1. Title Page

Title	Emulation of a phase 3 study comparing Rituximab with Dimethyl Fumarate in early Relapsing-Remitting Multiple Sclerosis
Research question & Objectives	The objective is to emulate the comparative clinical effectiveness of rituximab versus dimethyl fumarate in a real-world setting based on a previously published randomized controlled clinical trial
Protocol version	V2
Last update date	18 December 2024
Contributors	**Primary investigator contact information:** anna.glaser@ki.se **Contributor names:** Jan Hillert Anna Glaser Lars Forsberg Elena Flavia Mouresan
Study registration	**Site:** n/a **Identifier:** n/a
Sponsor	**Organization:** n/a **Contact:** n/a
Conflict of interest	n/a

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2. Abstract

Approved disease modifying therapies (DMTs) for multiple sclerosis (MS), mostly for relapsing forms of MS (RMS), have been around since the mid-1990s, each supported by a standard drug development process including large phase 3 randomised clinical trials (RCTs). In Sweden and elsewhere, off-label rituximab, a biological targeting B-cells has become the dominant MS treatment in the last decade. To fill the void of phase 3 data, clinical investigators performed a registry-based RCT, RIFUND-MS, using the Swedish MS registry (SMSreg) as its clinical report form (CRF), which showed superior effectiveness on relapses and MRI parameters compared to dimethyl fumarate (DMF) an approved MS DMT.

However, although significantly positive, RIFUND-MS was relatively small with less than 200 included patients. In addition, a draw-back of RIFUND-MS, like all RCTs, is that the inclusion and exclusion criteria limit the study cohort. For instance, older patients, secondary-progressive patients or patients with severe disability, were excluded, so knowledge of the effectivensee is missing for these patient groups.

We aim to use observational data from the SMSreg to evaluate the comparative effectiveness and safety of rituximab vs. dimethyl fumarate (DMF) on a variety of outcomes including relapses, MRI activity and disease progression. By comparing a strict scenario, where the inclusion/exclusion criteria mirror as closely as possible those of the published RIFUND-MS study, to a pragmatic scenario, which widens the inclusion/exclusion criteria to include subpopulations that are rarely included in traditional clinical trials (e.g. elderly, secondary-progressive MS), we seek to to evaluate the effect of these therapies on patients that are routinely treated in clinical practice. Moreover, outcomes that are difficult to evaluate in a traditional clinical trial either due to underpower or due to the limited follow-up of a trial, will be evaluated using observational data (e.g. disease progression).

This study will investigate the usefulness of observational data to support, confirm and extend the reported superiority of rituximab over DMF on disease activity and progression in a wider range of patients and real-world settings.

3. Amendments and updates

Version date	Version number	Section of protocol	Amendment or update	Reason
2024-09-22	V1	All	Protocol creation	Prepare draft version
2024-12-18	V2	All	Minor adjustments to whole protocol	Finalize protocol

4. Milestones

Table 1 Milestones

Milestone	Date
Finalize protocol	2024-12-18
Data access	2025-01-08
Finalize data analysis	2025-12-31

5. Rationale and background

What is known about the condition: Multiple sclerosis (MS) is an inflammatory disorder of the central nervous system that affects mainly adults of working age. The most common subtype is relapsing-remitting (RRMS) which is characterized by neurological symptoms that can significantly affect function, activity, quality of life and work capacity. Disability accumulation is mainly observed during the secondary-progressive (SPMS) phase which follows approximately 25 years after the RRMS phase.

What is known about the exposure of interest: Rituximab is a disease modifying therapy that has been used off-label in multiple sclerosis patients in Sweden to decrease the number of relapses since 2014. The only existing phase 3 data come from the registry-based randomized clinical trial (r-RCT) RIFUND-MS, comparing rituximab and DMF. There is limited evidence on the effect of rituximab on relapses in older populations, secondary-progressive MS patients or progression.

Dimethyl fumarate (DMF) is a first-line per oral disease modifying therapy included in the high-cost protection and subsidized in Sweden since 2014. Phase 3 studies have shown a reduction in relapse activity of over 50%.

Gaps in knowledge: The effectiveness of rituximab in the real world setting is unknown, as is the potential benefit of rituximab on relapses and MRI activity in populations not included in clinical trials, e.g. elderly populations. In addition a potential benefit over DMF on disability accumulation in general, is missing. Last, it is poorly shown how data from MS registries can most effectively be used to confirm, extend or refute results of phase 3 studies.

What is the expected contribution of this study? The emulation of the RIFUND-MS trial will allow the estimation of the effect of rituximab in populations not included in clinical trials by applying both strict and pragmatic scenarios of inclusion criteria. Moreover, this study will showcase the value of observational data in studying populations and outcomes that a typical clinical trial is underpowered to study, such as progression.

