NON-INTERVENTIONAL POST AUTHORISATION SAFETY STUDY (PASS) PROTOCOL

A NON-INTERVENTIONAL POST-AUTHORISATION SAFETY STUDY TO INVESTIGATE THE RISK OF MORTALITY IN MULTIPLE SCLEROSIS PATIENTS TREATED WITH ALEMTUZUMAB (LEMTRADA®) RELATIVE TO COMPARABLE MULTIPLE SCLEROSIS PATIENTS USING OTHER DISEASE MODIFYING THERAPIES: A COHORT STUDY

COMPOUND: Alemtuzumab

PRIME STUDY NUMBER: CSA0002

Version 4.0, April 2024

The Study is conducted by Parexel International hereinafter referred also as the Study Management.

NAMES AND ADDRESSES

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PASS Information

Title	**A non-interventional post-authorisation safety study to investigate the risk of mortality in multiple sclerosis patients treated with alemtuzumab (LEMTRADA®) relative to comparable multiple sclerosis patients using other disease modifying therapies: a cohort study**
Protocol version identifier	Version 4.0
Date of last version of protocol	April 2024
EU PAS register number	EUPAS42543
Active substance	Alemtuzumab (GZ402673): ATC L04AA34
Medicinal product	LEMTRADA
Product reference	EU/1/13/869/001
Procedure Number	EMEA/H/C/003718
Marketing authorisation holder(s)	Sanofi Belgium
Joint PASS	No
Research question and objectives	**Question:** What is the risk of mortality in multiple sclerosis patients treated with LEMTRADA as compared to multiple sclerosis patients treated with other highly efficacious disease modifying therapies (HE-DMT)? **Primary objective:** To ascertain whether multiple sclerosis patients treated with LEMTRADA have a higher risk of all-cause mortality than comparable multiple sclerosis patients treated with other HE-DMT.
Country(-ies) of study	Sweden, Denmark, United Kingdom, Czech Republic, Germany
Author	REDACTED Sanofi - Pharmacoepidemiology, REDACTED REDACTED REDACTED

Marketing authorisation holder(s)

Marketing authorisation holder(s)	Sanofi Belgium
MAH/MAH REPRESENTATIVE contact person	QPPV office -- Sanofi R&D- Global Pharmacovigilance & Epidemiology REDACTED Senior Medical Adviser -- QPPV deputy for Specialty Care Business Unit REDACTED REDACTED REDACTED

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2 LIST OF ABBREVIATIONS

aHR:	Adjusted hazard ratio
AIM-IMA:	L'Agence Intermutualiste - Het InterMutualistisch Agentschap (Belgian Social Security database)
AT:	As-treated
ATC:	Anatomical therapeutic chemical
ATT:	Average treatment effect among treated
BIPS:	Bremen Institute for Prevention Research and Social Medicine
BMI:	Body mass index
BMSD:	Big MS Data
CED:	Cohort entry date
CI:	Confidence interval
CIOMS:	Council of International Organization of Medical Sciences
CNK:	Code National/Kode National
Covid-19:	Coronavirus disease 2019
CS:	Corticosteroid
DAG:	Directed Acyclic Graph
DMSR:	Danish Multiple Sclerosis Registry
DMT:	Disease modifying therapy
EC:	European Commission
ECCS:	External Comparison Cohort Study
EDSS:	Expanded Disability Status Scale
EMA:	European Medicines Agency
ENCePP:	European Network of Centres for Pharmacoepidemiology and Pharmacovigilance
EU:	European Union
GePaRD:	German Pharmacoepidemiological Research Database
GP:	General practitioner
GPP:	Guidelines for Pharmacoepidemiology Practices
HE-DMT:	Highly efficacious DMT
HE-DMT_NL:	HE-DMT other than LEMTRADA
HR:	Hazard ratio
ICD:	International Classification of Diseases
IT:	Information technology
ITT:	Intent-to-treat
IV:	Intravenously
JCV:	John Cunningham virus
LEM:	LEMTRADA

MAH:	Marketing authorisation holder
MI:	Multiple imputation
MRI:	Magnetic resonance imaging
NHS:	National Health System
MS:	Multiple sclerosis
OP:	Operating protocol
PASS:	Post-authorisation safety study
P-DMT:	Platform DMT
PI:	Principal Investigator
PML	Progressive multifocal leukoencephalopathy
PPMS:	Primary progressive multiple sclerosis
PRAC:	Pharmacovigilance Risk Assessment Committee
PS:	Propensity score
PY:	Person-Years
ReMuS:	The Czech Multiple Sclerosis Registry
RR:	Relative Risk
RRMS:	Relapsing remitting multiple sclerosis
SAP:	Statistical analysis plan
SMR:	Standardised mortality ratio
SMSR:	Swedish Multiple Sclerosis Registry
SPMS:	Secondary progressive multiple sclerosis
UK:	United Kingdom
US:	United States

3 RESPONSIBLE PARTIES

3.1 RESPONSIBILITIES OF THE DATA SOURCE PROVIDER

1. To contribute to and collaborate with the study in accordance with the protocol.
2. To allow access to data sources relevant to the study.
3. To obtain ethical approval where necessary and adhere to legal requirements surrounding data protection.
4. To develop the operating protocol (OP) with the Study Management.
5. To organise training of staff in accordance with the Study Management.
6. To produce the working databases and analysis databases according to procedures outlined in OPs.
7. To participate in the meetings and other activities necessary for the good conduct of the study.
8. To participate in the feasibility study and review of final study reports.

List of collaborators at protocol stage:

Denmark	The Danish Multiple Sclerosis Registry (DMSR) Copenhagan University Hospital, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen, Denmark Principal Investigator: Prof Melinda Magyari
Sweden	The Swedish Multiple Sclerosis Registry (SMSR) Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden Principal Investigator: Prof Jan Hillert
Czech Republic	The Czech Multiple Sclerosis Registry (ReMuS) IMPULS Endowment Fund, Katerinska 30, 120 00 Prague 2, Czechia Principal Investigator: Dana Horakova Dpt. of Neurology and Center of Clinical Neuroscience, First Faculty of Medicine, Charles University in Prague and General University Hospital, Prague, Czechia
United Kingdom	University Hospital of Wales 4th Floor, B-C Link Corridor, Main Hospital Building, Heath Park, Cardiff, CF14 4XN Principal Investigator: Prof Neil Roberston

	Cambridge University Hospitals, Department of Clinical Neurosciences, University of Cambridge, England Principal Investigator: Dr William Brown Derriford Hospital/ Plymouth University Room N13, ITTC Building, Plymouth Science Park, Davy Road, PL68BX Principal Investigator: Prof Jeremy Hobart
Germany	The German Pharmacoepidemiological Research Database (GePaRD) Leibniz Institute for Prevention Research and Epidemiology – Bremen Institute for Prevention Research and Social Medicine (BIPS) GmbH Achterstraße 30, 28359 Bremen, Germany Principal Investigator: Prof Ulrike Haug

3.2 RESPONSIBILITIES OF THE STUDY MANAGEMENT

1. To write the study protocol.
2. To write OPs specific to each database.
3. To coordinate the implementation of the study in the various databases, with consideration to local administrative, legal and technical environment.
4. To define with database providers the practical modalities of participation in the study, including legal and financial aspects.
5. To monitor study progress, identify or predict problems and work with database providers to find suitable solutions.
6. To plan, supervise and report on the feasibility study.
7. To prepare statistical methods and computer software needed for data analysis.
8. To train database scientists on tasks related to data handling, cleaning and analyses.
9. To organise meetings between the database providers, the marketing authorisation holder (MAH) and the Study Management for discussing study progress and results.
10. To prepare study reports intended for submission to the Regulator.
11. To regularly inform the MAH on study advancement and issues to be addressed.

3.3 RESPONSIBILITIES OF THE MAH

The MAH is responsible for taking all reasonable steps and providing adequate resources to ensure the proper conduct of the study.

3.4 RESPONSIBILITIES OF INDEPENDENT SCIENTIFIC ADVISERS

Independent scientific advisers may be convened to review study documentation, e.g., the protocol, feasibility reports, interim reports, and the final report.

The independent scientific advisers do not have conflict-of-interest that could be associated with the study, nor be involved in the study conduct, nor have any direct link with data sources used for the study.

4 ABSTRACT

Version and date: Version 4.0, April 2024

Author: Sanofi, 450 Water St, Cambridge MA 02141, USA

Title: A non-interventional post-authorisation safety study to investigate the risk of mortality in patients treated with alemtuzumab relative to comparable patients using other disease modifying therapies: a cohort study

Rationale and background: Following a European Medicines Agency (EMA) Article 20 procedure (EMEA/H/A-31/1483/C/3718/0028) in 2019, an investigation into the risk of mortality in multiple sclerosis (MS) patients treated with LEMTRADA compared to a relevant MS patient population is required for years during which LEMTRADA has been in use.

Research objective/question: To ascertain whether MS patients treated with LEMTRADA have a higher risk of all-cause mortality than comparable MS patients treated with other highly efficacious disease modifying therapies (HE-DMT).

Study design: Observational comparative cohort study based on the secondary use of data held in MS registries, administrative database and other data sources in Europe.

Population: MS patients treated with a HE-DMT in usual clinical practice in multiple European countries.

Variables: The outcome will be all-cause mortality. Exposure will be exposure to LEMTRADA vs. exposure to HE-DMT other than LEMTRADA. Patient characteristics and known predictors of mortality in MS patients (e.g., severity of MS, comorbidities) will be used to control for confounding.

Data: Individual level data of MS patients held in population-based MS patient registries, administrative and prescription registries, as well as chart review data will be used. Data from the date of LEMTRADA approval/reimbursement (2013-2015) until last available data in each data source will be used. There will be no transfer of individual or of identifiable data to any recipient.

Data sources:

- MS registries in Denmark, Sweden and Czech Republic
- Chart review in the United Kingdom
- Prescription and administrative data in Germany

Sample size:

All MS patients treated with a HE-DMT will be included in the study. Expected estimates of precision surrounding the risk of mortality have been computed considering pooled analyses, a mortality rate of 0.24 per 100 person-years in HE-DMT treated patients, relative risks ranging from 1.2 to 2.0 and a ratio of 1:4 between patients treated with LEMTRADA and patients treated with another HE-DMT, a median follow-up of six years, and Type I error (α risk) of 5% (two-sided). A HR of 1.5 with a 95% confidence interval (CI) ranging from 1.00 to 2.25 could be possible with 1,000 LEMTRADA treated MS patients, pooled from the five contributing countries. Of note, precision estimates were re-calculated using the most up-to-date cohort sizes from 2023 feasibility analyses, as Belgium is no longer contributing to this study.

Data analysis: Statistical analyses will be performed separately in each data source. Within each data source, a propensity score (PS) will be used to control for confounding. Propensity score weighted Cox proportional hazards models will be used to calculate adjusted hazard ratios (aHRs) and corresponding 95% CIs. The weighting approach will generate the average treatment effect among LEMTRADA treated MS patients. To provide overall results for the study, aHRs found in each data source may be pooled using meta-analytic methods to obtain a summary estimate for the risk of mortality, if appropriate.

Milestones

Feasibility study report **Q3 2022**

Extended feasibility study report **Q4 2023**

Progress report with interim analyses **Q4 2023**

Final analysis study report **Q3 2024**

5 AMENDMENTS AND UPDATES

Protocol history: The first version of the protocol that was approved by the pharmacovigilance risk assessment committee (PRAC) was V1.4 (20 May 2021). A first protocol amendment occurred in 2022 and resulted in the approved protocol, which was numbered V1.5 (24 September 2022). The third protocol is the result of a second amendment and is numbered V3.0 (October 2023)[1]. The current protocol is the result of PRAC requests on V3.0 and is numbered V4.0 (April 2024).

Protocol Amendments: Summary of Changes

Section number and name	Description of change	Brief Rationale	Version number and date
na	Protocol revisions as part of initial approval procedure	Responding to pharmacovigilance risk assessment committee (PRAC) requests for revisions to protocol prior to final approval	1.0 – 1.4 July 2020 – May 2021
Section 4: Abstract Section 6: Milestones	Removal of tasks not representing direct regulatory commitments.	Sequential steps towards delivering direct regulatory commitments were removed because they are a marker of internal progress towards a formal regulatory commitments (e.g., submission of feasibility report).	1.5 June 2022
	Feasibility report submission changed from Q4 2021 to Q3 2022	Feasibility report changed to accommodate availability of data.	
	Interim report submission changed from Q4 2022 to Q2 2023.	First interim report changed to accommodate availability of data.	

[1] Note there is no protocol V2.0

Section number and name	Description of change	Brief Rationale	Version number and date
	Removal of interim report submission Q4 2023	Because the first interim report is moved to Q2 2023 the submission of two interim reports the same year (i.e., 2023) is not warranted as the differences between the two reports would be minimal.	
Section 9.8 Feasibility study	Dates for submission of interim analysis updated	See above	1.5 June 2022
	Removal of dates pertaining to feasibility analysis	See above	
	Removal of Gannt chart	The Gannt Chart has been removed because it served as a planning tool at the start of this project. Additions and revisions to the chart are out of scope to protocol amendments.	
Title page, Names and addresses, post-authorisation safety study (PASS) information, and Sections 4 and 12	Updated Study Management to Parexel International throughout the document, assigned document ownership to Sanofi only, and updated marketing authorisation holder (MAH) epidemiology representative contact address.	Due to a change of contract research organisation (CRO) and administrative update.	3.0 October 2023
Section 3.1	Updated Principal Investigator (PI) information for Cambridge University Hospitals and Bremen Institute for Prevention Research and Social Medicine (BIPS).	Administrative change	3.0 October 2023

Section number and name	Description of change	Brief Rationale	Version number and date
Sections 4, 6, and 12	Updated milestones information. Accordingly, dates of each milestone were modified throughout the protocol.	To reflect the current progress of this PASS.	3.0 October 2023
Section 7 and 9.3.5	Updated texts and footnotes to specify the External Comparison Cohort Study (ECCS) is currently subject to discussions with health authorities regarding feasibility; however, the data from the ECCS used to inform this protocol remain relevant.	To reflect the current status of ECCS.	3.0 October 2023
Sections 7.2, 9.1.2, 9.3.3, and Appendix 1	Added newly approved platform disease modifying therapy (P-DMT) (Appendix 1 only) and highly efficacious DMTs (HE-DMTs) to the list of comparator drugs. Drugs that are highly efficacious vs. moderately to highly efficacious were specified. References for the newly approved comparator drugs were added.	Additional drugs obtained market approval since the initial protocol was approved.	3.0 October 2023
Sections 7.2 and 9.5.1	Updated text regarding cumulative deaths and mortality rate reported in the ongoing PASS for LEMTRADA.	To reflect up-to-date data.	3.0 October 2023
Section 9.2	Added text to clarify the withdrawal of the data source from Belgium. Descriptions about Belgium as a data source (L'Agence Intermutualiste – Het InterMutualistisch Agentschap [AIM-IMA]) elsewhere were removed throughout the protocol.	The Belgian data source opted to no longer participate in early 2023.	3.0 October 2023
Sections 9.2.2.2 and 9.9.3	Updated text to clarify MS patients with missing values for age or gender will be excluded from the analysis.	To reflect that few missing data are expected for age and gender based on the feasibility analysis.	3.0 October 2023

Section number and name	Description of change	Brief Rationale	Version number and date
Sections 9.1.2, 9.3.3, 9.3.4, 9.3.5, 9.7.4, 9.7.6.3, 9.7.7.1, and Appendix 2	Updated text throughout the protocol to clarify that the treatment exposure variable and main analysis will be handled using time-fixed approach, instead of time-dependent approach. Rationale for this update was provided. Additional updates were made in Section 9.3.4 and Appendix 2 to specify the decision of using time-fixed "as-treated (AT)" approach on the overall analysis population for the main statistical analysis.	Changed design approach for the main statistical analysis based on the feasibility analysis in 2022 and 2023.	3.0 October 2023
Section 9.3.1	Modified the section heading and added text to include the updated look-back periods for each core variable according to the results of the feasibility analysis 2022. Cross-references were added in Sections 9.2.2.1, 9.2.3, 9.3.3, and 9.3.5 when discussing the look-back period of individual core variables.	To update the information for the look-back period based on the feasibility analysis; the pre-specified two-year period that was uniformly applied to all core variables was modified so that various look-back periods will be applied to different core variables.	3.0 October 2023
Sections 9.3.2, 9.4.1, and 9.10.5.3	Added text to include information of Danish Cause of Death registry, details of active tracing for vital status in Czech Republic, and a mixed linkages and active tracing for death data in the UK.	Responding to PRAC request to include more details on active tracing in the Czech database, and to align to the updates of linkage in operating protocols.	3.0 October 2023
Section 9.3.3	Added text for the censoring rules of AT approach.	As a time-dependent exposure model is not used for the main analysis, the censoring technique for the main analysis was added.	3.0 October 2023

