COVER PAGE

Official Title	An observational registry-based study to evaluate the long-term safety of tofersen in people with SOD1-ALS
Protocol ID	233AS401
Study Indication	Amyotrophic lateral sclerosis
Document Date	12 June 2025

PASS PROTOCOL

TITLE:	An observational registry-based study to evaluate the long-term safety of tofersen in people with *SOD1*-ALS
PROTOCOL VERSION IDENTIFIER:	233AS401, Version 3.0
DATE OF LAST VERSION OF PROTOCOL:	07 March 2025
HMA-EMA CATALOGUE NUMBER:	Study will be registered following Pharmacovigilance Risk Assessment Committee approval
ACTIVE SUBSTANCE:	Tofersen
MEDICINAL PRODUCT:	Qalsody
PRODUCT REFERENCE:	EMEA/H/C/005493
PROCEDURE NUMBER:	EMEA/H/C/PSP/S/0109
MARKETING AUTHORISATION HOLDER:	Biogen Netherlands B.V. Prins Mauritslaan 13 1171 LP Badhoevedorp The Netherlands
JOINT PASS:	No
RESEARCH QUESTION AND OBJECTIVES:	The study's research questions are related to characterizing the long-term safety and tolerability of tofersen in routine clinical practice among adults with amyotrophic lateral sclerosis (ALS) who have a mutation in the *SOD1* gene. All objectives will be stratified by tofersen treatment status except secondary objective 2 (assessed among tofersen users only). All data will be summarized by descriptive statistics. **Primary objectives** 1. To describe demographic and clinical characteristics of people with *SOD1*-ALS.

2. To describe the frequency of serious adverse events (SAE) among people with *SOD1*-ALS, including serious neurologic events previously reported in clinical trial participants (e.g., myelitis, radiculitis, aseptic meningitis, increased intracranial pressure, and/or papilloedema).

Secondary objectives

1. To describe the frequency of new comorbid conditions, pregnancy and pregnancy outcome among people with *SOD1*-ALS.
2. To describe the frequency of treatment discontinuation among people with *SOD1*-ALS treated with tofersen.

[redacted]

1. [redacted]
2. [redacted]
3. [redacted]

COUNTRIES OF THE STUDY:

Precision-ALS programme: participating TRICALS sites in multiple European countries including Ireland, Portugal, Netherlands, Belgium, Sweden, Switzerland, UK, Italy, Spain, Germany, and France.

ALS/ MND NHC: clinical centres in the United States.

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1. TABLE OF CONTENTS

LIST OF TABLES

2. LIST OF ABBREVIATIONS

Note: The following abbreviations and their definitions are considered first-use in this document. Please refer to this list for abbreviations used in this document.

AE	adverse event
ALS	amyotrophic lateral sclerosis
ALSFRS-R	Amyotrophic Lateral Sclerosis Functional Rating Scale – Revised
ALS/MND NHC	ALS/MND Natural History Consortium
BMI	body mass index
CRF	case report form
DALYS	disability adjusted life years
EMA	European Medicines Agency
EU	European Union
[redacted]	[redacted]
GVP	Good Pharmacovigilance Practice
HMA	Heads of Medicine Agency
HRQoL	health related quality of life
ICD	International Classification of Diseases
ICH	International Council for Harmonisation
LP	lumbar puncture
MA	marketing authorisation
MAH	marketing authorisation holder
MedDRA	Medical Dictionary for Regulatory Activities
MND	motor neuron disease
N/A	not applicable
PAS	post-authorisation study
PASS	post-authorisation safety study
PT	preferred term
QALYs	quality adjusted life years
RMP	risk management plan
SAE	serious adverse event
SAP	statistical analysis plan
SOC	system organ class
SOD1	superoxide dismutase 1
SOD1-ALS	amyotrophic lateral sclerosis with a confirmed superoxide dismutase 1 mutation
[redacted]	[redacted]
TRICALS	Treatment Research Initiative to Cure ALS
UK	United Kingdom
US FDA	United States Food and Drug Administration
WHO	World Health Organization

3. RESPONSIBLE PARTIES

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

4. ABSTRACT

Title:	An observational registry-based study to evaluate the long-term safety of tofersen in people with *SOD1*-ALS
Version Number:	3.0
Date of Protocol:	12 June 2025
Name and Affiliation of Main Author:	Dr. [redacted] Biogen
Rationale and Background:	Amyotrophic lateral sclerosis (ALS), also known as motor neuron disease (MND), is a rare, progressive, and ultimately fatal neurodegenerative disease that causes loss of motor neurons in the brain and the spinal cord that are responsible for controlling voluntary muscle movement. The prevalence of ALS in Europe is estimated to be approximately 6.55 per 100,000 persons. In approximately 2% of people living with ALS, the disease is attributed to mutation(s) in the gene encoding the enzyme superoxide dismutase 1 (SOD1), which results in the production and accumulation of a toxic form of SOD1 protein. This study is part of the planned pharmacovigilance activities for tofersen (tradename: Qalsody™). Qalsody has been approved in multiple regions, including by both the United States Food and Drug Administration (US FDA) (April 2023) and the European Medicines Agency (EMA) (May 2024). In Europe, Qalsody was granted marketing authorisation (MA) under exceptional circumstances with the indication of treatment of adults with ALS associated with a mutation in the *SOD1* gene. In the clinical studies, serious neurological events of myelitis, radiculitis, increased intracranial pressure, papilloedema, and aseptic meningitis have been reported in participants receiving tofersen and not in placebo treated participants. Details on these serious reactions are included in the European Union (EU) product information. One of the main aims of this registry-based study is to further evaluate these serious neurological events under routine medical care. Data from 2 existing networks of ALS disease registries, the Treatment Research Initiative to Cure ALS (TRICALS) network's Precision-ALS programme and the ALS/NMD Natural History Consortium (NHC), will be leveraged for this study. While the primary aim of this study is to evaluate long-term safety of tofersen among people with *SOD1*-ALS, [redacted]

Research Question and Objectives:	The study's research questions are related to characterizing the long-term safety and tolerability of tofersen in routine clinical practice among adults with ALS who have a mutation in the *SOD1* gene. All objectives will be stratified by tofersen treatment status except secondary objective 2 (assessed among tofersen users only). All data will be summarized by descriptive statistics. **Primary objectives** 1. To describe demographic and clinical characteristics of people with *SOD1*-ALS. 2. To describe the frequency of serious adverse events (SAE)[1] among people with *SOD1*-ALS, including serious neurologic events previously reported in clinical trial participants (e.g., myelitis, radiculitis, aseptic meningitis, increased intracranial pressure, and/or papilloedema). **Secondary objectives** 1. To describe the frequency of new comorbid conditions, pregnancy and pregnancy outcome among people with *SOD1*-ALS. 2. To describe the frequency of treatment discontinuation among people with *SOD1*-ALS treated with tofersen. [redacted] 1. [redacted] 2. [redacted] 3. [redacted]

[1] An SAE in this registry-based study protocol is any untoward medical occurrence, that, regardless of treatment status, meets the criteria listed in Section 16.