6. Research question and objectives

Table 2 Primary and secondary research questions and objective

A. Primary research question 1 and objective

Objective:	The objective is to emulate the comparative effectiveness of rituximab versus dimethyl fumarate in a real-world setting using strict inclusion/exclusion criteria based on the results of a randomized, registry based phase 3 study (RIFUND-MS).
Hypothesis:	The comparative effectiveness of rituximab versus dimethyl fumarate in the real-world setting using strict inclusion/exclusion criteria is similar to the comparative efficacy in the previously published phase 3 randomized study RIFUND-MS.
Population ***(mention key inclusion-exclusion criteria):***	Relapsing-remitting MS patients between 18 and 50 years of age with a disease duration less than 10 years at index date, with at least one relapse event or 2 new/enlarged T2 lesions or 1 Gd+ lesion within one year pre-index date, a score between 0 and 5.5 (inclusive) on the Expanded Disability Status Scale (EDSS), and treatment naïve or exposed only to Interferons or Glatiramer acetate at index date.
Exposure:	Rituximab: Initial infusion with 1000 mg iv and thereafter 500 mg iv every 6 months
Comparator:	Dimethyl fumarate: 240 mg orally twice daily with starting dose of 120 mg x2 for one week
Outcome:	Primary: Proportion of patients with relapse during the 24-month observational period Secondary: Time to first relapse Proportion of patients free from all MRI activity during the 24-month observation period EDSS-based 24 week Confirmed Disability Worsening (CDW) Change in EDSS from baseline to month 24 Drug persistence No evidence of disease activity NEDA-2/-3

Time *(when follow up begins and ends):*	Follow up starts on the same day as treatment initiation until the end of the 24-month period or until the date of death/loss to follow-up.
Setting:	Outpatient
Main measure of effect:	Relative risk, Time to first event

B. Primary research question 2 and objective

Objective:	The objective is to emulate the comparative effectiveness of rituximab and dimethyl fumarate in a real-world setting using a pragmatic scenario of inclusion/exclusion criteria based on the results of the RIFUND-MS, a randomized phase 3 study.
Hypothesis:	The comparative effectiveness of rituximab and dimethyl fumarate in the real-world setting using a pragmatic scenario of inclusion/exclusion criteria is similar to the comparative efficacy in the previously published phase 3 randomized study RIFUND-MS.
Population *(mention key inclusion-exclusion criteria):*	Relapsing-remitting or secondary-progressive MS patients between 18 and 60 years of age with a score between 0 and 5.5 (inclusive) on the Expanded Disability Status Scale (EDSS).
Exposure:	Rituximab: Initial infusion with 1000 mg iv and thereafter 500 mg iv every 6 months
Comparator:	Dimethyl fumarate: 240 mg orally twice daily with starting dose of 120 mg x2 for one week
Outcome:	Primary: Proportion of patients with relapse during the 24-month observational period Secondary: Time to first relapse Proportion of patients free from all MRI activity during the 24-month observation period EDSS-based 24 week Confirmed Disability Worsening (CDW) Change in EDSS from baseline to month 24 Drug persistence No evidence of disease activity NEDA-2/-3

Time *(when follow up begins and ends):*	Follow up starts on the same day as treatment initiation until the end of the 24-month period or until the date of death/loss to follow-up.
Setting:	Outpatient
Main measure of effect:	Relative risk, Time to first event

C. Secondary research question 1 and objective

Objective:	The objective is to emulate the comparative effectiveness of rituximab and dimethyl fumarate on progression in a real-world setting using strict inclusion/exclusion criteria based on the results of the RIFUND-MS, a randomized phase 3 study.
Hypothesis:	The comparative effectiveness of rituximab and dimethyl fumarate on the rate of disability progression in the real-world setting using strict inclusion/exclusion criteria is similar to the comparative efficacy in the previously published phase 3 randomized study RIFUND-MS.
Population *(mention key inclusion-exclusion criteria):*	Relapsing-remitting MS patients between 18 and 50 years of age with a disease duration less than 10 years at index date, with at least one relapse event or 2 new/enlarged T2 lesions or 1 Gd+ lesion within one year pre-index date, a score between 0 and 5.5 (inclusive) on the Expanded Disability Status Scale (EDSS), and treatment naïve or exposed only to Interferons or Glatiramer acetate at index date.
Exposure:	Rituximab: Initial infusion with 1000 mg iv and thereafter 500 mg iv every 6 months
Comparator:	Dimethyl fumarate: 240 mg orally twice daily with starting dose of 120 mg x2 for one week
Outcome:	EDSS-based 24 week Confirmed Disability Worsening (CDW) Time to confirmed, sustained EDSS 4 and 6 Time to SPMS
Time *(when follow up begins and ends):*	Follow up starts on the same day as treatment initiation until the end of a 48 or 72-month period or until the date of death/loss to follow-up.
Setting:	Outpatient
Main measure of effect:	Relative risk, Time to first event