Section number and name	Description of change	Brief Rationale	Version number and date
Section 9.3.5	Updated covariate list: re-name core-variable from “Date of cohort entry” to “Year of cohort entry”, add "statins" as a core variable, and update the MS types to be considered.	The variable name is updated according to the obtained and used data. Statin was added as core variable to reflect cardiovascular medication use together with antihypertensives. MS type was updated to reflect the available categories in the data sources.	3.0 October 2023
Sections 9.3.5 and 9.7.3	Added text to specify the situations where variable re-categorisations will be considered.	To clarify the need for re-categorisations.	3.0 October 2023
Section 9.3.5.1 (newly added)	Added subsection to clarify expanded disability status scale (EDSS) categories used in the analyses.	Responding to PRAC request for more details regarding basis for categorisation of EDSS.	3.0 October 2023
Sections 9.4.2 and 9.7.2 (Figure 2)	Incorporated analytic database into working database.	To align to the terminology used in SAP.	3.0 October 2023
Sections 9.5.1 and 9.5.2	Added texts to specify the mortality rate of 0.24/100 person-years was selected in the HE-DMT treated population for the precision calculations.	To reflect mortality data from the feasibility study in 2022.	3.0 October 2023
Section 9.5.2 and Appendix 5	Updated text and Table 5 for precision assessment of mortality risk estimates. Appendix 5 were replaced with supplementary information for crude and weighted patient numbers for each data source.	Up-to-date available patient numbers based on extended feasibility report in 2023.	3.0 October 2023

Section number and name	Description of change	Brief Rationale	Version number and date
Section 9.7.4	Updated text that re-specification of the propensity score (PS) models, including modification of covariate codification, was performed. Added text to clarify the average treatment effect among treated (ATT) approach still holds after excluding non-overlapping regions. Removed contents regarding high dimensional (hd)-PS model.	PS models were re-specified based on the imbalance observed for some variables during feasibility analysis 2022. Few LEMTRADA patients were removed from the non-overlapping regions in the feasibility analysis. hd-PS model was removed as it was deemed infeasible based on study progress and available data.	3.0 October 2023
Sections 9.7.4, 9.7.7.2, 9.10.3, and 9.10.5.4	Updated list of sensitivity analyses.	To reflect decisions made after the feasibility and extended feasibility analyses and feedback from PRAC.	3.0 October 2023
Section 9.7.6.1	Texts was updated to remove sex adjusted/standardised mortality rates, revise the unit of crude mortality rate (per 100,000 PYs), and specify that the mortality rate difference will be calculated.	Sex adjustment was omitted to be able to observe mortality rates by sex. The unit for crude mortality rate was amended to reflect the rare outcome event. Analysis of mortality rate difference was added in response to PRAC's request.	3.0 October 2023
Section 9.7.6.2	Modified texts to clarify only data-source specific Kaplan-Meier curve will be generated for the analysis of survival curves.	De-identified patient-level data cannot be transferred to the Study Management. Similarly, descriptions regarding de-identification were removed in Section 10.2.	3.0 October 2023
Section 9.7.6.3	Added text to specify a bootstrap estimator will be used to generate 95% confidence interval (CI) for the hazard ratios (HRs). A reference was added.	To specify the method of CI, and to assess the impact of the residual confounding on the risk estimate.	3.0 October 2023

Section number and name	Description of change	Brief Rationale	Version number and date
	Updated text to specify three Cox proportional hazard models will be applied, including unadjusted, PS weighted, and PS weighted with imbalanced covariates (main analysis).		
Section 9.7.6.4	Updated text to clarify meta-analysis will be performed to combine a minimum of two data sources and possibly all data sources of adjusted hazard ratios (aHRs) to obtain a summary hazard ratio (HR). Added text to specify when meta-analysis will be conducted.	To reflect the minimal requirement for meta-analysis based on statistical consideration and the fact that not all data sources may have data feasible for the analysis.	3.0 October 2023
Section 9.7.8	Updated text to specify how the death by cause analysis will be reported (by exposure group, by single, first instance of ICD-10 code, which may be aggregated) and to clarify cause of death might be assessed descriptively.	To specify the analysis for cause of death. The descriptive analysis is due to the variability and potentially low counts for specific causes of death as observed in feasibility study.	3.0 October 2023
Section 9.7.9	Added subsection (Section 9.7.9.2) for “incidence of switching in HE-DMT other than LEMTRADA (HE-DMT_NL) exposure group” as one of other analyses to be performed. Accordingly, a level-4 heading (Section 9.7.9.1) was added to specify the analysis of death rate over time that was originally included in this section.	To examine extent of bias that may be introduced as follow-up for HE-DMT_NL group is not censored at a switch to another HE-DMT_NL treatment, though LEMTRADA group is censored at a switch to HE-DMT_NL.	3.0 October 2023

Section number and name	Description of change	Brief Rationale	Version number and date
Section 9.8	Modified texts of feasibility study, including updates on objectives and removal of study details (Section 9.8.2 to 9.8.4). Conclusion of feasibility study, and objectives and analytic results of extended feasibility study were added. Heading was modified to reflect the updated contents.	The MAH has completed the feasibility analyses.	3.0 October 2023
Section 9.8.5 (now Section 9.8.2)	Modified texts for the analyses to be performed during the interim analysis.	Changes to main analysis and sensitivity analyses due to results of the feasibility analysis and extended feasibility analysis.	3.0 October 2023
Section 9.9.2	Updated contents regarding standard descriptive procedures of data quality control, e.g., for missing and erroneous data, at data source level. Removed the exploratory analysis for the follow-up completeness.	To clarify quality control will be carried out locally. Follow-up completeness was removed as it is no longer planned for the study.	3.0 October 2023
Section 9.9.3	Updated text to specify EDSS and MS type will be considered for multiple imputation, and clarify multiple imputation will be considered depending on the on missing data patterns.	Extended feasibility analysis has informed that missing data for EDSS and MS type are the most problematic. To clarify strategies used for handling missing data.	3.0 October 2023
Section 9.10.1	Limitations section expanded regarding imbalance on confounding variables, and the strategies used to mitigate residual confounding (i.e., doubly robust analyses and the use of restricted and stratified populations). A reference was added to support the use of doubly robust analyses.	The extended feasibility results indicate imbalance may remain on certain variables even after propensity score methods. Therefore, there is a need to acknowledge this limitation and discuss the implications.	3.0 October 2023

Section number and name	Description of change	Brief Rationale	Version number and date
Section 9.10.5.4	Removed E-values calibration and sensitivity analysis trimming both ends of the PS distribution in E-value computation.	E-value calibration was removed to simplify the analysis. The sensitivity analysis involving trimming was removed based on PRAC feedback suggesting to deprioritise trimming.	3.0 October 2023
Section 10.1	Removed contents regarding consent information.	Due to secondary use of data, consent is not required.	3.0 October 2023
Appendix 1	Added text to allow possible new HE-DMTs that entre the market during the study period to be included in the final analyses.	To allow MS drugs that are newly entering the market before the final cut-off to be considered in study analysis.	3.0 October 2023
Appendix 3	Added texts to clarify that PS model with “multiple sclerosis (MS) disease duration” was preferred over that with “year of MS onset”, and was thus carried forward. The MS disease duration was transformed into a categorical variable.	Responding to PRAC request to include newly defined PS model with MS disease duration, instead of year of MS onset.	3.0 October 2023
Appendix 4	Added details of proxy indicator of EDSS for GePaRD data.	To provide information about validity and comparability of all proxy variables to the original variables.	3.0 October 2023
Appendix 6 (Original)	Removed Appendix 6: Feasibility questionnaire. The numbering of the following appendices shifted accordingly.	No longer relevant at this stage of study.	3.0 October 2023
Sections 4, 9.5.2, and 9.7.7.2	Removed Type I error rate of 10% and updated precision table (Table 5) accordingly.	PRAC suggestion to remove.	4.0 April 2024
Sections 9.1.1, 9.5.2, 9.7.6.4, 9.7.7.2, and 9.10.3	General clarification added that the meta-analysis may be performed as appropriate.	Clarification.	4.0 April 2024

Section number and name	Description of change	Brief Rationale	Version number and date
Section 9.3.2	Added information to explain that active tracing for vital status in Czech Republic ReMuS database was carried out for patients who entered throughout the study period.	PRAC request to clarify time frame for active tracing in Czech Republic.	4.0 April 2024
Section 9.3.5.1	Added information on updated EDSS categorizations.	Clarified EDSS categorizations that will be used in final analysis.	4.0 April 2024
Section 9.4.1.3; Appendix 7	Added details regarding the variables selected for the two-stage sampling approach for the Czech Republic ReMuS database.	PRAC request for comparator patients to be included from the whole of Czech Republic population; therefore, a two-stage matching and sampling approach was introduced to optimize the selection of comparator patients.	4.0 April 2024
Section 9.7.4	Clarified that further PS model re-specifications were done after interim analysis.	Clarification.	4.0 April 2024
Section 9.7.6.1	Statistical analysis of mortality rates updated to remove age-related mortality rates.	Age-related mortality rates no longer assessed as part of the study.	4.0 April 2024
Section 9.7.6.2	Statistical analysis of survival curves amended to clarify that weighted Kaplan-Meier curves will be produced.	Clarification.	4.0 April 2024
Section 9.7.6.3	Clarified estimator to be used for generating 95% CI for the HRs.	Clarification.	4.0 April 2024
Section 9.7.6.5, 9.10.5, and 9.10.5.4	E-value information relocated from Section 9.10.5.4 to statistical analysis section. General language added to clarify that E-values may be evaluated if deemed appropriate to do so.	PRAC suggestion to relocate information.	4.0 April 2024
Section 9.7.7.1	For final analyses, SAP version updated to latest version (V7.0).	Clarification.	4.0 April 2024

Section number and name	Description of change	Brief Rationale	Version number and date
Section 9.7.7.2	Removed sensitivity analysis for analysis considering the same follow-up for all data sources.	PRAC suggestion to remove.	4.0 April 2024
Section 9.7.7.2	Removed sensitivity analysis for exclusion of patients with CED after LEMTRADA label change.	PRAC suggestion to remove.	4.0 April 2024
Section 9.7.8	Potential assessable databases for secondary analysis updated to include ReMuS.	Availability of ReMuS database confirmed during development of interim analysis report.	4.0 April 2024
Section 9.7.9.1	Added new stratified analysis (YCED-CAT) with demographics and mortality risks by categories of year of CED, and removed death rates examined at 2-year intervals.	Removed Kaplan-Meier curves at 2-year intervals.	4.0 April 2024
Section 9.7.9.2	Text revised as sensitivity analysis will not be performed.	Text aligned with SAP.	4.0 April 2024
Section 9.8.1	Brief overall conclusion of the first feasibility study added.	PRAC request to include brief conclusion.	4.0 April 2024
Section 9.8.2	Section revised to past tense. General conclusion of interim analysis results added.	General conclusion added as interim analysis has been completed.	4.0 April 2024
Section 13	Additional references added.	Completeness.	4.0 April 2024
Appendix 1	Text revised as list of DMTs have been finalized.	Clarification.	4.0 April 2024
Appendix 8	Appendix 7 (original) was moved out and now named Appendix 8.	List of stand-alone documents moved out as the last appendix.	4.0 April 2024

6 MILESTONES

Feasibility study report	**Q3 2022**
Extended feasibility study report	**Q4 2023**
Progress report with interim analyses	**Q4 2023**
Final analysis study report	**Q3 2024**

7 BACKGROUND AND RATIONALE

7.1 BACKGROUND

Disease modifying therapies (DMT) are immunomodulatory drugs used in the treatment of multiple sclerosis (MS). DMT are classified in two broad categories, the platform-DMT (P-DMT) and the highly efficacious DMT (HE-DMT) (Appendix 1) (1,2).

LEMTRADA® is a HE-DMT that received initial marketing authorisation by the European Medicine Agency (EMA) in September 2013[2]. The approved indication was: "treatment of adult patients with relapsing remitting multiple sclerosis (RRMS) with active disease defined by clinical or imaging features".

In 2019, LEMTRADA was subject to a European Commission (EC) triggered procedure under Article 20 of Regulation (EC) No 726/2004 in which new and cumulative safety data were assessed by the EMA pharmacovigilance risk assessment committee (PRAC)[3]. The procedure concluded that LEMTRADA is associated with new and emerging safety events; cardiovascular adverse events (temporally associated with infusion) and additional autoimmune events. The PRAC advised changes to the European Union (EU) Summary of Product Characteristics indication, the list of contraindications and safety monitoring recommendations to best ensure patient safety.

An outstanding safety question from the procedure was the evaluation of the risk of fatal events associated with exposure to LEMTRADA. In the Article 20 Assessment report, mortality rates from clinical trials (0.17 per 100 person-years [PY]) and post-marketing data (0.42 per 100 PY) were reviewed. However, these data were deemed insufficient to fully understand whether there may be an increased risk of mortality associated with exposure to LEMTRADA due to a lack of adequate comparative data.

Given this evidence gap, an investigation of the risk of mortality in patients treated with LEMTRADA compared to a relevant MS patient population is planned. This post-authorisation safety study (PASS) protocol outlines the investigational plan to address this request. The study will utilise real-world data retrieved from multiple sources in Europe including MS patient registries, administrative health care database, and chart reviews. This will enable a direct comparison of the risk of fatal events in LEMTRADA exposed patients and risk of fatal events in a population of clinically comparable MS patients from the same data source.

This PASS protocol has been written with background knowledge from the External Comparison Cohort Study (ECCS) initiated in 2015 which is a sub-study of the EUPASS7346 on LEMTRADA[4]. The ECCS was designed to assess the incidence of adverse events among LEMTRADA treated and MS patients treated with other DMT. In the ECCS study, data until the end of 2018-2019 provided an indication of prescription patterns for LEMTRADA:

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[2] Committee for Medicinal Products for Human Use (CHMP). Summary of opinion (initial authorisation). LEMTRADA (alemtuzumab). EMA/377379/2013, 27 June 2013.

[3] Pharmacovigilance Risk Assessment Committee (PRAC). Assessment report. Procedure under Article 20 of Regulation (EC) No 726/2004 resulting from pharmacovigilance data. LEMTRADA. EMA/682560/2019, 31 Oct 2019.

[4] The ECCS has been implemented in Denmark (Danish MS Registry) and in Belgium (Belgian MS Registry [BELTRIMS] and the Belgian social security database [AIM-IMA]). Currently, the ECCS is subject to discussions with health authorities regarding feasibility. Nonetheless, the data from the ECCS used to inform this protocol remain relevant.

- A peak in LEMTRADA prescriptions is noticeable in the year directly following LEMTRADA reimbursement with a stabilisation afterwards.
- For about one fifth of LEMTRADA recipients, this therapy was the first DMT ever received.
- Approximately 16% of LEMTRADA recipients were aged 50 years or more.
- Between 15% (in Denmark) and 37% (in Belgium) of LEMTRADA treated MS patients have an Expanded Disability Status Scale (EDSS)[5] of 4.0 or more at initiation of LEMTRADA.

Hence, there is a substantial proportion of young MS patients treated with LEMTRADA rapidly after MS diagnosis and a substantial proportion of older MS patients treated with LEMTRADA after a history of therapy with another DMT.

7.2 RATIONALE

LEMTRADA is a recombinant humanised monoclonal antibody for the treatment of patients with RRMS. LEMTRADA binds to CD52, a cell surface antigen present at high levels on T and B lymphocytes, and at lower levels on natural killer cells, monocytes, and macrophages. LEMTRADA is administered as two courses of 12 mg/day on five consecutive days at baseline and on three consecutive days 12 months later.

LEMTRADA has been demonstrated to be a highly efficacious agent in the treatment of RRMS with data from clinical trials demonstrating reduced relapse rates, reduced disability, reduced brain volume loss and improved EDSS scores (3,4,5). The efficacy of LEMTRADA in RRMS patients across multiple parameters of the disease is well established and maintained over long-term follow-up (6,7,8). This level of efficacy is present across a wide range of patient populations, as evidenced by the consistency of findings across various subgroups including baseline EDSS score, disease activity level, age, and history of prior DMT use in LEMTRADA clinical studies (4). However, LEMTRADA is associated with serious risks, including risk of cardiovascular events (with temporal relation to infusion), serious infections, and auto-immune-mediated conditions (9).

Several other therapies are considered to be highly efficacious: natalizumab (TYSABRI®), ocrelizumab (OCREVUS®), ofatumumab (KESIMPTA®), and cladribine (MAVENCLAD®), or moderately to highly efficacious: fingolimod (GILENYA®), ponesimod (PONVORY®), ozanimod (ZEPOSIA®), and siponimod (MAYZENT®). Of note there has also been significant off-label use of rituximab (anti-CD20) for the treatment of MS (10). In this protocol, the term HE-DMT includes both highly efficacious and moderately to highly efficacious therapies, if not otherwise specified. All of these agents are associated with a risk of serious adverse events including some fatal events, for example, via progressive multifocal leukoencephalopathy (PML) or infectious causes (9,11,12,13,14,15,16,17,18,19).

MS is a heterogeneous disease and selection of therapy is highly individualised. Important considerations should include clinical factors such as: disease severity and prognosis; comorbidities; prior therapy response or tolerability; as well as patient preferences including risk tolerance and important life factors such as employment status and family planning (1). Given the complex risk-benefit profiles for DMT there can be many patient-related factors that contribute to

[5] Expanded Disability Status Scale: standard scale used by neurologists for evaluating the level of disability associated with MS disease. The scale ranges from 0 (no disability) to 9.5 (10 is equivalent to death due to MS).

risks and safety outcomes for each drug. Thus, adequate comparative data is necessary for understanding the safety profiles of each DMT.

In the context of these considerations, it is unknown whether there is a difference in the occurrence of fatal events in LEMTRADA treated patients as compared to fatal events in clinically similar patients treated with other HE-DMT.

There are various sources of data inclusive of clinical trial data, extension studies, single arm observational studies, and pharmacovigilance data that provide some information on mortality rates in LEMTRADA treated patients, albeit all with their own unique limitations.

