PASS Protocol	
Active substance	Eplontersen sodium
Study number	D8450R00022
Version number	2.0
Date	*14 November 2025*

TITLE PAGE

Post-Authorisation Safety Study

A cohort event monitoring study to characterise the use of eplontersen in patients with prior liver transplant and pre-existing severe hepatic impairment and to assess long-term safety among all new users of eplontersen (sTTRing)

Marketing Authorisation Holder(s)

Marketing authorisation holder(s)	[redacted]
MAH contact person	[redacted]

PASS INFORMATION

Title	A cohort event monitoring study to characterise use of eplontersen in patients with prior liver transplant and pre-existing severe hepatic impairment and to assess long-term safety among all new users of eplontersen (sTTRing) Short title: Long-term safety of eplontersen treated aTTR patients and in liver transplant and severely hepatic impaired subpopulations
Protocol version identifier	V2.0
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Marketing authorisation holder(s)	[redacted]
Joint PASS	No
Research question and objectives	The aim of this observational cohort study is to characterise use of eplontersen in patients with prior liver transplant or with pre-existing severe hepatic impairment, as well as to assess long-term safety among all new users of eplontersen; all are areas of missing information Primary objectives are: 1. To describe demographic and clinical characteristics of patients at eplontersen initiation, including the prevalence of prior liver transplant

	(overall and by reason for liver transplant), and the prevalence of severe hepatic impairment; and to describe patients in these subgroups (prior liver transplant, severe hepatic impairment). 2. To describe long-term safety in patients who initiate eplontersen treatment, including onset of new clinical events, abnormal laboratory values and serious adverse events.
Country (-ies) of study	Canada, China, Germany, Spain, United Kingdom, United States
Author(s)	[redacted]

1. TABLE OF CONTENTS

TITLE PAGE 1
PASS INFORMATION 2
1. TABLE OF CONTENTS 4
2. LIST OF ABBREVIATIONS 6
3. RESPONSIBLE PARTIES 8
4. ABSTRACT 9
5. AMENDMENTS AND UPDATES 14
6. MILESTONES 15
7. RATIONALE AND BACKGROUND 15
8. RESEARCH QUESTION AND OBJECTIVES 17
8.1 Primary objectives 17
8.2 Secondary objectives 18
8.3 Exploratory objectives 18
9. RESEARCH METHODS 19
9.1 Study design 19
9.2 Setting 23
9.2.1 Inclusion criteria 23
9.2.2 Exclusion criteria 23
9.3 Variables 24
9.3.1 Eplontersen exposure 24
9.3.2 Non-Eplontersen Exposure 24
9.3.3 Outcomes 25
9.4 Data sources 30
9.4.1 D8450R00003 study data 30
9.4.2 D8450R00003 tokenized to real-world USA data 32
9.4.3 [redacted] Pharmacovigilance database Argus 33
9.5 Study size 34
9.5.1 Sample size expectation 34
9.5.2 Sample size considerations on precision of incidence/prevalence proportions 36
9.6 Data management 37
9.7 Data analysis 37
9.7.1 Overview of the analyses 37
9.7.2 Descriptive analysis 45
9.7.3 Safety events description 46
9.7.4 Cohort event monitoring analysis 47
9.7.5 Hazard plots 48
9.7.6 Qualitative review of serious adverse events 48

9.7.7 Comparative safety 48
9.8 Quality control 50
9.9 Limitations of the research methods 52
9.9.1 Bias and confounding 52
9.9.2 Challenges to interpretation 54
9.9.3 Generalisability of study findings 54
9.10 Other aspects 55
10. PROTECTION OF HUMAN SUBJECTS 55
10.1 Data protection 55
10.2 D8450R00003 data linkage with Argus PV data 56
10.3 Confidentiality of study/patient data 56
11. MANAGEMENT AND REPORTING OF ADVERSE EVENTS/ADVERSE REACTIONS 57
11.1 Collection of adverse events and special situations 57
11.2 Reporting of Adverse Events and Special Situations 57
12. PLANS FOR DISSEMINATING AND COMMUNICATING STUDY RESULTS 57
13. REFERENCES 58

LIST OF TABLES

Table 1. Amendments and updates 14
Table 2. Study milestones 15
Table 3. Demographic and clinical characteristics for analysis of sTTRing dataset 27
Table 5. Precision assessment (95% binomial exact confidence intervals) for expected prevalence/incidence proportions 36
Table 6. Outcomes mapped to objectives of the sTTRing study 39
Table 7. Effective sample sizes required to rule out incidence rate ratios of > 2 for AEs 2 and > 2 for AEs 4 with 80% power (Poisson regression) 50
Table 8. Power to detect a difference in incidence densities with sample size of 250 patients with mean follow-up of 6 months in each group 72

LIST OF APPENDICES

Appendix A List of stand-alone documents 60
Appendix B ENCePP checklist for Study protocols 61
Appendix C Additional information 68

2. LIST OF ABBREVIATIONS

Abbreviation or special term	Explanation
AE	Adverse Event
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
ASO	Antisense oligonucleotide
ATTR	Transthyretin amyloidosis
ATTR-CM	Transthyretin amyloidosis with cardiomyopathy
ATTRv-PN	Transthyretin amyloidosis with polyneuropathy
ATTRv	Transthyretin amyloidosis variant
ATTRwt	Transthyretin amyloidosis wild type
[redacted]	
BMI	Body Mass Index
CEM	Cohort Event Monitoring
CCI	Charlson Comorbidity Index
CI	Confidence Interval
eCRF	Electronic Case Report Form
EHR	Electronic Health Records
EMA	European Medical Agency
EU	European Union
ES	End of study
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GFR	Glomerular Filtration Rate
GGT	Gamma Glutamyl Transferase
GPP	Good Pharmacoepidemiology Practice
ICH	International Council for Harmonization
ICSR	Individual Case Safety Report
ID	Incidence Density
INR	International Normalised Ratio
LLT	Lowest Level Term
D8450R00003 (MaesTTRo)	Non-interventional prospective, multi-country study collecting real-world data on the characteristics, treatment patterns, and outcomes of patients with amyloid transthyretin (ATTR) amyloidosis
MedDRA	Medical Dictionary for Regulatory Activities

Abbreviation or special term	Explanation
MPR	Medication Possession Ratio
MHRA	Medicines and Healthcare products Regulatory Agency
NfL	Neurofilament Light Chain
NT-proBNP	N-terminal pro B-type Natriuretic Peptide
ODCP	Organized Data Collection Program
ODD	Orphan Drug Designation
PASS	Post Authorisation Safety Study
PDC	Proportion of Days Covered
PHI	Protected Health Information
PT	Preferred Term
PV	Pharmacovigilance
Q1, Q3	Interquartile Range
RMP	Risk Management Plan
SAP	Statistical Analysis Plan
SD	Standard deviation
SIN	Study Identification Number
SOC	System Organ Classification
sTTRing	A cohort event monitoring study to characterize use of eplontersen in patients with prior liver transplant and pre-existing severe hepatic impairment and to assess long-term safety among all new users of eplontersen
TTR	Transthyretin
UK	United Kingdom
UACR	Urine Albumin-Creatinine Ratio
UPCR	Urine Protein Creatinine Ratio
USA	United States of America

3. RESPONSIBLE PARTIES

Name	Professional title	Role in study	Affiliation	Email address
[redacted]				

4. ABSTRACT

Title: Post Authorisation Safety Study (PASS): A cohort event monitoring study to characterise use of eplontersen in patients with prior liver transplant and pre-existing severe hepatic impairment and to assess long-term safety among all new users of eplontersen (sTTRing)

Rationale and background:

As part of additional pharmacovigilance (PV) activities in the local United Kingdom (UK) standalone Risk Management Plan (RMP), [redacted] has agreed with Medicines & Healthcare products Regulatory Agency to perform a PASS in order to address areas of missing information arising from: exclusion of patients with prior liver transplant from clinical trials, a lack of evidence on how adverse effects of eplontersen treatment impacts patients with severe hepatic impairment and the limited number of patients in clinical trials receiving long term treatment for 12 months or more.

The study will utilise several existing data sources and will primarily be secondary data use of the non-interventional prospective, multi-country study collecting real-world data on the characteristics, treatment patterns, and outcomes of patients with amyloid transthyretin (ATTR) amyloidosis (known as 'MaesTTRo', [redacted] Study identifier: D8450R00003) study data.

Research question and objectives:

The aim of this observational cohort study is to characterise use of eplontersen in patients with prior liver transplant or with pre-existing severe hepatic impairment, as well as to assess long-term safety among all new users of eplontersen; all are areas of missing information as per the UK RMP.

The primary objectives of the study are as follows:

1. To describe demographic and clinical characteristics of patients at eplontersen initiation, including the prevalence of prior liver transplant (overall and by reason for liver transplant), and the prevalence of severe hepatic impairment; and to describe patients in these subgroups (prior liver transplant, severe hepatic impairment).

2. To describe long-term safety in patients who initiate eplontersen treatment, including onset of new clinical events, abnormal laboratory values and serious adverse events.

The secondary objectives of the study are as follows:

1. To describe eplontersen treatment use, including duration of treatment and reasons for discontinuation, overall and by sub-populations of individuals with prior liver transplant or individuals with pre-existing severe hepatic impairment.

2. To describe the incidence of safety events occurring in patients who initiate eplontersen treatment, including onset of new clinical events, abnormal laboratory values and serious

adverse events separately i) in patients with prior liver transplant, overall and by reason for liver transplant if feasible; and ii) in patients with pre-existing severe hepatic impairment

The study will also include several exploratory objectives which are described in the protocol.

Study design:

The sTTRing study is a retrospective observational cohort study of patients initiating eplontersen in the real-world (and compared to patients initiating other transthyretin amyloidosis [ATTR] treatments, if feasible) which combines data from several different data sources.

1. The D8450R00003 study is an ongoing retrospective observational cohort study of patients with ATTR and will be used for primary analyses.

2. In addition, USA claims data in tokenized patients enrolled into D8450R00003, if feasible, will be used for exploratory analyses.

3. [redacted] PV database (Argus), if linkage to D8450R00003 data is feasible, will be used for exploratory analyses.

Population:

The D8450R00003 study cohort includes individuals with confirmed diagnosis of ATTR, aged ≥18 years at the time of providing the informed consent. The sTTRing study population will be a subset of the D8450R00003 cohort who meet the additional criteria below.

Inclusion Criteria:

1. D8450R00003 participants who consented to have their data used for future related research studies.

2. D8450R00003 participants who initiated eplontersen treatment up to 1-year prior to enrolment into D8450R00003 study observation period, irrespective of ATTR phenotype or genotype. For the comparative analyses, patients unexposed to eplontersen treatment and who initiated another ATTR treatment during D8450R00003 study observation period will be included.

Exclusion criteria:

1. Patients with exposure to eplontersen more than 1-year prior to enrolment into D8450R00003 study.

2. Patients who participated in an interventional ATTR study in the 12-months prior to enrolment into D8450R00003 study.

Variables:

The primary endpoints of the primary objective of the study will be 1) the prevalence of patient with prior liver transplant and by reason for transplant; 2) the prevalence of patients with severe hepatic impairment. Reasons for prior liver transplant will be captured as follows:

- ATTR, deceased donor, not domino
- ATTR, deceased donor domino
- ATTR, living donor
- Other

Hepatic impairment will be coded in the D8450R00003 electronic Case Report Form (eCRF) as part of the Charlson Comorbidity Index information, as follows: None, Mild, Moderate to Severe. The Child-Pugh scoring system will further be used to distinguish moderately (Class B) from severely impaired patients (Class C).

Exposure to eplontersen will be defined using start and end date of treatment, and reason for discontinuation will also be captured.

Data sources:

1. D8450R00003 study data:

In D8450R00003, secondary structured de-identified data, including demographic, clinical, health care utilization and treatment information, are gathered from electronic health records or paper charts, at enrolment and approximately every 6 months via electronic case report form (eCRF). Medical history is coded by SOC (System Organ Class) and PT (Preferred <Term) using the Medical Dictionary for Regulatory Activities (MedDRA®, latest version at the time of the Electronic Data Capture build) lowest level term. Concomitant medications are coded with the World Health Organization Drug Dictionary.

Serious adverse events (SAEs) occurring during conduct of D8450R00003, regardless of causality, will also be actively collected for all ATTR treatment using an eCRF form, and eplontersen related SAEs will be reported to [redacted] according to standard procedures and summarised descriptively as part of the study objectives for D8450R00003. Study sites participating in D8450R00003 have been provided information for reporting non-serious eplontersen related AEs directly to [redacted] as part of passive surveillance methodology.

2. D8450R00003 tokenized to real-world USA data:

Feasibility of using USA tokenized data will be assessed. For USA patients that consent to tokenization, a process (creation of a unique, encrypted identifier called a token, in place of personal identifiable information) will be used to collect additional deidentified data including diagnoses (recorded as using ICD-9 or ICD-10 terminology) and laboratory test results from other sources that are part of patients' routine medical care (electronic medical, hospital, or pharmacy records). These data will be used for exploratory objectives.

3. [redacted]PV database (Argus):

Feasibility of linkage between de-identified D8450R00003 study data and de-identified Argus data will be assessed. Argus is the [redacted]global patient safety PV database with records of all adverse events reported to [redacted]. All adverse events (AEs) related to study drugs are reported through Argus. This includes serious and non-serious AEs, including potential side effects and safety concerns. If any SAE occurs during the D8450R00003 study for patients taking eplontersen, these will be entered into Argus. Potential variables considered for the probabilistic linkage may include age, sex and height and other variables as available but will not include personally identifiable information (including, but not limited to, patient and reporter names, addresses, phone numbers). A test population of eplontersen patients will be conducted to assess linkage feasibility using SAE data.

Study size:

The sTTRing study is descriptive in nature with no formal statistical testing. It will include all individuals meeting the study selection criteria, reflecting the real-world use of eplontersen. Over a period of 4.5 years from date first patient enrolled in Q2 2024, D8450R00003 study aims to enrol at least 1,850 individuals in its cohort, including ~1,000 patients with PN or mixed phenotype (284 patients with PN phenotype, 716 patients with mixed phenotype), and ~850 patients with cardiomyopathy phenotype. Based on several assumptions, the anticipated sample size of eligible D8450R00003 patients analysed in sTTRing will range from 320 to 380 patients, including 240 to 285 new eplontersen users and 80-95 prior eplontersen users.

Data analysis:

For the primary endpoints of the study, prevalence will be estimated and 95% confidence intervals will be computed using a Binomial exact distribution. The numerator will be either the number of cases of individuals with prior liver transplant or pre-existing severe hepatic impairment at start of eplontersen treatment. The denominator will be the overall sTTRing study population of eplontersen users.

Patient characteristics will be summarised using descriptive methods through the tabular display of the number of valid patients, means, standard deviations, medians and interquartile range (Q1 and Q3), minimum and maximum values, for continuous variables, and frequency distributions and percentages for categorical and binary variables. Descriptive safety analyses will be performed, frequency counts and percentages, incidence risk and event rate (e.g. at 0-6 months, 7- end of sTTRing study period at reporting), and so on for all follow-up data available for all clinical events (including new diagnoses, ATTR signs and symptoms or significant changes in laboratory marker). A cohort event monitoring analysis will be performed to identify adverse events that require further characterisation. If feasible, a comparator cohort of non-eplontersen exposed patients who initiated another ATTR treatment will be identified and matched eplontersen initiators to achieve balance, and comparative

analyses will be performed to evaluate long-term safety of eplontersen relative to other treatments.