7. Research methods

7.1. Study design

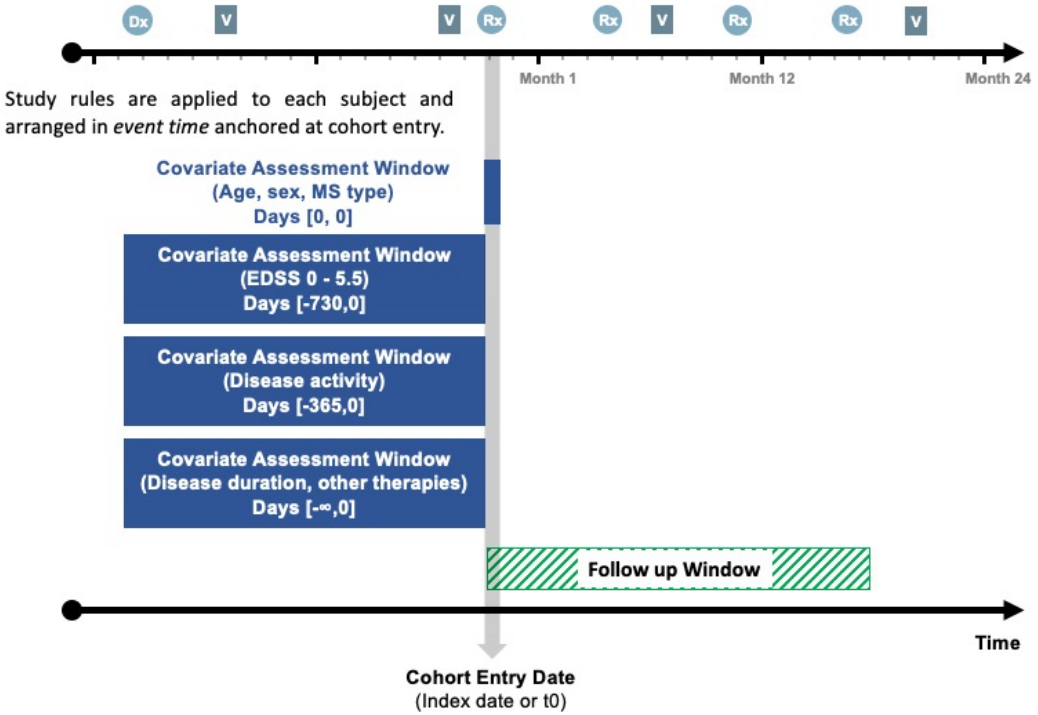
Research design (e.g. cohort, case-control, etc.): Retrospective new user active comparator cohort study

Rationale for study design choice: The active comparator design can help mitigate both measured and unmeasured confounding by increasing the overlap of characteristics between cohorts. The new user design ensures proper adjustment for confounding by clearly establishing the temporal sequence between pretreatment variables and drug exposure and reduces the risk of immortal time bias. The new user design mimics the concept of "treatment assignment" in RCTs, a key element of the target trial framework.

7.2. Study design diagram

Figure 1: From transactional data to study implementation*

Individual-patient data is retrieved from the SMSreg and documented as encounters from various sources, including diagnoses (Dx), drug dispensings (Rx), visits with EDSS measurement and relapse confirmation (V). It is arranged in months.



7.3. Setting

7.3.1 Context and rationale for definition of time 0 (and other primary time anchors) for entry to the study population

The start of the follow-up (time 0) is the day of initiation of Rituximab or Dimethyl Fumarate treatment. This mimics the time of initiation of treatment at randomization.

Table 3 Operational Definition of Time 0 (index date) and other primary time anchors

Study population name(s)	Time Anchor Description (e.g. time 0)	Number of entries	Type of entry	Washout window	Care Setting[1]	Code Type[2]	Diagnosis position	Incident with respect to…	Measurement characteristics/ validation	Source of algorithm
Exposure: Rituximab	Date of 1st dose administration	Single	Incident	[0,0]	n/a	ATC	n/a	Rituximab (iv, 500 mg every 6 months) or Dimethyl fumarate (oral, 240 mg twice daily)	No validation study	SMSreg
Comparator: Dimethyl Fumarate	Date of 1st prescription	Single	Incident	[0,0]	n/a	ATC	n/a	Rituximab (iv, 500 mg every 6 months) or Dimethyl fumarate (oral, 240 mg twice daily)	No validation study	SMSreg