Mortality rates observed in randomised trials with LEMTRADA and their follow-up studies (0.17 per 100 PY) do not suggest an excess risk of mortality compared to published data on mortality in MS populations (4,20,21,22). Similarly, early reports on LEMTRADA use in daily neurological practice in the United Kingdom (UK) did not allude to unexpected fatal outcomes (23). Two ongoing single arm safety studies for LEMTRADA have reported interim deaths. First, in TREAT-MS there have been two deaths in 779 patients as of 2018[6]. Second, in the ongoing PASS for LEMTRADA (GZ402673-OBS13434), there have been six deaths, as of December 2019, in 2,092 European MS patients, corresponding to a mortality rate of 0.12 per 100 PY (95% confidence interval [CI]: 0.05; 0.27[7].

However, patient selection criteria for controlled trials, and lack of comparator arms in long-term follow-up from controlled trials and post-marketing observational studies limit the comparability of these data to other MS populations including those treated with other HE-DMTs.

A review of adverse events reported to the EMA database of suspected adverse reactions related to medicinal products (EudraVigilance) indicated that fatal events associated with LEMTRADA treatment were reported to occur more frequently than in randomised trials and early clinical series (24). But it is difficult to derive a reliable estimate of mortality rates from pharmacovigilance data based on spontaneous reports given the unknown rate of under or over reporting and lack of complete information in many reports. Moreover, the absence of data on adverse events occurring in comparable MS patients not receiving LEMTRADA limits the interpretation of spontaneous reports.

Thus, current data from multiple sources are not sufficient to understand whether LEMTRADA is causally associated with mortality.

The study outlined in this protocol proposes an observational cohort study intended to compare the risk of mortality in LEMTRADA treated MS patients with the risk of mortality in comparable MS patients, i.e., those treated with other HE-DMT. The data sources will consist of MS patient registries, prescription/administrative data, and clinical chart data in European countries that hold data on patient characteristics, clinical parameters, therapies, vital status, as well as data on major potential confounders like cardiovascular or respiratory conditions and other co-morbidities.

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[6] https://onlinelibrary.ectrims-congress.eu/ectrims/2019/stockholm/279348/rocco.haase.treat-ms.study.of.real-world.effectiveness.of.alemtuzumab.in.rrms.html?f=listing%3D3%2Abrowseby%3D8%2Asortby%3D1%2Amedia%3D1, interim results of January 2014 to October 2018.

[7] EUPAS 7346 : http://www.encepp.eu/encepp/viewResource.htm?id=28499, update of March 2020.

8 RESEARCH QUESTION AND OBJECTIVES

8.1 RESEARCH QUESTION

What is the risk of mortality in MS patients treated with LEMTRADA as compared to MS patients treated with other HE-DMT?

8.2 PRIMARY OBJECTIVE

To ascertain whether MS patients treated with LEMTRADA have a higher risk of all-cause mortality than comparable MS patients treated with other HE-DMT.

8.3 SECONDARY OBJECTIVE

To examine the cause of death in data sources where this information is available and when the number of cases for a specific cause is sufficient for formal examination. This objective will be exploratory as it is anticipated that availability and quality of cause-specific mortality data will be variable across data sources.

9 RESEARCH METHODS

9.1 STUDY DESIGN

9.1.1 General study design

This PASS is an observational comparative cohort study based on secondary use of data held in various European real-world data sources. The primary endpoint is all-cause mortality, and the secondary endpoint is cause-specific mortality. The study will compare mortality risk between MS patients treated with LEMTRADA and comparable MS patients treated with another HE-DMT.

Some drug safety studies especially those concerning rare events (e.g., mortality) and rare exposures (e.g., LEMTRADA) require more data than is available in any single observational database. Therefore, it has become common in drug safety research to use data from multiple data sources, usually from different countries (25,26,27,28,29,30).

The approach is to develop a master protocol and share this protocol across all contributing data sources. Each data source will be required to adapt the master protocol as an operating protocol (OP) to their local data and to implement it in their own usual software. These OPs are written by Study Management and reviewed by the MAH and data sources. Thus, each data source will produce local effect estimates that may be ultimately combined by meta-analysis (31). This approach is akin to the Common Data Model concept (27), whereby all datasets are formatted in one pre-defined manner and thus, one programme can be run on all.

It is known that the format of several variables may differ across data sources. Whenever possible, the "statistical analysis plan" (SAP) and the OP (i.e., how the SAP is applied in each data source) will aim at harmonising the format of variables used in analyses, e.g., same categories implanted for categorising continuous variables.

9.1.2 Comparable MS patients

In studies of non-intended adverse events such as mortality, the risk of the outcome can be strongly correlated with the progression of the disease being treated. Therefore, it is important that the comparison treatment is for a similar indication and stage of disease as the treatment under study (32).

In 2013, the EMA approved LEMTRADA for adult patients with RRMS with active disease defined by clinical or imaging features[8]. Hence, LEMTRADA was the first HE-DMT approved by the EMA as a first line DMT. In this regard, LEMTRADA therapy could have been initiated shortly after diagnosis of highly active MS (i.e., as first line DMT), or after evidence that another DMT failed to control disease activity. In Q2 2019, the indication for LEMTRADA was restricted following reports of serious cardiovascular and immune mediated adverse events and the initiation of an Article 20 procedure (EMEA/H/A-31/1483/C/3718/0028). In January 2020, the EMA restricted the indication of LEMTRADA therapy to (i) MS patients with highly active disease, despite a full and adequate course of treatment with at least one DMT, or (ii) patients with rapidly evolving severe RRMS defined by two or more disabling relapses in one year, and with one or more Gadolinium

[8] LEMTRADA Public Assessment Report 25/09/2013. EMA/563018/2013. Available from https://www.ema.europa.eu/en/documents/assessment-report/lemtrada-epar-public-assessment-report_en.pdf

enhancing lesions on brain magnetic resonance imaging (MRI) or a significant increase in T2 lesion load as compared to a previous recent MRI[9].

The other HE-DMT that are available on the market have similar indications to LEMTRADA: natalizumab (TYSABRI®), ocrelizumab (OCREVUS®), ofatumumab (KESIMPTA®), mitoxantrone (NOVANTRONE®), cladribine (MAVENCLAD®), fingolimod (GILENYA®), ponesimod (PONVORY®), ozanimod (ZEPOSIA®), and siponimod (MAYZENT®) (9,11,12,13,14,15, 16,17,18,19). Of note there is also off-label use of rituximab (anti-CD20) for the treatment of MS (10). The literature indicates earlier recourse to HE-DMT in the last decade, suggesting use of LEMTRADA and other HE-DMT at similar stages of disease, especially those agents that are infused and cladribine (33,34,35).

By using an active comparator design, whereby the comparator population comprises patients treated with other HE-DMT, it is possible to better control for disease severity and prognosis at baseline. The aim is to achieve two treatment groups that have similar risk of the outcome related to their disease (32).

Apart from MS disease severity, which is addressable using an active-comparator design, the two treatment groups may be different in other ways that could potentially be associated with the outcome. For example, one treatment group could be older than the other, and thus more likely to die; or imbalanced in terms of other comorbidities, for example cardiovascular conditions which have been cited as a leading cause of death in MS patients (36).

To deal with potential imbalance of measured confounders, a propensity score (PS) will be developed (Section 9.7.4) and used in weighted analyses (Section 9.7.6.3).

During amendment of the study protocol (Protocol V3.0), a decision was made to update the design approach to have a time-fixed exposure. This approach was taken to better focus on measurement and control of confounding on one occasion, at CED. Additionally, few patients switched between LEMTRADA and HE-DMT in the extended feasibility study, further supporting this decision (see Section 9.3.4 for more details).

9.2 SETTING

The study will include MS patients treated with a HE-DMT after the date of LEMTRADA approval/reimbursement in usual clinical practice in European countries. Data from the data sources of the following separate countries will be used: Denmark, Sweden, Czech Republic, the UK, and Germany. Patients will be treated in routine care and will not receive any additional monitoring/intervention due to this study. Of note, as communicated to the PRAC in April 2023, the Belgian database, L'Agence Intermutualiste - Het InterMutualistisch Agentschap (AIM-IMA), which was previously included in the study, is no longer included. All references to Belgian data have been removed from this amended protocol.

9.2.1 Study duration

Cohort entry can occur from the date of LEMTRADA approval/reimbursement until six months prior to last available data in each data source. The date of approval/reimbursement varies from

[9] https://www.ema.europa.eu/en/medicines/human/EPAR/lemtrada#product-information-section.

country to country; it ranges from 2013 to 2015 in the countries. selected for this study. The last available data will range from 2021 to 2023 depending on the data source.

9.2.2 Eligibility criteria

9.2.2.1 Inclusion criteria

Eligible participants are MS patients who initiated LEMTRADA or other HE-DMT after the date of LEMTRADA approval/reimbursement. An MS diagnosis will be given by: presence in an MS registry/diagnosis of MS in MS registry data; International Classification of Diseases (ICD)-10 code G35 in administrative data; or a recorded diagnosis of MS in medical charts.

Included patients will have a pre-defined period of look-back data at cohort entry to facilitate covariate assessment. This period varies depending on the type of data (core variables) collected, and is chosen according to the results of the feasibility study conducted in Q3, 2022 (see Section 9.3.1 and Section 9.8, respectively).

For the German data source, a two-year period of continuous insurance before cohort entry date (CED) is required as an eligibility criterion.

9.2.2.2 Exclusion criteria

MS patients with missing age and/or gender will be excluded (Section 9.9.3).

9.2.3 Cohort entry date

The CED will be defined as the date of first prescription or of actual dispensing of a HE-DMT after the date of LEMTRADA approval/reimbursement. Initiation or switching status will be determined by using look-back data from maximum patient history, with a two-year minimum in Germany, prior to cohort entry to establish prior DMT history. For details about the look-back period of individual core variables, see Section 9.3.1.

Cohort entry can occur at any point between LEMTRADA approval/reimbursement and up until six months prior to end of available data in each data source. This approach allows for at least six months follow-up for those who enter late in the study.

9.2.4 Analysis population(s)

All MS patients meeting eligibility criteria will be considered for inclusion in analyses.

9.2.5 Modalities of recruitment

This cohort study relies on secondary use of health-related data from European MS patient registries, administrative claims database, and chart review. The study relies thus on health care data that are routinely collected for social security or epidemiological purposes.

9.3 VARIABLES

9.3.1 Period of variable assessment and look-back period

The data collection period for exposure and outcome variables spans from 2013-2015 (dependent on local date of LEMTRADA reimbursement) to 2021-2023 (dependent on timing of final data updates in the local data sources). A time-lag in data availability in data sources, varying from few

months to 24 months, needs to be taken into account. This means there will be seven to ten years of follow-up data across all sources to address the study objective.

Examination of medical history in the pre-defined look-back period before CED will enable measurement of variables at CED, for example, past use of DMT prescriptions, previous relapses, and comorbidities.

Based on medical, epidemiological, and MS specific criteria, the following look-back periods were selected measuring covariates at CED:

- HE-DMT before CED for cohort eligibility: maximum available patient history
- HE-DMT and P-DMT before CED: maximum available patient history
- History of diseases and of non-DMT medications: two years before CED
- EDSS: two-year look-back before and six months after CED
- MS type: maximum patient history

Other core variables (hospitalisations-related variables) measured at CED will be assessed on the basis of a two-year look-back period.

Note that in Germany, a two-year minimum of continuous enrolment is required for patients to be included into the study.

9.3.2 Outcome variable

The outcome will be all-cause mortality, given by recorded date of death. Wherever available, the cause of death will also be assessed.

MS registries typically update vital status data via linkage with vital status registries. Prescription registries typically update vital status data through receiving regular information on births, deaths, emigrations, and immigrations from vital status registries.

Practical aspects for vital status updates are specific to each data source and are detailed in OPs. *A-priori*, there is no indication from any data source that differential recording of mortality for any drug in particular could be expected.

In Denmark and Sweden, linkage with national vital status registries will be done using a unique national personal identifier.

In Sweden, vital status of patients is recorded through linkage with the Swedish Cause of Death Register. The Swedish Cause of Death Register is a high quality virtually complete register of all deaths that occurred in Sweden since 1952 (37).

In Denmark, deaths will be updated via linkage with the civil status registry. The Danish Civil Registration System keeps records on gender, date of birth, change of address, date of emigration, and changes in vital status since 1968. Daily updated information on migration and vital status allows for nationwide cohort studies with virtually complete long-term follow-up on emigration and death (38). The cause of death is available through a linkage with the Danish Cause of Death Register. The cause of death is recorded using ICD-10 codes, and is defined using the main cause of death.

In Czech Republic and the UK, data sources are not directly linkable to vital status registries and updates of vital status of MS patients need to be performed at a pre-determined time before using data for analyses.

In the Czech Republic ReMuS MS disease registry, active tracing of MS patients for vital status is carried out for those entering throughout the study period. This means that direct contact with patients, their relatives, or their general practitioner (GPs) is made to assess vital status. Active tracing is in parallel with the provision of routine care in the Czech Republic and, in line with the recording of follow-up data on patients in the ReMuS registry; as such, individual consent is not required. Hence, the date of death is directly available in the database. Starting October 2022, at each database update, patients who had no recorded visit in their MS referral centres in the six months before the database update are identified. This threshold of six months was chosen because most MS patients have follow-up visits every six months. Patients who did not visit their neurologist at the planned visit could potentially be deceased. Thus, their vital status is further investigated by local investigators, who schedule follow-up visits with the patients.

In the UK, a combination of established linkages and active ascertainment is used. For example, in Wales, a linkage between MS registry data and the Welsh Demographic Service in addition to the Welsh Clinical Portal exists (39). In the Cambridge and Plymouth sites, death data are obtained from primary care linkages or active ascertainment.

In Germany, deaths are recorded as a reason for end of insurance or if the death occurred in a hospital. Completeness and accuracy of vital registration in the GePaRD have been validated against reference mortality index databases (40,41,42).

9.3.3 Exposure variable

The exposure variable used for the main statistical analyses will be LEMTRADA vs. other HE-DMT. In the comparison group of other HE-DMT, no distinction will be made between the various HE-DMT. This lack of distinction between HE-DMT does not apply to the baseline period, where the number and type of prior DMT are measured.

For the main statistical analysis, the time-dependent exposure approach was used from Protocol V1.0 to V1.5. Due to the limitations identified from the feasibility analysis, however, it was determined that the time-fixed approach will be implemented instead because the value of a time-dependent approach may be lost if all covariates are not updated for all patients at switch times. Additionally, the switch rate to a different exposure group after CED was low (43), also reducing the value of a time-dependent approach. For the time-fixed approach, the exposure to LEMTRADA or HE-DMT will be determined from the date of treatment initiation (i.e., CED) after LEMTRADA reimbursement.

Treatment initiation is defined as the documentation in medical charts that a HE-DMT was prescribed or delivered for the first time to a MS patient, or in a database evidence of new use using a pre-defined look-back period to establish prior medication history. For details about the look-back period of individual core variables, see Section 9.3.1.

The date of switch is also captured to enable calculating follow-up time when patients were “on-treatment”, for the as-treated (AT) analyses. For the purposes of the working database, treatment switching is defined as the documentation in medical charts of the discontinuation of a treatment with a HE-DMT, and its replacement with another HE-DMT, or in a database as evidence

of initiation of a new HE-DMT without continuing the prior DMT. For the purposes of the AT analysis, which censors patients at first switch to a different exposure group, the censoring rules are as following:

- LEMTRADA to HE-DMT other than LEMTRADA (HE-DMT_NL) will end follow-up
- HE-DMT_NL to LEMTRADA will end follow-up
- HE-DMT_NL to HE-DMT_NL will not end follow-up

The administration schedule differs substantially between HE-DMT, for example, fingolimod, siponimod, ozanimod, ponesimod, and cladribine are taken orally whereas LEMTRADA, ocrelizumab, and natalizumab are given by intravenous infusion, and ofatumumab is administered subcutaneously. Therefore, doses or days of administration will not be used to calculate exposure duration. Rather, exposure will be considered as having occurred if a prescription or dispensing for a HE-DMT is found. Exposure continues until a switch occurs or until end of follow-up. Since dose and duration of use will not be taken into account, some exposure misclassification could occur (i.e., a patient was considered exposed in the study until switch although the treatment was already discontinued before the switch).

Where possible, information on the exposure will be extracted based on anatomical therapeutic chemical (ATC) codes of HE-DMT described in Table 1. If other types of codification are used in data sources, an adequate bridging will be made, with the ATC codes in Table 1 as reference.

It is anticipated that capture of HE-DMT exposure will be complete for all the data sources participating to the study.

Table 1 List of HE-DMT with their ATC codes

Description	Brand names	ATC code
Rituximab	MabThera®, Rixathon®, Riximyo®, Blitzima®, Ritemvia®, Rituneza®, Ruxience®[biosimilar], Truxima®[biosimilar]	L01XC02
Ocrelizumab§	Ocrevus ®	L04AA36
Cladribine§	Mavenclad ®	L04AA40
Mitoxantrone	Novantrone ®	L01DB07
Fingolimod*	Gilenya ®	L04AA27
Natalizumab§	Tysabri ®	L04AA23
Alemtuzumab§	LEMTRADA ®	L04AA34
Ofatumumab§	Kesimpta®	L04AA52
Ponesimod*	Ponvory®	L04AA50
Ozanimod*	Zeposia®	L04AA38
Siponimod*	Mayzent®	L04AA42

* Moderately to highly efficacious DMT

§ Highly efficacious DMT

ATC: anatomical therapeutic chemical code; HE-DMT: highly efficacious disease modifying therapy

9.3.4 Follow-up time and exposure

Follow-up time is calculated from the CED until last update of vital status in each administrative/MS registry data source, death, emigration, or end of data collection, whichever occurs first.

