All patients who switched to Tysabri from another DMT through 31 December 2020 will be included. The study will continue to follow-up patients until 31 December 2023 to allow for a minimal follow-up of 3 years.

Timelines may be extended for some MS registries that may not start prospectively collecting SAE data until later in the study period. A report on the patient numbers for

each data source and updates on the risk estimate of PML and the incidence of other serious OIs will be submitted according to the schedule outlined in Section 6, Table 2.

9.3. Variables

Information collected for analysis will include information as outlined in Annex 3, Table 5 and Table 6.

9.4. Data Sources

TOUCH and selected EU MS registries.

9.5. Study Size

It is expected that at least 16,095 patients switching from DMTs to Tysabri are projected to be captured over the study period (based on patients included as of 31 December 2020). To obtain the targeted threshold of 0.5 per 1000 person-years, a required sample size of 9449 patients is required to switch from newer DMTs to Tysabri, with 1754 from prior fingolimod, 4077 from prior DMF treatment, and 3618 from prior teriflunomide treatment. When the three newer DMTs are combined into one group, 3004 patients are required to switch from newer DMTs to Tysabri. To provide a contemporaneous reference group, the patients who switch to Tysabri from interferon beta and glatiramer acetate (approximately 64,000 patients, as projected) will also be included in the study.

Table 3 illustrates the estimated required sample sizes for the DMT groups.

As the occurrence of PML remains the most important serious adverse event affecting the benefit-risk assessment of Tysabri treatment, the sample size (or duration of accrual) considerations are focused on reaching the incidence precision threshold for the PML incidence rate (i.e., 0.5/1000 person-years). The required person-years for each prior DMT group was calculated based on the Clopper-Pearson method. For each prior DMT group, the average Tysabri exposure (in years) is calculated as the observed person-years (based on patients included as of 31 December 2020)/number of patients (based on patients included as of 31 December 2020). The required number of patients for each group was estimated based on the assumption that the average Tysabri exposure (in years) will be at least the same as the current observed Tysabri exposure (based on patients included as of 31 December 2020). Therefore, the required number of patients for each prior DMT group is calculated as the required person-years/average Tysabri exposure.

Table 3: Estimation of Required Sample Size for DMT Groups

	Established DMT	Newer DMTs			
	IFN-β, GA	Fingolimod	Dimethyl Fumarate	Teriflunomide	Pooled Newer DMTs[f]
Number of Patients	64232	5579	8478	2784	16095
Observed Person-Years	203784	13069	18065	5632	35447
Number of PML Cases	200	2	8	2	11
Incidence of PML per 1000 person-years[a]	0.98 (0.85 - 1.13)	0.15 (0.02 - 0.55)	0.44 (0.19 - 0.87)	0.36 (0.04 - 1.28)	0.31 (0.15 - 0.56)
Margin of Error[b]	0.14	0.27	0.34	0.62	0.20
Required Number of Patients[c]	5361	1754	4077	3618	3004
Average Exposure (person-years)[d]	3.17	2.34	2.13	2.02	2.20
Required Number of Person-Years[e]	17007	4108	8687	7319	6616

Source: CSR Interim Report 5 dated 27 September 2021

[a] Rate per 1000 person-years and 95% CIs calculated via F distribution to compute exact intervals.

[b] Margin of error ≤ 0.5 was considered acceptable, as outlined in the protocol.

[c] Required number of patients for each group is calculated as the required person-years/average Tysabri exposure (in years).

[d] Average Tysabri exposure (in years) for each group is calculated as the observed person-years in interim report 5 (based on patients included as of 31 December 2020)/number of patients in interim report 5 (based on patients included as of 31 December 2020).

[e] Required person-years for each group is calculated based on the Clopper-Pearson method.

[f] Patients who have multiple newer DMTs (concurrently) just prior to their switch to Tysabri in TOUCH database will appear in all relevant DMT strata based on their prior DMT use.

9.6. Data Management

Data management and statistical software to be used in the study, including procedures for data collection, retrieval, collection, and preparation are outlined in the Statistical Analysis Plan.

9.7. Data Analysis

It is not planned to pool patient-level data from TOUCH and EU MS registries. Established DMTs (IFN-β and GA) are pooled into one group, while newer DMTs (fingolimod, DMF, and teriflunomide) are both pooled into one group and analysed separately.

Furthermore, pooling of patient-level data from different EU registries is currently not planned (due to different minimal data elements/data capturing between the EU MS registries) but may be considered for later analysis time points (closer to the end of this observational study period).

The analysis of the EU MS registries follows as much as possible (if data capturing and actual patient and event numbers allow) the analysis of TOUCH.

Analysis of TOUCH

Descriptive analyses of TOUCH patients will be performed to characterise the study population. The distribution of age, gender, prior IS (yes/no), anti-JCV antibody status, and duration of Tysabri treatment will be summarised for each prior DMT group.

The PML risk estimate will be determined by the yearly exposure interval for each prior DMT, with and without prior IS exposure, among anti-JCV antibody positive patients, anti-JCV antibody positive and unknown anti-JCV antibody status, and across all patients (anti-JCV antibody positive, negative, and unknown status) using the life table method. Table 4 below illustrates the presentation of the planned analysis. The prior DMT refers to the DMT most recently used prior to switching to Tysabri. The number of patients with use of multiple newer DMTs will be determined. Due to the likely small sample size, no separate estimates of PML risk are currently planned for this group of patients.