[1] IP = inpatient, OP = outpatient, ED = emergency department, OT = other, n/a = not applicable

[2]See appendix for listing of clinical codes for each study parameter

7.3.2 Context and rationale for study inclusion criteria:

The study includes patients between 18 and 50 years of age at index date, diagnosed with relapsing-remitting multiple sclerosis according to the prevailing McDonald criteria at the time of diagnosis, with an expanded disability status score (EDSS) between 0 and 5.5 (inclusive) measured during a 2-year period pre-index, with documented disease activity defined as minimum one relapse event or 2 new or enlarging T2 lesions or one contrast-enhancing lesion during a one-year period pre-index, with an MS diagnosis less than 10 years before index date, and treatment naïve or exposed to only interferon or glatiramer acetate therapies.

In the pragmatic scenario we will drop the inclusion criteria to include otherwise neglected subpopulations such as elderly, patients with longer disease duration, patients with a low probability of experiencing disease activity, patients with a high EDSS score (>5.5) or diagnosed with other phenotypes of multiple sclerosis.

Table 4. Operational Definitions of Inclusion Criteria

Criterion	Details	Order of application	Assessment window	Care Settings[1]	Code Type[2]	Diagnosis position[3]	Applied to study populations:	Measurement characteristics/ validation	Source for algorithm
Age between 18 and 50 years		Before selection of index date	[0,0]	n/a	n/a	n/a	Exposure (rituximab), comparator (dimethyl fumarate)	No validation study	SMSreg
Diagnosed with relapsing-remitting multiple sclerosis (RRMS)		Before selection of index date	[0,0]	Any	ICD-10	Any	Exposure (rituximab), comparator (dimethyl fumarate)	No validation study	SMSreg
EDSS between 0 and 5.5 (inclusive)		Before selection of index date	[-730,0]	n/a	n/a	n/a	Exposure (rituximab), comparator (dimethyl fumarate)	No validation study	SMSreg
10 years or less since MS diagnosis		Before selection of index date	[-∞,0]	n/a	n/a	n/a	Exposure (rituximab), comparator (dimethyl fumarate)	No validation study	SMSreg
Documented evidence of disease activity	Defined as: minimum of one relapse, two new or enlarged T2 lesions, or one contrast-enhancing lesion	Before selection of index date	[-365,0]	n/a	n/a	n/a	Exposure (rituximab), comparator (dimethyl fumarate)	No validation study	SMSreg
Treatment naïve or only exposure to interferons or glatiramer acetate	Full treatment history evaluation	Before selection of index date	[-∞,-1]	n/a	n/a	n/a	Exposure (rituximab), comparator (dimethyl fumarate)	No validation study	SMSreg

[1] IP = inpatient, OP = outpatient, ED = emergency department, OT = other, n/a = not applicable
[2] See appendix for listing of clinical codes for each study parameter
[3] Specify whether a diagnosis code is required to be in the primary position (main reason for encounter)

7.3.3 Context and rationale for study exclusion criteria

Patients with a diagnosis of primary or secondary progressive multiple sclerosis and patients that participated in the RIFUND-MS clinical trial are excluded from this study. Patients with missing age or sex are excluded.

Patients receiving simultaneous treatment with other immunosuppressive drugs, had severe cardiac disorder, received vaccination within 4 weeks of index date, had a clinically relevant ongoing infection or somatic or psychiatric comorbidity or were pregnant/breastfeeding at index date are excluded if possible to identify in the SMSreg.

Table 5. Operational Definitions of Exclusion Criteria

Criterion	Details	Order of application	Assessment window	Care Settings[1]	Code Type[2]	Diagnosis position[3]	Applied to study populations:	Measurement characteristics/ validation	Source for algorithm
Diagnosis of progressive multiple sclerosis		Before the selection of index date	[-∞,0]	Any	n/a	Any	Exposure (rituximab), comparator (dimethyl fumarate)	No validation study	SMSreg
Participation to RIFUND-MS R-RCT		Before the selection of index date	[-∞,0]	n/a	n/a	n/a	Exposure (rituximab), comparator (dimethyl fumarate)	No validation study	SMSreg
Sex missing/unknown		Before the selection of index date	[0,0]	n/a	n/a	n/a	Exposure (rituximab), comparator (dimethyl fumarate)	No validation study	SMSreg
Age missing/unknown		Before the selection of index date	[0,0]	n/a	n/a	n/a	Exposure (rituximab), comparator (dimethyl fumarate)	No validation study	SMSreg
Pregnant/ breastfeeding		Before the selection of index date	[0,0]	n/a	n/a	n/a	Exposure (rituximab), comparator (dimethyl fumarate)	No validation study	-
Simultaneous treatment with other		Before the selection of index date	[0,0]	n/a	ATC	n/a	Exposure (rituximab), comparator	No validation study	-