Milestones:

Final approved protocol: Q2 2025

Start date of data collection: Q3 2026

Last date of data collection: Q1 2032

Submission of final report of study results: Q1 2033

5. AMENDMENTS AND UPDATES

Table 1. Amendments and updates

Number	Date	Section of study protocol	Amendment or update	Reason
2.0	14 November 2025	9, 10	Clarification of data governance for linkage of D8450R00003 study data to the Argus PV database to ensure it does not violate patient confidentiality.	At the request of MHRA (letter dated 30 April 2025)
2.0	14 November 2025	9.3.3.2	Removed laboratory markers and added eGFR	Based on laboratory markers available
2.0	14 November 2025	9.3.2	Addition of a non-eplontersen population definition	To clarify the definition of the non-eplontersen population for comparative analyses if performed
2.0	14 November 2025	9.4.3	Clarification of [redacted] Argus PV linkage database process	Process has been refined based on current knowledge
2.0	14 November 2025	7, 9.1, 9.4.2, 9.5.2, 9.9	Clarification of tokenization process	Process has been refined based on current knowledge
2.0	14 November 2025	9.5	Primary objectives are descriptive	Wording clarification
2.0	14 November 2025	9.1, 9.3, 9.7	Clarification of follow-up period for safety events following discontinuation of eplontersen and definition of non-eplontersen exposure and clarification of key design definitions (e.g. follow-up)	Follow-up post-discontinuation updated based on time needed for clearance of eplontersen
2.0	14 November 2025	8.3, 9.7	Removal of exploratory objective 7 as redundant with primary analysis	Redundancy removed

2.0	14 November 2025	4, 9.7	Clarification of reporting of incidence and follow up periods	Wording clarification

6. MILESTONES

Table 2. Study milestones

sTTRing Study Milestone	Planned date
Final approved protocol	Q2 2025
Registration in the EU PAS register	Q3 2025
Statistical analysis plan submission	Q4 2025
Progress Report on D8450R00003 sample size recruitment	Q2 2026
Start of data collection	Q3 2026
Interim report #1 submission, including reports on both feasibility of tokenized USA claims data linkage to claims data & PV data linkage	Q3 2027
Interim report #2 submission	Q3 2028
Interim report #3 submission	Q3 2029
Interim report #4 submission	Q3 2030
Interim report #5 submission	Q3 2031
End of data collection	Q1 2032
Final Study Report	Q1 2033

EU: European Union; PAS: Post-Authorisation Studies; PV: pharmacovigilance; USA: United States of America.

7. RATIONALE AND BACKGROUND

Transthyretin amyloidosis (ATTR) is a rare, progressive disease caused by the extracellular accumulation of misfolded mutated transthyretin (TTR) protein fibrils (1, 2). Primarily synthesised in the liver, TTR is a 127-residue homotetrameric protein responsible for transporting thyroxine and retinol-binding protein. The dissociation of TTR tetramers leads to the aggregation and misfolding of monomers and oligomers, resulting in the formation of insoluble amyloid fibrils that deposit systemically, contributing to peripheral and autonomic neuropathy as well as systemic manifestations, particularly cardiomyopathy (2). Genetically, ATTR is either hereditary caused by a mutation in the transthyretin gene (ATTRv) or wild-

type (ATTRwt) without a mutation and phenotypically, ATTR presents as either cardiomyopathy (ATTR-CM), polyneuropathy, or a mixture of the two (1).

Hereditary transthyretin amyloidosis with polyneuropathy (ATTRv-PN) is an autosomal dominant disease wherein misfolded TTR proteins accumulate in multiple systems, including the heart, gastrointestinal tract, and other organs, with a great variability in clinical presentation, and with a ten-year survival expectation (3). In a systematic literature review, Antonopoulos et al. reported for non-subtyped ATTR, the 2-year survival was 70.4% (95% CI 66.9–73.9), for ATTRwt it was 76.0% (95% CI 73.0–78.9), and for ATTRv it was 77.2% (95% CI 74.0–80.4) (4).

Data on incidence and prevalence of ATTRv-PN are scarce. Although the exact prevalence is difficult to determine (due to heterogeneity and scarcity of data and also due to the disease being a multisystemic disorder with heterogenous clinical presentation), the worldwide prevalence of ATTRv-PN is estimated to be between 10,000 and 50,000 patients, affecting men and women equally (5). ATTRv-PN is mostly diagnosed at 30-50 years of age and median age at diagnosis has been estimated at 39 years old. However, estimates vary geographically, with higher prevalence and younger ages of diagnosis in endemic areas, such as Portugal, Cyprus, Spain, France, Japan, Northern Sweden, and descendants from these regions, compared to the rest of the world (6, 7). In these endemic areas, liver transplant has traditionally been a treatment option, suggesting significant hepatic impairment among patients. Among symptomatic patients, liver transplantation was less frequently performed in the United States USA compared to the rest of the world (3.3% vs. 18.6%). However, cardiac transplantation was more common in the USA compared to the rest of the world (3.3% vs. 1.0%) overall, though this difference was not observed when comparing cardiology sites specifically.

Clinical management of ATTRv-PN patients warrants a multidisciplinary approach. Current available options for care include targeted anti-amyloid therapy to inhibit further production and/or deposition of amyloid aggregates, symptomatic therapy of sensorimotor and autonomic polyneuropathy, and treatment of cardiac, renal and ocular involvement. Targeted anti-amyloid drugs include: TTR tetramer stabilisers, which stabilise the TTR protein and therefore inhibit formation of amyloid fibrils, and silencer drugs, which reduce or block TTR synthesis in the liver, thereby also preventing formation of amyloid deposits.

[redacted] jointly developed eplontersen (WAINZUA™), a ligand-conjugated antisense oligonucleotide (ASO), which acts as a silencer reducing the production of TTR proteins. Eplontersen is marketed for the treatment of ATTRv-PN and is being studied for the treatment of patients with ATTR-CM. Eplontersen was first approved in the USA in December 2023, and was also approved on 14 October 2024 in the United Kingdom (UK) for the treatment of ATTRv in adult patients with Stage 1 and 2 polyneuropathy. As part of additional pharmacovigilance (PV) activities in the local UK standalone Risk Management Plan (RMP), [redacted] has agreed with Medicines & Healthcare

products Regulatory Agency (MHRA) to perform a Post Authorisation Safety Study (PASS) that will address areas of missing information arising from: exclusion of patients with prior liver transplant from clinical trials, a lack of evidence on how adverse effects of eplontersen treatment impacts patients with severe hepatic impairment and the limited number of patients in clinical trials receiving long-term treatment for 12 months or more.

To meet this requirement, [redacted] proposes the 'long-term safety of eplontersen treated aTTR patients and in liver transplant and severely hepatic impaired subpopulations', known as 'sTTRing'. The study will use the cohort event monitoring (CEM) PV method of intensive surveillance via a secondary data approach (8). Data for sTTRing will be sourced from the 'non-interventional prospective, multi-country study collecting real-world data on the characteristics, treatment patterns, and outcomes of patients with amyloid transthyretin (ATTR) amyloidosis' (known as 'MaesTTRo', [redacted] Study identifier: D8450R00003, ClinicalTrials.gov identifier: NCT06465810, more details in Annex 1). D8450R00003 aims to create a global cohort of patients with ATTR amyloidosis to longitudinally observe the natural course of the disease, patient characteristics, treatment patterns including eplontersen, health care utilisation and serious adverse events. The sTTRing dataset will extract data from patients enrolled in D8450R00003 and who initiated eplontersen, and if comparative analyses are performed, a balanced cohort of patients unexposed to eplontersen will also be identified. Additionally, claims data from tokenized patients in D8450R00003 in the USA will be analyzed, if feasible, and deidentified individual case safety report (ICSR) data from [redacted]PV database will also be used, if feasible, to link those data to deidentified D8450R00003 patients.

8. RESEARCH QUESTION AND OBJECTIVES

The aim of this observational cohort study is to characterise use of eplontersen in patients with prior liver transplant or with pre-existing severe hepatic impairment, as well as to assess long-term safety among all new users of eplontersen; all are areas of missing information as per the UK RMP.

8.1 Primary objectives

The primary objectives of the study are as follows:

1. To describe demographic and clinical characteristics of patients at eplontersen initiation, including the prevalence of prior liver transplant (overall and by reason for liver transplant), and the prevalence of severe hepatic impairment; and to describe patients in these subgroups (prior liver transplant, severe hepatic impairment).

2. To describe long-term safety in patients who initiate eplontersen treatment, including onset of new clinical events, abnormal laboratory values and serious adverse events.

8.2 Secondary objectives

The secondary objectives of the study are as follows:

1. To describe eplontersen treatment use, including duration of treatment and reasons for discontinuation, overall and by sub-populations of individuals with prior liver transplant or individuals with pre-existing severe hepatic impairment.

2. To describe the incidence of safety events occurring in patients who initiate eplontersen treatment, including onset of new clinical events, abnormal laboratory values and serious adverse events separately i) in patients with prior liver transplant, overall and by reason for liver transplant, if feasible; and ii) in patients with pre-existing severe hepatic impairment.

8.3 Exploratory objectives

The exploratory objectives include objectives that will further address the aims of the study by comparing eplontersen to other ATTR treatment, if feasible, including:

1. If feasible, to compare long-term safety of eplontersen to other treatments. This will involve the following subobjectives:
    1.1. To identify an adequately balanced group of patients unexposed to eplontersen and new users of other ATTR treatments during follow-up in D8450R00003 for matching to patients initiating eplontersen treatment.
    1.2. To characterise the matched group and describe patients' demographic and clinical characteristics at their index date.
    1.3. To compare the incidence of safety events that require further characterisation in those matched patients not exposed to eplontersen and new users of other ATTR treatments to patients initiating eplontersen treatment.

In addition, the exploratory objectives will also allow exploration of potential sources of bias due to missingness in adverse event collection in D8450R00003 Electronic Data Capture (EDC) and misclassification or missingness in severe hepatic impairment characterisation:

2. If feasible, to describe eplontersen treatment use in USA patients with tokenized data, including duration of treatment and reasons for discontinuation, and adherence to treatment.
3. To describe significant changes in hepatic impairment severity during follow-up, in patients with moderate to severe hepatic impairment at initiation of eplontersen.

4. If feasible, to describe long-term safety in patients who initiate eplontersen treatment using ICSRs of adverse drug reactions spontaneously reported to [redacted] a PV database.
5. If feasible, to describe non-serious safety events occurring in patients who initiate eplontersen treatment using clinical events identified using claims data in tokenized USA patients.
6. If feasible, to describe long-term safety in patients who initiate eplontersen, including onset of new clinical events, abnormal laboratory values and serious adverse events stratified by ATTR phenotype (mixed, ATTR-PN, ATTR-CM).

The exploratory objectives will also evaluate whether changes in key design features affect the findings of the study:

.

7. To describe safety of patients with moderate to severe hepatic impairment initiating eplontersen, including onset of new clinical events, abnormal laboratory values and serious adverse events.

As feasibility of the comparative analyses outlined in exploratory objective 1 are dependent on sample size accrual, a decision will be made at interim report 3 whether to retain that objective in the study.

9. RESEARCH METHODS

9.1 Study design

The sTTRing study is a retrospective observational cohort study and analyses of patients initiating eplontersen in the real-world, combining data from several different data sources. If feasible, patients treated with eplontersen will be compared to a matched cohort of patients unexposed to eplontersen and new users of other ATTR treatments. The data sources to be used in the sTTRing cohort are depicted in Figure 1:

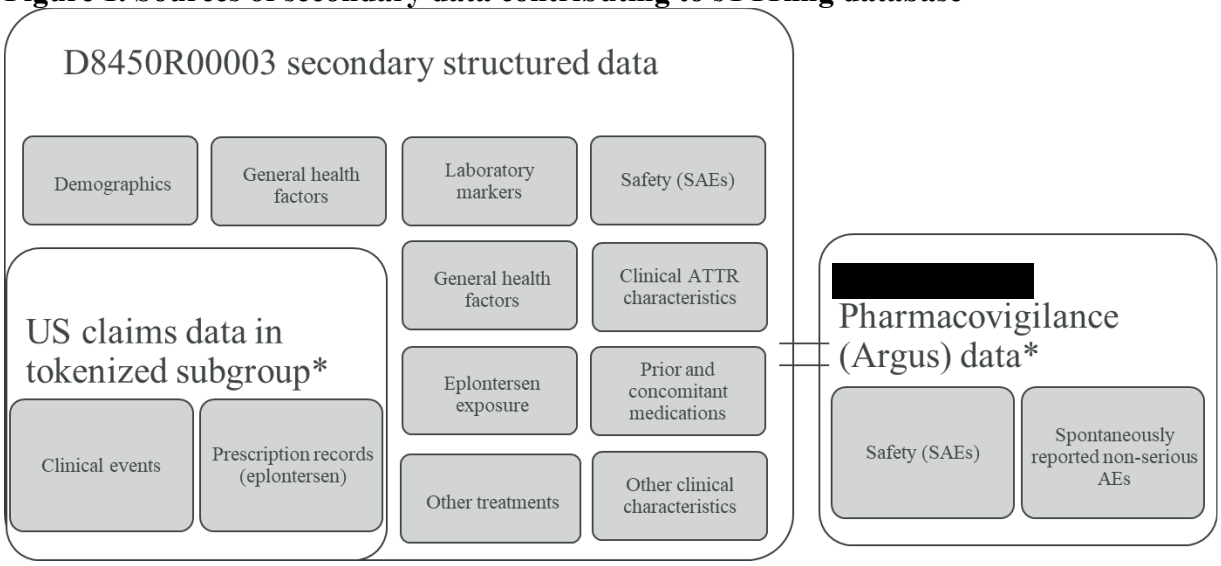
1. The D8450R00003 study is the ‘parent study.’. D8450R00003 is an ongoing prospective observational cohort study of patients with ATTR and will be used for primary analyses. The D8450R00003 study contains both primary and secondary data collection; sTTRing, the ‘child study’, will only reuse the secondary, structured, de-identified data collected in D8450R00003.

2. In addition, US claims data in tokenized patients enrolled into D8450R00003, if feasible, will be used for exploratory analyses.

3. [redacted]PV database (Argus), if linkage to de-identified D8450R00003 data is feasible, will be used for exploratory analyses.

Further information on the data collection process for each source of data is provided in Section 9.4. When performing linkage with Argus PV dataset, every effort will be made to avoid duplication of information; and specifically checks will be in place to ensure that adverse events are not counted twice.

Figure 1. Sources of secondary data contributing to sTTRing database



*Pending feasibility assessment. See Annex 1 for a full list of variables.

The following design definitions will apply:

- **D8450R00003 cohort entry (enrolment date):** the date of signature of the patient Informed Consent Form for entry into D8450R00003 cohort.

- **D8450R00003 follow-up**: period from D8450R00003 index date to the earliest of death, loss to follow-up, withdrawal from the D8450R00003 study, or end of the D8450R00003 study.

- Patients who initiated eplontersen treatment during the D8450R00003 study observation period will be labeled '**new eplontersen users**', and patients who initiated eplontersen treatment in the 1-year prior to D8450R00003 will be labeled '**prior eplontersen users**.'