Study Design:	This study is an observational, registry-based study of people with *SOD1*-ALS using data collected by 2 existing networks of ALS disease registries: 1) the TRICALS network's Precision-ALS programme with data from participating clinical centres across multiple European countries and 2) ALS/MND NHC with data collected from clinical centres in the United States. The data is collected via disease registry networks of prospective observational cohort design and consolidated in shared data platforms. All data are collected by the registry networks as part of their longitudinal data collection, and for the purposes of this study, the analysis of the data is considered secondary use of the registry data. As the data in the registries are collected according to routine clinical practice and their respective observational plans, there are no interventions proposed that might pose any additional risk. Participants are expected to be followed in the registry networks as per their respective protocols until death or loss to follow-up. The study will be based on the routine medical care in the geographies. The study will include data on all *SOD1*-ALS cases enrolled in the Precision-ALS platform and ALS/MND NHC, including prevalent *SOD1*-ALS cases at start of data collection and incident *SOD1*-ALS cases, irrespective of tofersen treatment status. All endpoints will be stratified by tofersen treatment status except secondary endpoint 2 (assessed among tofersen users only). All data will be summarized by descriptive statistics. **Primary endpoints** 1. Demographic and clinical characteristics, using descriptive statistics. 2. Frequency of SAEs. **Secondary endpoints** 1. Number and proportion of participants with new comorbid conditions, or pregnancy. 2. Number and proportion of participants with reported treatment discontinuation and listing of reported reasons for discontinuation. [redacted] 1. [redacted] 2. [redacted] 3. [redacted]
Population:	Adults with *SOD1*-ALS enrolled in TRICALS network's Precision-ALS programme with data from participating clinical centres across multiple European countries or at ALS/MND NHC with data from participating clinical centres in the United States (tofersen users and tofersen non-users). The 2 networks of ALS registry platforms/consortia are considered representative of people diagnosed with *SOD1*-ALS in Europe and the United States.

Variables:	Data from the registry networks will be provided for inclusion in this study, as available. Follow-up data is anticipated to be available from the registry networks as per their respective observational plans depending on local clinical practice. **Exposure** • Tofersen exposure (dose and duration), as reported in the registry data. **Primary endpoints** • Demographic and clinical characteristics at the corresponding index date for treated and untreated participants for each objective: demographics (e.g., age, sex, race/ethnicity or grandparental origin, weight, height, body mass index [BMI], family history of ALS), clinical characteristics (e.g., age at diagnosis, age at symptom onset, Revised El Escorial classification, phenotype, *SOD1* mutation type), medical history as available, concomitant medications (e.g., medication name, dose and unit, start and stop dates), disease history (e.g., ALSFRS-R scores, history of ventilation [invasive, non-invasive], tracheostomy, feeding tube), pregnancy status. • SAEs (e.g., event name, SAE start and stop date, whether SAE required hospitalization, outcome of SAE etc.). **Secondary endpoints** • Changes in medical history and new comorbid conditions, medications (new or changed medications during follow-up), vital status, pregnancy status and pregnancy outcome. • Treatment discontinuation and reported reasons for discontinuation. [redacted] • [redacted] • [redacted] • [redacted]
Data Sources:	This study focuses on the secondary use of data from 2 large networks of ALS disease registries, the TRICALS network's Precision-ALS programme and ALS/MND NHC. These data sources are networks of ongoing longitudinal ALS disease registries. Individual participant-level data will be collected at routine clinic visits by each registry network. In advance of initiation of this study, both networks have been engaged to implement treatment and SAE data collection in their registries.

Study Size:	Data will be extracted on all *SOD1*-ALS cases enrolled or entering in the Precision-ALS platform and ALS/MND NHC registry networks at study start and data on all incident *SOD1*-ALS cases enrolling in the registry during the first 5 years of the study. Thus, participants present or entering the registry networks during the first 5 years of the study may be included in the analyses. Given the difficulty in predicting enrolment of *SOD1*-ALS cases, the study will monitor enrolment and reassess the enrolment period duration while the study is ongoing. For a 5-year enrolment period, assuming a total of 25 *SOD1*-ALS cases per year could be enrolled, approximately 125 cases could be expected across both registry networks. Participants are expected to be followed in the registry networks as part of their respective observational plans until death or loss to follow-up, regardless of tofersen treatment status. Assuming a median follow-up time of approximately 2 years, an enrolment of 25 cases per year, and an estimate of 40% tofersen treatment proportion, 100 person-years of follow-up data could be expected among tofersen-treated participants which will allow evaluation of the incidence of serious neurological events. Therefore, the target study size is at least 100 person-years of follow-up from tofersen-treated participants or at least 125 total *SOD1*-ALS cases (tofersen-treated or untreated) during the first 5 years of the study. The enrolment period is currently planned for a minimum of 5 years, and the suitability of that time frame will be reassessed during the study. Using the target of 100 person-years of follow-up data collection by the registry networks, the confidence intervals widths and precision of the estimate of 0.033 serious neurologic events per person-year can be calculated based on a Poisson distribution. The expected study end date may be modified based on study progress, which will be reviewed annually as part of the reassessment procedure.

Data Analysis:	Data will be analyzed separately for each registry network. However, the analysis performed will be harmonized as much as possible, where data capture and actual participant and event numbers allow. Statistical analyses will generally be descriptive in nature and there is no *a priori* hypothesis to be tested. Continuous variables will be described with summary statistics including number of observations, mean, standard deviation, median, first quartile, third quartile, and minimum and maximum values. Categorical data will be described in terms of frequencies and percentages. An additional category of 'missing' will be added to account for missing data. Event rates may also be calculated as deemed appropriate. All efforts will be made to collect data as completely as possible but incomplete dates could occur and this must be considered when evaluating the event and censoring times. Partial dates with missing information will be imputed as detailed in the statistical analysis plan (SAP). Analyses will be conducted on all adults with *SOD1*-ALS enrolled in Precision-ALS and ALS/MND NHC, including participants treated with tofersen as well as untreated participants. All analyses will be stratified by tofersen treatment status except secondary objective 2 (assessed among tofersen users only). Further details on the exclusion of data from clinical trials and the impact on index dates will be detailed in the SAP. Comparisons between treated and untreated patients for outcomes related to safety, tolerability, or effectiveness are not planned, owing to the inability to address bias in the analyses, given the small sample size for *SOD1*-ALS and uncertainties around patient selection for tofersen treatment. All participants, including participants who discontinue tofersen, will be followed until death or loss to follow-up. Sensitivity analyses will be performed censoring data 150 days (5 half-lives) after tofersen discontinuation (after which data will then be excluded from the analysis). Data collected while a person with *SOD1*-ALS is participating in an interventional clinical trial (with tofersen or any other investigational medicinal product) will be excluded since this study will focus on real-world data and the results from interventional clinical trials will be available elsewhere. The analysis population and definition of index date for statistical analysis will differ by treatment status and by study objective. In general terms, index date will be the date of tofersen treatment for data from participants in the treated group or date of enrolment into the registry for data from participants in the untreated group [redacted]. Sensitivity analyses will be performed excluding retrospectively treated participants (i.e., those who initiated tofersen treatment prior to enrolment in the registry networks).
Milestones:	An interim progress report will be submitted annually as part of the annual reassessment procedure.