immunosuppressive drugs							(dimethyl fumarate)		
Severe cardiac disorder		Before the selection of index date	[-∞,0]	Any	ICD-10	Any	Exposure (rituximab), comparator (dimethyl fumarate)	No validation study	-
Vaccination within 4 weeks of index		Before the selection of index date	[-30,0]	n/a	n/a	n/a	Exposure (rituximab), comparator (dimethyl fumarate)	No validation study	-
Clinically relevant ongoing infection or clinically significant somatic or psychiatric comorbidity		Before the selection of index date	[0,0]	Any	ICD-10	Any	Exposure (rituximab), comparator (dimethyl fumarate)	No validation study	-

[1] IP = inpatient, OP = outpatient, ED = emergency department, OT = other, n/a = not applicable
[2] See appendix for listing of clinical codes for each study parameter
[3] Specify whether a diagnosis code is required to be in the primary position (main reason for encounter)

7.4. Variables

7.4.1 Context and rationale for exposure(s) of interest

The new treatment initiators strategy avoids bias related to depletion of susceptibles and confounding by time varying indication. The use of dimethyl fumarate as an active comparator allows for comparisons of patients that are treated with the most used disease modifying treatments during the study period and at a similar level of disease severity.

Algorithm to define duration of exposure effect:

Rituximab infusions: Initial infusion with 1000 mg iv and thereafter 500 mg iv every 6 months. Extended dose interval up to 24 months is allowed. A washout window of 6 months will be added after the last infusion and considered exposed time.
Dimethyl fumarate: 240 mg twice daily with starting dose of 120 mg x 2 for one week. Longer titration period is allowed. A washout window of 30 days will be added after the treatment end and considered exposed time.

Table 6. Operational Definitions of Exposure

Exposure group name(s)	Details	Washout window	Assessment Window	Care Setting[1]	Code Type[2]	Diagnosis position[3]	Applied to study populations:	Incident with	Measurement characteristics/ validation	Source of algorithm

								respect to...		
Exposure (rituximab)	Full treatment history evaluation	[-∞, -1]	[1, censor]	n/a	ATC	n/a	Exposure, control	Rituximab (iv), dimethyl fumarate (oral)	No validation study	SMSreg
Control (dimethyl fumarate)	Full treatment history evaluation	[-∞, -1]	[1, censor]	n/a	ATC	n/a	Exposure, control	Rituximab (iv), dimethyl fumarate (oral)	No validation study	SMSreg

[1] IP = inpatient, OP = outpatient, ED = emergency department, OT = other, n/a = not applicable
[2] See appendix for listing of clinical codes for each study parameter
[3] Specify whether a diagnosis code is required to be in the primary position (main reason for encounter)

7.4.2 Context and rationale for outcome(s) of interest

The primary outcome is proportion of patients with a relapse during a 24-month observation period. Secondary objectives include time to first relapse, proportion of patients free from all MRI activity in a 24-months period, EDSS-based 24 week Confirmed Disease Worsening (CDW), change in EDSS from baseline to month 24, drug persistence, and no evidence of disease activity (NEDA)-2 and NEDA-3..

The EDSS-based 24 week CDW is defined as the proportion of patients reaching a 1 point or greater EDSS worsening from a baseline score of 1 or greater, or an increase of 1.5 or more points from baseline score of 0, that was sustained at an EDSS evaluation at 24 weeks or later. NEDA-2 is defined as no relapse and no MRI activity throughout the entire 24-month trial period. NEDA-3 is defined as NEDA-2 plus no evidence of disability worsening that was sustained for 24 weeks or more.

Table 7. Operational Definitions of Outcome

Outcome name	Details	Primary outcome?	Type of outcome	Washout window	Care Settings[1]	Code Type[2]	Diagnosis Position[3]	Applied to study populations:	Measurement characteristics/ validation	Source of algorithm
Relapse		Yes	Risk ratio, Time-to-event	n/a	n/a	n/a	n/a	Exposure (rituximab), control (dimethyl fumarate)	No validation study	SMSreg
MRI activity		No	Risk ratio	n/a	n/a	n/a	n/a	Exposure (rituximab), control	No validation study	SMSreg