The time to PML following initiation of Tysabri therapy will also be assessed using a Kaplan-Meier approach for each prior DMT among anti-JCV antibody positive patients (with and without prior IS exposure).

The incidence and incidence rate (per 1000 person-years) of other OIs will be determined for each prior DMT. Kaplan-Meier curves may also be used to explore exposure and event relationship if the number of cases allows such analyses.

A sensitivity analysis may be performed for TOUCH, limited to patients who have switched to Tysabri from another DMT on or after 31 January 2012, when risk stratification was added to the PML estimated incidence table. An additional sensitivity analysis may also be performed for TOUCH, limited to patients who have switched to Tysabri from another DMT on or after 01 January 2016 to align with the EU registries.

Table 4: PML Risk Estimates per 1000 Patients (With 95% CIs) in Anti-JCV Antibody Positive Patients by Year of Natalizumab Exposure Using Life Table Method

Natalizumab Exposure	Anti-JCV Antibody Positive					
		Treatment Prior to Switch (Without Prior Use of IS)				
	With Prior Use of IS*	IFN-β, GA	Fingolimod	DMF	Teriflunomide	Pooled Newer DMTs**
1-12 Months						
13-24 Months						
25-36 Months						
37-48 Months						
49-60 Months						
61-72 Months						

*Prior IS group includes all patients who have prior IS exposure, regardless of their DMT switch status
**Newer DMTs include pooled data from fingolimod, DMF, and teriflunomide.

Analysis of EU MS Registries

Descriptive analyses of patients captured within EU MS registries will be performed to characterise the study population.

Patient and disease characteristics like country, age, gender, disease duration, prior IS usage (yes/no), disease activity (relapse and Expanded Disability Status Scale [EDSS]), if available and anti- JCV antibody status will be summarised at Tysabri initiation for each prior DMT group.

During Tysabri exposure, the anti-JCV antibody status and any concomitant medication (related or unrelated to MS) will be summarised if available.

Follow-up time (including frequency/ density of follow-up) and Tysabri exposure (including reasons for Tysabri discontinuation other than PML) will be analysed if available.

The PML risk estimate will be determined by yearly Tysabri exposure intervals for each prior DMT, with and without prior IS exposure, among anti-JCV antibody positive patients, anti-JCV antibody positive and unknown, and across all patients (anti-JCV antibody positive, negative, and unknown status), using the life table method. The prior

DMT refers to the DMT most recently used prior to switching to Tysabri. Table 4 above illustrates the presentation of the planned analysis.

The time to PML following initiation of Tysabri therapy will also be assessed using a Kaplan-Meier approach for each prior DMT among anti-JCV antibody positive patients (with and without prior IS exposure).

The incidence and incidence rate (per 1000 person-years) of other serious OIs will be determined for any respective each prior DMT. A Kaplan-Meier approach may also be used to explore exposure and event relationship (if event numbers allow).

For a subset of EU registries that can confirm that data capture of SAEs and DMT history was systematic prior to the date of the prospective component of this study (01 January 2016), a sensitivity analysis of the retrospective component (data captured prior to 01 January 2016) may be included.

9.8. Quality Control

Not applicable.

9.9. Limitations of the Research Methods

Not applicable.

9.10. Other Aspects

Not applicable.

10. PROTECTION OF HUMAN SUBJECTS

Not applicable.

11. MANAGEMENT AND REPORTING OF ADVERSE EVENTS/ADVERSE REACTIONS

Not applicable.

12. PLANS FOR DISSEMINATING AND COMMUNICATING STUDY RESULTS

The interim study reports will be available according to the schedule outlined in Section 6 Table 2. A final study report will be submitted to regulators by 30 June 2024.

13. REFERENCES

Berger JR, Pall L, Lanska D, et al. Progressive multifocal leukoencephalopathy in patients with HIV infection. J Neurovirol. 1998;4(1):59-68.

Bloomgren G, Richman S, Hotermans C, et al. Risk of natalizumab-associated progressive multifocal leukoencephalopathy. N Engl J Med. 2012;366(20):1870-80.

Cinque P, Scarpellini P, Vago L, et al. Diagnosis of central nervous system complications in HIV-infected patients: cerebrospinal fluid analysis by the polymerase chain reaction. AIDS. 1997;11(1):1-17.

Portegies P, Solod L, Cinque P, et al. Guidelines for the diagnosis and management of neurological complications of HIV infection. Eur J Neurol. 2004;11(5):297-304.

Safak M, Khalili K. An overview: Human polyomavirus JC virus and its associated disorders. J Neurovirol. 2003;9 Suppl 1:3-9.

Wattjes MP, Barkhof F. Diagnosis of natalizumab-associated progressive multifocal leukoencephalopathy using MRI. Curr Opin Neurol. 2014;27(3):260-70.

Wiendl H, Toyka KV, Rieckmann P, et al. Basic and escalating immunomodulatory treatments in multiple sclerosis: current therapeutic recommendations. J Neurol. 2008;255(10):1449-63. Epub 2008/10/29.

ANNEX 1: LIST OF STAND-ALONE DOCUMENTS

Not applicable.