- **sTTRing patient date of cohort entry (sTTRing baseline index date):** will be defined as the date of first exposure to eplontersen in eligible sTTRing patients. For "new eplontersen users" who start eplontersen during D8450R00003 study period, index date will be after D8450R00003 enrolment, whilst for 'prior eplontersen users' who start eplontersen treatment in the 1-year prior to D8450R00003 enrolment, index date will be prior to D8450R00003 enrolment. For the comparative analysis, index date will be defined as the date of first exposure to an alternative ATTR treatment initiated during D8450R00003 follow-up in patients unexposed to eplontersen.

- **sTTRing patient baseline period:** will be defined as the 1-year prior to first exposure to eplontersen (or other ATTR treatment for comparator group) during D8450R00003 follow-up. For patients who initiated eplontersen in the 1 year prior to enrolment into D8450R00003 study, less than 1 year of baseline period may be available (as D8450R00003 only collects some medical history data up to 1-year prior to enrolment). Information on prior liver transplant and ATTR diagnosis are collected in D8450R00003 without time limits, hence data prior liver transplant and time since diagnosis will be available even if more than 1-year prior to D8450R00003 enrolment.

- **sTTRing patient date of last data:** will correspond to the D8450R00003 date of last data for each patient and will be the date to the earliest of death, lost to follow-up, withdrawal from the D8450R00003 study, 115 days after eplontersen discontinuation, or end of the D8450R00003 study.

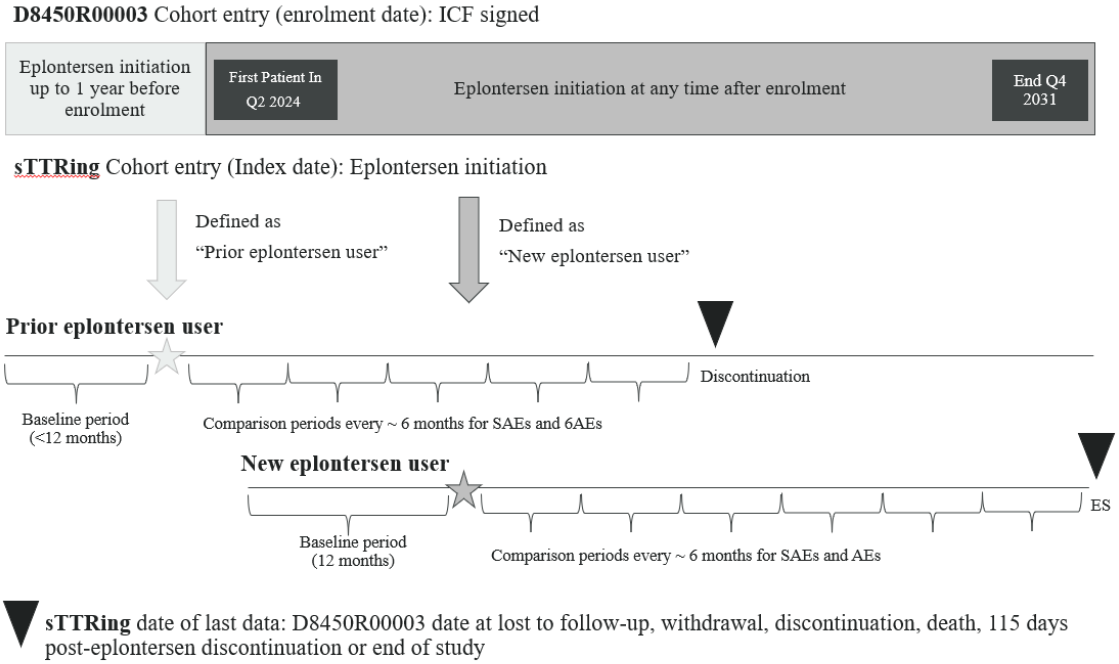
- **sTTRing study observation period:** will also correspond to D8450R00003 study observation period and include the baseline period of D8450R00003 if any prior eplontersen user is included in sTTRing. D8450R00003 was initiated in 2024, with first patient enrolled in Q2 2024, and will run until Q4 2031. The last patient is anticipated to be recruited in Q4 2028. Hence, the enrolment period is expected to last approximately 4.5 years, and patients may have from 3 and up to 7 years follow-up, if not withdrawn or censored beforehand, depending on when the patient is enrolled. Considering this, the sTTRing observation period will also be from Q2 2024 until Q4 2031. New eplontersen users enrolled in sTTRing may have up to 7 years follow-up depending on when they enter D8450R00003 study and when they initiate eplontersen treatment. Prior eplontersen users (who initiated treatment up to 1-year prior to D8450R00003 enrolment) may have up to 8 years of follow-up. As such, sTTRing's patients are anticipated to have length of follow-up similar or greater than published safety studies for alternative treatments and this will allow the sTTRing study to characterise long-term safety in the post-marketing setting (9-12).

- For cohort event monitoring analyses, **sTTRing reference 'at-risk' period** for safety events will be defined as the 0-6 months data collection for D8450R00003 period

during which first exposure to eplontersen occurs. **sTTRing comparisons periods** will be the subsequent 6-month interval and then all subsequent 12-month intervals (e.g., 7-12 months and 13-24 months) that are available for each patient during follow-up. For serious adverse events, optionally 3-month intervals in the first year and 6-month intervals after the first year may also optionally be used.

The study design definitions are depicted in Figure 2. In the rest of the protocol below, if not specified, references are to sTTRing index dates, follow-up and date of last data rather than to D8450R00003.

Figure 2. sTTRing study period and definitions



Note: Discontinuation refers to discontinuation of eplontersen. The figure shows the study design for sTTRing with two examples, one example of a prior eplontersen user who discontinues treatment during follow-up and is then censored before end of study (e.g. due to death; withdrawal from D8450R00003, 115 days after eplontersen discontinuation, or lost to follow-up); and one example of a new eplontersen user who does not discontinue and is censored at end of study.

9.2 Setting

The D8450R00003 study cohort includes individuals with confirmed diagnosis of ATTR[1], aged ≥18 years at the time of providing the informed consent. The overall sTTRing study population will be a subset of the D8450R00003 cohort who meet the additional inclusion and exclusion criteria below.

9.2.1 Inclusion criteria

Please refer to Annex 1 specific inclusion criteria for the D8450R00003 study.

The sTTRing study cohort will include those who meet the following additional inclusion criteria:

1. D8450R00003 participants who consented to have their data used for future related research studies.

2. D8450R00003 participants who initiated eplontersen treatment up to 1-year prior to enrolment into or during the D8450R00003 study observation period, irrespective of ATTR phenotype or genotype. For the comparative analyses, patients unexposed to eplontersen treatment and who initiated other ATTR treatments during D8450R00003 study observation period will be included.

9.2.2 Exclusion criteria

Please refer to Annex 1 specific exclusion criteria for the D8450R00003 study.

The sTTRing study cohort will exclude those to whom the following additional exclusion criteria apply:

3. Patients with exposure to eplontersen more than 1-year prior to enrolment into D8450R00003 study.

4. Patients who participated in an interventional ATTR study in the 12-months prior to enrolment into D8450R00003 study.

D8450R00003 will only collect medical history data for the 12-months prior to enrolment (apart from prior liver transplant and ATTR diagnosis date), hence those patients initiating eplontersen more than 1-year prior cannot be characterised. In addition, patients previously enrolled in trials are excluded to capture real-world patients treated as part of routine care.

[1] This does not include evidence of primary or light chain amyloidosis (AL) or serum protein A amyloidosis (AA), or asymptomatic patients with ATTR amyloidosis and asymptomatic ATTR allele carriers.

9.3 Variables

Variables used to fulfil the objectives of the study are described below. Further detail will be provided in the Statistical Analysis Plan (SAP).

9.3.1 Eplontersen exposure

Exposure to eplontersen will be ascertained from patients' medical records as documented in the D8450R00003 electronic Case Report Form (eCRF). Start and end of eplontersen treatment will be captured as well as reasons for discontinuation and will be described as part of secondary objective 1. Duration of treatment will be ascertained as number of days between start and the earliest of (i) end of treatment (temporary discontinuation will not be captured in D8450R00003 eCRF), and (ii) date of last data. Exposure will be assumed as continuous between start and end of treatment.

Patients' self-inject eplontersen in their home setting hence adherence to eplontersen will not be recorded in patients' medical notes. D8450R00003 eCRF does not capture repeat prescriptions nor temporary discontinuation, but does capture dates of treatment start and end with eplontersen which will be extracted. However, if feasible, linked data from claims will be used in USA patients may provide information on adherence, for e.g. using measures such as mean possession ratio, as part of exploratory objective 2. Further details will be provided in the SAP.

Safety objectives will be evaluated using follow-up time available from initiation of eplontersen treatment, including up to 115 days' time off-treatment or 115 days after discontinuation. The terminal elimination half-life of eplontersen is 25.5 days with >95% clearance by 115 days (13). Person-time observed will be calculated for every patient as number of days from index date until date of last data (earliest of death, loss to follow-up, withdrawal from the D8450R00003 study, or end of the D8450R00003 study). For the time to event analysis of safety outcomes requiring further characterisation, follow-up will also be censored at event date.

9.3.2 Non-Eplontersen Exposure

The non-eplontersen population will consist of all patients from of all D8450R00003 who meet the eligibility criteria for sTTRing and who have no record of treatment with eplontersen prior to the enrollment in D8450R00003 or any time during the study follow-up and who are new users of other ATTR treatments during follow-up. This comparison group will be used for the propensity score matching analysis for exploratory objectives.

9.3.3 Outcomes

9.3.3.1 Patients with prior liver transplant and severe hepatic impairment

The primary endpoints of the primary objective of the study are:

1. The prevalence of liver transplant prior to eplontersen initiation (overall and by reason for liver transplant)

2. The prevalence of severe hepatic impairment at eplontersen initiation

Patients with prior liver transplant and severe hepatic impairment (regarded as missing information) will be identified by investigators, or their delegates, using patients' medical records and paper charts and information will be recorded in eCRF. The following definitions will be used.

Prior liver transplant

Prior liver transplant will be ascertained if present in a patient' medical records anytime prior to index date and recorded in the eCRF.

Reason for liver transplant will also be captured as one of the following:

- Reason for transplant = ATTR, deceased donor, not domino
- Reason for transplant = ATTR, deceased donor, domino
- Reason for transplant = ATTR, living donor
- Other reason for liver transplant, specified as free-text

Hepatic Impairment

Hepatic impairment will be ascertained according to comorbidities and laboratory test results available in 12 months prior to and including index date (see Table 3). Note that if a patient initiates eplontersen during D8450R00003, as data is collected on clinical diagnoses every 6-months the 12-month period prior may need to be restricted to <12 months depending on when the patient initiated eplontersen. Laboratory test results will be recorded in D8450R00003 eCRF at baseline and each follow-up time points if available in patients' medical records, i.e., only if performed as part of routine care.

Within the D8450R00003 eCRF, liver disease is captured as one of the components of the Charlson Co-morbidity index (CCI), defined according to 3 categories (14):

- None
- Mild,
- Moderate to severe

In addition, to distinguish patients between moderate and severe hepatic impairment Child-Pugh class will be used whenever recorded or defined algorithmically using selected reported laboratory parameters, when available (15). If neither the Child Pugh class nor laboratory markers are available at baseline prior to eplontersen initiation, the carry-forward approach (also known as last observation carried forward) will be applied to the Child Pugh score and/or information used to calculate Child Pugh. Data recorded during earlier periods will be used instead, and if not available, data recorded in follow-up periods will be used. If data is missing at all time-points during follow-up, then it will not be possible to distinguish patients between moderate versus severe hepatic impairment, and safety in those patients will be described as part of exploratory objective 8.

The Child-Pugh scoring system categorises patients into three categories: A - good hepatic function, B - moderately impaired hepatic function, and C - advanced hepatic dysfunction. Their current scoring system uses an algorithm as follows (16):

- Encephalopathy: None = 1 point, Grade 1 and 2 = 2 points, Grade 3 and 4 = 3 points. Grading of encephalopathy will be recorded in D8450R00003 eCRF.
- Ascites: None = 1 point, slight = 2 points, moderate = 3 points. Grading of ascites will be recorded in D8450R00003 eCRF.
- Bilirubin: under 2 mg/ml = 1 point, 2 to 3 mg/ml = 2 points, over 3 mg/ml = 3 points. Bilirubin will be recorded in D8450R00003 eCRF.
- Albumin: greater than 3.5mg/ml = 1 point, 2.8 to 3.5mg/ml = 2 points, less than 2.8mg/ml = 3 points. Albumin will be recorded in D8450R00003 eCRF.
- International Normalised Ratio (INR): <1.7 = 1 point, 1.7-2.3=2 points, >2.3= 3 points. INR will be recorded in D8450R00003 eCRF.

The severity of cirrhosis is then categorised as follows:

- Mild hepatic impairment corresponds to Child-Pugh A: 5 to 6 points
- Moderate hepatic impairment corresponds to Child-Pugh B: 7 to 9 points
- Severe hepatic impairment corresponds to Child-Pugh C: 10 to 15 points

The primary and secondary analyses will use the narrow precisely defined phenotype (Child Pugh class C). Changes between moderate and severe hepatic impairment states will be evaluated as part of exploratory objective 3. An analysis will evaluate safety objectives as part of exploratory objective 8 using a modified broad phenotype definition of 'moderate to severe hepatic impairment' to explore potential impact of missing Child Pugh score or misclassification on prevalence estimates, defined as follows:

- Child Pugh classes B and C
- Child Pugh score ≥ 7
- or CCI category of moderate or severe hepatic disease.

9.3.3.2 Demographic and clinical characteristics

Baseline drug utilisation information as collected for D8450R00003 will be available to characterise eplontersen users within the sTTRing study cohort. See Annex 1 for complete listing of D8450R00003 data variables that will be collected at baseline and follow-up. For description of patients at eplontersen initiation, baseline data will be used and if multiple records, the most recent record prior to date of initiation of eplontersen will be used.

These drug utilization characteristics as identified at index date and at 6-month follow-up intervals in D8450R00003 are detailed in Table 3 and will include:

- Demographic characteristics
- General health factors
- Laboratory markers
- Clinical ATTR characteristics
- Medications
    - ATTR medications
    - Prior and concomitant medications at start of eplontersen treatment (as part of standard care for the indication)
    - Other treatments
- Other clinical characteristics

Table 3. Demographic and clinical characteristics for analysis of sTTRing dataset

Variable	Data collected at baseline and follow-up
Demographic characteristics	- age, - sex, - race/ethnicity, if allowed
General health	- height, - weight, - body mass index (BMI, calculated)

Laboratory markers	- serum TTR, - complete blood count, - hemoglobin, - creatinine, - albumin, - Cystatin C, - glomerular filtration rate (GFR), - liver enzymes: alkaline phosphatase (ALT), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyl transfers (GGT), total bilirubin, - brain natriuretic protein (BNP), - N-terminal pro B-type natriuretic peptide (NT-proBNP), - vitamin A level, - international normalised ratio (INR), - urine albumin-creatinine ratio (UACR), - urine protein creatinine ratio (UPCR)
Clinical ATTR characteristics	- ATTR diagnoses date, - duration since first diagnosis, - age at diagnosis, - phenotype (ATTR-PN, mixed, ATTR-CM) - genotype (ATTRv, ATTRwt, ATTR unknown) - Zygosity - TTR mutation and variant - clinical manifestations of ATTR amyloidosis, - familial amyloid polyneuropathy Coutinho staging, - Polyneuropathy disability score, - 6-minute walk test, - National Amyloidosis Center (NAC) ATTR staging, - New York Heart Association (NYHA) classification, - Charlson co-morbidity index (CCI) and CCI components
Medications	- ATTR: treatment start/end date, reason for discontinuation, posology (cumulative dose and treatment duration) (see list in Annex 1), - Prior and concomitant medications at start of eplontersen treatment (as part of standard care for the indication) including ATTR medications, heart failure and arrhythmia-related treatments, polyneuropathy-related treatments and other treatments (see full list in Annex 1)
Comorbidities	- Other clinical characteristics: prior procedures (e.g. surgical history), pre-existing and concurrent morbidities including CCI score and components and other

comorbidities captured (depression, fibromyalgia, paraproteinemia, other) (see full list in Annex 1).