5. AMENDMENTS AND UPDATES

Table 1: Changes to Protocol 233AS401

Version No.	Date	Section of Study Protocol	Amendment or Update	Reason
2.0[a]	07 March 2025	Global	Amendment to milestones, research objectives, endpoints, study location, variables, study size, data analysis, and limitations of the research methods	The study size and data analysis sections were amended to specify the targeted study size and to distinguish clearly between analyses proposed regarding safety objectives versus exploratory objectives, respectively, in accordance with the comments received from the Pharmacovigilance Risk Assessment Committee.
3.0	12 June 2025	Section 6 and Annex 2	Amendment to milestones table and Annex 2	The milestones table was amended to clarify that the potential continuation of the study will be determined based on the data available at the end of data collection (data extraction).

[a] This protocol amendment was implemented before the initiation of data collection.

6. MILESTONES

Table 2: Milestones for Protocol 233AS401

Milestone	Planned Date
Start of data collection (data extraction)[a]	30 January 2026
End of data collection (data extraction)[a, b]	30 July 2033
Interim progress reports	Annually (with reassessment)
Registration in HMA-EMA catalogue of real-world data studies[c]	31 October 2025
Report of study results	Within 12 months of end of data collection (data extraction)

[a] Per European Union (EU) Guideline on good pharmacovigilance practices ([EMA (EMA/330405/2012) 2012] Section A.1), the start and end of data collection for secondary use of data are the date from which data extraction starts and the date from which the analytical datasets are available.

[b] The end of data extraction for this study is set to 7 years from start of data extraction. The expected end date of data extraction may be modified based on study progress, which will be reviewed annually as part of the reassessment procedure. The potential continuation of the study will be determined based on the results obtained at the targeted end of data collection (data extraction).

[c] Study will be registered in Heads of Medicine Agency-European Medicines Agency (HMA-EMA) catalogue of real-world data studies following Pharmacovigilance Risk Assessment Committee approval of final protocol and prior to study initiation.

7. RATIONALE AND BACKGROUND

7.1. Introduction

Amyotrophic lateral sclerosis (ALS), also known as motor neuron disease (MND), is a rare, progressive, and ultimately fatal neurodegenerative disease that causes loss of motor neurons in the brain and the spinal cord that are responsible for controlling voluntary muscle movement. The progressive loss of motor neurons typically leads to paralysis and ultimately death from respiratory failure.

The prevalence in Europe is estimated to be approximately 6.55 per 100,000 persons based on the pooled prevalence estimates of individual studies in Europe excluding the United Kingdom (UK) [Brown 2021]. In approximately 2% of people living with ALS, the disease is attributed to mutation(s) in the gene encoding the enzyme superoxide dismutase 1 (SOD1) [Bunton-Stasyshyn 2015], hereafter referred to as *SOD1*-ALS.

Though the mechanisms by which mutations in *SOD1* cause degeneration in ALS are not fully understood, mutations in this gene are thought to cause an accumulation of toxic forms of SOD1 protein via a gain of function mechanism [Trist 2022; Trist 2021]. This neurotoxic property can arise from various primary causes, all of which increase the propensity of the SOD1 protein to aggregate [Allen 2003; Andersen 1995; Bruijn 1998; Ekhtiari Bidhendi 2018; Ilieva 2009; McCampbell 2018; Saccon 2013; Sau 2007]. Over 200 causative *SOD1* mutations associated with ALS have been identified to date [ALSoD 2023], with a median survival time of 2.3 years [Opie-Martin 2022], although heterogeneity exists between *SOD1* mutation types [Huang 2024].

7.2. Study Rationale and Regulatory Background

This study is part of the planned pharmacovigilance activities for tofersen (tradename: Qalsody™). Qalsody has been approved in multiple regions, including by both the United States Food and Drug Administration (US FDA) (April 2023) and the EMA (May 2024). In Europe, Qalsody was granted marketing authorisation (MA) under exceptional circumstances with the indication of treatment of adults with ALS associated with a mutation in the *SOD1* gene. As part of the conditions of the MA under exceptional circumstances, the marketing authorisation holder (MAH) agreed to conduct and submit the results of this observational registry-based study (Study 233AS401), to further evaluate the long-term safety of tofersen in people living with *SOD1*-ALS. This specific obligation is included as a Category 2 study in the EU risk management plan (RMP).

The safety and efficacy of tofersen was assessed in clinical trials and has a positive benefit/risk profile in the treatment of adults with *SOD1*-ALS. However, information on long-term use is limited. Given this and the rarity of the indication, this non-interventional registry-based study is being conducted as part of the post approval pharmacovigilance activities to further evaluate the long-term safety of tofersen among people with *SOD1*-ALS under routine medical care.

The safety profile is based on 166 participants with ALS (162 with *SOD1*-ALS) that were exposed to any dose of tofersen in the clinical development programs. Of these, a total of

147 people with *SOD1*-ALS received tofersen 100 mg, for 368.83 participant-years. The median exposure for this group was 148.4 weeks. Most adverse events (AE) reported during treatment with tofersen were consistent with the types and severities of AEs seen in people with *SOD1*-ALS or events observed in the context of the lumbar puncture (LP) procedure.

In the clinical studies, serious neurological events of myelitis, radiculitis, increased intracranial pressure, papilloedema, and aseptic meningitis have been reported in participants receiving tofersen and not in placebo treated participants. Details on these serious reactions are included in the EU product information.

One of the main aims of this registry-based study is to further evaluate these serious neurological events under routine medical care. Data from 2 existing networks of ALS disease registries, the TRICALS network's Precision-ALS programme [McFarlane 2023] and the ALS/NMD NHC [Berger 2023], will be leveraged for this study. In both registry networks, data collection is encounter-based with comprehensive assessments of healthcare received and health outcomes. This study will provide additional safety data to further characterize the safety profile of tofersen, both regarding the risks mentioned above and other serious events, during long-term use in routine clinical practice. As with many clinical studies, the tofersen clinical development programme employed specific exclusion criteria including female participants who were pregnant, as a result there are no clinical data from the use of tofersen in pregnant women. However, data from studies in animals in which tofersen is not pharmacologically active do not indicate direct or indirect harmful effects with respect to reproductive toxicity. This registry-based study provides an opportunity to collect frequency data on pregnancy and pregnancy outcome among people with *SOD1*-ALS and are included as a secondary study objective.