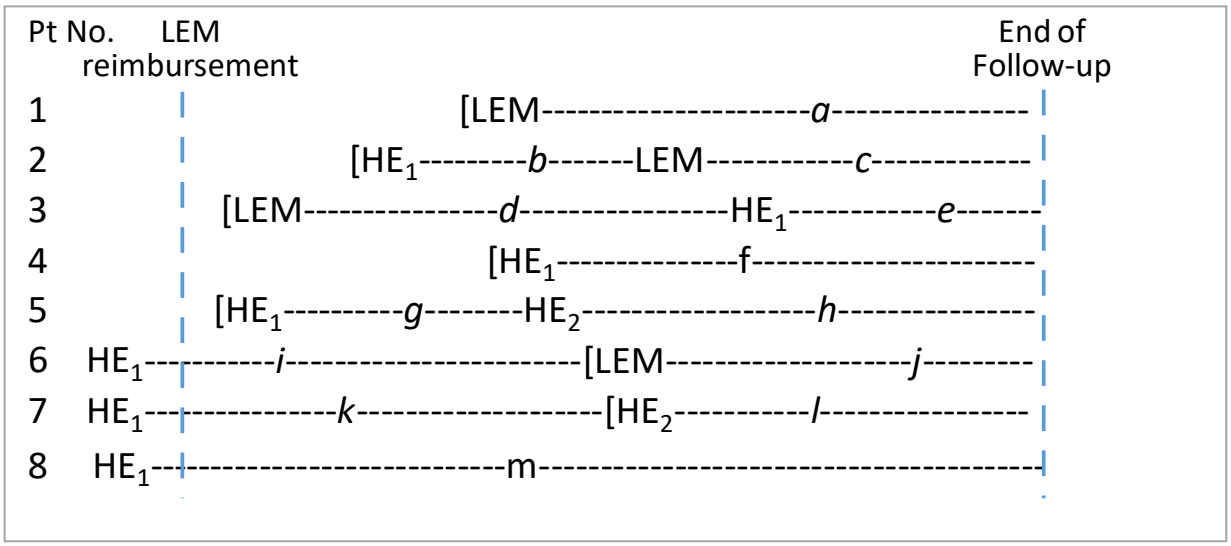
Various drug utilisation trajectories can be envisioned and are outlined in Figure 1. Although complex drug utilisation patterns may be possible, the time-fixed exposure approach aims to find the first LEMTRADA or HE-DMT exposure only, rather than allowing exposure to vary over time in one patient. The time-fixed exposure approach was chosen over time-dependent in order to simplify the measurement of covariates and potential confounders at one single CED, and thereby improve the interpretability of the results. Further, time-dependent exposure assessment would be more justified, if switching was frequent during the follow-up, as time-dependent analyses allow considering all treatment episodes during follow-up. However, this advantage of the time-dependent exposure assessment was not considered beneficial for the current study, because the extended feasibility analyses revealed relatively few patients switching between LEMTRADA and HE-DMT (1% to 36%) during the follow-up. Moreover, the benefits of considering all treatment episodes during follow-up (time-dependent exposure) could be lost, if it is impossible to accurately measure and account for all time-dependent confounders (44).

Two time-fixed exposure approaches are applied in this PASS: AT and intent-to-treat (ITT). The AT approach will be used as the main analysis of the study while the ITT approach is applied for sensitivity analysis only (see Sections 9.7.7.1 and 9.7.7.2, respectively); the selection of AT over ITT approach was based on the following reasons:

- AT approach more closely aligns with safety concerns relating to proximal exposure to LEMTRADA
- From the feasibility analyses, it is known that there was little difference between length of AT follow-up and ITT follow-up for each cohort for most countries

For the AT approach, a patient will be right censored if the patient switches treatment to the other exposure group (see Section 9.3.3 for detailed censoring rule). For the ITT, there will be no right censoring if a patient discontinues or interrupts treatment. The follow-up times for the different patients based on AT and ITT approaches are shown in Table 2 and Table 3, respectively. A more detailed comparison of the AT with ITT approaches is displayed in Appendix 2.

Figure 1 Examples of MS patient trajectories after prescription of HE-DMT



Outcomes are not displayed.
[represents cohort entry; italicised letters are durations of treatment.
LEM, LEMTRADA; MS, multiple sclerosis; HE, non-LEMTRADA HE-DMT; HE-DMT, highly efficacious highly efficacious disease modifying; pt, patient

Table 2 Group assignment and follow-up time for outcome assessment according to the AT approach (right censoring) – main analysis

Patient number according to Figure 1	Group assignment	Follow-up time for outcome assessment according to Figure 1
1	LEMTRADA	a
2	HE-DMT	b
3	LEMTRADA	d
4	HE-DMT	f
5	HE-DMT	g+h
6	LEMTRADA	j
7	HE-DMT	l
8	na	na

AT, as-treated; HE-DMT, highly efficacious disease modifying therapy; na, not applicable
Patient 8 is not included because they did not initiate a treatment during the study period

Table 3 Group assignment and follow-up time for outcome assessment according to the ITT approach – sensitivity analysis only

Patient number according to Figure 1	Group assignment	Follow-up time for outcome assessment according to Figure 1
1	LEMTRADA	a
2	HE-DMT	b+c
3	LEMTRADA	d+e
4	HE-DMT	f
5	HE-DMT	g+h
6	LEMTRADA	j
7	HE-DMT	l
8	na	na

HE-DMT, highly efficacious disease modifying therapy; ITT, intent-to-treat; na, not applicable
Patient 8 is not included because they did not enter the study.

9.3.5 MS patients' characteristics variables

Covariates in this PASS represent variables related to MS patients' demographic characteristics, disease and treatment history, and covariates known to be associated with mortality risk of MS patients.

There may be key differences in baseline characteristics between LEMTRADA and non-LEMTRADA treated MS patients, for example, age, MS disease severity, or comorbidities that increase the risk of mortality. The literature shows that LEMTRADA is often used as a "rescue therapy" when other DMTs have failed to control the disease activity (as indicated by clinical measures and/or MRI) (45,46,47). These studies indicate that compared to other HE-DMT treated MS patients, LEMTRADA treated MS patients tend to have higher EDSS scores, long duration of the disease, and history of one or more previous DMTs. Data from ECCS[10] show that young MS patients can also be treated with LEMTRADA.

The active comparator design, using comparator drugs that have a similar indication to LEMTRADA, helps to mitigate the impact of confounding by indication at baseline. Nonetheless, an imbalance in unmeasured disease-related or other characteristics between the two groups could lead to confounding, and may bias the relationship between the exposure group and mortality, independently of the exposure (48). It is therefore important to obtain data on as many relevant covariates as possible to safeguard against unmeasurable confounding.

A large body of literature exists on risk factors for mortality in MS patients, which provides guidance about variables required to control for confounding (39,49,50,51,52,53). These variables are listed below. The strategy for dealing with confounding is outlined in Section 9.7.4. We complemented this literature review by creating Directed Acyclic Graphs (DAGs). DAGs have been designed in function of knowns and unknowns on relationships between exposure, mortality, and

[10] The External Comparative Cohort Study (ECCS) aims to compare the incidence of a selection of adverse events among alemtuzumab treated MS patients vs. MS patients treated with other DMTs.

other variables (see Appendix 3). They represent how potential confounders could act on the relationship between the exposure and the outcome.

In instances where a direct measure of the variable cannot be assessed, proxy measures are considered, if such an approach is possible. Proxy measures are used for EDSS and relapses in the German administrative data (see Appendix 4).

Measurement of variables occurs at CED. A pre-defined look-back period is required as the covariate assessment period, not including the CED. For details about the look-back period of individual core variables, see Section 9.3.1.

Variables that are core to the successful conduct of the study are indicated by an asterisk (*). These core variables are those known to be associated with mortality among MS patients. Where multiple recordings of variables exist for each patient (e.g., EDSS or body mass index (BMI) or smoking status), the value closest to the CED is chosen. Some variables may be re-categorised for analysis, based on available categories in a data source, or the distribution of the data with particular attention on zero to few observations per variable category. Details of the variable names and formats can be found in the SAP. Covariates are listed below:

- **Year of cohort entry***;
- **Sex***;
- **Age at cohort entry***;
- **Treatment history before cohort entry***: number and type of DMT (P-DMT or HE-DMT) received before cohort entry;
- **Year of MS diagnosis*** (year of first P- or HE-DMT prescription may be used as a proxy);
- Number of relapses in preceding year;
- **Type of MS***: relapsing remitting (RRMS), secondary progressive (SPMS), primary progressive (PPMS), clinically isolated syndrome, undetermined (39,50,52,53);
- **EDSS*** (53); (see Section 9.3.5.1 for more details on categorisation)
- Time between MS diagnosis and EDSS 4 (assessed whenever EDSS 4 is reached); patients who did not reach EDSS 4 at the time of variable assessment will be identified as such (39);
- **Co-morbidities***: e.g., auto-immune diseases, cardiovascular disease, cancer, respiratory disease, urinary tract infection (36,49);
- Neuropsychological disorders: mood disorders (ICD-10 F30.# to F39.#), mental and behavioural disorders (F60.# to F69.#) and epilepsy (G40.#) (36,54). Information on these disorders are rarely reported in data sources. However, these disorders may be captured via proxy variables such as psycho-tropic and anti-epileptic drugs received by MS patients (see prescribed medications below).
  NOTE: # denotes the wildcard in this protocol
- **Hospital stays with number of stays and duration***;
- Prescribed medications other than DMT* e.g.,:
  - Statins*,
  - Anti-hypertensives*,
  - Anti-platelets*,
  - Proton pump inhibitors,
  - Immunosuppressants,

  - Anti-depressors*
  - Benzodiazepines*,
  - Anti-epileptic*,
  - Anti-diabetic*,
  - Prescribed nutritional supplements and vitamins.

- Indicator of socio-economic status (e.g., education level, deprivation index, area of residence);
- Smoking status (e.g., current smoker, past smoker, never smoker, unknown smoking status);
- Adiposity (e.g., body mass index);
- MRI:
  -MRI done before LEMTRADA or HE-DMT treatment;
  -Results of MRI exam;
- John Cunningham virus (JCV) status;
- Cerebrospinal fluid analyses.

9.3.5.1 Expanded Disability Status Scale (EDSS)

As of the feasibility analysis conducted in 2022, EDSS is used as a categorical variable. The following categories are used: [0 - 2.25]; [2.25 - 3.75]; [3.75 - 5.75]; [5.75 - 6.25]; [6.25 - 7.75]; [7.75 - 10].

The cut-points of the EDSS categories were decided based on known relationships between the EDSS and poor prognosis (39,55) and PI expert knowledge, with the aim of addressing, as best as possible, confounding due to EDSS.

Clinically relevant cut-points for EDSS categories were identified: 2, 4, 6, and 8. A cut-point at EDSS 4 is important because life expectancy of MS patients with an EDSS < 4 is not necessarily similar to the life expectancy of the general population (39,55). The category 0 - 4 was further divided in 0 - 2 and 2.5 - 3.5, because the differential risk of MS progression between EDSS 2 and 4 should be accounted for, and 1 point increments at EDSS < 4 are clinically relevant (56). Cut-points at 6 and 8 are important because there is an important difference in disability and mortality risk between EDSS 6 (standardised mortality ratio [SMR] 3.85, 95% CI 2.63 – 5.47) and 8 (SMR 22.17, 95% CI 18.20 – 26.75) (39). Therefore finer categories 4 - 5.5, 6.5 - 7.5, and 8 - 9.5 were created to address changing risk across smaller unit changes in EDSS. Based on the above, the following categories were selected: 0 - 2, 2.5 - 3.5, 4 - 5.5; 6; 6.5 - 7.5, 8 - 9.5. However, because the EDSS is computed by averaging the values over the period ranging from two years before CED to 6 months after CED, these categories were adapted into the categories listed in the 2022 feasibility report, to account for possible 0.25 and 0.75 values due to averaging. A larger category was required at the top end of the scale to accommodate sparse data. Despite differences in EDSS distribution among patients in data sources (for those data sources that collect EDSS values: SMSR, DMSR, ReMuS, and UK), harmonisation of EDSS categories across the countries was considered appropriate.

Patient counts were sparse for higher levels of EDSS categorizations for SMSR, DMSR, ReMuS, and UK, and thus were unfit for many of the PS models during interim analysis. Therefore, for final analysis an additional variable will be created and used in the PS models (and Cox PH models if

necessary) that combines the last 3 levels: 5.75 – 10. Final categories are [0 – 2.25]; [2.25 – 3.75]; [3.75 – 5.75]; [5.75 – 10] (126).

9.4 DATA SOURCES

We will use data from countries that:

- have frequent use of LEMTRADA in order to increase the size of exposed cohort;
- represent as much geographical spread as is practicable;
- have data sources in which key variables can be measured.

MS registry data from Denmark, Sweden, and the Czech Republic are used. These registries are part of the Big MS Data (BMSD) network (57). This network encompasses data from six European MS registries and is a collaboration coordinated by Karolinska Institute. The collaboration has close links with pharmaceutical companies and is currently being used for other PAS studies in the area of MS[11]. The BMSD network undertook a quality approval process by the EMA, with an application submitted in October 2020. Submitted documentation included a presentation of the individual MS registries and BMSD as well as a description of similarities and differences between the registries including number of MS patients and coverage; data collection; serious adverse events; quality control; analysis methods; PASS (ongoing, planned, and BMSD coordinated). Karolinska Institute is currently developing a webpage for this consortium. A review paper outlining the details of the BMSD network is in development. The qualification procedure is progressing.

This PASS also utilises hospital chart data from three study sites in the UK (Cambridge, Plymouth, and Cardiff) and administrative data from Germany (GePaRD database). More details on each contributing data source are presented below in Table 4 and Section 9.4.1.

[11] The CONFIDENCE study: Safety and Effectiveness of Ocrelizumab under Real World Conditions: a Non-Interventional Post Authorisation Safety Study in Patients Diagnosed with Relapsing or Primary Progressive Multiple Sclerosis. http://www.encepp.eu/encepp/viewResource.htm?id=33758

Table 4 Description of expected data sources

Country	Cumulative number of patients exposed to LEMTRADA*	Date of LEMTRADA reimbursement	Description of Data Source
Sweden	56	September 2013	National MS Patient registry that is linked to national registries for mortality, prescriptions, and hospital data
Denmark	126	September 2013	National MS Patient registry that is linked to national registries for mortality, prescriptions, and hospital data
Czech Republic	179	January 2015	National MS patient registry – good follow-up and good data on death due to incentive-based data collection. Death data are directly available in register through active ascertainment. Non-MS related data on comorbidities and comedications are collected separately from patient charts.
UK	445	May 2014	Collaboration established for this project amongst three large UK MS academic clinical centres that manually extract data from chart reviews. Register linkages are also utilised, whenever available.
Germany	~407	October 2013	Administrative data: prescriptions, outpatient and inpatient services + diagnoses

*The presented numbers of exposed patients are based on numbers available in the extended feasibility study in 2023 (Section 9.8.1).
MS: multiple sclerosis; UK, United Kingdom; NA: not available.

9.4.1 Description of individual data sources

9.4.1.1 The Swedish Multiple Sclerosis Registry (SMSR)

The Swedish MS Registry (SMSR)[12] started in 1996 (58). It has been collecting DMT prescriptions data since 2001. The SMSR is used by all neurology departments, and by most neurologists in Sweden. There are around 14,500 MS patients alive and registered in the SMSR, which corresponds to approximately 80% coverage of the total Swedish MS patient population.

As patients are identified with a unique national personal identifier, it is possible to link data from the SMSR with external registries, including the National Cause of Death Registry and the National Patient Registry.

9.4.1.2 The Danish Multiple Sclerosis Registry (DMSR)

The DMSR[13] was created in 1956 and collects data on all Danish MS patients since then (59). Registration of MS patients is compulsory in Denmark. Data on therapies are collected since 1996. The main goal of the DMSR is to track the impact and improve the quality of immunomodulatory and immunosuppressive treatments in patients with MS.

Each treatment site (e.g. hospital, MS referral centre, etc.) enters their data directly into the DMSR. The DMSR contains follow-up data on relapses, side effects, EDSS, and treatments. The database includes around 18,000 MS patients who are currently alive, of which about one third are treated with a DMT.

As patients are identified with a unique national personal identifier, it is possible to link data from the DMSR with external registries, including the national patient registry (collecting prescription data), the national civil status registry (collecting immigration/emigration data and vital statistics) (60), and cause of death registry. The strength of the DMSR is that MS patient notification is nationwide and virtually complete. Its weakness is that follow-up usually stops when the patient discontinues treatment, however this is mitigated by follow-up continuing in linked data sources.

9.4.1.3 The Czech Multiple Sclerosis Registry (ReMuS)

The ReMuS is the Czech national MS registry that is operated by the Endowment Foundation Impuls[14] in cooperation with the Czech Neuroimmunological Society (61). The main goals of the ReMuS are:

- to describe the real prevalence and incidence of MS in the Czech Republic,
- to provide to the health authorities data about severity of MS, treatment allocation, and employment and social situation of MS patients,
- to serve as a basis for research at national and international level.

The registry started in January 2013 and has been collecting data from 15 MS referral centres across the whole Czech Republic. The estimated number of patient records as of 2016 was 8,353. The software iMed has been used in all MS centres as a tool for data collection. Data are summarised

[12] http://www.neuroreg.se/en.html/multiple-sclerosis
[13] https://www.rigshospitalet.dk/english/departments/neuroscience-centre/department-of-neurology/research/the-danish-multiple-sclerosis-registry/about-the-registry/Pages/about-the-danish-multiple-sclerosis-registry.aspx)
[14] https://www.nfimpuls.cz/index.php/en/czech-ms-registry/about-the-registry

centrally twice a year and summary output is provided publicly[15]. The number of LEMTRADA patients treated as part of routine care versus as part of LEMTRADA clinical trials was investigated in a feasibility study (see Section 9.8). Vital status is directly available in ReMuS via active ascertainment (the routine schedule for MS patients in the Czech Republic).
A two-stage matching and sampling approach is used to optimize the selection of comparator patients in the Czech Republic ReMuS disease registry. The methodological details are presented in Appendix 7.