Estimated glomerular filtration rate (eGFR) will also be estimated using the CKD Epi equation (17).

9.3.3.3 Safety endpoints

The sTTRing study will use the Cohort Event Monitoring (CEM) methodology. CEM methodology typically uses the definition below for an event which a) captures both beneficial and harmful health-related events and b) removes the overarching requirement for causality and medical confirmation (18).

Adverse Event definition for CEM: any new diagnosis, any reason for referral to a consultant or admission to hospital, any unexpected deterioration (or improvement) in a concurrent illness, any suspected drug reaction, any alteration of clinical importance in laboratory values or any other complaint which was considered of sufficient importance to enter into the patient's notes occurring within 115 days of last eplontersen dose.

Adverse Event definition for sTTRing: this definition has been adapted to the sTTRing study, and co-primary outcomes for long-term safety surveillance (primary objective 2, secondary objective 2) will include any AEs recorded for the sTTRing study cohort exposed to eplontersen including:

- All SAEs identified
- Other AEs: any new diagnosis reported as a co-morbidity, a new sign or symptoms, or new abnormal laboratory value within 115 days of last eplontersen dose

sTTRing is a secondary data study based on use of D8450R00003 study data, as well as, if feasible, i) claims data for a subset of USA patients with tokenized data and ii) de-identified ICSRs from Argus pharmacovigilance (PV) database. Events that are duplicated in D8450R00003 and linked datasets will be identified as part of the linkage with a reasonable probability. Further details on the sources of data are provided in Section 9.4. Primary and secondary analyses will be conducted using data from D8450R00003 only, and exploratory analyses will be conducted using linked PV data and tokenized claims data, if feasible.

D8450R00003 actively collects adverse events for all ATTR treatment considered serious by the study investigator using an eCRF. Additionally, sites are provided instructions to report all non-serious adverse events to [redacted] using passive reporting. Data on co-morbidities, ATTR signs and symptoms, and laboratory parameters are requested at baseline and at each six-month follow-up interval.

For the sTTRing study the focus will be describing adverse events which may be defined using data listed in Table 3 as any untoward medical occurrence i.e., any unfavorable and unintended symptom or sign (including an abnormal laboratory finding) or disease temporally associated with starting the medication under surveillance, irrespective of causality. Thus, any new diagnosis reported as a co-morbidity, a new sign or symptoms, or new abnormal laboratory value can be considered an adverse event.

To facilitate a systematic and comprehensive listing of all AEs, clinical data in D8450R00003 (ATTR signs and symptoms, co-morbidities, selected investigations, and laboratory results) in addition to serious adverse events, will be coded using the Medical Dictionary for Regulatory Activities (MedDRA (19)). Clinical data that cannot be mapped to medDRA will also be considered in the analyses. What constitutes an abnormal laboratory value will be further specified in the SAP for each laboratory test listed in Table 3 and may include medDRA codes for abnormal laboratory value when available (e.g. AST abnormal), or bespoke definitions (e.g. 3 times upper limit of normal range). These data collectively will be systematically evaluated to generate more comprehensive AE profiles as applicable to the study objectives including identification of signals of previously unlisted AEs that may not have been reported. However, CEM is dependent on data collected for D8450R00003 for purposes of its own specific study objectives, and there is no guarantee that all necessary data for safety surveillance will be complete or systematically available.

9.3.3.4 Follow-up

Overall distribution of total duration of follow-up (person time observed) in sTTRing will be described to provide context to primary safety analyses. The proportion of patients lost to follow-up due to withdrawal or discontinuation from D8450R00003 and reasons for loss to follow-up will be reported.

9.4 Data sources

This sTTRing study ('the child study') will reuse secondary data collected from the parent D8450R00003 study and, if feasible, data from tokenized USA patient data and deidentified PV data from [redacted] safety database (Argus). As such, sTTRing will be regarded as a secondary data use observational cohort study.

9.4.1 D8450R00003 study data

The non-interventional prospective, multi-country study collecting real-world data on the characteristics, treatment patterns, and outcomes of patients with amyloid transthyretin (ATTR) amyloidosis (known as 'MaesTTRo', [redacted] Study identifier: D8450R00003, ClinicalTrials.gov identifier: NCT06465810, more details in Annex 1) study aims to create a global cohort of patients with ATTR amyloidosis to longitudinally observe the natural course

of the disease, patient characteristics, treatment patterns including eplontersen, health care utilisation and serious adverse events.

As a hybrid study, the parent D8450R00003 study contains primary and secondary structured data from routine clinical visits collected into an electronic data capture (EDC) system. Primary data consists of patient-reported outcome questionnaires completed at enrolment and every six months (±3 months) only during routine on-site clinical visits. Secondary structured data consists of demographic, clinical, health care utilisation and treatment collected as per routine clinical practice. These data are abstracted directly from the electronic health records (EHR) or review of paper charts for each patient and entered in the electronic data capture (EDC) system using electronic case report forms (eCRFs), at enrolment and every 6 months (±14 days). Medical history is coded by SOC (System Organ Class) and PT (Preferred Term) using the Medical Dictionary for Regulatory Activities (MedDRA®, latest version at the time of the EDC build) lowest level term (LLT). Concomitant medications are coded with the World Health Organization (WHO) Drug Dictionary. No site visits are required for D8450R00003 study, and patients are not contacted for data collection outside of routine clinic visits. sTTRing will only reuse the secondary structured data collected in D8450R00003 study.

Data from sites within the United Kingdom, Germany, Canada, Spain, China and United States are included with a variety of ATTR phenotypes and genotypes. D8450R00003 site selection has focused on including centers of excellence specialised in ATTR care. For example, in the UK, patients will be referred to the study via the National Amyloidosis Centre (NAC) which is the standard referral pathway in clinical practice in the UK. The D8450R00003 cohort timelines are as follows (Table 4. D8450R00003 cohort timelinesTable 4):

Table 4. D8450R00003 cohort timelines

D8450R00003 cohort accrual milestone	Planned date
First D8450R00003 patient in	Q2 2024
Last D8450R00003 patient in	Q4 2028
Last D8450R00003 patient out	Q4 2031
Final D8450R00003 database lock	Q1 2032

To facilitate enrolment into the study, D8450R00003 is implementing several measures. Patient data are collected in the EDC by the Investigator and/or qualified designee at study enrolment (after informed consent has been signed) and throughout the follow-up period. No mandatory visits, tests, or assessments are required for this study. Apart from confirmation of inclusion/exclusion criteria, which must be collected, all data are collected only if available in the medical records. The eCRF has also been designed to minimise site burden and ensure

maximal participation at each site. Sites selected for the D8450R00003 study are known to treat a large proportion of patients with ATTR in their respective countries and have been selected to enable representativeness of the ATTR population and enhance generalisability of study results.

To gain further insights into the safety of ATTR treatments, including eplontersen, all serious adverse events (SAEs) regardless of causality or ATTR treatment are obtained from the patients' medical records and collected in D8450R00003 DEC. Serious adverse events (SAEs) for eplontersen occurring during conduct of D8450R00003, regardless of causality, will therefore be collected using an eCRF form, and will also be reported to [redacted] according to standard procedures and summarised descriptively as part of the study objectives for D8450R00003. Study sites participating in D8450R00003 have been provided information for reporting non-serious AEs directly to [redacted] as part of passive surveillance methodology.

9.4.2 D8450R00003 tokenized to real-world USA data

For patients enrolled in the USA, a tokenization process (creation of a unique, encrypted identifier called a token, in place of personal identifiable information) will be used to collect additional de-identified data including diagnoses (recorded as using ICD-9 or ICD-10 terminology). Typical Data Elements in medication claims (both open and closed claims) are also anticipated to include drug information: NDC Code (National Drug Code), dosage, strength, formulation, quantity dispensed, days supply, and refills. Prescription data will be utilised to calculate adherence, if feasible.

Only de-identified data will be analysed. Patients will be given a choice within the informed consent form to opt in or opt out of participating in the tokenization process.

Feasibility assessment is the process that relies upon uploading the defined sample of study generated tokens, e.g. Datavant^{TM} tokens into Datavant marketplace to check the patient data match rate (overlap rate) with secondary datasets available within Datavant set of tokenized databases like for example Optum.

Open claims data contains patient treatment history compiled from different insurance providers (Medicare, Medicaid etc.), while closed claims data contains patient treatment history from a defined insurance provider. Open claims data will be less complete, might have some gaps in comparison with closed claims that will have detailed information.

D8450R00003 currently is within the process of defining scientific variables of interest to develop the customised analytics and assess the cost and timelines of expert determination with ICON. In sTTRing, a feasibility assessment will be performed to evaluate whether USA tokenized data use for exploratory outcomes is feasible. Feasibility assessment will consist of

evaluating whether the tokenized data for D8450R00003 is suitable for sTTRing, i.e., includes relevant data including clinical diagnoses and prescription information, and whether that data use will pass the independent data privacy assessment.

Further details on the planned linkage approach will be provided in the SAP. Results of the feasibility assessment will be provided in interim Report 1.

9.4.3 [redacted] Pharmacovigilance database Argus

Argus is the [redacted] global patient safety PV database with records of all adverse events reported to [redacted]. Reporters are encouraged to spontaneously report all adverse events related to study drugs through Argus, including serious and non-serious adverse events, special situations, potential side effects and safety concerns. All ICSRs are then processed per the [redacted] standard operational procedures.

As per the D8450R00003 study protocol, SAEs occurring during the study period will be systematically collected, and SAEs for eplontersen will also be reported [redacted] Argus PV. For non-serious AEs, investigators and patients are informed of the option and means to report non-serious AEs to [redacted] or national health authority via spontaneous reporting systems. Considering this, additional data gathered as part of existing PV ICSR handling of non-serious AEs may be obtained from [redacted] Argus PV for use in the sTTRing study.

- Since the D8450R00003 study database and the PV Argus database are not directly linked, a feasibility study will be performed for the sTTRing study to determine if de-identified non-serious adverse event (AE) reports can be linked to de-identified D8450R00003 records. Invalid ICSRs, nullified ICSRs and ICSRs linked to other studies will be excluded. This will enable inclusion of a longitudinal evaluation of non-serious AEs (where spontaneously reported) to enhance the representativeness of the AE data.

SAEs should have a study identification number (SIN), a unique number through which patient data will be coded and rendered indirectly identifiable (pseudonymized), included in the report if they arise from an Organized Data Collection Program (ODCP) such as the D8450R00003 study which will be explored for facilitating record linkage. Whilst a SIN is not mandated for non-serious AEs arising from an ODCP, it is recommended. A sensitivity analysis will be conducted to ascertain impact of linkage on the AE estimations. Where feasible, these SIN will also be used to assist in deduplication of AE reports.

9.4.3.1 D8450R00003 study data linkage with Argus data: feasibility assessment

Potential variables considered for the probabilistic linkage of de-identified records may include age, sex and height and other variables as available. Linkage datasets will not include identifiable data such as patient and reporter names, addresses (postal, fax or email), phone

numbers or other related identifiers (e.g., institution ID). SAEs will have been captured in the D8450R00003 data set as well as reported to PV and present in the Argus dataset. Data on these SAEs will be analysed to further assess data quality and the degree of agreement between entries from the two datasets. Any insights obtained from this subgroup analysis of D8450R00003 patients with SAEs will be considered to adjust the general linkage approach for non-serious AEs as needed. Further details on the planned linkage approach will be provided in the SAP. Results of the feasibility assessment will be provided in interim Report 1.

The results of the feasibility assessment will be reported to the MHRA.

9.5 Study size

The primary endpoints of the sTTRing study (prevalence of prior liver transplant and prevalence of severe hepatic impairment at eplontersen initiation) are descriptive, therefore power calculations are not performed. The study will include all individuals meeting the study selection criteria, reflecting the real-world use of eplontersen. Sample size may be estimated based on several assumptions and expectations are detailed below and described in Figure 3. D8450R00003 sample size accrual as well as number of patients in D8450R00003 not consenting to have their data used for future related research will be provided as part of the progress, interim and final sTTRing study reports, based on ongoing recruitment into D8450R00003 study. The projections presented in Section 9.5.1 below may also be revised over time if any assumption markedly departs to what is observed in already available data.

9.5.1 Sample size expectation

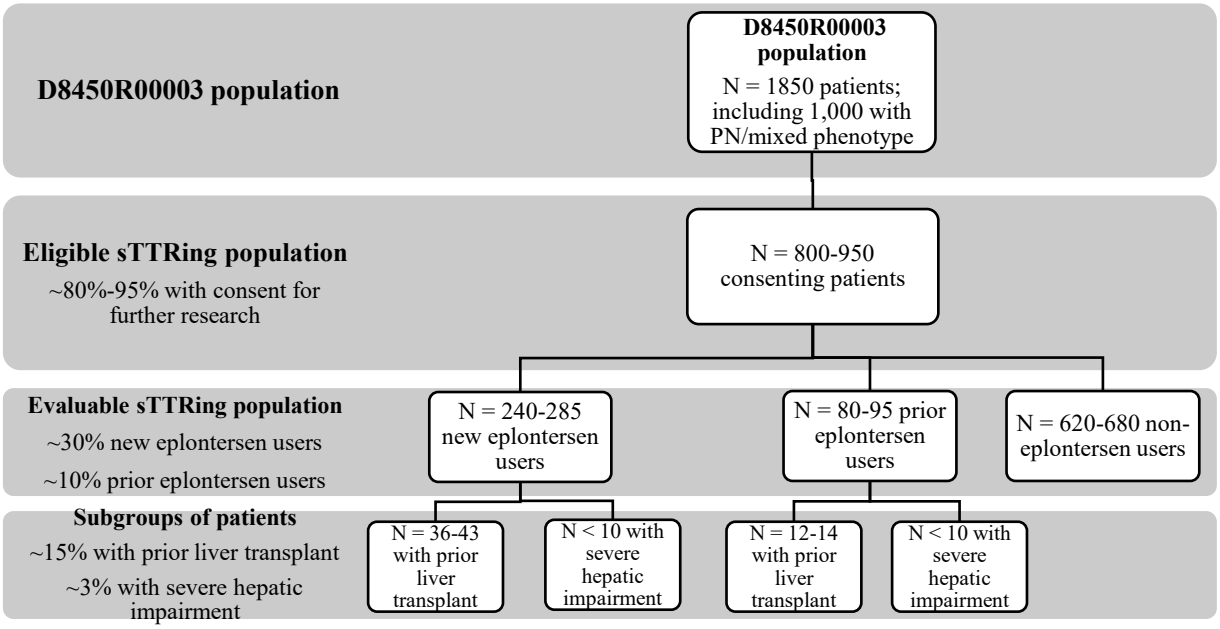
Over a period of 4.5 years from date first patient enrolled in Q2 2024, D8450R00003 study aims to enrol at least 1,850 individuals in its cohort. It is anticipated that the approximate distribution of the 1,850 patients will be 284 patients with PN phenotype, 716 patients with mixed phenotype, and 850 patients with cardiomyopathy phenotype.