While the primary aim of this study is to evaluate long-term safety of tofersen among people with *SOD1*-ALS, [redacted]

8. RESEARCH QUESTION AND OBJECTIVES

8.1. Research Question

The study's research questions are related to characterizing the long-term safety and tolerability of tofersen in routine clinical practice among adults with ALS who have a mutation in the *SOD1* gene.

All objectives will be stratified by tofersen treatment status except secondary objective 2 (assessed among tofersen users only). All data will be summarized by descriptive statistics.

8.2. Primary Objectives

1. To describe demographic and clinical characteristics of people with *SOD1*-ALS.
2. To describe the frequency of SAEs[1] among people with *SOD1*-ALS, including serious neurologic events previously reported in clinical trial participants (e.g., myelitis, radiculitis, aseptic meningitis, increased intracranial pressure, and/or papilloedema).

8.3. Secondary Objectives

1. To describe the frequency of new comorbid conditions, pregnancy and pregnancy outcome among people with *SOD1*-ALS.
2. To describe the frequency of treatment discontinuation among people with *SOD1*-ALS treated with tofersen.

[redacted]

1. [redacted]
2. [redacted]
3. [redacted]

[1] An SAE in this registry-based study protocol is any untoward medical occurrence, that, regardless of treatment status, meets the criteria listed in Section 16.

9. RESEARCH METHODS

9.1. Study Design

This study is an observational, registry-based study of people with *SOD1*-ALS using data collected by 2 existing networks of ALS disease registries: 1) the TRICALS network's Precision-ALS programme with data from participating clinical centres across multiple European countries and 2) ALS/MND NHC with data collected from clinical centres in the United States. The data is collected via disease registry networks of prospective observational cohort design and consolidated in shared data platforms. All data are collected by the registry networks as part of their longitudinal data collection, and for the purposes of this study, the analysis of the data is considered secondary use of the registry data. As the data in the registries are collected according to routine clinical practice and their respective observational plans, there are no interventions proposed that might pose any additional risk.

Participants are expected to be followed in the registry networks as per their respective protocols until death or loss to follow-up. The study will be based on the routine medical care in the geographies. The study will include data on all *SOD1*-ALS cases enrolled in the Precision-ALS platform and ALS/MND NHC, including prevalent *SOD1*-ALS cases at the start of data collection and incident *SOD1*-ALS cases, irrespective of tofersen treatment status. The assessment of incident drug users is a key feature to avoid immortal time bias [Suissa 2008] and depletion of susceptibles in real-world evidence studies [Moride and Abenhaim 1994]. However, in the case of ultra-rare diseases (< 1 case per 50,000 population) like *SOD1*-ALS [Brown 2021], the inclusion of prevalent *SOD1*-ALS cases including tofersen users at the start of data extraction will allow the collection of as much data as possible.

All endpoints will be stratified by tofersen treatment status except secondary endpoint 2 (assessed among tofersen users only). All data will be summarized by descriptive statistics.

9.1.1. Primary Endpoints

1. Demographic and clinical characteristics, using descriptive statistics.
2. Frequency of SAEs.

9.1.2. Secondary Endpoints

1. Number and proportion of participants with new comorbid conditions, pregnancy, and pregnancy outcome.
2. Number and proportion of participants with reported treatment discontinuation and listing of reported reasons for discontinuation.

[redacted]

1. [redacted]

2. [redacted]
3. [redacted]

9.2. Setting

9.2.1. Selection Criteria

Adults with *SOD1*-ALS enrolled in TRICALS network's Precision-ALS programme with data from participating clinical centres across multiple European countries or at ALS/MND NHC with data from participating clinical centres in the United States (tofersen users and tofersen non-users). The 2 networks of ALS registry platforms/consortia are considered representative of people diagnosed with *SOD1*-ALS in Europe and the United States.

9.2.2. Study Location

This study will leverage data on people with *SOD1*-ALS collected by the TRICALS network's Precision-ALS programme in multiple European countries including Ireland, Portugal, Netherlands, Belgium, Sweden, Switzerland, UK, Italy, Spain, Germany, France and ALS/MND NHC in the United States. In the case that the registry networks expand, any new countries and sites contributing data to the Precision-ALS platform may be considered for inclusion in this study. New countries and sites contributing data to ALS/MND NHC may be considered for inclusion in this study, if the contributing countries do not overlap with participating countries already included in this study as part of the Precision-ALS programme to avoid duplicate reporting on the same person with *SOD1*-ALS.

9.2.3. Overall Study Duration and Follow-Up

All participants with an ALS diagnosis and a confirmed *SOD1* mutation from contributing registry networks will be considered for inclusion in the study. Although this specific obligation currently has an unlimited duration, for the purpose of planning (e.g., final report), the study protocol plans for the end of data extraction for this study to be 7 years after the study start. Participants present or entering the registries during the first 5 years of the study may be included in the analyses, and the 7-year study duration allows the possibility for 2 years of follow-up for patients entering in Year 5. The expected end date may be modified based on study progress, which will be reviewed annually as part of the reassessment procedure.

Follow-up data are collected by the registry networks as part of their longitudinal data collection. All data are collected according to routine clinical practice in routine encounter-based clinic visits per the registry networks' protocols, which may occur at differing intervals, depending on the local standards for patient care. Participants are expected to be followed in the registry networks as per their respective observational

plans until death or loss to follow-up, regardless of tofersen treatment status. The contributing registries will not include data from participants who have withdrawn the required consents. For this study, all available data will be obtained and reported as part of the annual reassessment procedure throughout the study period.

9.3. Variables

Data from the registry networks will be provided for inclusion in this study, as available. Follow-up data is anticipated to be available from the registry networks as per their respective observational plans depending on local clinical practice.

Exposure

- Tofersen exposure (dose and duration), as reported in the registry data.

Primary Endpoints

- Demographic and clinical characteristics at the corresponding index date for treated and untreated participants for each objective:
  - Demographics (e.g., age, sex, race/ethnicity or grandparental origin, weight, height, BMI, family history of ALS).
  - Clinical characteristics (e.g., age at diagnosis, age at symptom onset, Revised El Escorial classification, phenotype, *SOD1* mutation type).
  - Medical history as available.
  - Concomitant medications (e.g., medication name, dose and unit, start and stop dates).
  - Disease history (e.g., ALSFRS-R scores, history of ventilation [invasive, non-invasive], tracheostomy, feeding tube).
  - Pregnancy status.
- SAEs (e.g., event name, SAE start and stop date, whether SAE required hospitalization, outcome of SAE etc.).

Secondary Endpoints

- Changes in medical history and new comorbid conditions, medications (new or changed medications during follow-up), vital status, pregnancy status and pregnancy outcome.
- Treatment discontinuation and reported reasons for discontinuation.