ANNEX 2: ENCEPP CHECKLIST FOR STUDY PROTOCOLS

ENCePP Checklist for Study Protocols (Revision 4)

Adopted by the ENCePP Steering Group on 10/15/2018

The European Network of Centres for Pharmacoepidemiology and Pharmacovigilance (ENCePP) welcomes innovative designs and new methods of research. This Checklist has been developed by ENCePP to stimulate consideration of important principles when designing and writing a pharmacoepidemiological or pharmacovigilance study protocol. The Checklist is intended to promote the quality of such studies, not their uniformity. The user is also referred to the ENCePP Guide on Methodological Standards in Pharmacoepidemiology, which reviews and gives direct electronic access to guidance for research in pharmacoepidemiology and pharmacovigilance.

For each question of the Checklist, the investigator should indicate whether or not it has been addressed in the study protocol. If the answer is "Yes", the section number of the protocol where this issue has been discussed should be specified. It is possible that some questions do not apply to a particular study (for example, in the case of an innovative study design). In this case, the answer 'N/A' (Not Applicable) can be checked and the "Comments" field included for each section should be used to explain why. The "Comments" field can also be used to elaborate on a "No" answer.

This Checklist should be included as an Annex by marketing authorisation holders when submitting the protocol of a non-interventional post-authorisation safety study (PASS) to a regulatory authority (see the Guidance on the format and content of the protocol of non-interventional post-authorisation safety studies). The Checklist is a supporting document and does not replace the format of the protocol for PASS presented in the Guidance and Module VIII of the Good pharmacovigilance practices (GVP).

Study title: An observational study utilising data from the US Tysabri TOUCH programme and select EU MS Registries to estimate the risk of progressive multifocal leukoencephalopathy (PML) and other serious opportunistic infections among patients who were exposed to an MS disease modifying treatment prior to treatment with Tysabri

Study reference number: 101MS411

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]	☒	☐	☐	4,6,9.1,9.2.3
1.1.2 End of data collection[2]	☒	☐	☐	4,6,9.1,9.2.3,9.5
1.1.3 Study progress report(s)	☐	☒	☐	
1.1.4 Interim progress report(s)	☒	☐	☐	4, 6,12
1.1.5 Registration in the EU PAS register	☒	☐	☐	Title page
1.1.6 Final report of study results	☒	☐	☐	4, 6,12

Comments:

1.1.5-EU PAS Register number is 19800

[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.

Section 2: Research question	**Yes**	**No**	**N/A**	**Section Number**
2.1 Does the formulation of the research question and objectives clearly explain:	☒	☐	☐	
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)	☒	☐	☐	4, 7
2.1.2 The objective(s) of the study?	☒	☐	☐	4, 8
2.1.3 The target population? (i.e. population or subgroup to whom the study results are intended to be generalised)	☒	☐	☐	4, 7
2.1.4 Which hypothesis(-es) is (are) to be tested?	☐	☒	☐	
2.1.5 If applicable, that there is no *a priori* hypothesis?	☐	☒	☐	

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)	☒	☐	☐	4, 9.1
3.2 Does the protocol specify whether the study is based on primary, secondary or combined data collection?	☒	☐	☐	4, 9.7
3.3 Does the protocol specify measures of occurrence? (e.g. rate, risk, prevalence)	☒	☐	☐	4, 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)	☐	☒	☐	
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:

3.5- Secondary data is used for this study.

Section 4: Source and study populations	**Yes**	**No**	**N/A**	**Section Number**
4.1 Is the source population described?	☒	☐	☐	4, 7
4.2 Is the planned study population defined in terms of:				
4.2.1 Study time period	☒	☐	☐	4,6,9.1,9.2.3,9.5
4.2.2 Age and sex	☒	☐	☐	4, 9
4.2.3 Country of origin	☒	☐	☐	4, 9
4.2.4 Disease/indication	☒	☐	☐	4 ,7
4.2.5 Duration of follow-up	☒	☐	☐	4,9.1, 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)	☒	☐	☐	4, 9

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)	☒	☐	☐	4, 6, 9.1, 9.2.1, 9.2.3, 9.5
5.2	Does the protocol address the validity of the exposure measurement? (e.g. precision, accuracy, use of validation sub-study)	☒	☐	☐	4, 9.5
5.3	Is exposure categorised according to time windows?	☐	☒	☐	
5.4	Is intensity of exposure addressed? (e.g. dose, duration)	☒	☐	☐	4, 9
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?	☒	☐	☐	4, 8, 9

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?	☒	☐	☐	4, 7, 8, 9
6.2	Does the protocol describe how the outcomes are defined and measured?	☒	☐	☐	4, 7, 8, 9
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)	☒	☐	☐	4, 9.5
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)	☒	☐	☐	4, 9.7
7.2	Does the protocol address selection bias (e.g. healthy user/adherer bias)	☒	☐	☐	4, 9.7
7.3	Does the protocol address information bias? (e.g. misclassification of exposure and endpoints, time-related bias)	☒	☐	☐	4, 9.7

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)	☒	☐	☐	9.7

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)	☒	☐	☐	4, 9
	9.1.2 Outcomes? (e.g. clinical records, laboratory markers or values, claims data, self-report, patient interview including scales and questionnaires, vital statistics)	☒	☐	☐	4, 8, 9
	9.1.3 Covariates and other characteristics?	☒	☐	☐	4, 9.7
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)	☒	☐	☐	4, 9, Annex 3
	9.2.2 Outcomes? (e.g. date of occurrence, multiple event, severity measures related to event)	☒	☐	☐	4, 8, 9, Annex 3
	9.2.3 Covariates and other characteristics? (e.g. age, sex, clinical and drug use history, co-morbidity, co-medications, lifestyle)	☒	☐	☐	4, 9.7, Annex 3
9.3	Is a coding system described for:				
	9.3.1 Exposure? (e.g. WHO Drug Dictionary, Anatomical Therapeutic Chemical (ATC) Classification System)	☐	☒	☐	
	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:

9.3.2- MedDRA specified in the SAP

Section 10: Analysis plan		**Yes**	**No**	**N/A**	**Section Number**
10.1	Are the statistical methods and the reason for their described?	☒	☐	☐	4, 9.7
10.2	Is the study size and/or statistical precision estimated?	☒	☐	☐	4, 9.5
10.3	Are descriptive analyses included?	☒	☐	☐	4, 9.7
10.4	Are stratified analyses included?	☒	☐	☐	4, 9.7
10.5	Does the plan describe methods for analytic control of confounding?	☒	☐	☐	4, 9.7

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?	☒	☐	☐	4, 9.7

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)	☐	☒	☐	
11.2	Are methods of quality assurance described?	☐	☒	☐	
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)	☒	☐	☐	4, 9

Comments:

Limitations have been discussed in the interim reports.

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?	☐	☐	☒	

Comments:

Secondary data is being used for this study.

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?	☐	☒	☐	

Comments:

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

Date:

Signature: _____

ANNEX 3: ADDITIONAL INFORMATION

DATA ELEMENTS

Table 5: Data elements available in the TOUCH database

Data Elements	Baseline[1]	Tysabri Treatment Period[2]
Eligibility criteria[3]	X	
Demographic characteristics (age, gender) [3]	X	
Prior Disease Modifying Therapy (DMT)[3]	X	
Prior IS (including duration)[3]	X	
Tysabri exposure (number of infusions)[3]		X
Anti-JCV antibody status[3]	X	X
Progressive Multifocal Leukoencephalopathy (PML)[4]		X
Opportunistic infection[4]		X

1 Baseline time period includes pre-Tysabri treatment period through initiation of Tysabri treatment.
2 Length of follow-up time varies by patient
3 Data available in TOUCH
4 Data available in the Biogen safety database.

JCV=John Cunningham virus; PML=progressive multifocal leukoencephalopathy; DMT=disease modifying therapy

Table 6: Minimum[1] data elements in EU MS registries

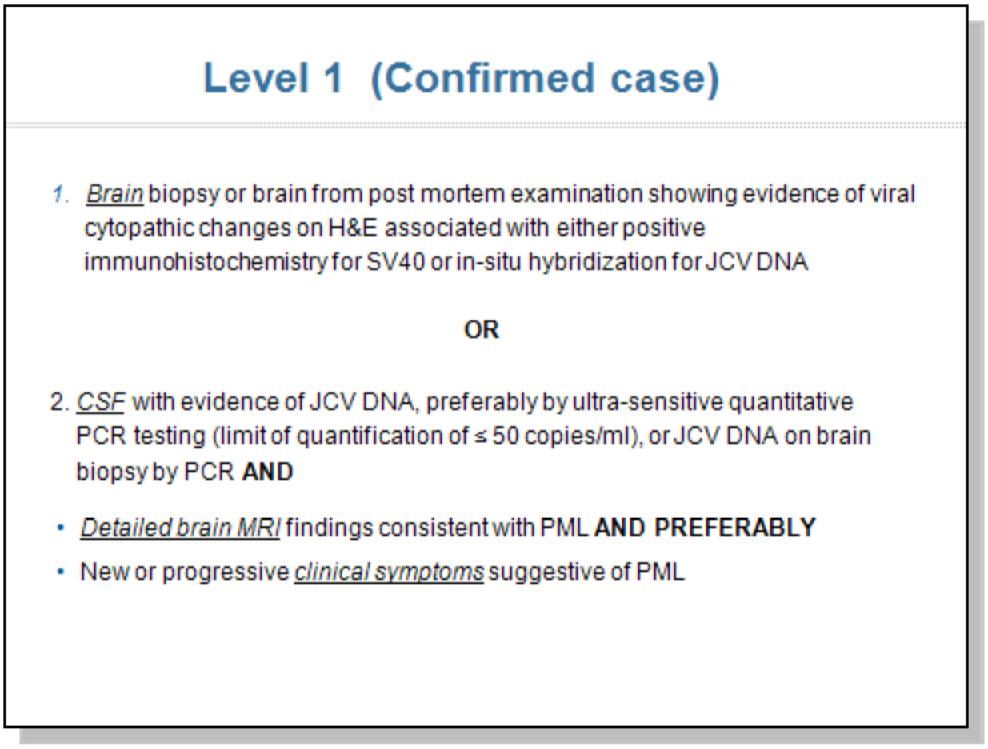
Data Elements	Baseline[2]	Tysabri Treatment Period[3]
Eligibility criteria	X	
Demographic characteristics (age, gender, country)	X	
Disease characteristics (disease duration, relapse, EDSS)	X	
Anti- JCV antibody status	X	X
Prior DMT (including duration)	X	
Prior IS (including duration)	X	
Prior medications not related to MS (including duration)	X	
Concomitant and subsequent DMT (including duration)		X
IS (including duration)		X
Concomitant medications not related to MS (including duration)		X
Tysabri exposure (Number of infusions)		X
Progressive Multifocal Leukoencephalopathy (PML)		X
Other serious opportunistic infection		X