For the purposes of sTTRing, all patients initiating eplontersen in D8450R00003 who consent for their data to be used for further research will be included irrespective of phenotype or genotype. In the countries where D8450R00003 sites are located (Canada, China, Germany, Spain, United Kingdom, United States) eplontersen is currently approved or under review for ATTRv-PN only. As PN indication includes mixed PN and CM phenotype, sample size calculations are revised based on PN plus mixed target. Early data from patients enrolled in D8450R00003 as of Q1 2025 suggest that between 80% and 95% of the cohort is expected to consent to having their data used for future research. Although the exact proportion of new eplontersen users during the study period is not yet known due to the recent market launch in several countries, it is anticipated that approximately 30% of enrolled patients will be new users during the study, with a further 10% expected to have had recent prior exposure. On this basis, the eligible sample size for the sTTRing study is estimated to range from 320 to 380

patients, which includes approximately 240 to 285 new eplontersen users and 80 to 95 prior eplontersen users.

In addition, data regarding liver transplant have been considered. Published literature report liver transplant procedures in 3.3% of ATTR patients in the USA (20)., and 18.6% of ATTR patients in rest of the world combined (Maurer et al., 2016). For the purpose of sample size estimation, a midpoint value of 15% has been applied, given the geographic mix of D8450R00003 sites (USA vs. rest of the world). Consequently, the subset of new eplontersen users with prior liver transplant is anticipated to range from 48 57 patients (with roughly 36 to 43 new users and 12-14 prior users). There is limited literature on the prevalence of severe hepatic impairment in patients with ATTR. One study in untreated patients with ATTR-CM reported a prevalence of 2.4% of liver disease (21). Consequently, the subset of new eplontersen users with ATTR-PN or mixed phenotype and prior severe hepatic impairment may be anticipated as less than 10 patients.

Figure 3: Sample size estimation for sTTRing study



Note: New eplontersen users are those who initiated eplontersen treatment during D8450R00003 study period; and prior eplontersen users are those who initiated eplontersen treatment in the 1-year prior to enrolment into D8450R00003 study. * including those who initiated eplontersen more than 1-year prior to enrolment into D8450R00003 study.

Furthermore, in the United States, a tokenization process is planned for patients enrolled in D8450R00003 who consent to the use of tokenization. D8450R00003 targets to enrol ~780 patients at US sites, ~330 with mixed / PN phenotype. Based on early results in the first 90 enrolled USA patients, anticipated sample size of tokenized subset of eligible sTTRing patients is derived with the following assumptions: ~83% consenting to have their data used for further research, 92% consenting to tokenization. Assuming ~30% new eplontersen users and ~10% prior eplontersen users, approximately 76 new users and 25 prior users are anticipated to have tokenized data. A feasibility assessment will be performed to evaluate whether using USA tokenized data is feasible and suitable real-world data can be for D8450R00003 tokenized patients (see Section 9.4.2).

9.5.2 Sample size considerations on precision of incidence/prevalence proportions

This is a descriptive study and the primary outcomes of the primary objective are:

1. The prevalence of liver transplant prior to eplontersen initiation (overall and by reason for liver transplant).

2. The prevalence of severe hepatic impairment at eplontersen initiation.

The goal is to estimate the incidence or prevalence of adverse events as precisely as possible given the final sample size. For illustration purposes Table 5 displays the 95% binomial exact confidence intervals across a range of expected prevalence/incidence estimates and possible over the first 12 months of exposure. For example, if the observed proportion is 5% in a sample of 300 ATTR patients treated with eplontersen, the 95% confidence interval would be 5.0% [2.8%, 8.1%]. If no event is observed in a sample of 300 patients, the upper limit of the 95% confidence interval would be 1.2%.

Table 5. Precision assessment (95% binomial exact confidence intervals) for expected prevalence/incidence proportions

Observed prevalence /incidence proportion	n = 100	n = 200	n = 300	n = 400
0%	[0.0, 3.6]	[0.0, 1.8]	[0.0, 1.2]	[0.0, 0.9]
1%	[0.0, 5.4]	[0.1, 3.6]	[0.2, 2.9]	[0.3, 2.5]
2%	[0.2, 7.0]	[0.5, 5.0]	[0.7, 4.3]	[0.9, 3.9]
3%	[0.6, 8.5]	[1.1, 6.4]	[1.4, 5.6]	[1.6, 5.2]
4%	[1.1, 9.9]	[1.7, 7.7]	[2.1, 6.9]	[2.3, 6.4]

5%	[1.6, 11.3]	[2.4, 9.0]	[2.8, 8.1]	[3.1, 7.6]
10%	[4.9, 17.6]	[6.2, 15.0]	[6.8, 14.0]	[7.2, 13.4]

9.6 Data management

The sTTRing database will be comprised of secondary de-identified data sources including the D8450R00003 study data, and, if feasible, de-identified [redacted]PV data (Argus), and tokenized US Claims data. These data sources will be utilised to build a comprehensive dataset that will be analyzed to address the study objectives.

All data collected in D8450R00003 will be stored and evaluated in accordance with regulatory and local requirements and applicable guidance for electronic records. Specific processes used to manage the data throughout the study and ensure the accuracy and completeness of the data collected is documented in the D8450R00003 Data Management Plan and will be added to the sTTRing Data Management Plan.

For D8450R00003, programmed automated edit checks (e.g., value range, units, internal consistency) are implemented in the EDC system to provide controls for data entry accuracy. Additional manual edit checks are also implemented to identify data inconsistencies that are not detected by automated edit checks. In case of inconsistent, erroneous, or missing data, queries will be generated electronically and sent to the site staff for correction.

9.7 Data analysis

9.7.1 Overview of the analyses

The table below (

Table 6) describes the statistical estimates relative to each objective and further details on the methodology is provided in the following subsections. Further detail will be provided in the SAP which will include a list of statistical estimates relative to each objective per outlined analyses as well as table shells.

Analyses for primary and secondary objectives will be performed at interim and final analyses, and analyses for the exploratory objectives will only be performed for the final analyses and reported in the final report. A description of the safety events, incidence risk and event rate for defined time periods (e.g., 0-6 months, and 7-end of follow-up available at the time of reporting) and for the entire study follow-up will be provided as part of primary and secondary objectives in the interim reports. The cohort event monitoring analysis will be performed to identify events that require further characterisation for primary and secondary objectives in the final report. The cumulative hazard rate will be estimated if >10 events for database events. To maximise sample size, the comparative analysis will be performed, if feasible, for the final analysis and reported in the final report. As feasibility of the comparative analyses outlined in exploratory objective 1 are dependent on sample size accrual, a decision will be made at interim report 3 whether to retain that objective in the study. Sample size considerations are provided in Section 9.5.1.

Primary analyses will be performed separately in new eplontersen users during D8450R00003 study and prior eplontersen users. Other analyses will be performed in new eplontersen users unless specified.

Subgroups of interest within the sTTRing study cohort will include individuals identified with (a) prior liver transplant, and separately b) pre-existing severe liver impairment at start of eplontersen treatment. Whilst it is highly unlikely that individuals will have both conditions at the start of eplontersen treatment, should this occur, each individual will contribute data to both subgroups (which are analyzed independently of each other).

Table 6. Outcomes mapped to objectives of the sTTRing study

No.	Objective	Outcome	Reporting
Primary objectives and outcomes:			
1.	To describe demographic and clinical characteristics of patients at eplontersen initiation, including the prevalence of prior liver transplant (overall and by reason for liver transplant), and the prevalence of severe hepatic impairment; and to describe patients in these subgroups (prior liver transplant, severe hepatic impairment).	1. prevalence of liver transplant prior to eplontersen initiation (overall by reason for liver transplant), 2. prevalence of severe hepatic impairment prior to eplontersen initiation These will be summarised as prevalence proportion and 95% CI. Secondary outcomes: Demographic characteristics (e.g. age, sex), general health factors (e.g. BMI), indication related characteristics (e.g. ATTR diagnoses dates and duration since first diagnosis, type, severity), prior and concomitant medications (as part of standard care of ATTR at eplontersen initiation), pre-existing and concurrent relevant morbidities will be described using descriptive statistics of counts, proportions and/or distribution characteristics (mean (SD), median (range)). These characteristics will be described in new eplontersen users; overall and by subgroups of interest, i.e., patients with prior liver transplant and patients with severe hepatic impairment at eplontersen initiation. Furthermore, these characteristics will also be described in new and prior eplontersen users together, and by subgroup of prior liver transplant.	Interim and final reports

2.	To describe long-term safety in patients who initiate eplontersen treatment, including onset of new clinical events, abnormal laboratory values and serious adverse events.	Onset of new clinical events, abnormal laboratory values and serious adverse events will be analysed as follows: a) Counts and frequency of first events and total number of events, incidence rate and incidence risk of events per time periods (e.g., 0-6, 7-end of follow-up available at time of reporting). b) Cohort event monitoring analysis including a. Incidence densities per 6-months (in the first year) or 12-months (after one year) intervals will be estimated. b. Differences in incidence densities per 6- or 12-months intervals c. Nelson-Aalen estimator of the cumulative hazard rate function over time. c) Further, for SAEs, qualitative case reports of clinical course and patients' characteristics and comorbidities will be provided. Analyses will be performed in new eplontersen users overall and by subgroups of interest. Furthermore, analyses will also be performed together for new and prior eplontersen users for SAEs and abnormal laboratory values.	Interim and final reports for a) and c) Final report only for b)

No.	Objective	Outcome	Reporting
Secondary objectives and outcomes:			
1.	To describe eplontersen treatment use, including duration of treatment and reasons for discontinuation, overall and by sub-populations of individuals with prior liver transplant or individuals with pre-existing severe hepatic impairment.	For eplontersen posology (e.g. cumulative dose and treatment duration), and discontinuation including reasons for discontinuation, descriptive statistics including counts, proportions and/or distribution characteristics (mean (SD), median (range) will be summarised. Duration of eplontersen treatment and reasons for its discontinuation will be described. These will be summarised in new eplontersen users overall and by sub-populations of individuals with prior liver transplant, reasons for liver transplant and/ or pre-existing severe hepatic impairment.	Final report

No.	Objective	Outcome	Reporting
2.	To describe the incidence of safety events occurring in patients who initiate eplontersen treatment, including onset of new clinical events, abnormal laboratory values and serious adverse events separately i) in patients with prior liver transplant, overall and by reason for liver transplant if feasible; and ii) in patients with pre-existing severe hepatic impairment.	As in primary objective 2 in subpopulations of interest.	Final report
Exploratory objectives and outcomes:			

No.	Objective	Outcome	Reporting
1.	1. If feasible, to compare long-term safety of eplontersen to other treatments. This will involve the following subobjectives: 1.1. To identify an adequately balanced group of patients unexposed to eplontersen and new users of other ATTR treatments during follow-up in D8450R00003 for matching to patients initiating eplontersen treatment. 1.2. To characterise the matched group and describe patients' demographic and clinical characteristics at pseudo-index date (corresponding to the date of matched eplontersen initiation) 1.3. To compare the incidence of safety events that require further characterisation in those matched patients not exposed to eplontersen and new users of other ATTR treatments to patients initiating eplontersen treatment.	In events identified for further characterisation as per primary objective 2 and secondary objective 2, if sufficient sample size and balanced group, comparative analysis will be performed. 1.1 An adequately balanced group will be identified by propensity score matching methodology 1.2 The non-eplontersen exposed cohort will be described prior and post matching Incidence rate ratios will be estimated to compare the new eplontersen users and non-eplontersen cohorts.	Final report

No.	Objective	Outcome	Reporting
2.	If feasible, to describe eplontersen treatment use in USA patients with tokenized data, including duration of treatment and reasons for discontinuation, and adherence to treatment.	As in secondary objective 1 using additional AEs identified using tokenized USA data. Adherence to treatment using refill records will be described using medication possession ratio and proportion of days covered. Adherence is defined as sum of the days' supply for all fills of eplontersen in a particular time period, divided by the number of days in the time period. Analyses will be performed for new eplontersen users	Final report
3.	To describe significant changes in hepatic impairment severity during follow-up in patients with moderate to severe hepatic impairment at initiation of eplontersen.	Descriptive statistics including counts and proportion of patients changing from moderate to severe and vice-versa during follow-up. Analyses will be performed for new eplontersen users.	Final report
4.	If feasible, to describe long-term safety in patients who initiate eplontersen treatment using ICSRs of adverse drug reactions spontaneously reported to [redacted] PV database.	As in primary objective 2 using additional AEs identified using Argus PV data. The data collected on AEs will be used to describe the frequency and severity of these adverse events. Analyses will be performed for new eplontersen users.	Final report

No.	Objective	Outcome	Reporting
5.	If feasible, to describe safety events occurring in patients who initiate eplontersen treatment using clinical events identified using claims data in tokenized USA patients.	As in primary objective 2. The data collected on clinical events will be used to describe the frequency and severity of these clinical events. Analyses will be performed for new eplontersen users.	Final report
6.	If feasible, to describe long-term safety in patients who initiate eplontersen, including onset of new clinical events, abnormal laboratory values and serious adverse events stratified by ATTR phenotype (mixed, ATTR-PN, ATTR-CM).	As in primary objective 2 in subpopulations of interest. The data collected on clinical events will be used to describe the frequency and severity of these clinical events. Analyses will be performed for new eplontersen users.	Final report
7.	To describe safety of patients initiating eplontersen with moderate to severe hepatic impairment, including onset of new clinical events, abnormal laboratory values and serious adverse events.	As in primary objective 2 in subpopulations of interest. Analyses will be performed for new eplontersen users.	Final report

9.7.2 Descriptive analysis

Prevalence of overall patient population (Binomial exact 95% CI) and subgroups of interest will be estimated. The numerator will be either the number of cases of individuals with prior liver transplant or pre-existing severe hepatic impairment at start of eplontersen treatment. The denominator will the overall sTTRing study population of eplontersen users.

Patient characteristics will be summarised using descriptive methods through the tabular display of the number of valid patients, means, standard deviations (SD), medians and interquartile range (Q1 and Q3), minimum and maximum values, for continuous variables, and frequency distributions and percentages for categorical and binary variables.

The analysis will be performed for primary objective 1 (interim and final report), for exploratory objective 1.2 (final report).

9.7.3 Safety events description

Follow-up time will be described using estimates such as mean, standard deviation, median, interquartile range, minimum, maximum, for all patients included in the analyses, and among specified subgroups (prior liver transplant, prior severe hepatic impairment).

Descriptive safety analyses will be performed for all clinical events (e.g., change in comorbidity, AATR signs and symptoms), abnormal lab values, and SAEs estimating frequency counts of events and patients experiencing events, incidence risk and incidence rate per time period (e.g., 0-6 months, 7- end of follow-up period available at time of reporting) incidence densities per 6- and 12-month intervals and difference in incidence densities. Summary tabulations will be presented on all AE MedDRA term counts, overall by rank and by body system. This will include any event where event date is missing. Frequency estimates will include the following:

- **i)** **Incidence risk**: the proportion of individuals with an incident event divided by total number of patient at start of time period t 95% confidence intervals of incidence risks will be computed using a Binomial Exact Distribution.
- **ii)** **Incidence rate**: total number of incident events during period t divided by total person time at risk. The total person-time at risk will end at the earliest of end of period t and event date for database events (e.g., SAE, abnormal laboratory test result) and at the end of the 6 months-data collection period for events collected in 6-month periods in D8450R00003 (e.g., comorbidities)

Incidence densities (IDs) will be defined per 100 person-years at period t as follows:

Incidence Densities ($ID_{t)}$ = Number of first reports of an event during period t /number of patient-years of observation for period t*100

Patient-months of follow-up will account for censoring of patients. The 95% confidence interval of the IDs will be computed using a Poisson Exact distribution. For SAEs, date of the event will be known and incidence rates will be estimated using those dates, whilst for other AEs data collection will be as per D8450R00003 protocol every 6 months ((±14 days).