[redacted]

- [redacted]
- [redacted]
- [redacted]

9.4. Data Sources

Disease registries have been previously used to assess the long-term safety in post marketing commitments requested by the US FDA and the EMA. This study focuses on the secondary use of data from 2 large networks of ALS disease registries, the TRICALS network's Precision-ALS programme [McFarlane 2023] and ALS/MND NHC [Berger 2023]. These data sources are networks of ongoing longitudinal ALS disease registries. Individual participant-level data will be collected at routine clinic visits by each registry network (Section 9.3). In advance of initiation of this study, both networks have been engaged to implement treatment and SAE data collection in their registries. The Precision-ALS data platform was developed by a consortium comprising international clinicians from the European ALS Research entity TRICALS working closely with computational data and scientists based in the Science Foundation Ireland-funded ADAPT research centre in Ireland [ADAPT 2024]. TRICALS is the world's largest network of specialty ALS centres, collecting and combining participant data from centres in multiple countries across Europe. The Precision-ALS programme is a research platform, consisting of a longitudinal data collection infrastructure based on electronic medical records as well as primary data collection for some variables. Precision-ALS includes ALS referral centres across multiple European countries. Precision-ALS has collated data from 21,000 people living with ALS from 9 participating sites [McFarlane 2023; Precision ALS 2024]. As the Precision-ALS platform expands, additional countries may be considered for inclusion in the platform and study. ALS/NMD NHC is an established multidisciplinary clinic-based registry network that prospectively and longitudinally captures important clinical information about the disease process from people living with ALS. The ALS/NMD NHC consists of academic medical centres in the United States and Europe. However, to avoid potential duplication of data from national registries already included in the Precision-ALS programme, only data from centres in the United States will be included. Over 2100 people living with ALS have enrolled in this natural history study. Data collection, curation, and analysis is through NeuroBANK, a flagship clinical research platform which is run by the Neurological Clinical Research Institute at Massachusetts General Hospital [Berger 2023; data4cures 2024].

9.5. Study Size

Data will be extracted on all *SOD1*-ALS cases enrolled or entering in the Precision-ALS platform and ALS/MND NHC registry networks at study start and data on all incident *SOD1*-ALS cases enrolling in the registry during the first 5 years of the study. Thus, participants present or entering the registry networks during the first 5 years of the study may be included in the analyses. Given the difficulty in predicting enrolment of

SOD1-ALS cases, the study will monitor enrolment and reassess the enrolment period duration while the study is ongoing.

For a 5-year enrolment period, assuming a total of 25 *SOD1*-ALS cases per year could be enrolled, approximately 125 cases could be expected across both registry networks. Participants are expected to be followed in the registry networks as part of their respective observational plans until death or loss to follow-up, regardless of tofersen treatment status. Assuming a median follow-up time of approximately 2 years [Opie-Martin 2022], an enrolment of 25 cases per year, and an estimate of 40% tofersen treatment proportion, 100 person-years of follow-up data could be expected among tofersen-treated participants which will allow evaluation of the incidence of serious neurological events. Therefore, the target study size is at least 100 person-years of follow-up from tofersen-treated participants or at least 125 total *SOD1*-ALS cases (tofersen-treated or untreated) during the first 5 years of the study. The enrolment period is currently planned for a minimum of 5 years, and the suitability of that time frame will be reassessed during the study.

Limited data are available on the expected incidence of serious neurologic events. Using findings from the 147 tofersen treated (100 mg) participants in the Study 233AS101 (EudraCT number: 2015-004098-33) and Study 233AS102 (EudraCT Number: 2016-003225-41) integrated population over approximately 2.5 years exposure, a total of 12 serious neurologic events in 10 participants were reported for a combined rate of 0.033 events per person-year.

Using the target of 100 person-years of follow-up data collection by the registry networks, the confidence intervals widths and precision of the estimate of 0.033 serious neurologic events per person-year can be calculated based on a Poisson distribution. Table 3 also presents precision for scenarios with higher person-years of follow-up, which may occur depending on actual enrolment into the registry networks, the proportion of patients treated with tofersen treatment, and/or the median follow-up time. In each scenario the precision is reasonably tight.

Table 3: Study Size Precision Table for Neurological SAEs Based on Observing 0.033 Events Per Person-year

	Person-years follow-up	Expected Number of Events[a]	Lower 95	Upper 95	CI width	Precision (CI width/2)
Any Neurologic SAE	100	4	0.0109	0.1024	0.0915	0.0458
	150	5	0.0108	0.0778	0.0670	0.0335
	200	7	0.0141	0.0721	0.0580	0.0290
	250	9	0.0165	0.0683	0.0519	0.0259
	300	10	0.0160	0.0613	0.0453	0.0227

[a] Rounded up to the nearest integer

The expected study end date may be modified based on study progress, which will be reviewed annually as part of the reassessment procedure.

9.6. Data Management

Data on participants are collected during routine encounter-based clinical visits and are entered using electronic case report forms (CRF) by sites participating in the registry networks. Data management is maintained at each registry network according to their internal processes. Provided datasets and tables will be managed according to Biogen processes and in line with all regulations. A summary of the information collected on SAEs, pregnancy, and tofersen treatment is included in Annex 4 (Section 17).

9.7. Data Analysis

Planned analyses are summarized below and will be described in more detail in a separate statistical analysis plan (SAP).

Data will be analyzed separately for each registry network. However, the analysis performed will be harmonized as much as possible, where data capture and actual participant and event numbers allow.

Statistical analyses will generally be descriptive in nature and there is no *a priori* hypothesis to be tested. Continuous variables will be described with summary statistics including number of observations, mean, standard deviation, median, first quartile, third quartile, and minimum and maximum values. Categorical data will be described in terms of frequencies and percentages. An additional category of 'missing' will be added to account for missing data. Event rates may also be calculated as deemed appropriate.

All efforts will be made to collect data as completely as possible but incomplete dates could occur and this must be considered when evaluating the event and censoring times. Partial dates with missing information will be imputed as detailed in the SAP.

Analyses will be conducted on all adults with *SOD1*-ALS enrolled in Precision-ALS and ALS/MND NHC, including participants treated with tofersen as well as untreated participants. All analyses will be stratified by tofersen treatment status except secondary objective 2 (assessed among tofersen users only). Further details on the exclusion of data from clinical trials and the impact on index dates will be detailed in the SAP.

Comparisons between treated and untreated patients for outcomes related to safety, tolerability, or effectiveness are not planned, owing to the inability to address bias in the analyses, given the small sample size for *SOD1*-ALS and uncertainties around patient selection for tofersen treatment.

All participants, including participants who discontinue tofersen, will be followed until death or loss to follow-up. Sensitivity analyses will be performed censoring data 150 days (5 half-lives) after tofersen discontinuation (after which data will then be excluded from the analysis) [Benet and Zech 1981; FDA 2003; Rowland and Tozer 2010].