9.4.1.4 Academic clinical MS centres in England (Cambridge and Plymouth) and Wales (Cardiff)

A tripartite collaboration has been established for the purposes of this study across three academic-clinical MS centres in England (Cambridge and Plymouth) and Wales (Cardiff). These institutions have sizeable MS practices, including regular use of LEMTRADA as a treatment option. Data on key comorbidities and drug utilisation are available from patient charts and patient notes. Variables needed for the study are extracted from patient charts of eligible patients, and entered into a local database. Linkages to primary care databases and national demographic data, at the local level, are also available (39). Data from each local database are sent to lead PI (Prof Neil Roberston, Cardiff) to be combined into one dataset for analysis.

The tripartite collaboration in the UK involves medical institutions that have similar working patterns and operate within the National Health System (NHS). MS patients' management follows UK NHS recommendations and follow-up of MS patients (via active tracing) is similar in all three institutions. Data on MS patient's characteristics, clinical course (e.g., EDSS and relapses) and treatment received are collected in the same way. Therefore, the tripartite collaboration will be considered as a single source of data.

9.4.1.5 The German Pharmacoepidemiological Research Database (GePaRD)

Since 2004, the Leibniz Institute for Prevention Research and Epidemiology – Bremen Institute for Prevention Research and Social Medicine (BIPS) has been working on the establishment and maintenance of the project-based German Pharmacoepidemiological Research Database (GePaRD)[16]. This database contains claims data from four statutory health insurance providers and covers about 20 million insured Germans since 2004 (~17% of the population of Germany). GePaRD data fairly represent the German general population with respect to age, sex, region of residence, overall hospitalisation rates, disease-specific admission rates (62), and medication dispensations (63). Patients with middle to higher socioeconomic status may be overrepresented in GePaRD, since three of the four statutory health insurance providers are more likely to insure patients of middle to higher socioeconomic status (41).

GePaRD includes demographic characteristics for each person, information on drug prescriptions, outpatient/inpatient hospital contacts and diagnoses since 2004. Prescription data include reimbursable drugs and include dates of prescription and of actual dispensing, dates of delivery, and the amount of drug prescribed. All diagnoses are based on the German Modification of the International Classification of Diseases, 10th revision (ICD-10-GM).

[15] https://www.nfimpuls.cz/index.php/en/czech-ms-registry/final-reports
[16] https://www.bips-institut.de/en/research/research-infrastructures/gepard.html

This database is updated on an annual basis with pseudonymised and validated data. Of note, the entire process from data delivery to availability for studies can take up to two years. BIPS can be commissioned to carry out drug utilisation or drug safety studies that are requested by health authorities such as the EMA.

9.4.2 Study implementation in data sources

In each data source, a "working database" specific to the study will be created and hosted in the local information technology (IT) system. All data relevant to the study derived from the local IT system will be stored in this working database. Resolution of anomalous data, variable recodifications, computations of exposure and follow-up times, and if needed, linkages with external databases will be performed within this working database. When all data handling procedures are terminated and the working database is final, scripts will produce results such as descriptive statistics and risk estimates in tables and figures based on the working database. These tables and figures with aggregated results will be transferred to the Study Management where further analyses may be carried out using meta-analytic techniques.

All procedures specific to a data source that are needed for producing the working database are detailed in an OP. A main goal of OPs is to harmonise as far as possible the most important variables to be included in analyses, in order to minimise heterogeneity between data sources. OPs will be developed in collaboration with each local data source to accommodate local variations in variable definitions, and ways to apply statistical scripts on datasets.

9.5 STUDY SIZE

9.5.1 Background information

Sample size calculations require a mortality rate in the unexposed population. We outline below the mortality rates that we have reviewed before performing our calculations.

Mortality rates for MS populations, generated using MS registry data, are 2.5 to 3.5 higher than in general populations (52,64,65,66). These data refer to a heterogeneous MS population; those treated with DMT and those not treated with DMT inclusive of those who may not attend neurology services regularly.

As regards mortality rates in DMT users: a study in the United States (US) Veterans found that use of DMT was associated with a 42% reduced risk of mortality in comparison to never use of a DMT, after adjustment for age, sex, and many other confounders (53). Articles reporting all-cause mortality rates in mainly DMT treated MS patients show rates of 0.32 per 100 PY in Denmark (from 1996 to 2015) (67) and of 0.24 to 0.37 per 100 PY in France (from 1976 to 2004 and 1990 to 2009) (52,64).

The randomised trials on LEMTRADA enrolled MS patients 18 to 55 years of age and demonstrated mortality of 0.17 per 100 PY[17]. Unpublished interim data for an ongoing LEMTRADA PASS[18]

[17] Pharmacovigilance Risk Assessment Committee (PRAC). LEMTRADA INN/active substance: alemtuzumab. PRAC assessment report, , 31 October 2019
[18] EUPAS 7346 : http://www.encepp.eu/encepp/viewResource.htm?id=28499

reports six deaths in 2,092 LEMTRADA treated patients with 4813.4 PY in Europe as of December 2019, giving a mortality rate of 0.12 deaths per 100 PY (95% CI: 0.05; 0.27).

Based on a scarcity of data for mortality rates in HE-DMT users as an MS subgroup, we use the above rates along with unpublished data from an on-going PASS on another DMT, teriflunomide (Aubagio®) [19] to select a range of plausible morality rates for our computations. In the Teriflunomide PASS, all-cause mortality rates in HE-DMT treated patients in Denmark and France were 0.31 per 100 PY (based on 2014-2018 data).

Data from feasibility report 2022 for this PASS (EMEA/H/C/003718/ANX/009.1) indicate that the mortality rate in the combined population (LEMTRADA + HE-DMT) ranged from 0.22/100 PY to 0.28/100 PY.

Thus, using all the above data, a mortality rate of 0.24/100 PY was selected as a single plausible mortality rate in the HE-DMT treated population for the below precision calculations.

9.5.2 Determination of sample size

All patients meeting inclusion criteria will be considered for inclusion in the study. Because sample size is *a priori* given for this study, we have estimated the precision of mortality risk using the following key assumptions and analysis choices:

- Mortality rates in the HE-DMT group of 0.24 per 100 PY (see Section 9.5.1).
- Mortality rate 1.2 to 2.0 times higher in LEMTRADA treated patients than in patients treated with other HE-DMT
- A ratio of exposed/unexposed of 1:4 i.e., one patient treated with LEMTRADA with four patients treated with HE-DMT
- Median follow-up of six years. This value was chosen because median follow-up in the ECCS was approximately four years as of 2019, based on data collected between 2013-2015 and 2017-2018. The follow-up will be longer in the current study since MS patients will be followed from 2013-2015 until 2021-2023.
- Type I error (α risk) of 5% (two-sided).

The method for calculating precision is based on Rothman and Greenland (68). Sensitivity analyses show how precision changes with respect to study size, assumed mortality and relative risk of mortality between the two groups (Table 5).

Results in Table 5 indicate that if the mortality rate in HE-DMT treated MS patients was 0.24 per 100 PY and if the relative risk of LEMTRADA vs. HE-DMT were 1.5, the 95% CI would range from 1.00 to 2.25 if 1,500 LEMTRADA treated patients were included in a pooled analysis. The possibility to gather relevant data on 1,500 LEMTRADA treated MS patients is communicated in Table 4. As Belgium is no longer contributing to this study, the precision estimates were re-calculated using the most up-to-date cohort sizes from 2023 feasibility analyses and are presented in Table 5.

[19] EUPAS 19610: http://www.encepp.eu/encepp/viewResource.htm?id=26074

Table 5 Re-assessment of precision for mortality risk estimates with the uptodate cohort size

Description		Original protocol with 1,000 LEMTRADA patients	Original protocol with 1,500 LEMTRADA patients	Up-to-date crude available numbers	Up-to-date available numbers from weighted analyses
Scenarios		**1**	**2**	**3**	**4**
Mortality rate in comparison (non-LEM) group (deaths/100 PYs)		0.24	0.24	0.24	0.24
Mean follow-up (years)		6	6	6	6
Number of LEM patients in a pooled analysis		1,000	1,500	1,167	1,167
Number of non-LEM patients in a pooled analysis		4,000	6,000	15,757	6,045
RR = 1.2	95% CI	0.70 – 2.05	0.77 – 1.86	0.76 - 1.89	0.74 - 1.95
RR = 1.5	95% CI	0.91 – 2.46	1.00 – 2.25	0.99 - 2.26	0.96 - 2.34
RR = 1.7	95% CI	1.06 – 2.73	1.16 – 2.50	1.15 - 2.51	1.11 - 2.59
RR = 2.0	95% CI	1.28 – 3.13	1.39 – 2.88	1.39 - 2.87	1.34 - 2.98

Scenarios 3 and 4: Up-to-date available numbers are based on extended feasibility report in 2023
Details about crude and weighted patient numbers for each data source are presented in Appendix 5
CI, confidence interval, LEM, LEMTRADA; non-LEM, non-LEMTRADA HE-DMT; RR, relative risk; PY, person-years

9.6 DATA MANAGEMENT

The data management procedures are specific to each data source and are thus detailed in data source specific OPs. OPs will be finalised for each data source after the results of the feasibility analysis inform on available data and variable format (see Section 9.8). An essential goal of OPs will be to harmonise variable formats and handling of missing data across data sources, in order to reduce heterogeneity between data sources. Each data source will produce a working database containing the same variable names and definitions as the other data sources, wherever possible, as detailed in the SAP and OPs.

Each data source employs selected software for data management, sometimes locally developed and not commercially available. Statistical packages are usually R or SAS. These technical items are described in the OPs.

In general, local procedures include checking electronic files, maintaining security and data confidentiality, following analysis plans, and performing quality-control checks of all programs. Each data source maintains patient-identifying information securely on site according to internal/local standard operating procedures or guidance documents. Security processes are in place to ensure the safety of all systems and data. Every effort is made to ensure that data are kept secure so that they cannot be accessed by anyone except authorised staff.

Appropriate data storage and archiving procedures are followed, with periodic backup of files. Standard procedures are in place at each data source to restore files in the event of a hardware or software failure.

9.7 DATA ANALYSIS

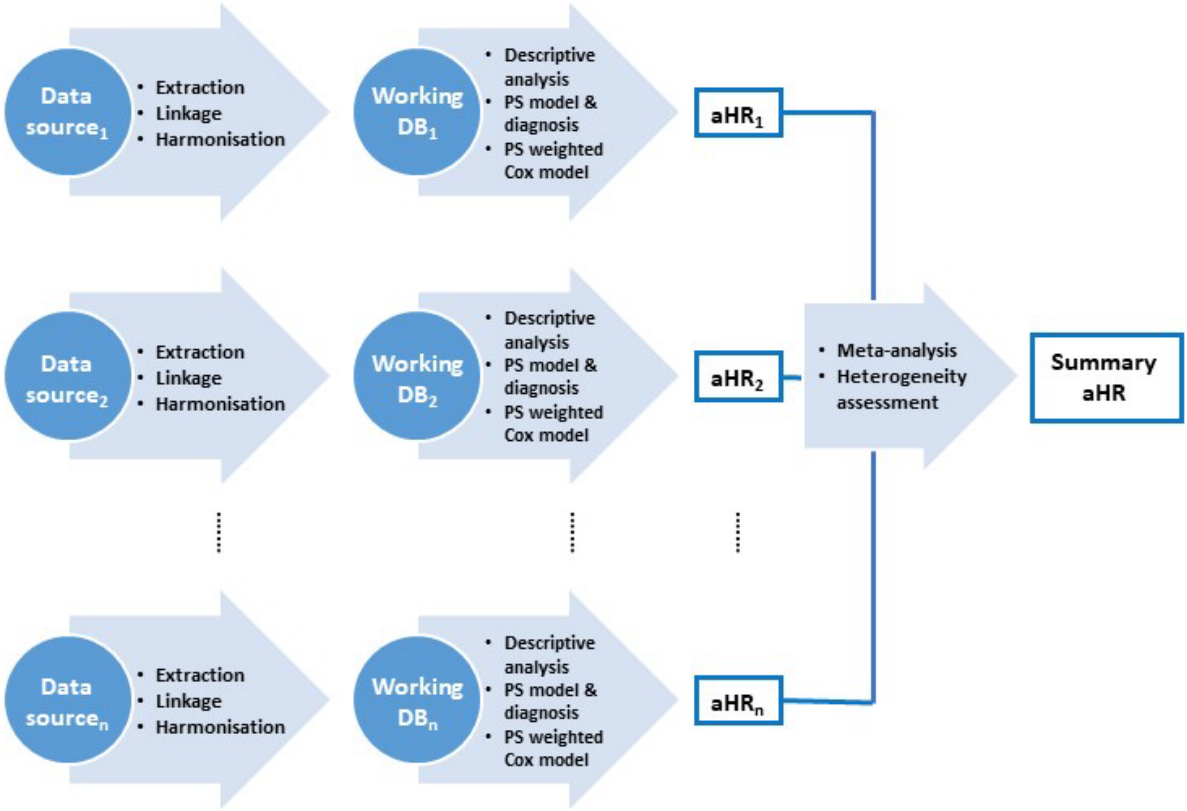
9.7.1 Patient data

The study is based on secondary use of patient data, i.e., data that are generally collected for administrative or routine care purposes.

9.7.2 Data flow

The data flow and analysis steps are summarised in Figure 2.

Figure 2 Data flow of the PAS study of LEMTRADA use and mortality risk



DB, database; aHR, adjusted hazard ratio; PAS, post-authorisation safety; PS, propensity score

In each data source, the data collection period regarding DMT use and vital status spans from the date of approval/reimbursement of LEMTRADA in the country until end of follow-up. A working database is created in each data source, and descriptive analyses, PS building, and Cox Proportional Hazard model analyses will be conducted in each data source. These data source specific hazard ratios (HRs) may then be meta-analysed to compute a summary HR (31).

Seven steps can be delineated for the data analysis process:

1. Descriptive analyses of exposure, confounding variables, and outcomes;
2. PS construction for predicting the likelihood of LEMTRADA treatment;
3. Analysis of PS density function and of covariate balance;
4. Statistical analyses of mortality risk of MS patients treated with LEMTRADA vs. MS patients treated with other HE-DMT;
5. Sensitivity analyses;
6. Meta-analysis of results on mortality risks with assessment of heterogeneity across data sources;
7. Estimation of the possible influence of unmeasured confounders.

During descriptive analyses and PS model construction (steps 1 to 3), no link will be made between exposure/covariates, and fatal outcomes.

9.7.3 Descriptive analyses

Because of the expected complexity of data at hand, a descriptive analysis step will be required for defining the optimal way to construct PS models and perform subsequent analyses (69).

The descriptive analysis will be done for all eligible MS patients included in each data source and for each variable. This will allow specifying the group of patients receiving LEMTRADA or another HE-DMT in each data source, and will be done before PS modelling. The descriptive analysis will compare how variables are distributed between LEMTRADA and HE-DMT patients. The descriptive analysis will thus assess how LEMTRADA treated and HE-DMT treated compare to each other in terms of sex, age, disease duration, age at disease onset, EDSS at CED, relapse history, comorbidities, past therapies, follow-up duration, and censored data (i.e., death, emigration, or loss to follow-up). Particular attention will be devoted for finding out whether for some variable categories there would be zero LEMTRADA treated MS patients, while there would be HE-DMT treated MS patients, and vice-versa (70). Variable re-categorisations will be considered in the event of zero to few observations for a variable category. Missing data will also be evaluated for each variable. Variability across data sources, in terms of variable availability and data format, will be examined. Whenever possible, harmonisation of variable format between data sources will be done.

Attention will be devoted to follow-up time between LEMTRADA treated and HE-DMT treated groups in order to detect the possibility of imbalance in outcome ascertainment between groups. Imbalance could occur for two reasons: 1) deaths occurring more in one group than another reflecting a true differential in mortality rates and a result that is valuable to report or 2) closer follow-up of MS patients in one group as compared to the other group, for instance, because health professionals are aware of hazards associated with a particular drug (detection bias). The latter reason could lead to better detection of deaths in one group over the other leading to a biased result. This bias is however unlikely because of the seriousness of the outcome. In any event, detection bias will be minimised by ensuring that end of follow-up is recorded in the same way for each group, with extensive effort to update death data in each data source regularly and comprehensively. Further details regarding the study's approach to addressing bias are provided in Section 9.10.5.

Descriptive analyses will appraise how LEMTRADA treated MS patients in routine care resemble MS patients included in phase III trials on LEMTRADA (age, MS type, EDSS, relapse history, disease duration, comorbidities, and DMT prior to first LEMTRADA course).

Descriptive analyses will be based on data organised in tabular format (without individual or identifiable data) that will be sent by each data source to the Study Management.

9.7.4 Propensity score (PS) model

A PS is a value ranging from 0.0 to 1.0 which represents the probability of being exposed vs. being non-exposed to the treatment being evaluated. The exposure probability is based on variables measured at initiation (CED) of LEMTRADA or another HE-DMT. PS-based methods have the ability to inform on and control for confounding, in particular confounding by indication as long as data exist for all relevant confounders (71,72,73). PS modelling is recommended for confounder adjustment when the outcome is rare and when many factors may confound the exposure-outcome relationship (73,74,75).