Analyses will be performed at interim and final reporting period for primary and secondary objectives, and at final reporting period for exploratory objectives. Analyses will be performed in all eligible patients for primary objective 2. For secondary objective 2, analyses will be performed in subsets of i) patients with prior liver transplant and by reason for liver transplant, and ii) patients with prior severe hepatic impairment. For exploratory objective 4, analyses will be performed using additional adverse events observed in linked PV data, if

feasible. For exploratory objective 5, the analyses will be performed using additional clinical events recorded in claims data in tokenized USA patients, and for exploratory objective 6 analyses will be performed in subsets of patients by phenotype (PN, mixed, CM). For exploratory objective 7, the analysis be performed expanding the phenotype definition to include moderate to severe hepatic impaired patients.

9.7.4 Cohort event monitoring analysis

A CEM analysis will be performed to identify adverse events that require further characterisation. The analysis will be performed for primary objective 2 and secondary objective 2. Sample size considerations are provided in Appendix C.

9.7.4.1 Incidence density disproportionality analysis

ID disproportionality analysis will be performed to identify adverse events that require further characterisation. Further, evaluation will be undertaken for any listed and unlisted AEs where the disproportionality analysis suggests a signal of a difference in rate over time and all SAEs.

IDs will be calculated by aggregate time-period. The time-period for aggregation will be:

- 6-month interval for all AEs (and optionally 3 months for SAEs) in the first year after initiation of eplontersen,
- 12-month interval for all AEs (and optionally 6 months for SAEs) in the following years.

These periods may be revised during the evolution of the study as data become available on sample size, number of cases of AEs and length of follow-up. The analysis will test the hypothesis that IDs are constant between the reference ‘at-risk’ periods (0-6 months) and the comparison periods (7-12 months, 13-24 months, etc. for AEs). A definition of reference ‘at-risk’ and comparison periods is provided in Section 9.1.

Incidence density difference (IDD_{t2-t1}): for each coded AE the arithmetic difference of IDs (IDD) between the first ‘at risk’ period t1 and a comparison period t2 with Poisson exact 99% CI.

Significant adverse events with $IDD_{t2-t1}>0$ and lower 99% confidence interval (CI) bound excludes null during any follow-up period will be regarded as possible signal associated with starting drug treatment (e.g. Type A adverse drug reaction). Significant adverse events with $IDD_{t2-t1}<0$ and upper 99% CI bound excludes the null will be regarded as possible signal with delayed onset following first exposure (e.g. Type D adverse drug reaction). Possible signal AEs will require further characterisation, as explained in the next sections.

IDs will only be calculated for intervals during which at least 1 event occurs, and differences in incidence densities will only be estimated if at least 3 incidence events occur in one of the

intervals. Since a large number of analyses are performed systematically and iteratively, appropriate adjustment will be considered to account for multiplicity (such as False Discovery Rate adjustment) (22).

9.7.5 Hazard plots

For events identified as events for further characterisation (i.e., possible signal), the crude risk function will be estimated.

Crude risk function: probability of first AE before time point t.

The crude risk function will be calculated based on the estimated cumulative hazard over time using Nelson-Aalen cumulative hazards. For these analyses, observation time of cases will be censored at the time of the first adverse event. Smoothed hazard plots to describe how the baseline risk of an AE changes over time will also be presented. Time to event models require a sufficient number of observations for analysis purposes so such models will not be fit where ≤ 10 incident events are reported. No adjustment for covariates is planned.

9.7.6 Qualitative review of serious adverse events

SAEs will be qualitatively reviewed with case report provided in the Appendix of the interim reports.

9.7.7 Comparative safety

If feasible, comparative analyses will be performed to compare safety events between exposed and matched non-eplontersen exposed cohort that have been identified as requiring further characterisation based on results of the cohort event monitoring analysis, i.e. if:

- a significant IDD is observed during follow-up and ≥3 events were observed during follow-up
- SAEs

The comparative safety analyses will be performed if the sample size allows for sufficient statistical power and if any differences in patient characteristics can be addressed through appropriate statistical methods.

9.7.7.1 Identification and characterisation of matched cohort

A new user design will be used to match new eplontersen users to patients that have not yet been exposed to eplontersen and initiate another ATTR treatment during follow-up in a 1:1 ratio based on propensity scores of initiating eplontersen.

The first step of the feasibility assessment will be to assess ability to identify a comparator cohort amongst patients enrolled into D8450R00003. As D8450R00003 enrols patients

irrespective of their treatment, it is anticipated that ~60% of patients will have been and remain unexposed to eplontersen throughout D8450R00003 study follow-up. Patients initiating TTR stabiliser or silencer treatments and remaining unexposed to eplontersen during follow-up will be considered for inclusion in the comparator group, including patients starting:

- TTR stabiliser: VYNDAMAX™ or VYNDAQEL® (tafamidis), DOLOBID™ (diflunisal), ATTRUBY™ (acoramidis)
- TTR silencer: AMVUTTRA™ (vutrisiran), ONPATTRO™ (patisiran), TEGSEDI™ (inotersen)

A comparator cohort will be identified that will seek to achieve balance in demographics (e.g.: age, gender), prior treatment (e.g.: liver transplant) and key clinical characteristics (e.g.: phenotype of ATTR, time since diagnosis, pre-existing comorbidities), as well as obtain a match for a sufficient proportion of the subjects. Index date will be the date of initiation of the other ATTR treatment.

In a second step, patients' characteristics in the matched group will be summarised descriptively at index date, i.e. date of initiation of the ATTR treatment. Summary will be as per Section 9.7.2.

9.7.7.2 Comparative analyses

Thirdly, AEs that require further characterization will be used in comparative analyses if the two conditions are met:

i) the observed AE incidence rate (i.e. ID) for all follow-up is available in eplontersen and matched groups
ii) eplontersen and matched groups sample sizes allow to rule out an incidence rate ratio of ≥ 2 with at least 80% power for AEs, or an incidence rate ratio of ≥ 4 with at least 80% power for SAEs.

Assumptions about the incidence rates will be based on the observed incidence rates across both treatment groups. Table 7 provides the required sample size to rule out a risk ratio of ≥ 2 and ≥ 4 based on a Poisson regression model for different expected incidence rates per person-year. The type 1 error rate is set to 2.5% for the one-sided test and power is set to 80%. The number of patients as well as the incidence rates are assumed to be equal in both treatment groups.

For example, if the incidence rate of an AE is 4 per 100 person-year and mean follow-up time is 3 years, an effective sample size of 273 patients in both eplontersen and comparator groups would be required to rule out an incidence rate ratio of ≥ 2 for AEs, and a sample size of 69 patients in each group would be required to rule out an incidence rate ratio of ≥ 4 for SAEs

with 80% power. Sample size expectations are of 240-285 new eplontersen users (Section 9.5.1).

Table 7. Effective sample sizes required to rule out incidence rate ratios of ≥ 2 for AEs 2 and ≥ 2 for AEs 4 with 80% power (Poisson regression)

Sample size per group to rule out a risk ratio	Mean follow-up (years)	Expected incidence rate per 100 patient-year					
		2	**4**	**7**	**10**	**15**	**20**
Risk ratio of ≥ 2 for AEs 2	**1**	1634	817	467	327	218	164
	2	817	409	234	164	109	82
	3	545	273	156	109	73	55
Risk ratio of ≥ 2 for AEs 4	**1**	409	205	117	82	55	41
	2	205	103	59	41	28	21
	3	137	69	39	28	19	14

If these conditions are met, the incidence rate ratio of eplontersen versus the comparator group will be estimated and reported with 95% CI for the AEs.

9.7.8 Change in hepatic impairment severity

Counts and proportions of patients changing from moderate to severe categories of hepatic impairment during follow-up, and vice-versa, will be provided.

9.7.9 Adherence to treatment estimates

If using US claims data in tokenized patients is feasible, adherence to eplontersen treatment will be estimated comparing expected number of doses issued according to monthly administration versus actual supplied using e.g. medication possession ratio (MPR) and proportion of days covered (PDC) metrics will be used to define mean possession ratio. Adherence will be defined as sum of the days' supply for all fills of eplontersen in a particular time period, divided by the number of days in the time period.

9.8 Quality control

9.8.1 Monitoring of D8450R00003 data collection

sTTRing is a secondary data study based on reuse of D8450R00003 study data. All data collected in both child (sTTRing) and parent (D8450R00003) studies will be stored and evaluated in accordance with regulatory and local requirements and applicable guidance for primary and secondary data collection methods. Specific processes used to manage the data throughout the conduct of those studies and ensure the accuracy and completeness of the data collected will be documented in their respective Data Management Plans.

D8450R00003 has put in place a range of measures to ensure data quality and completeness. Site staff will complete D8450R00003 eCRFs according to instructions from the sponsor and/or designee. Designated study personnel will participate in a training program that will encourage consistency of process and procedures at the investigative site and ensure collection of high-quality data for this study. Programmed automated edit checks (e.g., value range, units, internal consistency) will be implemented in the EDC system to provide controls for data entry accuracy. Additional manual edit checks will also be implemented to identify data inconsistencies that are not detected by automated edit checks. In case of inconsistent, erroneous, or missing data, queries will be generated electronically and sent to the site staff for correction. A continuous monitoring process will be in place to evaluate the progress of recruitment at all participating sites. This will allow early identification of any sites that are underperforming in terms of recruitment and allow for timely intervention.

Data will be stored for the required period as mandated by local regulations. Once the retention period has ended, data should be securely destroyed, ensuring that it cannot be reconstructed or used again.

9.8.2 USA tokenized data

For patients enrolled in the USA who consented to participate in the tokenization process, certain patient details (e.g., name, sex, date of birth, zip code) will be submitted to the tokenization system. A unique token (string of characters) will be generated for each patient. A secure database will maintain a mapping between the original personal identifiable information and the corresponding tokens. Mapping is encrypted and stored in a way that ensures that only authorised personnel or systems can access it. The tokens will be matched with data from administrative claims, which will be collected as a separate de-identified dataset. Depending on the type of claims database chosen by D8450R00003 for the USA tokenization process, if feasible, quality control measures will be described and further specified in the SAP.

9.8.3 Argus PV data collection

The [redacted] pharmacovigilance database is subject to rigorous data quality and integrity processes to ensure compliance with regulatory requirements.

Argus PV data undergo automated validation checks to ensure that key fields are correctly populated, such as the event's seriousness, product information, and patient demographics. Automated checks also ensure that no conflicting or incomplete data is entered. These checks include verifying that data such as age, dates, and lab results fall within acceptable ranges, and ensuring consistency in units across all entries. The system also checks for logical alignment between related data fields, such as ensuring that the event date does not precede the date of birth. Additionally, the checks confirm that all mandatory fields are completed, helping to maintain the integrity and consistency of the data.

A manual review is conducted on all AE reports in Argus. This process includes checking for consistency, completeness, and proper classification of the event. If discrepancies or missing information are identified, the cases are queried and followed up for clarification. ese checks include verifying that data such as age, dates, and lab results fall within acceptable ranges, and ensuring consistency in units across all entries. The system also checks for logical alignment between related data fields, such as ensuring that the event date does not precede the date of birth. Additionally, the checks confirm that all mandatory fields are completed, helping to maintain the integrity and consistency of the data.

9.9 Limitations of the research methods

9.9.1 Bias and confounding

The sTTRing study has limitations common to other secondary database observational studies including being subject to various forms of bias.

This study is prone to the same information bias that applies both to the D8450R00003 study and spontaneous reporting PV data. This bias occurs due to variability in data collection (including missing data elements), recording and interpreting, which can lead to inaccuracies, measurement errors and misclassifications of study information. The D8450R00003 study (from which data for the sTTRing study are to be derived) attempts to minimise this bias by implementing measures to improve internal validity standard measurement instruments, data entry manual and training material, providing appropriate training for site staff (including for pharmacovigilance reporting), and implementation of automated edit checks and queries in the EDC system for patient data capture in eCRF. Misclassification of outcomes, covariates and exposure is commonly present in observational studies. For example, for this study there is potential for misclassification of the co-primary outcome of pre-existing severe hepatic impairment since this variable is based on Child-Pugh class which may have to be defined algorithmically using diagnostic codes and selected reported laboratory parameters. Whilst the primary analyses will use the narrow precisely defined phenotype (Child Pugh class C), exploratory analysis will evaluate safety objectives as part of exploratory objective 8 using a modified broad phenotype definition (Child Pugh class B and C, Child Pugh score ≥ 7, or CCI category of moderate or severe hepatic disease).

The sTTRing study relies on a secondary data approach, systematically using all data collected during D8450R00003 including reported safety events, other clinical events and laboratory results. All clinical data (ATTR signs and symptoms, co-morbidities, selected investigations and laboratory results) in addition to serious adverse events will be coded according to MedDRA to facilitate systematic evaluation of all available clinically related information. In addition, active data collection for SAEs will be performed and D8450R00003 investigators/patients will also be encouraged to report non-serious AEs of eplontersen to [redacted] or national health authorities via spontaneous reporting systems and via US

claims data using tokenization process. As for all spontaneous reporting schemes, under-reporting of non-serious AE can be anticipated such that the AE risk profile as derived from such schemes may not be fully representative of all known and unknown risks. To mitigate this limitation and obtain a more representative AE risk profile that encompasses both serious and non-serious AEs, the following measures are proposed:

1) A feasibility assessment of linking D8450R00003 data to Argus PV data will be undertaken and, if feasible, safety of eplontersen using linked data will be evaluated in exploratory objective 4

2) A feasibility assessment of using tokenized claims data from D8450R00003 patients in the USA t will be performed and, if feasible, safety of eplontersen using linked data will be evaluated in exploratory objective 5

All data will be de-identified prior to linkage to preserve patients' confidentiality. Therefore, probabilistic rather than deterministic methods will be used to link data between de-identified D8450R00003 and de-identified Argus PV data. Claims data from tokenized patients in D8450R00003 will be presented separately from the rest of the analysis and in aggregate which will limit the ability to identify cases between data sources.

Selection bias is another common issue, when individuals included in the study differ from the true population in terms of characteristics or probability of experiencing an outcome. Within D8450R00003 the number of individuals who will consent for future use of their data, the number of them exposed to eplontersen, and the amount of loss of follow-up is unknown. Thus, selection bias may be introduced to this CEM study through non-probability sampling of D8450R00003 subjects. However, enrolment within D8450R00003 is planned across multiple countries in various specialist sites known to treat a high proportion of patients with ATTR. Therefore, it is anticipated that patients enrolled will be as representative as possible and the findings as generalisable as possible.

Immortal time bias may be introduced with the inclusion of patients with prior exposure to eplontersen, and these patients may differ from patients who initiate eplontersen after enrolment into D8450R00003. Characterisation of patients who were prior eplontersen users may be limited as D8450R00003 only collects some medical history data up to 1-year prior to enrolment, hence there is also a potential for differential missingness in baseline characteristics and outcomes between prior and new eplontersen users. Therefore, data analyses for the primary objectives will be performed separately in new eplontersen users as well as new and prior combined eplontersen users.