Data collected while a person with *SOD1*-ALS is participating in an interventional clinical trial (with tofersen or any other investigational medicinal product) will be excluded since this study will focus on real-world data and the results from interventional clinical trials will be available elsewhere.

The analysis population and definition of index date for statistical analysis will differ by treatment status and by study objective. In general terms, index date will be the date of tofersen treatment for data from participants in the treated group or date of enrolment into the registry for data from participants in the untreated group [redacted] Sensitivity analyses will be performed excluding retrospectively treated participants (i.e., those who initiated tofersen treatment prior to enrolment in the registry networks).

9.7.1. Primary Objectives

9.7.1.1. Demographic and Clinical Characteristics

Demographic and clinical characteristics will be summarized by descriptive summary statistics for continuous variables and frequency distribution and proportions for categorical variables.

Demographic and clinical characteristics will be summarized for each of the analysis populations using the corresponding index date for treated and untreated participants as defined for each of the other study objectives.

9.7.1.2. SAEs

Frequency of all reported SAEs will be summarized and presented by Medical Dictionary for Regulatory Activities (MedDRA) system organ class (SOC)/preferred term (PT) or International Classification of Diseases (ICD), as appropriate. Event rates may also be calculated if deemed appropriate.

The analysis of SAEs will be based on data collected only after implementation of SAE reporting forms and thereby the index date will be defined as the latter of tofersen initiation, registry enrolment, or implementation of SAE forms as appropriate, and the analysis population will consist of participants who are still alive at SAE form implementation.

9.7.2. Secondary Objectives

9.7.2.1. New Comorbid Conditions, Pregnancy and Pregnancy Outcome

Frequency of new comorbid conditions, pregnancy and pregnancy outcomes will be reported. Event rates may also be calculated if deemed appropriate.

9.7.2.2. Treatment Discontinuation

Summary statistics of the duration of treatment use and prescribed dosing frequency will be provided among people with *SOD1*-ALS receiving tofersen. Frequency and proportion of participants with treatment discontinuation will be reported. Reported reason for treatment discontinuation will be tabulated.

[redacted]

9.8. Quality Control

This study will use data from 2 existing networks of ALS disease registries. All data in the registry networks are collected according to routine clinic visits. Each registry network will use its own standard operating procedures, internal policies, or process guidance. The registry processes may include rules for data entry, data storage, methods to maintain and archive project and study documents, quality-control procedures for programming, standards for writing analysis plans, review of analysis programs and study documents by senior staff as well as internal audits if applicable.

The level of completeness for study variables will be monitored in each registry network throughout the course of the study. Biogen may work with the registry networks on supplementing their processes on an as-needed basis, and in accordance with registry governance.

The registry networks were selected for this study based on feasibility assessments, including review of protocols, processes, and relevant agreements between Biogen and the data source owners. Biogen will work with the registries to develop methods to enhance the quality of data and reporting for this protocol in line with guidances, where applicable (e.g., EMA data quality framework for EU medicines regulation [EMA 2023]).

9.8.1. Quality Assurance

Quality control procedures will be implemented at each stage of data handling. Prior to data delivery to Biogen or a third party on behalf of Biogen, the data holders will collect and manage data according to their own standards and in alignment with contracted agreements with Biogen. Quality control on the deliverables will be performed by Biogen or a third party acting on behalf of Biogen. Data anomalies will be communicated by Biogen to the registries for clarification and resolution, as appropriate. Any data entries that need to be resolved will be communicated by the registries to the impacted sites and corrected at site and central registry levels.

9.9. Limitations of the Research Methods

Although measures will be taken to ensure that the study is methodologically robust, certain limitations should be acknowledged, and all results should be interpreted considering these limitations.

Limitations of study setting and observational design

While a registry-based study allows data extraction from a broad population of people with *SOD1*-ALS, there are some limitations associated with this approach. Specifically, the data collected by the registry networks reflect local regulations and clinical practice, as well as registry governance and processes. This may result in some heterogeneity and inconsistency across sites and associated data quality concerns (e.g., missingness or loss to follow-up patterns). As the data in the registry networks are collected according to routine clinical practice, the frequency of clinical assessments, such as the ALSFRS-R, may vary by region or clinical practice.

Registry-based studies may have potential selection bias and uncertain generalizability. The registries might not represent the population of all people with *SOD1*-ALS. For example, people with *SOD1*-ALS living in more rural areas might be under-represented. However, participants in ALS/MND NHC have been shown to be more heterogeneous compared to anonymized clinical trial data from the Pooled Resource Open-Access ALS Clinical Trials database [Berger 2023].

As the registry networks are of observational design, treatment assignment is non-random. There will likely be systematic differences in the demographic and clinical characteristics, follow-up, and clinical outcomes between tofersen treated and untreated participants that preclude comparisons between tofersen treated and untreated participants. Statistical approaches to address these differences like matching require larger sample sizes than feasible for studies of ultra-rare diseases like *SOD1*-ALS (< 1 case per 50,000 population) [Brown 2021]. Therefore, valid comparisons cannot be drawn between the tofersen treated and untreated participants in this study. The analysis is focused on descriptive statistics, and the study includes untreated participants with *SOD1*-ALS to help improve understanding of tofersen drug utilization in the context of this ultra-rare disease.

This study will include data from both prevalent and incident tofersen users. The assessment of incident drug users is a key study design feature to avoid immortal time bias [Suissa 2008] and depletion of susceptibles in real-world evidence studies [Moride and Abenhaim 1994]. However, in the case of ultra rare diseases like *SOD1*-ALS, the inclusion of participants with prevalent *SOD1*-ALS, including tofersen users at the start of data extraction, will allow the collection of as much data as possible. The potential biases resulting from inclusion of prevalent users is acknowledged, and the analysis will take these potential biases into consideration (e.g., reporting results separately for prevalent and incident tofersen users if deemed appropriate). The safety profile of tofersen will be characterized through a review of the SAE data reported from individual participants during the study with a particular focus on the relationship assessment recorded on the SAE forms, temporal relationship of SAEs to tofersen dosing, the

consistency of SAE reports with tofersen clinical trial data and published literature, biologic plausibility, and confounding factors including known comorbidities of ALS.

Limitations of missing data

Missing reporting on exposure, covariates, and outcomes has the potential for misclassification of these variables. All efforts will be made by the registry networks to collect data as completely as possible. The data capture platforms of both registry networks have systems and data management processes that highlight and query for missing data in an attempt to minimize missingness. However, missing data or partial dates could still occur. To address this, an additional category of 'missing' will be added to account for missing data in analyses and partial dates will be imputed for analysis purposes as detailed in the SAP. Further, there is potential for unmeasured confounding (unmeasured third variable influencing the exposure and outcome). All analyses will be descriptive and there will be no assessment of treatment effects.