Using the PS with the SMR-weighted estimator, allows to compare the risk of mortality in LEMTRADA treated MS patients to a population treated with HE-DMT other than LEMTRADA

whose distribution of risk factors is similar to that of the LEMTRADA treated population. In this approach, the target of inference is the average treatment effect among LEMTRADA treated MS patients (ATT) (76).

Heterogeneity is expected in data between data sources, as well as differences in availability of variables involved in confounding, both of which may contribute to heterogeneity in mortality risk estimates across data sources. PS models including all variables available in each data source probably exert a better control of confounding, but probably also entail more heterogeneity. One way to minimise heterogeneity is to start with a parsimonious PS model including the same core variables in all data sources identified during literature review and assessment of confounding potential (see Section 9.3.5 and Appendix 3). The primary PS model is based on core variables and is obtained through fitting a logistic regression model.

The way the PS models were selected, and the exact statistical analysis strategy described hereafter depended on a thorough descriptive analysis of data at hand (see Sections 9.7.3 and 9.8). The PS models were investigated in the feasibility analysis 2022 and the extended feasibility analysis 2023.

The PS are computed only at CED, following a time-fixed exposure approach. The balance of variables between the two groups are then evaluated through computation of standardised mean differences after PS weighting (see Section 9.7.5) (71,77). To improve covariate balance, re-specification of the PS models, including modification of covariate codification (72), was performed after seeing the results of the feasibility analysis 2022 (see Section 9.8.1). The specification and rationale for the modification of certain covariates is detailed in the SAP V5.0 in Section 14.2.1. Further re-specifications were made after interim analysis and are detailed in SAP V7.0.

The PS density functions obtained for each data source are graphically displayed, and PS distributions among LEMTRADA and other HE-DMT treated MS patients are compared. Non-overlapping regions between LEMTRADA treated and other HE-DMT treated patients are checked. When non-overlapping (or poorly overlapping) regions are observed, the characteristics of MS patients populating these regions are examined so that factors determining the “non-positivity” of LEMTRADA treated and non-LEMTRADA treated MS patients are identified. Exclusion of non-overlapping regions at both ends of the PS distribution addresses non-positivity, and is recommended as the default prior to any PS implementation (78). It also avoids having MS patients who had practically no chance to have received LEMTRADA (78,79,80). From the feasibility analyses, it is known that few LEMTRADA patients were removed from non-overlapping regions; hence, the ATT approach still holds. Therefore, the main analysis will be based on the primary PS model using all data after exclusion of non-overlapping regions. The most appropriate final PS models identified from the extended feasibility analysis will be used for interim and final analysis.

9.7.5 Computation of PS weights

Data analysis methods based on PS weighting are often used for controlling the influence of confounding factors on the association between an exposure and an outcome in the setting of pharmacoepidemiology studies (73,76). PS weighting has several advantages over traditional multivariable regression analyses, like the ability to provide a global comparison of covariate

distribution between groups being compared, and the possibility to clearly define the target population of inference (i.e., the population onto which study findings apply) (81).

The SMR method (or "weighting by odds" method) for computing weights will be used. The method for computing PS weights has been selected considering that LEMTRADA is indicated for MS patients characterised by MS severity and progression. Thus, we aim to compare the risk of mortality in LEMTRADA treated MS patients to a population treated with another HE-DMT whose distribution of risk factors is similar to that of the LEMTRADA treated population. In this approach, the target of inference is the ATT (76).

Weighting in the LEMTRADA treated group is set to 1.0. Weighting in the comparison group is done using the odds of the PS attached to each MS patient included in comparison groups, i.e., PS/(1-PS). SMR weighting reweights the control patients to be representative of the treated population. SMR weighting thus results in an estimate of ATT. Approaches for dealing with potential extreme weights will be outlined in the SAP.

9.7.6 Statistical analysis

9.7.6.1 Mortality rates

In each data source, mortality rates will be calculated after the latest update of vital status. Crude mortality rates in the LEMTRADA and other HE-DMT treated groups will be calculated for all MS patients. Mortality rates will be expressed as number of deaths per 100,000 PY with 95% CI. Moreover, mortality rate difference will be calculated for the crude mortality rates. Comparisons between data sources will allow an appreciation of variability in mortality rates.

9.7.6.2 Survival curves

Weighted Kaplan-Meier curves from each data source will be produced. This will depict the survival of each data source, incorporating PS weighting.

9.7.6.3 Cox proportional hazard model

Statistical analyses will be conducted in each data source in order to compute the risk of mortality using the PS weighted time-fixed Cox proportional hazard model. The proportional hazards assumptions underpinning Cox regression will be checked via visual inspection of Schoenfeld residuals. Cox model specifications will be the same across data sources. What may vary are the PS models and subsequent weighting owing to variations in data availability and variable codification across data sources. Hence, heterogeneity in PS model is susceptible to introduce heterogeneity in results across data sources.

Cox proportional hazards models will be used to generate HRs. For the HRs, 95% CI will be generated using the robust sandwich-type variance estimator (82). Three Cox models will be performed with varying levels of adjustment. The rationale is to provide an indication of the ability to adjust for confounding. The first model will be unadjusted. The second model will be PS weighted. The third model will be PS weighted and additionally include any covariates that were not considered sufficiently balanced between the two exposure groups at CED based on standardised mean differences of that covariate after PS score weighting. This is detailed in the SAP.

9.7.6.4 Meta-analysis

Following the Council of International Organisation of Medical Sciences Working group X (CIOMS X) recommendations (31), a meta-analysis may be performed to combine all adjusted hazard ratios (aHRs) estimated in each data source to obtain a summary HR across data sources, if appropriate (86). Since some analyses may not be feasible for all data sources, e.g., due to limited sample size, a minimum of two data sources will be required to perform a meta-analysis (83). Interpretation of the summary HR will depend on heterogeneity of results across data sources.

Should a MA be performed, HRs will be pooled together by the Study Management using a random-effects model (84), in order to account for the presence of heterogeneity across included data sources. This considers that the differences between sources are random. If conducted, 95% CI for the pooled HR will be calculated.

Statistical heterogeneity will be assessed using Higgins' I^2 (85). Heterogeneity will be informed by the Cochrane Handbook on Systematic Reviews (86) and will be interpreted according to Borenstein (87). If substantial or considerable heterogeneity is detected ($I^2 \geq 50\%$), potential sources of heterogeneity will be explored. Clinical heterogeneity could be explained by the type of data (e.g., administrative database vs. MS registry), by differences in prescribing patterns for LEMTRADA and patient profiles (e.g., age, sex, EDSS, comorbidities) across databases and countries, and by variations in covariates in data source-specific PS models.

Results may be represented graphically with forest plots. Sensitivity analyses may be conducted, for example, by removing one data source at a time in order to evaluate the influence of each data source on the overall result (i.e., "leave-one-out" analysis).

The meta-analysis may be conducted on the main analysis and two of the sensitivity analyses: RRMS restricted and treatment-naïve restricted analyses. The leave-one-out analysis may be performed only on the main analysis.

9.7.6.5 E-values

E-values are an effective tool to assess the impact unmeasured confounding could have on a given analysis as it is defined as the minimum strength of association, on the risk ratio scale, that an unmeasured confounder would need to have with both the treatment and outcome, conditional on the measured covariates, to explain away a treatment–outcome association (115). Therefore the larger the E-value, the stronger the confounder associations would have to be to explain away the effect.

The E-value will be computed for the mortality risk obtained through fitting the main Cox model, as well as for its CI.

If E-values are evaluated, the associations between a known unmeasured confounder (e.g., smoking) and the exposure (LEMTRADA) and mortality are explored from the literature. These associations would be compared to the E-value when drawing conclusions (127).

In instances where the null (i.e., HR=1) is not in the CI, the E-value for the limit of the CI closest to the null would also be computed, as indicated by VanderWeele (116). This would inform on the strength of association an unmeasured confounder would be required to have in order to render the risk estimate statistically non-significant.

However, given the concerns over the interpretability of the HR found during the interim analysis, stemming from low event rates, the E-values may not be calculated if the HRs at final analysis are also deemed uninterpretable. Also, the E-values will not be produced if the meta-analysis is not conducted.

9.7.7 Primary analysis

9.7.7.1 Main analysis

The primary analysis will be based on an AT time-fixed approach (Section 9.3.4) using a weighted Cox proportional hazard model (see SAP V7.0) on the overall analysis population. Weights will be derived from the primary PS model fitted with core variables. Follow-up is determined as outlined in Section 9.3.4, Figure 1 and Table 2.

9.7.7.2 Sensitivity analyses

The sensitivity analyses that are essential for the main analysis interpretation are listed below. Analyses performed at interim analysis for a data source will also be performed at final analysis. Only the analyses deemed feasible for that particular data source will be performed (i.e., enough LEMTRADA patients and enough deaths within the restricted populations for analyses 2 and 3). These sensitivity analyses include:

1. An ITT censoring approach on the overall analysis population: Follow-up is determined as outlined in Section 9.3.4, Figure 1 and Table 3
2. RRMS only patients using an AT censoring approach
3. HE-DMT naïve patients using an AT censoring approach

Additional sensitivity analyses that are foreseen are described below:

4. Repeat of the main analysis restricting the HE-DMT exposure group to agents that reflect the highest levels of MS severity e.g., infused and/or subcutaneous agents and/or oral agents i.e., cladribine. This restriction may improve comparability of patients in terms of severity at CED.
5. PS models constructed with data from MS patients without missing data in core variables will also be constructed. This is called complete case analysis (88). This approach is used for the extended feasibility analysis and the interim analysis. For final analysis, this approach will be considered a sensitivity analysis.
6. Leave-one-out analysis may be performed to test the robustness of the main meta-analysis, if meta-analysis is conducted (see Section 9.7.6.4).

9.7.8 Secondary analysis

In secondary analyses we will investigate the cause of death in data sources where this information is available and when the number of cases for a specific cause is sufficient. The number of deaths by cause will be reported by exposure group by single, first instance of ICD-10 code (or other coded term), for each data source. Where feasible and appropriate, the codes may be aggregated. Several key points need to be taken into account for an analysis focusing on causes of death:

- For 40% to 60% of MS patients who die, the cause of death is recorded as “multiple sclerosis” (50,52,89,90,91,92,93,94).
- Coding practices differ between countries and between health professionals (95), with, for instance, the trade-off for assigning the cause of death to “multiple sclerosis” or to another cause of death (96).

For these reasons, the results from the cause of death analysis may not be informative and will have to be considered with caution.

During the feasibility study, availability of cause of death data was assessed; it was found four databases (ReMuS, DMSR, SMSR and UK centres) are potentially assessable for this analysis, provided that number of deaths for a given cause is sufficient. Given the variability and potentially low counts for specific causes of death, assessment of cause of death might only be possible as a descriptive analysis.

9.7.9 Other analyses

9.7.9.1 Death rates over time

A temporal analysis of fatal outcomes will be implemented as suggested by the PRAC. The PRAC Assessment Report on the Article 20 procedure for LEMTRADA raised the question of whether the number of fatalities stratified by age and disease severity within different time periods might exceed the expected rate. There is thus a need to examine whether rates of fatal events change with the year LEMTRADA treatment has been initiated.

There are two challenges to studying this. First, because the number of events over the study period is anticipated to be low, stratifying by time period would result in even less events per time period.

Second, patients treated with LEMTRADA from 2018-2019 onwards will have less available follow-up for an event to occur.

The 2023 interim analysis showed that the patient counts were sparse for LEMTRADA in later years of CED for Sweden, Denmark, and Germany. Also, there were few deaths in general. Therefore, crude mortality rates will be examined in broad groupings of years. The following year of CED groupings will be used to create demographics tables to examine changes to patient characteristics and crude mortality rates over time:

- UK: 2013 – 2016, 2017 – 31 March 2019, 01 April 2019+
- Czech Republic: 2015 – 2016, 2017 – 31 March 2019, 01 April 2019+
- Sweden, Denmark, and Germany: 2013 – 2016, 2017 – 31March2019

Further details are available in the SAP.

9.7.9.2 Incidence of switching in HE-DMT_NL exposure group

As a change from HE-DMT_NL to another HE-DMT_NL does not end follow up, though a change from LEMTRADA to another HE-DMT_NL does end follow up, it is of interest to examine how many patients in the HE-DMT_NL exposure group at CED change to another HE-DMT_NL. This will help determine the extent of bias that may have been introduced due to allowing the HE-DMT_NL group to continue to be followed past a treatment switch. Deaths counts and mortality rates will be calculated using an updated as-treated censoring approach which additionally censors patients when switching from HE-DMT_NL to another HE-DMT_NL, and not just HE-DMT_NL to LEMTRADA. These results will be described in the interpretation of the results and limitations.

9.8 FEASIBILITY STUDIES AND INTERIM ANALYSIS

9.8.1 Feasibility studies

A feasibility study was planned in the original protocol (Protocol V1.0). The objectives for the feasibility study were as follows:

1. Objective 1: assess availability and quality of data in each data source;
2. Objective 2: describe the number of patients and the average length of follow-up in each exposure group;
3. Objective 3: estimate the mortality rate of MS patients treated with HE-DMT in this PASS;
4. Objective 4: decide on the optimal length of the look-back period for assessing HE-DMT initiation and history of diseases at CED considering numbers of patients included in the cohort and comprehensiveness of data;
5. Objective 5: evaluate if MS patients treated with LEMTRADA are comparable to MS patients treated with other HE-DMT, and if balanced cohorts can be obtained.

Briefly, the overall conclusion of the first feasibility study was that, in its current form, there was a lack of balance on measurable confounders between the compared LEMTRADA and HE-DMT cohorts after PS weighting had been applied. After the first feasibility study (43), it was deemed that the study could not proceed due to remaining confounding between the LEMTRADA and HE-DMT cohorts. Thus, it was agreed with the PRAC that further feasibility analyses would be carried out to assess if several design and analytical changes could improve feasibility.

The objectives of the extended feasibility analysis are described below:

- The main objective was to achieve sufficient balance across PS weighted cohorts, i.e., the reduction of confounding. Confounding was addressed in the extended feasibility analysis with the following design and analysis changes, along with additional assessments:
    - Throughout the extended feasibility analysis, a time-fixed exposure approach was used which considers only the exposure status at CED rather than allowing patients who switch contribute to more than one cohort.
    - Variables were re-specified and then re-modelled in the PS model (e.g., categorising continuous variables) to evaluate if this would ameliorate imbalance on key covariates in PS-weighted cohorts. Several core variables were found to be consistently imbalanced between the weighted LEMTRADA and other HE-DMT cohorts in the feasibility analysis 2022. The variables were re-defined to assess whether the PS model and subsequent weighting could be improved. The changes were as follows:

        1. *c_HEDMT_before_rate, c_PDMT_before_rate* included in the feasibility PS model were replaced with binary variables which indicated whether a HE-DMT or P-DMT had been used before (*c_HEDMT_bin and c_PDMT_bin)*
        2. The continuous variables *c_disease_dur* and *c_YCED* included in the feasibility PS model were replaced with categorical variables *c_disease_dur_cat* and c_YED_cat

    - Re-specification of the PS models for the Czech Republic, Sweden, and Denmark to have covariate-reduced models. In these countries with few included patients, overfitting of the PS model was of concern (97). Hence, two composite variables were created: number of selected comorbidities at CED (*c_n_comorbid*) and number of selected previous medications at CED (*c_n_prev_med*). These variables were intended to replace, but still control for, all the binary variables used to create them.
    - Balance on core covariates was re-assessed following restriction to patients who were treatment naïve at CED and restriction on RRMS as MS type as an attempt to address confounding by indication (98) and disease severity. Apart from restriction to treatment naïve patients, the models were also restricted to other categories of line of therapy (1 previous HE-DMT at CED and 2 or more HE-DMT at CED) (99).

- Secondly, the extended feasibility analysis measured length of follow-up time according to an AT censoring approach versus an ITT approach to explore the appropriateness of a right censored approach.
- Thirdly, per communication from PRAC (EMEA/H/C/003718/ANX/009.2), the feasibility of examining short-term mortality (death within 30 days of exposure) as a comparative safety outcome in this PASS was assessed.

The conclusion of the extended feasibility analysis was that the study is feasible and will progress to the interim and final analyses. Some confounding remains; however, this may be addressable in outcome models by using doubly robust analyses (i.e., the addition of persistently imbalanced

variables to the PS weighted Cox proportional hazard models, where this is possible). Furthermore, the restricted analyses, while reduced in sample size, may reflect the "least biased" cohorts in terms of confounding by indication. Thus, the results of these analyses may help the interpretation of the results from the main analysis, and the overall study conclusion.

The AT approach was selected as the main analytic approach (see Section 9.3.4 of the protocol). Thirty-day mortality was deemed infeasible. A protocol amendment (V3.0) reflects the design and analytical updates now required for progression to the interim and final analyses.

9.8.2 Interim analysis

Based on the data obtained in the extended feasibility study 2023, interim analyses were completed in Q4 2023. The primary analyses were:

- Main analysis: all patients meeting inclusion criteria (AT)
- Sensitivity analysis: main analysis but with ITT follow-up
- Sensitivity analysis: no prior use of HE-DMT (AT)
- Sensitivity analysis: restricted to RRMS patients only (AT)

PS-weighted HRs were computed using complete case data, i.e., patients without missing core variables. The analyses are outlined in detail in the SAP V5.0 Section 15.

Data for the interim analysis encompassed the timeframe of 2013 to 2023 (depending on time-lag for data availability in each data source). These data are considered to represent the majority of the evaluable LEMTRADA population considering low utilisation rates from 2019 onwards (restricted labelling related to the Article 20 procedure followed by the coronavirus disease 2019 [Covid-19] pandemic).