9.9.2 Challenges to interpretation

This study involves multiple endpoints, which will be evaluated using several analytic approaches, including evaluation in several subgroups. The probability of false positive results increases as the number of comparisons increases. Any positive association will be interpreted in the context of the totality of evidence. The study size will be limited by the number of subjects who receive eplontersen. Precision of the estimates for some AEs may be low, especially in subgroup analyses or for very rare endpoints. Small sample size may also limit the study ability to identify suitable comparator group and perform comparative analyses. To address these limitations, the published literature will be considered when interpreting the results, and qualitative comparisons will be performed, if relevant.

D8450R00003 does not actively collect information on all adverse events, and although the EDC contains a comprehensive list of clinical events corresponding to the Charlson Comorbidity Index, and ATTR signs and symptoms, some events may be missed. To address those limitations, two feasibility analyses will be conducted, one linking de-identified D8450R00003 data to Argus PV data, and one using claims data from tokenized D8450R00003 patients in the USA. In case of success, additional data may allow identification of additional clinical events which will be taken into account in the safety analyses. The availability of tokenized data will also permit an estimation of adherence to eplontersen treatment. In case of failure of either of these feasibility assessments, the limitations of D8450R00003 will apply, i.e. the potential for missed clinical events, and this will be discussed when interpreting the results of the analysis. In case of failure of privacy assessment for tokenized USA data, adherence will not be estimable, however, reasons for discontinuations will be described from D8450R00003.

9.9.3 Generalisability of study findings

The sTTRing study is secondary data study based on the use of D8450R00003 data. D8450R00003 has been set-up as real-world observational study and is likely to encompass a more heterogeneous population of patients than the population of patients enrolled in interventional studies. Treatment decisions and outcomes in the context of routine care are likely to be more generalizable and complementary than those based on clinical trial data. D8450R00003. Every effort has been made by the D8450R00003 team to set-up sites at diverse locations and across multiple countries, at hospitals and centres known to diagnose and manage patients with ATTR amyloidosis to maximise recruitment at those sites. The rarity of the disease has also been taken into account by the D8450R00003 team when designing D8450R00003 and deciding on sample size targets. Considering this, it is anticipated that sTTRing will allow characterisation of real-world patients and their long-term safety outcomes with eplontersen treatment and results will be generalisable to future patients initiating eplontersen treatment as part of routine care.

sTTRing is a secondary use of D8450R00003 study data wherein it is possible that some of the patients included may have previously been enrolled into eplontersen clinical trials (e.g. ION-682884-CS3 and ION-682884-CS13) and other ATTR trials. Those patients will be excluded from the study to prevent selection bias and to reflect real-world users who initiate treatment as part of routine care practice. The exclusion of such patients will limit sample sizes and potentially impact the sTTRing study ability to perform analyses. Based on results from the first 6 months of D8450R00003, the impact on sample size appears limited as only ~10% of patients had prior eplontersen exposure and it is not known yet what subset of this 10% were enrolled into clinical trials. It is also anticipated that this number will decrease over time as recruitment for these trials are completed; and new patients will be diagnosed and initiated on eplontersen as part of routine care.

9.10 Other aspects

The study will adhere to guidance by the European Medicines Agency Guideline on good pharmacovigilance practices (GVP) Module VIII – Post-authorisation safety studies[2]; ENCePP Guide on Methodological Standards in Pharmacoepidemiology[3], the ENCePP Checklist for Study Protocols (Appendix B) and the Guidelines for Good Pharmacoepidemiology Practices of the International Society of Pharmacoepidemiology (ISPE GPP)[4]. The study will be registered on the EMA-HMA Catalogue of real-world data sources and studies[5].

10. PROTECTION OF HUMAN SUBJECTS

This observational study will be performed in accordance with ethical principles that are consistent with the Declaration of Helsinki, International Council for Harmonization (ICH) Good Clinical Practice (GCP), Good Pharmacoepidemiology Practice (GPP) and the applicable legislation on non-interventional studies and/or observational studies.

10.1 Data protection

Patients' personal data and investigators' personal data will be handled in compliance with all relevant local laws and regulations. The data collected for this study will be stored in a secure computer database, ensuring confidentiality in accordance with local data protection laws. All

[2] www.ema.europa.eu/en/documents/scientific-guideline/guideline-good-pharmacovigilance-practices-gvp-module-viii-post-authorisation-safety-studies-rev-3_en.pdf
[3] http://www.encepp.eu/standards_and_guidances/methodologicalGuide.shtml
[4] http://www.encepp.eu/standards_and_guidances/checkListProtocols.shtml
[5] https://catalogues.ema.europa.eu

patients have provided the necessary authorisations as required by local regulations, including authorisation for protected health information (PHI) in the USA, where applicable.

Sensitive data will be encrypted both at rest and during transmission to prevent unauthorized access and maintain confidentiality. Additionally, strict access controls will be enforced, ensuring that only authorized personnel can access the de-identified or anonymized data. This includes user authentication and role-based access within the system.

10.2 D8450R00003 data linkage with Argus PV data

Data collected in D8450R00003 are stored in a secure, access-controlled environment and evaluated in accordance with the Health Insurance Portability and Accountability Act (HIPAA), regulatory and local requirements and applicable guidance for electronic records. Patient names are not recorded in the EDC system. Patient data are coded and rendered indirectly identifiable (pseudonymized) using the unique study identification number in the EDC database.

During enrolment to D8450R00003 investigators enrol patients who meet the eligibility criteria, agree to participate and provide written informed consent (ICF). Participants who consent to participate in D8450R00003 and who also voluntarily provide consent to have their de-identified information used in future research studies will be used in the STTRing study.

Data collected from pharmacovigilance incident reports are stored and evaluated in Argus in accordance with regulatory requirements and following 21 CFR § 314.80 for Postmarketing reporting of adverse drug experiences[6] and Good Pharmacovigilance Practices and Pharmacoepidemiologic Assessment.[7]

De-identified participant data from D8450R0003 and de-identified case data from Argus will be matched using probabilistic linkage methods. Personal identifiers will not be used (including names, addresses, phone numbers and other related information). Linkage will be made based on age, sex and height and other variables as available.

10.3 Confidentiality of study/patient data

All personal identifiers such as patient and reporter names, addresses (postal, fax or email), phone numbers or other related identifiers (e.g., institution ID) will be removed or replaced

[6] https://www.ecfr.gov/current/title-21/chapter-I/subchapter-D/part-314/subpart-B/section-314.80].

[7] https://www.fda.gov/regulatory-information/search-fda-guidance-documents/good-pharmacovigilance-practices-and-pharmacoepidemiologic-assessment

https://www.fda.gov/regulatory-information/search-fda-guidance-documents/good-pharmacovigilance-practices-and-pharmacoepidemiologic-assessment

with pseudonyms or codes to ensure that individuals cannot be directly identified from the datasets. Where possible, further measures such as anonymisation may be applied to prevent any possibility of re-identifying individuals. Linkage of Argus PV data will only take place if it does not violate patient confidentiality. Probabilistic linkage between datasets will be conducted using age, sex and height and other variables as available to match de-identified patients between D8450R00003 and Argus PV database. These datasets will not include identifiable data.

11. MANAGEMENT AND REPORTING OF ADVERSE EVENTS/ADVERSE REACTIONS

11.1 Collection of adverse events and special situations

Study data are derived through secondary use of data from D8450R00003. For observational studies based on secondary data collection, individual AE reporting is not required.

11.2 Reporting of Adverse Events and Special Situations

No safety event data is required to be actively reported as these events are considered to have been reported through other sources when first collected. If the study collects safety events as part of the objectives these events will be described within the study report.

12. PLANS FOR DISSEMINATING AND COMMUNICATING STUDY RESULTS

The results of this study will be summarised in annual interim reports and a final study report that will be communicated to the applicable health authorities within the agreed timeframe. Study reports will be prepared using a template following the Guideline on Good Pharmacovigilance Practices (GVP) Module VIII Section B.6.3 (EMA 2017b). The interim reports will include status updates (i.e., progress against milestones) and will report and address any challenges in the progress of the project. Protocol details and an abstract of results will be posted on the EU-PAS register as per guidelines for studies meeting the criteria for PASS.

The final results of the study may be disseminated through submission of manuscripts for publication and guided by the Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication of the International Committee of Medical Journal Editors. Selected interim and final results may also be disseminated through publication or presentation at scientific meetings with input from the study scientific committee and other investigators serving as authors.

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Appendix A List of stand-alone documents

None.

Appendix B ENCePP checklist for Study protocols

Study title:
A cohort event monitoring study to characterise use of eplontersen in patients with prior liver transplant and pre-existing severe hepatic impairment and to assess long-term safety among all new users of eplontersen (sTTRing)

EU PAS Register® number: To be assigned when the study is registered
Study reference number (if applicable): D8450R00022

Section 1: Milestones	**Yes**	**No**	**N/A**	**Section Number**
1.1 Does the protocol specify timelines for				
1.1.1 Start of data collection[8]	☒	☐	☐	6
1.1.2 End of data collection[9]	☒	☐	☐	
1.1.3 Progress report(s)	☒	☐	☐	
1.1.4 Interim report(s)	☒	☐	☐	
1.1.5 Registration in the EU PAS Register®	☒	☐	☐	
1.1.6 Final report of study results.	☒	☐	☐	

Comments:

Section 2: Research question	**Yes**	**No**	**N/A**	**Section Number**
2.1 Does the formulation of the research question and objectives clearly explain:	☒	☐	☐	
2.1.1 Why the study is conducted? (e.g. to address an important public health concern, a risk identified in the risk management plan, an emerging safety issue)	☒	☐	☐	7
2.1.2 The objective(s) of the study?	☒	☐	☐	8
2.1.3 The target population? (i.e. population or subgroup to whom the study results are intended to be generalised)	☒	☐	☐	9.1, 9.2
2.1.4 Which hypothesis(-es) is (are) to be tested?	☐	☐	☒	

[8] 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.

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

Section 2: Research question	**Yes**	**No**	**N/A**	**Section Number**
2.1.5 If applicable, that there is no *a priori* hypothesis?	☐	☐	☒	

Comments:

Section 3: Study design	**Yes**	**No**	**N/A**	**Section Number**
3.1 Is the study design described? (e.g. cohort, case-control, cross-sectional, other design)	☒	☐	☐	9.1
3.2 Does the protocol specify whether the study is based on primary, secondary or combined data collection?	☒	☐	☐	9.1, 9.4
3.3 Does the protocol specify measures of occurrence? (e.g., rate, risk, prevalence)	☒	☐	☐	9.3, 9.7.2
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))	☒	☐	☐	9.7.2, 9.7.3, 9.7.4, 9.7.5
3.5 Does the protocol describe the approach for the collection and reporting of adverse events/adverse reactions? (e.g. adverse events that will not be collected in case of primary data collection)	☐	☐	☒	

Comments:

Study data are derived through secondary use of de-identified data from D8450R00003 and thus, individual AE reporting is not required. Measures of occurrence include prevalence, incidence risk, proportion of patients with an event during period t, incidence density, crude risk and, if feasible, comparative safety.

Section 4: Source and study populations	**Yes**	**No**	**N/A**	**Section Number**
4.1 Is the source population described?	☒	☐	☐	7, 9.4, Appendix C-(Annex 1)
4.2 Is the planned study population defined in terms of:				
4.2.1 Study time period	☒	☐	☐	9.1, 9.4.1
4.2.2 Age and sex	☒	☐	☐	9.4.1
4.2.3 Country of origin	☒	☐	☐	9.4.1

Section 4: Source and study populations	**Yes**	**No**	**N/A**	**Section Number**
4.2.4 Disease/indication	☒	☐	☐	9.4.1
4.2.5 Duration of follow-up	☒	☐	☐	9.4.1 Annex 1
4.3 Does the protocol define how the study population will be sampled from the source population? (e.g. event or inclusion/exclusion criteria)	☒	☐	☐	9.4.1

Comments:

This study is the secondary use of data collected in D8450R00003. D8450R00003 aims to create a global cohort of patients with transthyretin amyloidosis, irrespective of treatments received, age or sex.

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)	☒	☐	☐	9.3.1
5.2 Does the protocol address the validity of the exposure measurement? (e.g. precision, accuracy, use of validation sub-study)	☒	☐	☐	9.3.1
5.3 Is exposure categorised according to time windows?	☐	☒	☐	
5.4 Is intensity of exposure addressed? (e.g. dose, duration)	☒	☐	☐	9.3.1
5.5 Is exposure categorised based on biological mechanism of action and taking into account the pharmacokinetics and pharmacodynamics of the drug?	☐	☒	☐	
5.6 Is (are) (an) appropriate comparator(s) identified?	☒	☐	☐	9.7.7.1

Comments:

Section 6: Outcome definition and measurement	**Yes**	**No**	**N/A**	**Section Number**
6.1 Does the protocol specify the primary and secondary (if applicable) outcome(s) to be investigated?	☒	☐	☐	9.3.3
6.2 Does the protocol describe how the outcomes are defined and measured?	☒	☐	☐	9.3.2, 9.7.1

Section 6: Outcome definition and measurement	**Yes**	**No**	**N/A**	**Section Number**
6.3 Does the protocol address the validity of outcome measurement? (e.g. precision, accuracy, sensitivity, specificity, positive predictive value, use of validation sub-study)	☐	☒	☐	
6.4 Does the protocol describe specific outcomes relevant for Health Technology Assessment? (e.g. HRQoL, QALYs, DALYS, health care services utilisation, burden of disease or treatment, compliance, disease management)	☐	☒	☐	

Comments:

Section 7: Bias	**Yes**	**No**	**N/A**	**Section Number**
7.1 Does the protocol address ways to measure confounding? (e.g. confounding by indication)	☒	☐	☐	9.9.1
7.2 Does the protocol address selection bias? (e.g. healthy user/adherer bias)	☒	☐	☐	9.9.1
7.3 Does the protocol address information bias? (e.g. misclassification of exposure and outcomes, time-related bias)	☒	☐	☐	9.9.1

Comments:

Section 8: Effect measure modification	**Yes**	**No**	**N/A**	**Section Number**
8.1 Does the protocol address effect modifiers? (e.g. collection of data on known effect modifiers, sub-group analyses, anticipated direction of effect)	☒	☐	☐	9.7

Comments:

A primary objective of this observational study is to characterize subgroups of interest, including individuals with a) prior liver transplant and separately, b) pre-existing severe hepatic impairment at the start of eplontersen treatment.

Section 9: Data sources	**Yes**	**No**	**N/A**	**Section Number**
9.1 Does the protocol describe the data source(s) used in the study for the ascertainment of:				
9.1.1 Exposure? (e.g. pharmacy dispensing, general practice prescribing, claims data, self-report, face-to-face interview)	☒	☐	☐	9.3.1

Section 9: Data sources	**Yes**	**No**	**N/A**	**Section Number**
9.1.2 Outcomes? (e.g. clinical records, laboratory markers or values, claims data, self-report, patient interview including scales and questionnaires, vital statistics)	☒	☐	☐	9.3.2, Appendix C - Annex 1
9.1.3 Covariates and other characteristics?	☒	☐	☐	9.3.2.2 (Table 3)
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)	☒	☐	☐	9.3.1, 9.4.1
9.2.2 Outcomes? (e.g. date of occurrence, multiple event, severity measures related to event)	☒	☐	☐	9.3.2, Appendix C Annex 1
9.2.3 Covariates and other characteristics? (e.g. age, sex, clinical and drug use history, co-morbidity, co-medications, lifestyle)	☒	☐	☐	9.3.2.2 (Table 3)
9.3 Is a coding system described for:				
9.3.1 Exposure? (e.g. WHO Drug Dictionary, Anatomical Therapeutic Chemical (ATC) Classification System)	☒	☐	☐	9.4.1
9.3.2 Outcomes? (e.g. International Classification of Diseases (ICD), Medical Dictionary for Regulatory Activities (MedDRA))	☒	☐	☐	9.3.2.3, 9.4.1
9.3.3 Covariates and other characteristics?	☒	☐	☐	9.4.1
9.4 Is a linkage method between data sources described? (e.g. based on a unique identifier or other)	☒	☐	☐	9.4.2, 9.4.3

Comments:

This study relies on the secondary use of de-identified data from D8450R00003 and thus the methods utilizes for ascertaining of data, data sources and coding systems are described for D8450R00003. Data linkage and of D8450R00003 with de-identified data in the Argus PV and US claims data is described.