Limitations of sample size

As *SOD1*-ALS is an ultra-rare disease (< 1 case per 50,000 population) [Brown 2021], the available sample size across the 2 registry networks is expected to remain insufficient to allow hypothesis testing or subgroup analyses for this study [redacted]. While the protocol describes a target sample size, the target was estimated based on recent numbers of participants in the registries, which may not accurately predict the future number of *SOD1*-ALS participants during the study period. To maximize the sample size of people with *SOD1*-ALS, data from 2 networks of ALS disease registries are planned to be leveraged and no exclusion criteria will be applied by the study. The number of people with *SOD1*-ALS included in analyses will depend on a number of factors including differences in the prevalence of *SOD1*-ALS between regions/ethnic groups, availability of genetic testing, and rate of enrolment of people with *SOD1*-ALS in the registries.

9.10. Other Aspects

9.10.1. Study Funding

Biogen is the MAH of Qalsody, and as the sponsor of this study is funding the secondary data analysis of registry data. All financial details are provided in the separate contracts between the institutions, Investigators, and Biogen.

10. PROTECTION OF HUMAN PARTICIPANTS

Biogen must comply with this protocol and applicable International Conference on Harmonisation and GVP guidelines and conduct the study according to local regulations.

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

10.1. Participant Information and Consent

Biogen will only source data from registries that demonstrate protection of human participants and that have appropriate ethics approval and informed consent processes.

The data in the registry networks are collected based on participants' informed consent with the relevant registry network informed consent form or equivalent. The registry data will only be provided for use in this study in alignment with the existing registry consents and ethics approvals.

10.2. Participant Data Protection

This is a non-interventional study using secondary data that poses minimal risks for participants, related only to data privacy. To address this risk, all data used in the study will be de-identified with processes in place to ensure there is no breach of confidentiality regarding personal identifiers or health information. Data protection and privacy regulations will be observed in collecting, forwarding, processing, and storing data from study participants by both the registry networks and Biogen.

10.3. Compensation for Injury

Not applicable for secondary data study.

10.4. Conflict of Interest

The contributing registries should address any potential conflicts of interest (e.g., financial interest in the Sponsor) as part of their approved study consent processes as appropriate.

11. MANAGEMENT AND REPORTING OF ADVERSE EVENTS/ADVERSE REACTIONS

Not applicable. This study is secondary use of data and will follow relevant regulations. SAEs are collected per local and/or regional guidelines and requirements in the contributing registries.

12. PLANS FOR DISSEMINATING AND COMMUNICATING STUDY RESULTS

The interim study reports will be available according to the schedule outlined in Section 6.

12.1. Notification of Study Completion or Termination

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

12.2. Registration of Study and Disclosure of Study Results

Biogen will register the study and post study results, regardless of outcome, in the HMA-EMA catalogue of real-world data studies and on other publicly accessible websites in accordance with the applicable laws and regulations.

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14. ANNEX 1: LIST OF STAND-ALONE DOCUMENTS

None.

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

Study title: An observational registry-based study to evaluate the long-term safety of tofersen in people with *SOD1*-ALS

HMA-EMA catalogue number: Study not registered **Study reference number (if applicable):** Not Applicable

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

Comments:

1.1.3) As annual interim reports will be provided, other progress reports will not be delivered for this report.

Section 2: Research question	**Yes**	**No**	**N/A**	**Section Number**
2.1 Does the formulation of the research question and objectives clearly explain:	☒	☐	☐	8.1
2.1.1 Why the study is conducted? (e.g., to address an important public health concern, a risk identified in the risk management plan, an emerging safety issue)	☒	☐	☐	7.2
2.1.2 The objective(s) of the study?	☒	☐	☐	8.2 8.3
2.1.3 The target population? (i.e., population or subgroup to whom the study results are intended to be generalised)	☒	☐	☐	9.2.1
2.1.4 Which hypothesis(-es) is (are) to be tested?	☐	☒	☐	
2.1.5 If applicable, that there is no *a priori* hypothesis?	☒	☐	☐	9.7

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

Comments:

2.1.4) Statistical analyses will generally be descriptive in nature and there is no *a priori* hypothesis to be tested.

Section 3: Study design	**Yes**	**No**	**N/A**	**Section Number**
3.1 Is the study design described? (e.g., cohort, case-control, cross-sectional, other design)	☒	☐	☐	9.1
3.2 Does the protocol specify whether the study is based on primary, secondary or combined data collection?	☒	☐	☐	9.1
3.3 Does the protocol specify measures of occurrence? (e.g., rate, risk, prevalence)	☒	☐	☐	9.1.1 9.1.2
3.4 Does the protocol specify measure(s) of association? (e.g., risk, odds ratio, excess risk, rate ratio, hazard ratio, risk/rate difference, number needed to harm [NNH])	☐	☒	☐	
3.5 Does the protocol describe the approach for the collection and reporting of adverse events/adverse reactions? (e.g., adverse events that will not be collected in case of primary data collection)	☒	☐	☐	11

Comments:

3.4) Statistical analyses will generally be descriptive in nature and no measures of association will be calculated.

Section 4: Source and study populations	**Yes**	**No**	**N/A**	**Section Number**
4.1 Is the source population described?	☒	☐	☐	9.4
4.2 Is the planned study population defined in terms of:				
4.2.1 Study time period	☒	☐	☐	9.2.3
4.2.2 Age and sex	☒	☐	☐	9.2.1
4.2.3 Country of origin	☒	☐	☐	9.2.2
4.2.4 Disease/indication	☒	☐	☐	9.2.1
4.2.5 Duration of follow-up	☒	☐	☐	9.2.3
4.3 Does the protocol define how the study population will be sampled from the source population? (e.g., event or inclusion/exclusion criteria)	☒	☐	☐	9.2.1

Comments:

None.

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
5.2 Does the protocol address the validity of the exposure measurement? (e.g., precision, accuracy, use of validation sub-study)	☐	☒	☐	
5.3 Is exposure categorised according to time windows?	☐	☒	☐	
5.4 Is intensity of exposure addressed? (e.g., dose, duration)	☒	☐	☐	9.3
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?	☐	☒	☐	

Comments:

5.2, 5.3, 5.5) Details on the specifics of exposure data capture in each registry network will be outlined in the SAP.

5.6) Tofersen is the recommended first-line treatment for people with progressive ALS caused by pathogenic mutations in *SOD1* [van Damme 2023] and an acceptable active comparator is lacking. There are currently no other approved therapies indicated for the treatment of *SOD1*-ALS.