1 Some EU MS registries may not have all the minimum data element requirements.
2 Baseline time period includes pre-Tysabri treatment period through initiation of Tysabri treatment.
3 Length of follow-up time varies by patient (as captured within the respective MS registry)

JCV=John Cunningham virus; PML=progressive multifocal leukoencephalopathy; DMT=disease modifying therapy; IS=immunosuppressant; EDSS= Expanded Disability Status Scale; MS=multiple sclerosis

PML CASE DEFINITIONS

Following the Brighton Collaboration methodology, the new proposal for PML case definitions now includes five categories (Level 1 to Level 5) ranging from the highest to lowest level of diagnostic certainty, a category for insufficient data available, and a category with specific exclusion criteria. Several published Brighton Collaboration adverse event guidelines (see references 1-7 at the end of this Appendix) were reviewed to assist the MAH in the development of this new case definition, as well as input from external PML experts.



Level 1 – Confirmed Case

Level 1 represents the highest level of diagnosis certainty. In order to meet Level 1 criteria, the case must have one of the following:

- Brain biopsy or brain from post mortem examination showing evidence of viral cytopathic changes on hematoxylin and eosin (H & E) staining associated with either positive immunohistochemistry for SV40 or in-situ hybridization for JCV DNA **OR**

  All of the following criteria:

- CSF with evidence of JCV DNA, preferably by ultra-sensitive quantitative PCR testing (limit of quantification of ≤ 50 copies/ml), or JCV DNA on brain biopsy

by PCR **AND** detailed description of brain MRI findings that are consistent with PML

AND PREFERABLY new or progressive clinical symptoms suggestive of PML.

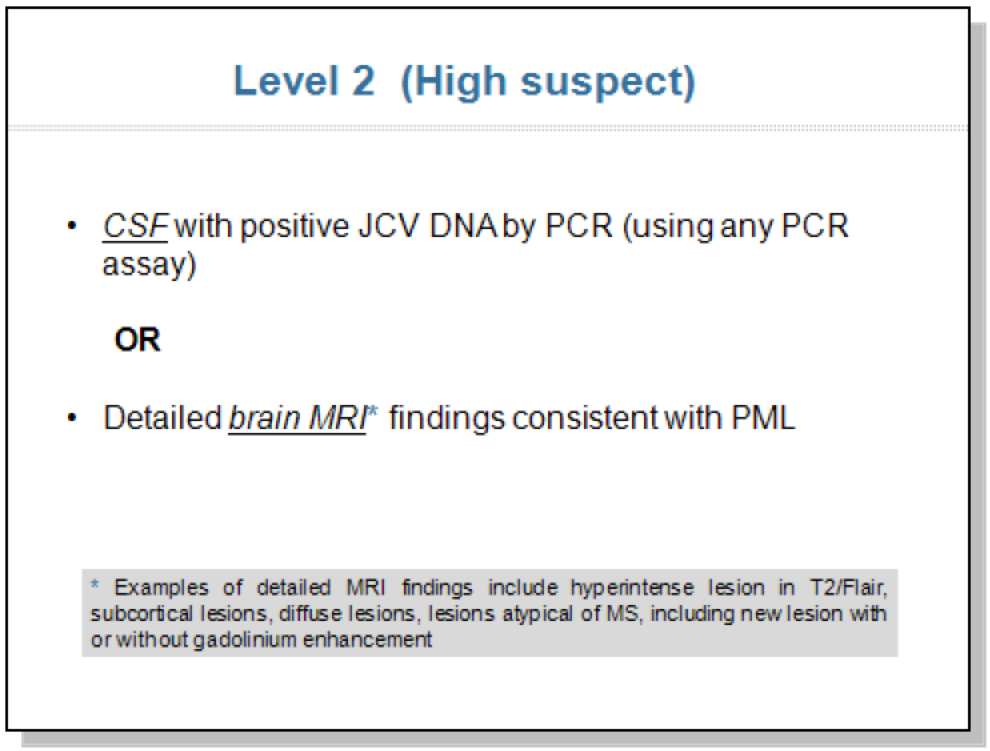
Examples:

1. A reported suspect PML case would be classified as Level 1 if the histopathology alone on brain biopsy or post mortem examination shows evidence for JCV infection by immunohistochemistry or in situ hybridization, even if the JCV DNA in CSF was negative.
2. A reported suspect PML case would be classified as Level 1 if the CSF is positive for JCV DNA (regardless of the type of PCR assay utilized, either qualitative or quantitative, ultra-sensitive or not ultra-sensitive) **and** there is a detailed description of a brain MRI that is consistent with PML.

Comments:
The use of ultra-sensitive JCV DNA PCR testing is preferable, however not mandatory. Most commercial assays for JCV DNA have a sensitivity of approximately 500 copies/mL. Ultra-sensitive quantitative JCV DNA PCR testing has a lower limit of quantification of 50 copies/mL (NIH has a sensitivity of 10 copies/mL). To date, approximately half of the confirmed Tysabri-treated PML cases have had JCV DNA copy numbers in the CSF of less than 500 copies/mL at the time of diagnosis. While the MAH acknowledges that ultra-sensitive quantitative JCV DNA PCR testing is the preferred test, it is not available globally, and therefore the presence of JCV DNA by PCR based on either qualitative, or quantitative, but not ultra-sensitive, assays is also acceptable.