The results of the interim analysis were of limited interpretability due to a small number of deaths which lead to unreliable HRs with wide 95% CI. Furthermore, due to varying prescribing practices for LEMTRADA across the contributing countries it is believed there is residual confounding that cannot be dealt with PS weighting or doubly robust methods. Based on descriptive results, the emergent prescribing patterns for LEMTRADA and contextualisation with mortality rates from the literature, it was determined that the results of the interim analysis did not provide evidence for increased mortality with respect to LEMTRADA use. It was anticipated that some changes between the interim analysis and final analysis (such as more follow-up, some adjustment of PS methods, and additional sensitivity analyses) may ameliorate the situation, although it is likely that limitations will persist.

The report on the final analyses will be available in Q3 2024.

9.9 QUALITY CONTROL

9.9.1 Data collection, validation, and data quality control at Study Management level

The Study Management will produce standard descriptive procedures, outlined in the OPs, to be applied on datasets for identifying missing and erroneous data and verify the consistency of the dataset. For instance, variables with values exceeding typical ranges and missing values will be flagged.

9.9.2 Data quality control at data source level

Data cleaning and quality control are the responsibility of each data source as outlined in the OPs and will follow the standard procedures specific to each data source as outlined by the data source's protocols. Follow-up and outcome ascertainment modalities specific to each data source will be detailed in OPs.

9.9.3 Handling of missing data

Descriptive analysis in each data source will examine frequency of missing data. Special attention will be devoted to core variables that will be used for the primary PS model. Descriptive analyses will evaluate whether missing data are random processes or could be linked to key characteristics such as age (100).

Regarding the core variables with missing data, and dependent on the pattern of missingness observed, the general approach will be the use of multiple imputations (MIs) (101), based on the R package Multivariate Imputation by Chained Equations (mice) (102,103). In SAS, the procedures MI and MIANALYZE will be used.

For age and gender, MS patients with missing value on these variables will be excluded. This was decided after the feasibility analysis was conducted when all data sources reported complete data for age and gender, except GePaRD in which one patient had a missing gender and was excluded. In GePaRD, the standard procedure is to exclude patients with missing values on age and gender, thus this procedure will be used, though may be only applicable for GePaRD only. Multiple imputation will be considered for the remaining core variables that may be missing: EDSS and MS type. Imputations will be considered depending on the on missing data patterns in the data sources (detailed in the SAP).

Of note, apart from the PS used for weighting, the main Cox model will include treatment (LEMTRADA vs. other HE-DMT), so that no additional missing data are anticipated for variables in the main Cox model. In a sensitivity analysis, the main Cox model will be fitted with a new PS weight after discarding all observations with missing data in core variables (88).

9.10 LIMITATIONS OF THE RESEARCH METHODS

9.10.1 Global study limitations

This study aims to investigate the risk of death associated with exposure to LEMTRADA relative to other HE-DMT. The main limitations are:

- The anticipated small number of fatal events, which may preclude reaching sufficient statistical precision.
- Imbalance in measured confounders and the potential influence of unmeasured confounder(s).

Ideally, the cohorts would be fully balanced on all measured confounding variables following the PS weighting. Based on the results of the extended feasibility analysis, some confounding will persist after PS weighting. However, doubly robust analyses will be used, which can potentially reduce residual confounding (104).

The use of restricted analyses (e.g., RRMS and HE-DMT naïve) aims to reduce confounding by indication, at the cost of reduced sample sizes and precision.

A further possibility in observational studies is the risk of bias due to unmeasured confounding. We will investigate how strong an unmeasured confounder must be for causing a spurious result (see Section 9.10.5.4).

9.10.2 Absence of similar examples in the literature

We did not find published literature investigating mortality associated with specific DMT. However, the methodological challenges in this study (e.g., low number of events, numerous confounders, complex exposure patterns, etc.) have been studied in other disease areas. Therefore, we have used some of these solutions (105,106,107).

9.10.3 Variability in data collection between data sources

There will be differences between data sources in available data and in data collection/recording procedures. For instance, depending on data source, initiation of HE-DMT will be captured as the date of prescription or of actual dispensing. The EDSS may be collected as a continuous variable or as a proxy based on use of assistive devices. Consequently, there may be heterogeneity in results between data sources because of differences in confounder adjustment. However, the SAP and OPs will aim at harmonising variable codification and format across data sources.

In addition, descriptive analyses within each data source, evaluation of the PS distribution, and a leave-one-out analysis, if appropriate, at the meta-analysis stage may be performed to first understand sources of heterogeneity.

9.10.4 Criteria for MS diagnosis

MS diagnosis criteria may differ according to age of MS patients at cohort entry. While McDonald criteria (108) has been in use for two decades, older MS patients may have been diagnosed according to Poser or Schumacher criteria (109), which may impact on age at MS onset or at MS diagnosis. However, there is no *a-priori* reason to believe that changes in diagnosis criteria may introduce bias because first, the assessment of the disease severity at CED is independent of diagnosis criteria, and second, the selection of MS patients considers the age at first prescription or dispensing of a HE-DMT.

9.10.5 Bias

This section presents several types of bias that could occur in the study. The biases potentially encountered include selection bias, information or detection bias, and unmeasured confounding.

Quantitative bias analysis methods will be used to explore and quantify the impact of such biases on study results. The complexity of these methods ranges from simple, with sensitivity analyses, to more intricate, with probabilistic and multiple bias modelling (110). In our case, we will perform sensitivity analyses and may compute E-values (unmeasured confounding bias), considering limitations of the latter method.

9.10.5.1 Selection bias

According to the Good Pharmacoepidemiology Practice from the European Network of Centres for Pharmacoepidemiology and Pharmacovigilance (ENCePP) definition, selection bias entails the selective recruitment into the study of subjects that are not representative of the exposure or outcome pattern in the source population. In a scenario characterised by selection bias, more outcomes might be present in one group more than the other as a direct result of differential selection or retainment (e.g., more intense medical surveillance in one group over the other) as opposed to a truthful over-occurrence. Minimal selection bias is expected in this study as all MS patients initiating a HE-DMT after LEMTRADA approval/reimbursement within each data source will be considered for inclusion.

In terms of generalisability, it is noted that not all countries in the EU will be represented, and in some data sources not all patients within a country will be represented, for example, the UK. Thus, the generated results will be applicable to countries included in this study and to countries that use LEMTRADA in a clinically similar way.

9.10.5.2 Misclassification bias in exposure and confounders

The possible influence of misclassification of drug exposed time will be addressed by sensitivity analyses.

Core variables defined in this protocol may be subject to misclassification, but the misclassification will likely be non-differential. In other words, we expect any measurement error will occur to the same extent for the LEMTRADA and the other HE-DMT groups. Thus, the results will not be biased in favour of one group or another, but could be biased towards the null (111).

9.10.5.3 Misclassification bias in outcome ascertainment

Incomplete follow-up may introduce detection bias mainly if medical surveillance of MS patients differs between treatment groups (112,113). For instance, health professionals could be more alert about vital status of LEMTRADA treated MS patients than of other HE-DMT treated MS patients. This contrast in surveillance may lead to mortality being observed more accurately or in a more timely way for LEMTRADA patients versus other HE-DMT patients (114). However, this bias is unlikely because of the seriousness of the outcome.

In Sweden and Denmark, the national MS registries are linked with national population registries that exhaustively record deaths and migrations of all people living in the country. Thus, follow-up will be complete in these countries. As mentioned in Section 9.3.2, the completeness and accuracy of vital registration in the GePaRD database (Germany) have been validated against reference mortality index databases, and there is no expectation that validity would differ across exposed groups in this study (40,41,42). In Sweden, Denmark and Germany, the recording of death is independent of the conduct of the PASS, and MS treatment status.

In countries, which contribute chart data to this PASS (Czech Republic and the UK), “active tracing” of MS patients will be used (see Section 9.3.2). Detection bias would be introduced if active tracing was carried out to different levels of completeness for each treatment group. In this regard, active tracing of MS patients in both treatment groups until study end is crucial. As stated above, given the gravity of the outcome it is expected that surveillance for the outcome would be equal in both groups.

In order to avoid misclassification bias on the outcome, analyses will be carried out only after the last vital status update.

9.10.5.4 Unmeasured confounding

An association between LEMTRADA use and mortality risk could be due to residual or unmeasured confounding not accounted for in the PS model. E-values may be used to quantify the impact of unmeasured confounding (Section 9.7.6.5).

10 PROTECTION OF HUMAN SUBJECTS

This study will be conducted in accordance with the guidelines for Good Pharmacoepidemiology Practice published by the European Network of Centres for Pharmacoepidemiology and ENCePP and the International Society of Pharmacoepidemiology (117,118).

10.1 CONSENT

Not Applicable

10.2 DATA PROTECTION

All data handling and hosting will be done in the data sources. Due to data protection legislation, exclusively the sources have access to the full individual-level data. No individual data will be transmitted to the Study Management or the MAH. Transfer of data to the Study Management will be done as aggregated, de-identified data in table or figure format via the use of secure sites.

11 MANAGEMENT AND REPORTING OF ADVERSE EVENTS/ADVERSE REACTIONS/OTHER MEDICALLY IMPORTANT EVENTS

For non-interventional studies that are based on use of secondary or routine health care data, reporting of adverse events/adverse drug reactions/other medically important events beyond the aim of the study is not required as laid out in the EMA guidelines for good pharmacovigilance practices modules VI and VIII and as also referenced in ENCePP guidelines for Good Pharmacoepidemiological Practice (13,117,119).

12 PLANS FOR DISSEMINATING AND COMMUNICATING STUDY RESULTS

In accordance with the 2010 EU pharmacovigilance legislation, the protocol of this study was entered into the publicly available EU PAS register after initial PRAC approval. A completed ENCePP Checklist for study protocols is included in Appendix 6: ENCEPP Checklist. Updates to the study protocol in case of substantial amendments and the final study report will also be entered in the register.

A report on the interim analysis was submitted to the Regulator in Q4 2023. The final report will be submitted in Q3 2024.

The Study Group will be comprised of Parexel International which is in charge of the PASS coordination, the PIs of individual data sources, and MAH representation. The Study Group will have access to the final results allowing for appropriate analysis, interpretation, and reporting of the study results. All involved parties from individual data sources give full authority to the Study Group for primary presentation and/or primary publication (i.e., the final study report) of results. No other publication is allowed before the primary publication. Any subsequent presentation or publication by a study participant (including for sub-studies) must be approved by the Study Group and reference the study and primary publication (i.e., the final study report). Independent scientific advisors may also be convened to review. The final decision to publish any manuscript/abstract/presentation will be made by the Study Group after prior notice to the MAH allowing for its internal review and comments. All manuscript/abstract/presentations must be submitted for internal review by the MAH at least forty-five (50) calendar days in advance of submission. The MAH may request that the name and/or names of one or several of its employees appear or do not appear in such publication. Any publication in a peer reviewed journal will be disclosed onto the ENCePP site within 2 weeks of publishing by journal.

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14 APPENDIX 1: DISEASE MODIFYING THERAPIES (DMT)

List of DMTs approved in Europe.

- The Platform DMT (P-DMT) include:
  - Dimethyl fumarate (Tecfidera®, Vumerity®),
  - Glatiramer acetate (Copaxone®),
  - Interferon beta 1-a (Avonex®, Rebif®),
  - Interferon beta 1-b (Betaferon®, Extavia®),
  - Peginterferon beta 1-a (Plegridy®),
  - Teriflunomide (Aubagio®).

- The Highly Efficacious DMT (HE-DMT) include:
  - Cladribine§ (Mavenclad®),
  - Fingolimod* (Gilenya®),
  - Mitoxantrone (Novantrone®),
  - Natalizumab§ (Tysabri®),
  - Alemtuzumab§ (LEMTRADA®),
  - Ocrelizumab§ (Ocrevus®),
  - Rituxumab (MabThera®, Rixathon®, Riximyo®, Blitzima®, Ritemvia®, Rituneza®, Ruxience®[biosimilar], Truxima®[biosimilar]).
  - Ofatumumab§ (Kesimpta®)
  - Ponesimod* (Ponvory®)
  - Ozanimod* (Zeposia®)
  - Siponimod* (Mayzent®)

The classification of P-DMT and HE-DMT in this PASS may differ from the classification encountered in some countries or adopted by some neurologists and scientists. DMT usually starts with a P-DMT.

* Moderately to highly efficacious DMT

§ Highly efficacious DM

15 APPENDIX 2: FOLLOW-UP SCENARIOS, PROS AND CONS

Scenarios	**1**		**2**	
	AT (Right-censoring analysis) - Main analysis		ITT - Sensitivity analysis only	
Patients	Exposure group	Follow-up	Exposure group	Follow-up
1	LEM (LEMTRADA)	a	LEM	a
2	HE-DMT	b	HE-DMT	b + c
	-	-	-	-
3	LEM	d	LEM	d + e
	-	-	-	-
4	HE-DMT	f	HE-DMT	f
5	HE-DMT	g+h	HE-DMT	g + h
	-	-	-	-
6	LEM	j	LEM	j
7	HE-DMT	l	HE-DMT	l
8	Not eligible	-	Not eligible	-
	1/ Pt 2: LEM exposure is ignored and thus death occurring during time c is also ignored. 2/ Pt 3: death after HE_1 is ignored. 3/ Loss of statistical precision because deaths occurring after switch are ignored.		1/ Pt 2: death during time c is attributed to HE_1, because HE-DMT index treatment carried forward. 2/ Pt 3: death during time e is attributed to LEM, because LEM index treatment carried forward.	

AT, as-treated; HE-DMT, highly efficacious disease modifying therapy; ITT, intent-to-treat; LEM, LEMTRADA
More data on the follow-up approaches (e.g., the length of follow-up and number of deaths) are provided in the extended feasibility report.

16 APPENDIX 3: DIRECTED ACYCLIC GRAPHS (DAG) FOR THE ASSOCIATION BETWEEN TREATMENT WITH LEMTRADA (LEM) OR ANOTHER HE-DMT (HE) AND MORTALITY

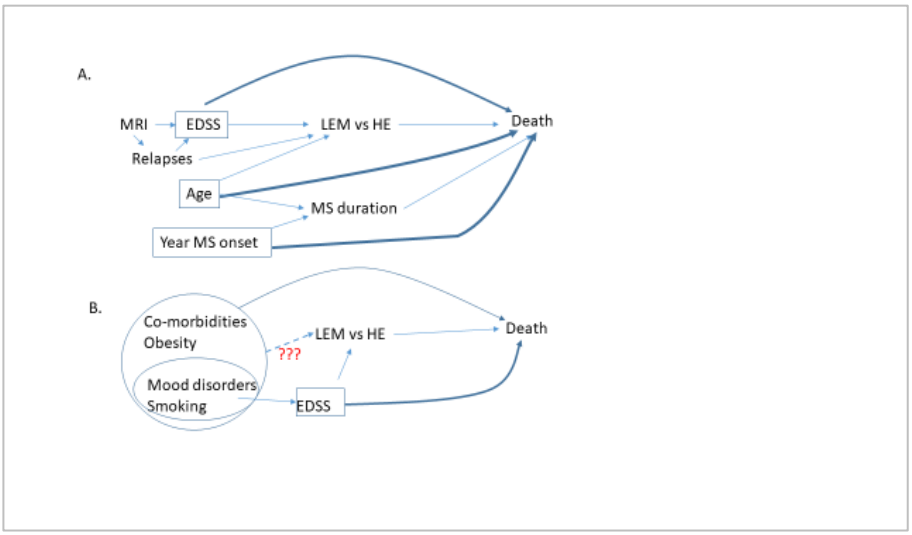
The DAGs have been designed in function of knowns and unknowns on relationships between exposure, mortality, and other variables.

In DAG A, EDSS is a backdoor path and needs to be controlled for. MRI and relapses affect EDSS, and thus their influence on mortality is mediated via EDSS. The few available data in the literature do not support an association between relapses and risk of death.

In DAG A, treatment with LEMTRADA or another HE-DMT depends on MS patient age, and age at LEMTRADA treatment initiation is not similar to age at other HE-DMT treatment initiation. Age is thus a backdoor path that needs to be controlled for. MS duration is correlated with year at MS onset and patient age. Many studies have shown that recent MS onset is associated with better survival than onset many years prior. Hence, MS duration is also a mediator for year of MS onset. During the first feasibility analysis, two PS models were fit. One with 'year of MS onset' and one with 'MS disease duration'. After analysis, the model with MS disease duration was preferred as it indicated disease severity and it was continuous, so it was carried forward to the extended feasibility. To further increase chances of achieving balance, MS disease duration was transformed into a categorical variable, with the following cut points: < 1 year, [1 – 5[years, [5 - 10[years, ≥ 10 years.

DAG B displays known risk factors for premature death. However, no literature exist informing on relationships between these variables and treatment with LEMTRADA or with another HE-DMT. The descriptive analysis will help shed light on these relationships. Of note, smoking and mood disorders exerts their influence on death through their negative influence on EDSS. But this is independent of their association with treatment.

Two directed acyclic graphs to depict confounding relationships between exposure drug groups and outcome mortality:



EDSS, expanded disability status scale; HE, HE-DMT; LEM, LEMTRADA; MRI, magnetic resonance imaging; MS, multiple sclerosis

17 APPENDIX 4: VARIABLES THAT REQUIRE PROXY INDICATORS

Relapses

The treatment with high doses of corticosteroid (CS) over short periods (e.g., 5 days) may be used as proxy indicator for relapses. These proxy indicators are based on knowledge that MS relapses are typically treated with high doses CS administered intravenously (IV) or taken orally. Relapses may be clinical or radiological (i.e., based on MRI change). Proxy indicators specific to data sources will be formulated according to ways by which data on CS therapies and MRI imaging are collected by data sources (to be defined in OPs).