								(dimethyl fumarate)		
EDSS-based CDW		No	Risk ratio	n/a	n/a	n/a	n/a	Exposure (rituximab), control (dimethyl fumarate)	No validation study	SMSreg
Drug persistence		No	Time-to-event	n/a	n/a	n/a	n/a	Exposure (rituximab), control (dimethyl fumarate)	No validation study	SMSreg
No evidence of disease activity NEDA-2/-3		No	Risk ratio	n/a	n/a	n/a	n/a	Exposure (rituximab), control (dimethyl fumarate)	No validation study	SMSreg
Time to confirmed sustained EDSS 4 and 6, SPMS		No	Time-to-event	n/a	n/a	n/a	n/a	Exposure (rituximab), control (dimethyl fumarate)	No validation study	SMSreg

[1] IP = inpatient, OP = outpatient, ED = emergency department, OT = other, n/a = not applicable
[2] See appendix for listing of clinical codes for each study parameter
[3] Specify whether a diagnosis code is required to be in the primary position (main reason for encounter)

7.4.3 Context and rationale for follow up

The analysis of all outcomes are done as intention-to-treat with patients included in the analysis according to their treatment initiation

Table 8. Operational Definitions of Follow Up

	Select all that apply	Specify
Follow up start	Week 0, Day1	
Follow up end[1]		
Date of outcome	Yes	For time-to-event outcomes
Date of death	Yes	Registry recorded death
End of observation in data	Yes	As recorded in registry

Day X following index date *(specify day)*	Yes	Month 24
End of study period (specify date)	No	n/a
End of exposure *(specify operational details, e.g. stockpiling algorithm, grace period)*	No	n/a
Date of add to/switch from exposure *(specify algorithm)*	Yes	Date the patient switches from exposure treatment to control treatment and vice versa
Other date *(specify)*	No	n/a

[1] Follow up ends at the first occurrence of any of the selected criteria that end follow up.

7.4.4 Context and rationale for covariates (confounding variables and effect modifiers, e.g. risk factors, comorbidities, comedications)

Covariates include variables that are expected a priori to be related to both DMT use and outcomes to mitigate against confounding.

Table 9. Operational Definitions of Covariates

Characteristic	Details	Type of variable	Assessment window	Care Settings[1]	Code Type[2]	Diagnosis Position[3]	Applied to study populations:	Measurement characteristics/ validation	Source for algorithm
Age at baseline		Continuous	[0,0]	n/a	n/a	n/a	Exposure (rituximab), control (dimethyl fumarate)	No validation study	SMSreg
Sex		Binary	[0,0]	n/a	n/a	n/a	Exposure (rituximab), control (dimethyl fumarate)	No validation study	SMSreg
Disease duration		Continuous	[0,0]	n/a	n/a	n/a	Exposure (rituximab), control (dimethyl fumarate)	No validation study	SMSreg
Treatment history		Categorical	[-∞,0]	n/a	n/a	n/a	Exposure (rituximab), control	No validation study	SMSreg

Characteristic	Details	Type of variable	Assessment window	Care Settings[1]	Code Type[2]	Diagnosis Position[3]	Applied to study populations:	Measurement characteristics/ validation	Source for algorithm
							(dimethyl fumarate)		
EDSS score		Continuous	[-730,0]	n/a	n/a	n/a	Exposure (rituximab), control (dimethyl fumarate)	No validation study	SMSreg
Number of relapses		Categorical	[-365,0]	n/a	n/a	n/a	Exposure (rituximab), control (dimethyl fumarate)	No validation study	SMSreg
T2 brain lesions		Categorical	[-365,0]	n/a	n/a	n/a	Exposure (rituximab), control (dimethyl fumarate)	No validation study	SMSreg
Contrast-enhancing lesions		Categorical	[-365,0]	n/a	n/a	n/a	Exposure (rituximab), control (dimethyl fumarate)	No validation study	SMSreg

[1] IP = inpatient, OP = outpatient, ED = emergency department, OT = other, n/a = not applicable
[2] See appendix for listing of clinical codes for each study parameter
[3] Specify whether a diagnosis code is required to be in the primary position (main reason for encounter)

7.5. Data analysis

7.5.1 Context and rationale for analysis plan

The proportion of patients with relapse during a 24 month observation period, the proportion of patients free from all MRI activity and the proportion of patients with confirmed EDSS score worsening will be analysed by log-binomial regression model similar to the RIFUND-MS trial. Time to 1st relapse, drug persistence, time to NEDA-2/-3 and time to confirmed sustained EDSS 4 and 6 and SPMS will be analysed by Cox Proportional Hazards regression. Inverse probability of treatment weighting (IPtW) will be applied to all analysis to mitigate confounding based on covariates described in Table 9.