Examples of a detailed description of brain MRI findings consistent with PML include: hyperintense lesion on T2/flair, subcortical lesions, or lesions atypical for MS, including new lesion with or without Gadolinium enhancement.

Evidence of clinical symptoms is preferred however it is not mandatory. The reason for this is that the MAH has examples of asymptomatic suspected PML cases identified during routine MRI screening in certain Tysabri-treated patients. These patients tested positive for JCV DNA in CSF and had a brain MRI that was consistent with PML and were thus assessed as confirmed cases of PML, despite any evidence of clinical symptoms.



Level 2 – High Suspect

In order to meet Level 2 criteria, the case must have one of the following:

- CSF with positive JCV DNA by PCR (using any PCR assay) **or**
- detailed MRI findings consistent with PML

Examples:

1. A reported suspect PML case would be classified as Level 2 if the CSF is positive for JCV DNA using any PCR assay and either no MRI results are reported or the MRI is not consistent with PML.

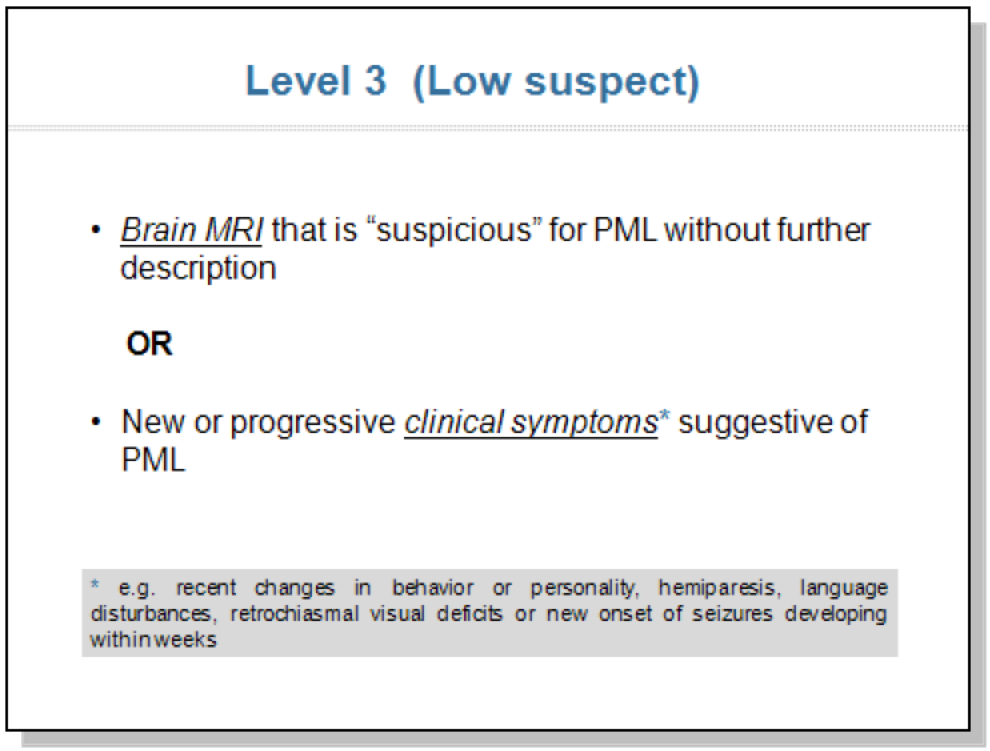
   Note: If further evaluation of PML is performed by the treating physician including additional tests of CSF which are negative for JCV DNA, then the classification of the case would depend on the quality of the assays used, the nature of the MRI findings and the clinical course of the patient and may need to be discussed with external experts to enable classification. For example, if the initial CSF result was performed using a qualitative assay and was positive (or borderline), but subsequent CSF result performed with an ultra-sensitive quantitative PCR assay was negative, and the brain MRI was not consistent with PML.

2. A reported suspect PML case would be classified as Level 2 if a detailed/descriptive brain MRI is provided which is consistent with PML with no report of CSF results or the CSF JCV DNA result is negative.

Comments:

For classification of a case as a Level 2, the use of ultra-sensitive JCV DNA PCR testing is not required. Any laboratory result using any commercial JCV DNA PCR assay is accepted for this level of diagnostic certainty.

Examples of a detailed description of brain MRI findings consistent with PML include: hyperintense lesion on T2/flair, subcortical lesions, or lesions atypical for MS, including new lesion with or without Gadolinium enhancement.