High doses of CS over a few days are often administered for acute conditions, such as lumbago, lumbar hernia, sciatic, dentistry/stomatology on jaws (e.g., placement of dental implant). In these cases, however, daily CS doses rarely exceed 50 mg.

The inclusion/exclusion criteria hereafter are in the setting of MS patients treated with a DMT (hence there is no need for algorithms to find MS patients in data sources). These criteria have been derived from a paper by Quantum Black report for Sanofi-Genzyme, as well as from the literature (47,120,121,122) and discussions with neurologists.

Inclusion:

1. high dose methylprednisolone during a short period, i.e., IV injection or oral intake of 500 mg/day or more for 3 to 5 days, or,
2. Methylprednisolone or prednisone per os 50 mg/day or more for 3 to 5 days, or,
3. Oral dexamethasone 16 mg per day for 5 consecutive days (123).

If CS are administered in 30 days following a first administration, it will be considered as the same relapse episode, and as a new relapse episode otherwise.

Exclusion:

1. CS in five days around the administration of a DMT;
2. CS around the time of IV administration of immuno-globulins;
3. CS doses less than 50 mg per day (IV or oral).

Searches in databases may be based on ATC codes or on local custom codes.

ATC codes: H02AB02: Dexamethasone; H02AB04: Methylprednisolone; H02AB06: Prednisolone; H02AB07: Prednisone.

Expanded Disability Status Scale

The EDSS is the standard scale used by neurologists for evaluating the level of disability associated with MS disease. The scale ranges from 0 (no disability, normal neurological exam) to 9.5 (10 is equivalent to death due to MS). Possible proxy indicators are:

- Acquisition or reimbursement of aids for disabled people (with dates), such as:
    - walking aids (cane, crutch, walker): EDSS 5 to < 7
    - wheel chairs: EDSS 7 to < 9
    - medicalised beds and/or physiotherapy for bedridden patients: EDSS 9 or more.
- Information on ambulatory status, e.g.,
    - Ambulatory without an assistive device: EDSS < 5
    - Usually walks with an assistive device (cane, crutch, walker): EDSS 5 to < 7
    - Usually uses wheelchair: EDSS 7 to < 9
    - Usually confined to bed: EDSS 9 or more

The equivalence between proxy indicators and EDSS is based on Kurtzke (124).
EDSS is not captured in administrative data source, such as the German GePaRD data. Proxy indicators of EDSS in administrative data have been developed and the use of these algorithms was investigated in the feasibility analysis (122,125).

Creation of the EDSS proxy creation of this proxy in GePaRD data was based on (124) and on ICD-10-GM codes corresponding to diseases affecting the following functional systems: pyramidal, extrapyramidal, central, sensory, bowel and bladder, cerebral and mental, and mobility. This proxy EDSS is based on the pyramidal functional system and on mobility functions. It is a categorical variable with four categories ranging from 1 to 4 (*c_EDSS_FS1*): 1 indicating the lowest level of disability and 4 indicating the highest level of disability of a patient.

The construction of this variable is based on ICD-10-GM codes as defined in the table below:

List of ICD-10-GM codes used to construct the proxy EDSS variable

Category	c_EDSS_FS1
1	none of the codes listed for categories 2 to 4
2	G83.1, G83.2, G83.3, G83.9, R26.2 or R26.8
3	G81.#, G83.0, R26.0, R26.1, R29.6 or Z74.0
4	G82.#, R26.3 or M62.3

EDSS, expanded disability status scale
# denotes the wildcard in this protocol

In order to construct *c_EDSS_FS1*, the time window of two years before and six months after CED is taken into account. Values for *c_EDSS_FS1* is attributed as follows:

- 1: if a patient has no code reported in the before-mentioned time-window under categories 2, 3 and 4
- 2: if a patient has one of the codes reported in the before-mentioned time-window under category 2

- 3: if a patient has one of the codes reported in the before-mentioned time-window under category 3
- 4: if a patient has one of the codes reported in the before-mentioned time-window under category 4

If a patient has a code in several categories, the category with the highest rank (i.e., largest value) is chosen.

18 APPENDIX 5: NUMBER OF LEMTRADA AND HE-DMT USERS IN EXTENDED FEASIBILITY REPORT

	LEM-unweighted	HE-DMT-unweighted	LEM-weighted	HE-DMT-weighted
BIPS	407	7,093	407	3,258
Danish	125	3136	125	379.6
Sweden	55	4397	55	2257.7
CR	154	577	154	56.6
UK	426	554	426	92.8
Total	1167	15757	1167	6044.7

LEM, LEMTRADA; HE-DMT: highly efficacious disease modifying therapies
The unweighted numbers reflect the numbers that remain in each cohort after removal of non-overlap patients.

19 APPENDIX 6: ENCEPP CHECKLIST

ENCePP Checklist for Study Protocols (Revision 4)

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

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 (https://www.encepp.eu/standards_and_guidances/checkListProtocols.shtml), 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:
A NON-INTERVENTIONAL POST-AUTHORISATION SAFETY STUDY TO INVESTIGATE THE RISK OF MORTALITY IN PATIENTS TREATED WITH ALEMTUZUMAB (LEMTRADA®) RELATIVE TO COMPARABLE PATIENTS USING OTHER DISEASE MODIFYING THERAPIES: A COHORT STUDY

EU PAS Register® number: EUPAS42543
First registered on: 26 August 2021
Last updated on: April 2024

Section 1: Milestones	**Yes**	**No**	**N/A**	**Section Number**
1.1 Does the protocol specify timelines for				4 & 6 & 12
1.1.1 Start of data collection[20]	☒	☐	☐	
1.1.2 End of data collection[21]	☒	☐	☐	
1.1.3 Progress report(s)	☒	☐	☐	
1.1.4 Interim report(s)	☒	☐	☐	
1.1.5 Registration in the EU PAS Register®	☒	☐	☐	
1.1.6 Final report of study results.	☒	☐	☐	

Comments:

All listed items in Abstract (Section 4) and Section 6, except 1.1.5 1.1.5 (registration in EU PAS register only) in Section 12

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)	☒	☐	☐	7 & 8 & 9
2.1.2 The objective(s) of the study?	☒	☐	☐	
2.1.3 The target population? (i.e., population or subgroup to whom the study results are intended to be generalised)	☒	☐	☐	
2.1.4 Which hypothesis(-es) is (are) to be tested?	☐	☐	☒	
2.1.5 If applicable, that there is no *a priori* hypothesis?	☐	☐	☒	

Comments:

2.1.1 in Section 7 2.1.2 in Section 8.2 & 8.3 2.1.3 in Sections 9.2, 9.2.4 and 9.4

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)	☒	☐	☐	9.1.1

[20] 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.
[21] Date from which the analytical dataset is completely available.

Section 3: Study design	**Yes**	**No**	**N/A**	**Section Number**
3.2 Does the protocol specify whether the study is based on primary, secondary or combined data collection?	☒	☐	☐	9.1.1
3.3 Does the protocol specify measures of occurrence? (e.g., rate, risk, prevalence)	☒	☐	☐	9.7.6.1
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))	☒	☐	☐	9.7.6.3
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)	☒	☐	☐	11

Comments:

Section 4: Source and study populations	**Yes**	**No**	**N/A**	**Section Number**
4.1 Is the source population described?	☒	☐	☐	9.2.4
4.2 Is the planned study population defined in terms of:				9.2, 9.3.1, 9.3.4, 9.4
4.2.1 Study time period	☒	☐	☐	
4.2.2 Age and sex	☐	☒	☐	
4.2.3 Country of origin	☒	☐	☐	
4.2.4 Disease/indication	☒	☐	☐	
4.2.5 Duration of follow-up	☒	☐	☐	
4.3 Does the protocol define how the study population will be sampled from the source population? (e.g. event or inclusion/exclusion criteria)	☐	☐	☒	No sampling, all relevant patients included

Comments:

4.2.1 in Section 9.2.1 and 9.3.1
4.2.3 in Section 9.4
4.2.4 in Section 9.2.4
4.2.5 in Section 9.3.4

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)	☒	☐	☐	9.3.3
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?	☒	☐	☐	Figure 1
5.4 Is intensity of exposure addressed? (e.g. dose, duration)	☐	☒	☐	9.3.3
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?	☒	☐	☐	9.1.2

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?	☒	☐	☐	9.3.2
6.2 Does the protocol describe how the outcomes are defined and measured?	☒	☐	☐	9.3.2
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)	☒	☐	☐	9.3.2
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)	☒	☐	☐	9.1.2 9.3.5, 9.7.4, 9.10.5
7.2 Does the protocol address selection bias? (e.g. healthy user/adherer bias)	☒	☐	☐	9.10.5.1
7.3 Does the protocol address information bias? (e.g. misclassification of exposure and outcomes, time-related bias)	☒	☐	☐	9.10.5.2, 9.10.5.3

Comments:

Confounding is addressed in several sections: 9.1.2 (Comparable MS patients), 9.3.5 (Variables for the study), 9.7.4 (Propensity Score Model), 9.10.5.4 (unmeasured confounding)

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)	☒	☐	☐	Appendix 3

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)	☒	☐	☐	9.3.3 & 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)	☒	☐	☐	9.3.2
9.1.3 Covariates and other characteristics?	☒	☐	☐	9.3.5
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)	☒	☐	☐	9.3.3
9.2.2 Outcomes? (e.g. date of occurrence, multiple event, severity measures related to event)	☒	☐	☐	9.3.2
9.2.3 Covariates and other characteristics? (e.g. age, sex, clinical and drug use history, co-morbidity, co-medications, lifestyle)	☒	☐	☐	9.3.5

Section 9: Data sources	**Yes**	**No**	**N/A**	**Section Number**
9.3 Is a coding system described for:				
9.3.1 Exposure? (e.g. WHO Drug Dictionary, Anatomical Therapeutic Chemical (ATC) Classification System)	☒	☐	☐	9.3.3
9.3.2 Outcomes? (e.g. International Classification of Diseases (ICD), Medical Dictionary for Regulatory Activities (MedDRA))	☒	☐	☐	9.3.2 Cause of death codes
9.3.3 Covariates and other characteristics?	☐	☒	☐	9.3.5 Additional lists of codes to be developed for SAP
9.4 Is a linkage method between data sources described? (e.g. based on a unique identifier or other)	☒	☐	☐	9.4

Comments:

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?	☒	☐	☐	9.7.3
10.4 Are stratified analyses included?	☒	☐	☐	9.7.9.1
10.5 Does the plan describe methods for analytic control of confounding?	☒	☐	☐	9.7.4
10.6 Does the plan describe methods for analytic control of outcome misclassification?	☐	☒	☐	
10.7 Does the plan describe methods for handling missing data?	☒	☐	☐	9.9.3
10.8 Are relevant sensitivity analyses described?	☒	☐	☐	9.7.7.2

Comments:

Sections 9.7.2 outlines the various stages in the statistical analyses plan from descriptive analyses to inform propensity score development to the final outcome model.

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)	☒	☐	☐	9.6

Section 11: Data management and quality control	**Yes**	**No**	**N/A**	**Section Number**
11.2 Are methods of quality assurance described?	☒	☐	☐	9.9
11.3 Is there a system in place for independent review of study results?	☒	☐	☐	3.4 & 12

Comments:

The role of the independent scientific advisers is outlined in Section 3.4 and Section 12

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?	☒	☐	☐	9.10.5.1
12.1.2 Information bias?	☒	☐	☐	9.10.5.2-9.10.5.3
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).	☒	☐	☐	9.10.5.4
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)	☒	☐	☐	9.8

Comments:

Misclassification of exposure and confounders in Section 9.10.5.2 Misclassification of outcome in Section 9.10.5.3

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?	☒	☐	☐	10.2

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: _____

Date: dd/Month/year

Signature: _____

20 APPENDIX 7: THE CZECH MULTIPLE SCLEROSIS REGISTRY SAMPLING STRATEGY

The PRAC has requested comparator patients to be included from the whole of the Czech Republic, with a 1:4 random sample. Including comparator patients outside of the Prague region requires additional manual data collection from patient charts because variables not related to MS disease (and needed to address the research question of the LEMTRADA Mortality PASS) are currently not collected or included in the ReMuS database. The manual extraction of data from patient charts by neurologists is a time-intensive and resource-intensive process. Further, in previous feasibility analyses, a substantial number of comparator patients were removed from the analysis set due to non-overlapping propensity scores. The loss of so many patients minimizes the utility of the effort put into chart abstraction. Therefore, it is advantageous to optimize the selection of comparator patients from the whole of the Czech Republic, for whom data will be collected manually and whose inclusion in the final analytical models can be optimised.

A two-stage sampling strategy was implemented:

- Stage 1 – Matching without replacement (aimed for 1:4 matches) on a set of variables outlined in Table 6 to identify a pool of potential comparator patients for each LEMTRADA-treated patient. This maximized the efficient selection of (comparable) comparator patients for whom data were manually extracted for. The intent was that more patients could be retained after removal of non-overlapping regions of PS, and in this way the efforts needed to extract data not wasted.
- Stage 2 – PS weighting to control for confounding and aim to establish exchangeability of cohorts.

For Stage 1, a parsimonious set ($\{X_{DAG}\}$) of covariates (a *minimally-sufficient* subset of confounders) was identified via the clinically-informed DAGs in Appendix 3 (1). No risk of overadjustment was anticipated.

The purpose of matching was to achieve a balanced distribution in the sampled (matched) data, and this balance could be disrupted by adjusting for additional variables. Thus, failure to account for matching variables while adjusting for additional unmatched variables could result in residual confounding and biased estimates (2–5).

There is precedent for this approach in the prior registered PASS (6), and in the published literature (2,4,5).

Table 6 Variables Used for the Czech Multiple Sclerosis Registry (ReMuS) Sampling Strategy

	Variable Name	Format	Matching On	Justification
Included core variables[1]				
Sex	c_gender	Binary: male/female	Exact category	Core variable as per protocol
Age	c_age	Continuous	Mahalanobis distance (defined caliper limit)	Core variable as per protocol
Duration of MS disease	c_disease_dur_cat	Categorical: < 1 year 1 year to < 5 years 5 years to < 10 years ≥ 10 years	Exact category	Core variable as per protocol
EDSS	c_EDSS_cat_4	Categorical: [0-2.25] [2.25-3.75] [3.75-5.75] [5.75-10]	Exact category	The variable is derived from the core variable (c_EDSS_cat) as per protocol, with the last 3 categories collapsed based on the observed distribution of EDSS.
Prior HE-DMT use	c_HEDMT_bin	Binary: yes/no	Exact category	Core variable as per protocol
Additional matching variable				
Region of MS centre	region_cat	Categorical: 1, 2, 3, 4	Exact category	To consider regional variation characteristics related to MS disease and mortality
Excluded variables[1]				
Year of cohort entry	c_YCED_cat	NA for matching	NA	Potential instrumental variable
Prior P-DMT use	c_PDMT_bin	NA for matching	NA	Considered of less priority than prior HE-DMT use
MS type	c_MS_type	NA for matching	NA	Misclassification

EDSS: Expanded Disability Status Scale; HE-DMT: highly efficacious disease-modifying therapies; NA: not applicable; P-DMT: platform disease-modifying therapy; ReMuS: Czech Multiple Sclerosis Registry

[1] From core variables already established as key confounders.

Source: ReMuS Sampling Strategy

References

1. Shrier I, Platt RW. Reducing bias through directed acyclic graphs. BMC Med Res Methodol. 2008;8(1):70.
2. Sjölander A, Greenland S. Ignoring the matching variables in cohort studies – when is it valid and why? Stat Med. 2013;32(27):4696–708.
3. Stuart EA. Matching methods for causal inference: A review and a look forward. Stat Sci. 2010 Feb 1;25(1):1–21.
4. Iwagami M, Shinozaki T. Introduction to Matching in Case-Control and Cohort Studies. Ann Clin Epidemiol. 2022;4(2):33–40.

5. Bian Y, Le Y, Du H, Chen J, Zhang P, He Z, et al. Efficacy and Safety of Anticoagulation Treatment in COVID-19 Patient Subgroups Identified by Clinical-Based Stratification and Unsupervised Machine Learning: A Matched Cohort Study. Front Med (Lausanne). 2021;8:786414.
6. Dupilumab and Pregnancy Outcomes: A Retrospective Cohort Study Using Administrative Healthcare Databases (Dupi PODS) | HMA-EMA Catalogues of real-world data sources and studies [Internet]. [cited 2024 Apr 15]. Available from: https://catalogues.ema.europa.eu/node/2829/administrative-details

21 APPENDIX 8: LIST OF STAND-ALONE DOCUMENTS

Not Applicable

Signature Page for VV-PV-0549858 v1.0

lemtrada-mortality[csa002]-protocol-amendment-v4.0-apr2024-clean

Approve & eSign	Sarah-Jo Sinnott Pharmacovigilance 17-Apr-2024 14:25:21 GMT+0000

Approve & eSign	Jian-Yu E Pharmacovigilance 17-Apr-2024 14:34:58 GMT+0000

Approve & eSign	Magdalena Chirieac Pharmacovigilance 17-Apr-2024 14:35:13 GMT+0000

Approve & eSign	George Diamantidis Regulatory 17-Apr-2024 15:13:58 GMT+0000

Approve & eSign	Leslie DONDEY-NOUVEL Pharmacovigilance 18-Apr-2024 10:59:19 GMT+0000