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
6.2 Does the protocol describe how the outcomes are defined and measured?	☒	☐	☐	9.3
6.3 Does the protocol address the validity of outcome measurement? (e.g., precision, accuracy, sensitivity, specificity, positive predictive value, use of validation sub-study)	☐	☒	☐	
6.4 Does the protocol describe specific outcomes relevant for Health Technology Assessment? (e.g., HRQoL, QALYs, DALYS, health care services utilisation, burden of disease or treatment, compliance, disease management)	☒	☐	☐	9.3

Comments:

6.3) The protocol does not directly address the validity of outcome measurement. Nonetheless, the registry networks have harmonized common data elements to

increase validity, and each registry network has appropriate quality control to ensure the reliability of the data collected in them.

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 9.7 9.9
7.2 Does the protocol address selection bias? (e.g., healthy user/adherer bias)	☒	☐	☐	9.1 9.7 9.9
7.3 Does the protocol address information bias (e.g., misclassification of exposure and outcomes, time-related bias)	☒	☐	☐	9.1 9.7 9.9

Comments:

None.

Section 8: Effect measure modification	**Yes**	**No**	**N/A**	**Section Number**
8.1) Does the protocol address effect modifiers? (e.g., collection of data on known effect modifiers, sub-group analyses, anticipated direction of effect)	☒	☐	☐	9.9

Comments:

None.

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)	☒	☐	☐	9.1
9.1.2 Outcomes? (e.g., clinical records, laboratory markers or values, claims data, self-report, patient interview including scales and questionnaires, vital statistics)	☒	☐	☐	9.1
9.1.3 Covariates and other characteristics?	☒	☐	☐	9.1
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
9.2.2 Outcomes? (e.g., date of occurrence, multiple event, severity measures related to event)	☒	☐	☐	9.3

Section 9: Data sources	**Yes**	**No**	**N/A**	**Section Number**
9.2.3 Covariates and other characteristics? (e.g., age, sex, clinical and drug use history, co-morbidity, co-medications, lifestyle)	☒	☐	☐	9.3
9.3 Is a coding system described for:				
9.3.1 Exposure? (e.g., WHO Drug Dictionary, Anatomical Therapeutic Chemical [ATC] Classification System)	☒	☐	☐	9.3
9.3.2 Outcomes? (e.g., International Classification of Diseases (ICD), Medical Dictionary for Regulatory Activities (MedDRA))	☒	☐	☐	9.7.1
9.3.3 Covariates and other characteristics?	☒	☐	☐	9.7.2
9.4 Is a linkage method between data sources described? (e.g., based on a unique identifier or other)	☐	☒	☐	

Comments:

9.4) No linkage between data sources.

Section 10: Analysis plan	**Yes**	**No**	**N/A**	**Section Number**
10.1 Are the statistical methods and the reason for their choice described?	☒	☐	☐	9.7
10.2 Is study size and/or statistical precision estimated?	☒	☐	☐	9.5
10.3 Are descriptive analyses included?	☒	☐	☐	9.7
10.4 Are stratified analyses included?	☒	☐	☐	9.7
10.5 Does the plan describe methods for analytic control of confounding?	☒	☐	☐	9.9
10.6 Does the plan describe methods for analytic control of outcome misclassification?	☒	☐	☐	9.9
10.7 Does the plan describe methods for handling missing data?	☒	☐	☐	9.9
10.8 Are relevant sensitivity analyses described?	☐	☒	☐	

Comments:

10.2) *SOD1*-ALS is a rare disease and as much data as possible will be collected but statistical analyses will be descriptive in nature. 10.8) Further details will be provided in the SAP.

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)	☒	☐	☐	10.2

Section 11: Data management and quality control	**Yes**	**No**	**N/A**	**Section Number**
11.2 Are methods of quality assurance described?	☒	☐	☐	9.8
11.3 Is there a system in place for independent review of study results?	☐	☒	☐	9.8

Comments:

11.3) Of note, independent review of the study results is typically not required for studies of this nature.

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?	☒	☐	☐	9.9
12.1.2 Information bias?	☒	☐	☐	9.9
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)	☒	☐	☐	9.9
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:

None.

Section 13: Ethical/data protection issues	**Yes**	**No**	**N/A**	**Section Number**
13.1 Have requirements of Ethics Committee/ Institutional Review Board been described?	☒	☐	☐	10.1
13.2 Has any outcome of an ethical review procedure been addressed?	☒	☐	☐	10.1
13.3 Have data protection requirements been described?	☒	☐	☐	10.2

Comments:

None.

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:

None.

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?	☒	☐	☐	12.2

Comments:

None.

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

Date:

This protocol was approved using an electronic workflow generated by a 21 CFR Part 11-compliant document management system.

Signature: The dated electronic signature page can be found on the final page of this document.

16. ANNEX 3: ADDITIONAL INFORMATION

Definition of an SAE

An SAE in this registry-based study protocol is any untoward medical occurrence, that, regardless of treatment status:

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

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

17. ANNEX 4: DATA COLLECTED FOR SERIOUS ADVERSE EVENTS, PREGNANCY INFORMATION, AND TOFERSEN TREATMENT

SAE Data

Variables	Data type / categories and responses
Report type	Initial
	Follow-up
SAE identifier[1]	
Report date	[date]
SAE date	[date]
Event name/term	Text field
SAE criteria	Death
	Life-Threatening event (this does not include an event that, had it occurred in a more severe form, might have caused death)
	Persistent or significant disability/incapacity
	Congenital anomaly/birth defect[2]
	Important Medical Events
	Hospitalization or prolongation of existing hospitalization
	Admission date: [date]
	Discharge date: [date]
Relationship to Tofersen	Not related
	Related
	Unknown
	Not applicable
Action taken with Tofersen treatment	No action
	Permanently withdrawn
	Temporarily suspended
	Unknown
	Other, Specify:
Outcome of SAE	Recovered/Resolved
	Recovered/Resolved with sequelae (prompts Text Field for Sequelae)
	Not recovered/not resolved
	Fatal
	Unknown

Variables	Data type / categories and responses
Date of outcome	[date]
Sequelae	Text field

[1]Event identifier/ID for ease tracking event longitudinally and cross-form reference
[2] Prompt pregnancy form

Pregnancy data

Variables	Data type / categories and responses
Date pregnancy confirmed	[date]
Data of last menstrual period	[date]
Ectopic pregnancy	Y/N
Date of outcome	[date]
Outcome	Live birth without congenital anomaly
	Live birth with congenital anomaly
	Premature birth
	Spontaneous abortion/miscarriage
	Elective termination
	Stillbirth
	Unknown

Tofersen treatment log

Variables	Data type / categories and responses
Treatment start date	[date]
Infusion dates	[date]
Dose	[numeric]
Dose unit	[Text field, values predefined]
Dose change	Y/N
Reason for dose change	[Text field]
Treatment discontinuation	Y/N
Treatment discontinuation date	[date]
Reason for discontinuation	Death[1]
	SAE[2]
	Healthcare provider decision
	Patient preference
	Other Specify [text field]
	Unknown

[1]Details captured on mortality form
[2]Prompt to SAE form