Table 10. Primary, secondary, and subgroup analysis specification

A. Primary analysis 1

Hypothesis:	The comparative effectiveness of Rituximab versus Dimethyl fumarate in the real-world setting using strict inclusion/exclusion criteria is similar to the comparative efficacy in the previously published phase 3 randomized study RIFUND-MS.
Exposure contrast:	Rituximab vs. dimethyl fumarate
Outcome:	Primary: Proportion of patients with relapse during the 24-month observational period Secondary: Time to first relapse Proportion of patients free from all MRI activity during the 24-month observation period EDSS-based 24 week Confirmed Disability Worsening (CDW) Change in EDSS from baseline to month 24 Drug persistence No evidence of disease activity NEDA-2/-3
Analytic software:	R
Model(s): ***(provide details or code)***	Log-binomial regression model Cox proportional hazards model
Confounding adjustment method	***Name method and provide relevant details, e.g. bivariate, multivariable, propensity score matching (specify matching algorithm ratio and caliper), propensity score weighting (specify weight formula, trimming, truncation), propensity score stratification (specify strata definition), other.***
	The log-binomial and Cox proportional hazards models will be applied on the IPtW population, using a term for treatment and adjusting for any baseline covariates that are not balanced in the IPtW population.
Missing data methods	***Name method and provide relevant details, e.g. missing indicators, complete case, last value carried forward, multiple imputation (specify model/variables), other.***
	Patients with missing or unknown age or sex will be excluded. Patients with a missing EDSS score within 2 years prior the index date will be excluded. If too many patients are missing baseline EDSS a categorical EDSS variable will be considered with a “Missing” level included. Lack of documented relapse in the SMSreg is interpreted as no relapse occurrence.
Subgroup Analyses	***List all subgroups***
	n/a

B. Primary analysis 2

Hypothesis:	The comparative effectiveness of Rituximab versus Dimethyl fumarate on MRI activity in the real-world setting using a pragmatic scenario of inclusion/exclusion criteria is similar to the comparative efficacy in the previously published phase 3 randomized study RIFUND-MS.
Exposure contrast:	Rituximab vs. dimethyl fumarate
Outcome:	Primary: Proportion of patients with relapse during the 24-month observational period Secondary: Time to first relapse Proportion of patients free from all MRI activity during the 24-month observation period EDSS-based 24 week Confirmed Disability Worsening (CDW) Change in EDSS from baseline to month 24 Drug persistence No evidence of disease activity NEDA-2/-3
Analytic software:	R
Model(s): ***(provide details or code)***	Log-binomial regression model Cox proportional hazards model
Confounding adjustment method	***Name method and provide relevant details, e.g. bivariate, multivariable, propensity score matching (specify matching algorithm ratio and caliper), propensity score weighting (specify weight formula, trimming, truncation), propensity score stratification (specify strata definition), other.***
	The log-binomial and Cox models will be applied on the IPtW population, using a term for treatment and adjusting for any baseline covariates that are not balanced in the IPtW population.
Missing data methods	***Name method and provide relevant details, e.g. missing indicators, complete case, last value carried forward, multiple imputation (specify model/variables), other.***
	Patients with missing or unknown age or sex will be excluded. Patients with a missing EDSS score within 2 year prior the index date will be excluded. If too many patients are missing baseline EDSS a categorical EDSS variable will be considered with a “Missing” level included. Lack of documented relapse in the SMSreg is interpreted as no relapse occurrence.
Subgroup Analyses	***List all subgroups***

	n/a

C. Secondary Analysis 2

Hypothesis:	The comparative effectiveness of Rituximab versus Dimethyl fumarate on disease progression in the real-world setting using strict inclusion/exclusion criteria is similar to the comparative efficacy in the previously published phase 3 randomized study RIFUND-MS.
Exposure contrast:	Rituximab vs. dimethyl fumarate
Outcome:	EDSS-based 24 week Confirmed Disability Worsening (CDW) Time to confirmed sustained EDSS 4 and 6 Time to SPMS
Analytic software:	R
Model(s): ***(provide details or code)***	Log-binomial regression model Cox proportional hazards model
Confounding adjustment method	***Name method and provide relevant details, e.g. bivariate, multivariable, propensity score matching (specify matching algorithm ratio and caliper), propensity score weighting (specify weight formula, trimming, truncation), propensity score stratification (specify strata definition), other.***
	The Log-binomial and Cox models will be applied on the IPtW population, using a term for treatment and adjusting for any baseline covariates that are not balanced in the IPtW population.
Missing data methods	***Name method and provide relevant details, e.g. missing indicators, complete case, last value carried forward, multiple imputation (specify model/variables), other.***
	Patients with missing or unknown age or sex will be excluded. Patients with a missing EDSS score within 2 year prior the index date will be excluded. If too many patients are missing baseline EDSS a categorical EDSS variable will be considered with a “Missing” level included. Lack of documented relapse in the SMSreg is interpreted as no relapse occurrence.
Subgroup Analyses	***List all subgroups***
	n/a