Level 3 - Low Suspect

In order to meet Level 3 criteria, the case must have one of the following criteria:

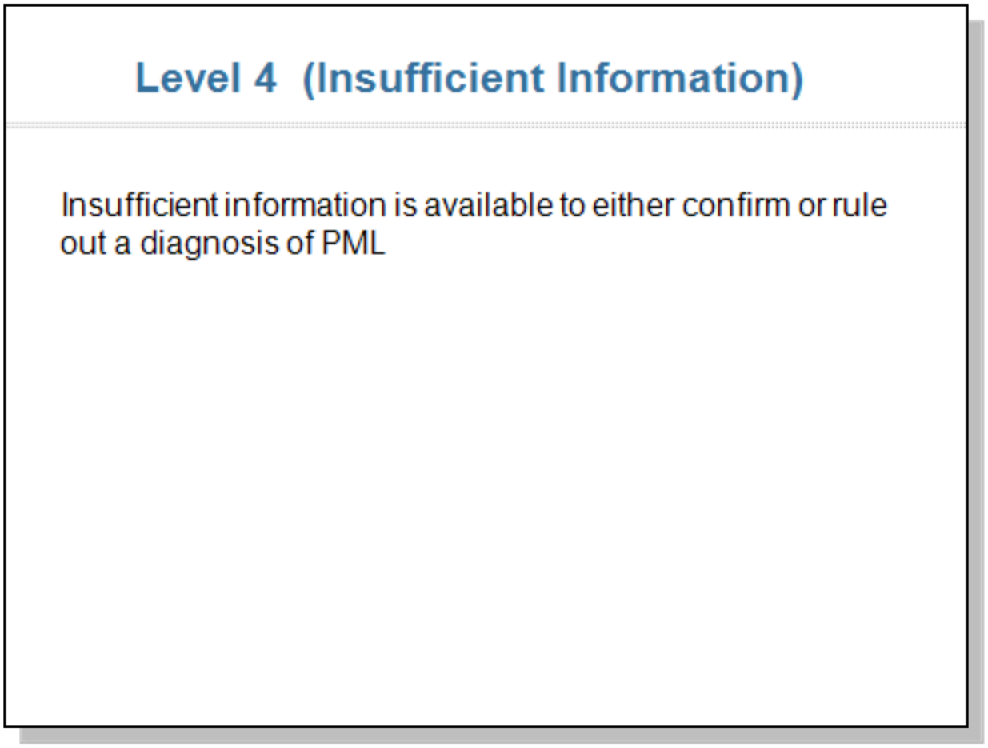
- Either a brain MRI that was considered "suspicious" for PML as stated by the treating physician without any further description **or**
- New or progressive clinical symptoms, which are suggestive of PML

Examples:

1. A reported suspect PML case would be classified as Level 3 if a brain MRI that is "suspicious" for PML is reported without any further details or description and either no CSF results are reported or the CSF is negative for JCV DNA.

2. A reported suspect PML case would be classified as Level 3 if the patient is reported to have new or progressive symptoms which are suggestive for PML and neither MRI nor CSF results are provided.

Comments:
Examples of clinical symptoms suggestive of PML may include but are not limited to recent changes in behavior or personality, hemiparesis, language disturbances, retrochiasmal visual deficits or new onset of seizures. These symptoms may have developed over the course of weeks.



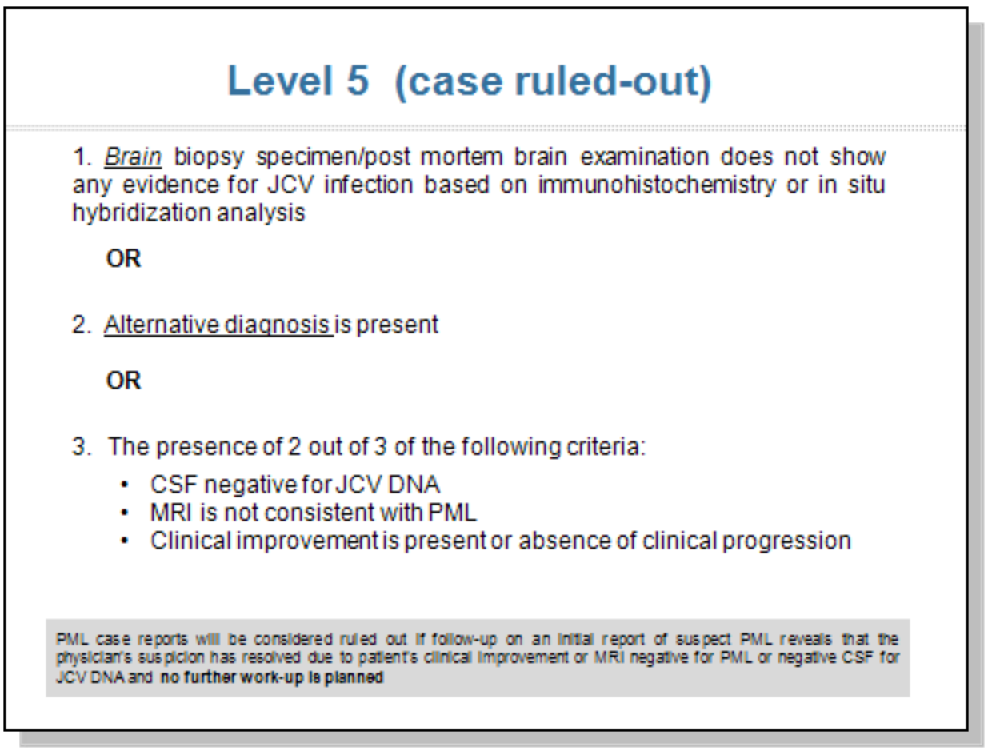
Level 4 - Insufficient Information

Level 4 is used for cases where there is insufficient objective information available to either confirm or exclude a diagnosis of PML, despite exhaustive due diligence.

If the evidence available for the case is insufficient due to missing information, the case will be classified as a "Reported suspect case of PML with insufficient evidence to meet the case definition".