Section 10: Analysis plan	**Yes**	**No**	**N/A**	**Section Number**
10.1 Are the statistical methods and the reason for their choice described?	☒	☐	☐	9.7.1
10.2 Is study size and/or statistical precision estimated?	☒	☐	☐	9.5
10.3 Are descriptive analyses included?	☒	☐	☐	9.7.2
10.4 Are stratified analyses included?	☒	☐	☐	9.7.1

Section 10: Analysis plan	**Yes**	**No**	**N/A**	**Section Number**
10.5 Does the plan describe methods for analytic control of confounding?	☐	☒	☐	
10.6 Does the plan describe methods for analytic control of outcome misclassification?	☒	☐	☐	8.3, 9.8.2, 9.8.3
10.7 Does the plan describe methods for handling missing data?	☒	☐	☐	9.6
10.8 Are relevant sensitivity analyses described?	☒	☐	☐	9.7.1

Comments:

This study will be descriptive in nature and relies on the secondary use of data from D8450R00003 and thus the methods for addressing missing data are addressed in the data management of D8450R00003. Exploratory objective outcomes address data sensitivity analyses (section 9.7.1).

Section 11: Data management and quality control	**Yes**	**No**	**N/A**	**Section Number**
11.1 Does the protocol provide information on data storage? (e.g. software and IT environment, database maintenance and anti-fraud protection, archiving)	☒	☐	☐	9.6, 9.8
11.2 Are methods of quality assurance described?	☒	☐	☐	9.8.1
11.3 Is there a system in place for independent review of study results?	☐	☒	☐	

Comments:

This study is based on the secondary use of data collected in D8450R00003.

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?	☒	☐	☐	9.9.1
12.1.2 Information bias?	☒	☐	☐	9.9.1
12.1.3 Residual/unmeasured confounding? (e.g. anticipated direction and magnitude of such biases, validation sub-study, use of validation and external data, analytical methods).	☐	☒	☐	
12.2 Does the protocol discuss study feasibility? (e.g. study size, anticipated exposure uptake, duration of follow-up in a cohort study, patient recruitment, precision of the estimates)	☒	☐	☐	9.5.1, 9.5.2, Appendix C-Annex 2

Comments:

This study is observational and descriptive in nature with no formal statistical testing. However, sample size considerations were explored for incidence/prevalence proportions as well as for cohort event monitoring (Annex 2).

Section 13: Ethical/data protection issues	**Yes**	**No**	**N/A**	**Section Number**
13.1 Have requirements of Ethics Committee/ Institutional Review Board been described?	☐	☐	☒	
13.2 Has any outcome of an ethical review procedure been addressed?	☐	☐	☒	
13.3 Have data protection requirements been described?	☒	☐	☐	10

Comments:

This study is a secondary use of data and includes data from patients who have consented in D8450R00003 to the use of their data for future related research studies (section 9.2.1).

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

Comments:

Section 15: Plans for communication of study results	**Yes**	**No**	**N/A**	**Section Number**
15.1 Are plans described for communicating study results (e.g. to regulatory authorities)?	☒	☐	☐	6, 12
15.2 Are plans described for disseminating study results externally, including publication?	☒	☐	☐	12

Comments:

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

Date: 09/April/2025

Electronically Signed

Appendix C Additional information

Annex 1. Protocol synopsis of D8450R00003 study

Title	A Non-interventional, Prospective, Multi-country Study Collecting Real-world Data on the Characteristics, Treatment Patterns, and Outcomes of Patients with Amyloid Transthyretin (ATTR) Amyloidosis
Identifiers	[redacted] study identifier[10]: D8450R00003 ClinicalTrials.gov identifier: NCT06465810
Status	Recruiting (first patient in: 25 June 2024)
Countries	Canada, China, Germany, Spain, United Kingdom, United States
Rationale	This study aims to create a global cohort of patients with ATTR amyloidosis to longitudinally observe the natural course of the disease and describe real-world treatment patterns and outcomes. In addition, information on the effectiveness of ATTR amyloidosis treatments, including eplontersen, is collected. This study is intended to increase knowledge about different genotypes and phenotypes of ATTR, diagnosis, management, and treatment of patients with ATTR amyloidosis in a real-world setting. Several features distinguish this study from existing registries and real-world studies: • Eligibility for enrollment of all patients with ATTR amyloidosis, irrespective of treatments received • Good representation of different ATTR amyloidosis phenotypes • Hybrid design, which incorporates both primary data (patient-centric measures) and secondary data collection • Targeted collection of study variables to minimise study site workload • Collection of data available as per routine care • Early and frequent collaboration with key external experts on study design, study conduct, data interpretation, and publication planning and authorship
Objectives	Primary objectives: 1. To describe the demographic and clinical characteristics of patients with ATTR amyloidosis at baseline. 2. To describe treatment patterns for patients with ATTR amyloidosis at baseline and describe longitudinal changes in treatment over time. 3. To examine the effectiveness, including health-related quality of life (HRQoL), clinical measures related to ATTR amyloidosis, healthcare resource utilization (HCRU), and mortality, of ATTR amyloidosis treatments. 4. To describe the demographic and clinical characteristics of patients at their first prescription of eplontersen, filled at any time during the observation period. 5. To describe treatment patterns of patients after initiation of eplontersen and the association of these treatment patterns with effectiveness, including HRQoL, clinical measures related to ATTR amyloidosis, HCRU, and mortality. Secondary objectives: 1. To compare the demographic and clinical characteristics of patients prescribed eplontersen at any time during the observation period to those who are not prescribed eplontersen during that time period. 2. To compare the effectiveness (including HRQoL, clinical measures related to ATTR amyloidosis, HCRU, and mortality) of eplontersen versus other ATTR amyloidosis treatments. NB: Comparative assessments only performed if sufficient data are available, allowing for adequate cohort balance and statistical power. 3. To describe HCRU for patients with ATTR amyloidosis. Exploratory objectives:

[10] [redacted]

	1. To identify the risk factors for worsening ATTR progression. 2. To describe healthcare costs for patients with ATTR amyloidosis. NB: Description of healthcare costs performed if sufficient data are available. 3. To evaluate the safety (serious adverse events [SAEs]) during ATTR amyloidosis treatments.
Design	International, longitudinal, non-interventional study of adult patients with transthyretin ATTR amyloidosis.
Study period	Study period: Q2/2024 to Q2/2031 (~7 years) Enrollment: Q2/2024 to Q2/2028 (~4 years) Follow-up: Q2/2024 to Q2/2031 up to 7 years)
Population	Patients with a diagnosis of amyloid transthyretin (ATTR) amyloidosis
Selection criteria	Inclusion Criteria: • Patient willing and able to provide written informed consent to participate in the study • Confirmed diagnosis of amyloid transthyretin (ATTR) amyloidosis • Aged ≥18 years at the time of signing the informed consent • Patient willing and able to participate in collection of electronic patient reported outcomes (PROs) Exclusion Criteria: • Concurrent participation in any interventional trial for ATTR amyloidosis • Involvement in the planning and/or conduct of the current study • Patients with evidence of primary or light chain amyloidosis (AL) or serum protein A amyloidosis (AA) • Asymptomatic patients with ATTR amyloidosis and asymptomatic ATTR mutation carriers
Countries	United Kingdom, Germany, Canada, Spain, United States and China
Sample size	Plans to enroll a minimum of 1,850 patients with ATTR amyloidosis, including 274 patients with PN phenotype, 706 patients with mixed phenotype, and 850 patients with cardiomyopathy phenotype (plus 20 patients in either category)
Data source	Includes both primary and secondary data. Primary data consists of patient-reported outcome (PRO) questionnaires. Patients are asked to complete electronic PRO questionnaires at enrolment and every 6 months (±3 months) only during routine visits. Secondary data consists of demographic, clinical, and treatment information, and is collected as per routine clinical practice. These data are abstracted directly from the electronic health record or review of paper charts for each patient and entered in the electronic data capture system. No site visits are required for this study, and patients are not be contacted for patient reported outcomes collection outside of routine clinic visits. For patients enrolled in the United States, a tokenization process (creation of a unique, encrypted identifier called a token, in place of personal identifiable information) is used to collect additional de-identified data (e.g., healthcare resource use, healthcare costs) from other sources that are part of patients' routine medical care (electronic medical, hospital, or pharmacy records). Only de-identified data is analyzed. Patients are given a choice within the informed consent form to opt in or opt out of participating in the tokenization process.
Variables	Demographics and patient characteristics: o Age, sex as determined by the investigator (male/female), race and ethnicity (where allowed) o Insurance (US only): uninsured; self-pay; Medicaid; Medicare; commercial; other government program; don't know; other o Weight, height, blood pressure, heart rate o Physiological measurements (heart rate, blood pressure) o Medical history within 12 months prior to baseline o Family history of ATTR amyloidosis Clinical characteristics of ATTR amyloidosis

o Date and nature of the first symptoms, signs of ATTR amyloidosis including cardiac (abnormal ECG: heart block/ventricular tachycardia, leg/ankle/abdominal swelling) and symptoms (fatigue, shortness of breath with activity, palpitations, orthostatic hypotension, angina pectoris, atypical chest pain, lightheadedness)
o Date of diagnosis of ATTR amyloidosis
o TTR genetic test results: zygosity, mutation, variant
o Clinical manifestations (signs and symptoms) of ATTR amyloidosis: ischemic heart disease, acute myocardial infarction, heart failure, atrial fibrillation, arrhythmias, conduction system disease, aortic valve stenosis, polyneuropathy, carpal tunnel syndrome, carpal tunnel syndrome surgery, autonomic neuropathy, nephrotic syndrome, subnephrotic proteinuria, gastrointestinal dysfunction, chronic kidney disease/acute kidney injury, spinal stenosis, spinal stenosis surgery, hepatomegaly, ascites, oedema, any other amyloidosis related manifestations (e.g., Popeye's syndrome, tendon rupture)
o New York Heart Association (NYHA) classification (most recent if multiple values are available): I, II, III, IV
o National Amyloidosis Centre (NAC) ATTR staging or Mayo staging, if available: 1, 2, 3
o Familial amyloid polyneuropathy (FAP) (Coutinho) staging: 0, 1, 2, 3
o Polyneuropathy disability (PND) score: 0, I, II, IIIA, IIIB, IV
o 6-minute walk test
o Charlson comorbidity index (CCI) (most recent score if multiple scores are available) and CCI components (myocardial infarction, congestive heart failure, peripheral vascular disease, cerebrovascular disease, dementia, chronic pulmonary disease, rheumatic disease, peptic ulcer disease, mild liver disease, diabetes (with and without chronic complications), hemiplegia or paraplegia, renal disease, any malignancy, moderate or severe liver disease, metastatic solid tumor, acquired immunodeficiency disease (AIDS)/ human immunodeficiency virus (HIV)
Other comorbidities of interest: depression, fibromyalgia
Liver disease (for patients with mild, moderate or severe liver disease: Child Pugh score, Child Pugh class, ascites, encephalopathy)
Liver transplantation (reason for transplant, type of transplant)
o Other comorbidities of interest like depression and fibromyalgia

Diagnostic procedures
o Biopsy (fat tissue, skin, gastrointestinal tract, lip, bone marrow, endomyocardial, other)
o Imaging (echocardiography variables, cardiovascular magnetic resonance imaging, bone tracer cardiac scintigraphy)
o Electrocardiogram (ECG) variables
o Sural nerve and tibial nerve amplitude

Treatments
o Prior and current ATTR amyloidosis treatment/procedures: tafamidis (TTR stabiliser), diflunisal (TTR stabiliser, non-steroidal anti-inflammatory), acoramidis (TTR stabiliser), vutrisiran (TTR silencer), patisiran (TTR silencer), inotersen (TTR silencer), eplontersen (TTR silencer), other AATR amyloidosis treatments introduced during the course of the study, doxycycline and taurodesoxycholic acid, liver transplant
o Concomitant treatments: heart failure/arrhythmia-related treatment (diruetics, angiotensin converting enzyme inhibitor, angiotensin receptor blocker, angiotensin receptor-neprilysin inhibitor, anticoagulant, beta-blockers, sodium-glucose co-transporter-2 inhibitor, mineralocorticoid receptor antagonist, digoxin, pacemaker use, implantable cardioverter-defibrillator, left ventricular assist device, cardiac transplant, trancatheter aortic valve replacement, surgical aortic valve replacement), polyneuropathy-related treatment (antiepileptics, antidepressants, topical pain treatments, opiods, tetrahydrocannabinol), other treatments (medications for gastrointestinal symptoms, vitamin A supplementation, dialysis)

Laboratory tests

	o Biomarkers: serum TTR levels, complete blood count, hemoglobin, troponin I, cystatin (where available), creatinine, reported glomerular filtration rate (GFR), albumin, liver enzymes, N-terminal pro-brain natriuretic peptide (NT-proBNP), vitamin A level, neurofilament light chain (NfL), international normalised ratio (INR) test o Urine test results: urine albumin-creatinine ratio (UACR), urine protein creatinine ratio (UPCR) Healthcare resource utilisation (outpatient visits, inpatient visits, emergency department visits, urgent care visits [where available], hospitalisations) Healthcare costs, when available PROs (SF-36v2, Norfolk QOL-DN, KCCQ-23)
Safety information	Safety data (SAEs) All SAEs regardless of causality or ATTR treatment are collected. SAEs are obtained from the from patients' medical records and recorded in the eCRF. SAEs are also reported to [redacted] pharmacovigilance reporting system. Non-serious AEs are not collected (into study eCRF). Instead, investigators/patients are informed of the option/means to report non-serious AEs to [redacted] or national health authority via spontaneous reporting systems.

Annex 2. Sample size considerations for the identification of adverse events requiring further characterisation based on cohort event monitoring analysis

The Table 8 below provides calculations to indicate the power to detect an IDD between two 6-months periods in a sample of 250 patients. The calculations are based on the z-test with the square root transform to test whether the difference between two independent Poisson rates is different from zero. The type 1 error rate is set to 5% for the two-sided test. As an example, if the incidence density in period 1 is 0.05 and the incidence density in period 2 is 0.10, the IDD is 0.05 and Rate ratio is 2, the power is approximately 54% to detect the difference in a sample of 250 patients.

Table 8. Power to detect a difference in incidence densities with sample size of 250 patients with mean follow-up of 6 months in each group

Backround ID (per 100 person-years)	**ID ratio > 1.5**	**ID ratio > 2**	**ID ratio > 3**	**ID ratio > 4**
1	5%	10%	21%	35%
5	12%	31%	74%	94%
10	20%	54%	96%	100%

ID: incidence density

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