Table 11. Sensitivity analyses – rationale, strengths and limitations

What is being varied? How?	Why? (What do you expect to learn?)	Strengths of the sensitivity analysis compared to the primary	Limitations of the sensitivity analysis compared to the primary

Sensitivity analysis 1	Expansion of inclusion period to 2014/01/01 – 2021/12/31	This will allow for larger population size that covers a longer time period and therefore cover more accurately the real world clinical practice.	Larger population size	Patients included during later years may differ in characteristics compared to those included in the earlier years. Adjustment for year of inclusion may be necessary.
Sensitivity analysis 2	Patients diagnosed with secondary-progressive multiple sclerosis (SPMS) will be included in the analysis.	The diagnosis of SPMS is set retrospectively after a transitioning period of observation. During this period the distinction of RRMS and SPMS may not be possible.	Bigger sample size and inclusion of patients that typically are not included in clinical trials but are treated in clinical practice	Deviation from the RIFUNS-MS protocol
Sensitivity analysis 3	Removal of "typical" RIFUND-MS population from the study population (patients that meet the strict inclusion/exclusion criteria)	Information on efficacy of the target therapy in populations not typically included in clinical trials is missing.	Analysis of patients that do not resemble the typical patient included in the clinical trial but are treated with the target therapies in everyday clinical practice. More accurate estimates for this subgroup of patients.	Deviation from the RIFUND-MS trial, smaller sample size, difficult to compare estimates.

7.6. Data sources

7.6.1 Context and rationale for data sources

Reason for selection: We will use the Swedish Multiple Sclerosis Registry (SMSreg)

Strengths of data source(s): The SMSreg is a nation-wide disease registry with a coverage of around 85% of the prevalent cases in Sweden. The registry routinely collects information on treatments, relapses, EDSS scores and other relevant variables and it is therefore of high quality.

Limitations of data source(s): Not all covariates may be collected in the SMSreg (eg. comorbidities).

Data source provenance/curation: The SMSreg has been widely used in research with more than 180 scientific reports based in whole or in part on data from the SMSreg.

Table 12. Metadata about data sources and software

	Data 1
Data Source(s):	Swedish Multiple Sclerosis Registry (SMSreg)
Study Period:	2014-01-01 to 2019-12-31
Eligible Cohort Entry Period:	2014-01-01 to 2017-12-31

Data Version (or date of last update):	n/a
Data sampling/extraction criteria:	All patients in the SMSreg
Type(s) of data:	Disease registry
Data linkage:	n/a
Conversion to CDM*:	Yes
Software for data management:	R

*CDM = Common Data Model

7.7. Data management

Data will be pseudo-anonymized and stored to the secured system that Karolinska Institutet (KI) provides to all it's researchers (OneDrive). Data will be analysed locally at KI. After the completion of the study all data will be documented and archived according to KI rules.

7.8. Quality control

Patients with missing age and sex will be excluded. Treatment records with missing end dates will be assumed as ongoing treatments or the end dates will be imputed to the day before the start of the next treatment. Overlapping treatments are not be allowed. Records with missing dates will be removed. All variables will be checked for logical intervals (eg. EDSS 0-10). Any recording outside expected values will be removed. In case of missing values no imputation will be applied but a categorization of the variables including a "missing" category will be considered.

7.9. Study size and feasibility

The same reasoning as in the RIFUND-MS trial will be followed. Assuming 72% of the DMF patients and 90% of the rituximab patients to be relapse-free over two years, a significance level of 5% and power of 80% and 15% of the DMF patients changing treatment before the endpoint is reached results in a sample size of 99 patients per treatment group.

Table 13. Power and sample size

See 7.9

8. Limitation of the methods

There is a degree of underreporting of relapses in the SMSreg.

EDSS score may not be available at index date. The one used in this study (-2 years from index date) may therefore differ from the real one. Moreover, EDSS scores are not measured at each clinical visit in every-day practice as the patients' needs have priority over data collection. This may create a missingness pattern that is not at random.

Transition from RRMS to SPMS is a slow process that can only be identified at a later stage. This can result in SPMS patients treated as RRMS patients during this transition period.

9. Protection of human subjects

Patient data will be anonymized and analysed locally at KI.

10. Reporting of adverse events

n/a

11. References

Svenningsson A., et al., *Safety and efficacy of rituximab versus dimethyl fumarate in patients with relapsing-remitting multiple sclerosis or clinically isolated syndrome in Sweden: a rater-blinded, phase 3. Randomized controlled trial*. The Lancet Neurology, 2022. 21(8): p. 693-703 https://doi.org/10.1016/S1474-4422(22)00209-5

12. Appendices