Example:

1. A reported suspect PML case would be classified as Level 4 if a suspect PML case is reported without any further details (no report of clinical symptoms, MRI results, or CSF results) and despite due diligence, we are unable to obtain any more detailed information. This has occurred when, for example, the treating physician wishes to remain anonymous or if the treating physician refuses to provide the requested information.



Level 5 - Case Ruled-Out

In order to meet Level 5 criteria, the case must have one of following criteria:

1. Brain biopsy specimen/post mortem brain examination does not show any evidence for JCV infection based on immunohistochemistry or in situ hybridization analysis **or**
2. An alternative diagnosis is present **or**
3. Presence of 2 out of the 3 following criteria:

    - CSF negative for JCV DNA
    - MRI is not consistent with PML
    - Clinical improvement is present or absence of clinical progression

Examples:

1. A reported suspect PML case would be classified as Level 5 if a brain biopsy has been performed which histologically shows no evidence of JCV infection by immunohistochemistry or in situ hybridization.

2. A reported suspect PML case would be classified as Level 5 if the treating physician has evaluated the patient and has provided an alternative diagnosis, which is not PML.

3. A reported suspect PML case would be classified as Level 5 if two out of three of the following have occurred: the patient has shown clinical improvement or absence of clinical progression, the MRI is not consistent with PML, or the CSF is negative for JCV DNA.

Comments:
Cases will also be considered ruled out if the treating physician's suspicion has resolved due to clinical improvement of the patient, or an MRI that is negative for PML, or CSF that is negative for JCV DNA **and** there is no further planned work-up of the patient.

Note: The MAH performs intensive due diligence on all potential cases of PML and may continue to receive additional follow-up information for weeks to months after initial receipt of a case. The implications of this are as follows:

- Cases that are initially classified in Level 2 or Level 3 may be later re-classified based on new information received. For example, a case initially classified as a Level 2 case may become a Level 1 case based on receipt of new information, or a case initially classified as a Level 3 case may become a Level 5 case with further follow-up.

- The Level 4 category will be utilized only after exhaustive due diligence has failed to provide sufficient information to classify a case.

- In general, once a case is classified as a Level 5 case, it remains in this category (unless new information is received which may prompt a re-classification).

- Finally, once a case has been classified as a Level 1 case, it remains a Level 1 case.

Additional References – Annex 3

1. https://brightoncollaboration.org/public/what-we-do/standards/case-definitions.html
2. Encephalitis, myelitis, and acute disseminated encephalomyelitis (ADEM): case definitions and guidelines for collection, analysis, and presentation of immunization safety data. *James J.Sejvar, Katrin S. Kohl, Roman Bilynski, Dean Blumberg, Therese Cvetkovich, Jochem Galama, Jane Gidudu, Lakshmi Katikaneni, Najwa Khuri-Bulos, James Oleske, Terhi Taipiainen, Max Wiznitzer. The Brighton Collaboration Encephalitis Working Group.* VACCINE 2007;25: 5771-5792.
3. Guillain-Barre syndrome and Fisher syndrome: Case definitions and guidelines for collection, analysis, and presentation of immunization safety data. *James Seivar, Katrin Kohl, Jane Gidudu, Anthony Amato, Nandini Bakshi, Roger Baxter, dale Burwen, David Cornblath, Jan Cleerbout, Kathryn Edwards, Ulrich Heininger, Richard Hughes, Najwa Khu-Bulus, Rudolf Korinthenberg, Barbara Law, Ursula Munro, Helena Maltezou, Patricia Nell, James Oleske, Robert Sparks, Priscilla Velentgas, Patricia Vermeer, Max Wizintzer, The Brighton Collaboration GBS Working Group.* VACCINE 2011;29: 599-612.
4. Anaphylaxis: Case definition and guidelines for data collection, analysis, and presentation of immunization safety data. *Jens Rueggeberg, Michael Gold, Jose-Maria Bayas, Michael Blum, Jan Bonhoeffer, Sheila Friedlander, Glacus de Souza Brito, Ulrich Heininger, Babatude Imoukhuede, Ali Khamesipour, Michel Erlewyn-Lajeunesse, Susana Martin, Mika Maekelae, Patricia Nell, Vitali Pool, Nick Simpson, The Brighton Collaboration Anaphylaxis Working Group.* VACCINE 2007; 25: 5675-5684.
5. Abscess at injection site: Case definition and guidelines for collection, analysis, and presentation of immunization safety data. *Katrin Kohl, Leslie Ball, Jane Gidudu, Sandra Hammer, Scott Halperin, Paul Heath, Renald Hennig, Jerry Labadie, Edward Rothstein, Anne Schuind, Frederick Varricchio, Wikke Walop, The Brighton Collaboration Abscess Working Group.* VACCINE 2007; 25: 5821-5838.
6. Diarrhea: Case definition and guidelines for collection, analysis, and presentation of immunization safety data. *Gidudu J et al, The Brighton Collaboration Diarrhea Working Group. (reference to -* https://brightoncollaboration.org/public/what-we-do/standards/case-definitions.html)
7. Generalized convulsive seizure as an adverse event following immunization: case definition and guidelines for data collection, analysis, and presentation. *Jan Boenhoffer, john Menkes, Michael Gold, Glacus Souza-Brito, Mageret Fisher, Neal Halsey, patricia Vermeer, The Brighton Collaboration Seizure Working Group.* VACCINE 2004;22: 557-562.

DEFINITION OF A 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 subject 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