Protocol B3461087

GLOBAL PREVALENCE OF TRANSTHYRETIN AMYLOID CARDIOMYOPATHY (ATTR-CM) IN PARTICIPANTS WITH HEART FAILURE WITH PRESERVED EJECTION FRACTION (HFpEF)

Statistical Analysis Plan (SAP)

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TABLE OF CONTENTS

LIST OF TABLES 3
APPENDICES 3
1. VERSION HISTORY 4
2. INTRODUCTION 8
2.1. Study Objectives, Endpoints, and Estimands 8
2.2. Study Design 10
3. ENDPOINTS AND BASELINE VARIABLES: DEFINITIONS AND CONVENTIONS 10
3.1. Primary Endpoint(s) 10
3.2. Secondary Endpoint(s) 11
CCI [redacted]
3.4. Baseline Variables 13
3.5. Safety Endpoints 13
4. ANALYSIS SETS (POPULATIONS FOR ANALYSIS) 13
5. GENERAL METHODOLOGY AND CONVENTIONS 13
5.1. Hypotheses and Decision Rules 13
5.2. General Methods 14
5.2.1. Analyses for Prevalence Endpoints 14
5.2.2. Analyses for Continuous Endpoints 14
5.2.3. Analyses for Categorical Endpoints 14
5.3. Methods to Manage Missing Data 14
6. ANALYSES AND SUMMARIES 14
6.1. Primary Endpoint(s) 14
6.1.1.1. Sensitivity/Supplementary Analysis 15
6.2. Secondary Endpoint(s) 15
CCI [redacted]
6.4. Safety Summaries and Analyses 17
6.4.1. Adverse Events 17
6.4.2. Physical Examination 17
7. INTERIM ANALYSES 17
7.1. Interim Analyses and Summaries 17
8. REFERENCES 17

9. APPENDICES 18

LIST OF TABLES

Table 1. Summary of Changes.....4
Table 2. Summary of Major Changes in SAP Amendment 1.....5
Table 3. Summary of Major Changes in SAP Amendment 2.....8

APPENDICES

Appendix 1. Summary of Primary, Secondary, CCI Analyses.....18
Appendix 2. List of Abbreviations.....20

1. VERSION HISTORY

This Statistical Analysis Plan (SAP) for Study B3461087 is based on the protocol amendment 2 dated 20 August 2020 and the latest PACL (08Oct 2021).

Table 1. Summary of Changes

Version/ Date	Associated Protocol Amendment	Rationale	Specific Changes
1/ 10 Mar 2020	Original 20 Dec 2019	Not applicable (N/A)	N/A
2/ 10 Sep 2020	Protocol Amendment 2 20 Aug 2020	Study simplified to improve study feasibility and re-focus plan to achieve study objectives.	• Sample size was decreased to 2000. • Visit 2 deleted from the protocol and therefore also from analyses. • CCI [redacted] • The following assessments were deleted: electrocardiogram (ECG), transthyretin (TTR) concentration, banked biospecimens. • Echocardiogram at screening only required if there has not been one performed ± 6 months of Screening Visit. • Results for Troponin I and/or T will be collected if performed ± 6 months of Screening Visit. • Participants with scintigraphy grade 1 may not have biopsy confirmation of transthyretin amyloid cardiomyopathy (ATTR-CM) therefore, clarification added to perform genotyping on participants with scintigraphy grades 1-3. Also, clarity provided for sequencing. • Participants with grade 1 scintigraphy that were diagnosed with ATTR-CM using a criteria other than cardiac biopsy will be summarized as “Indeterminate”. • Appendix 1 updated to include sensitivity analysis and to remove ECG,

			TTR concentration, banked biospecimens details.
3/24 Jan 2022	Protocol Amendment 2 20 Aug 2020/ Latest PACL: 08Oct 2021		• The Primary, sensitivity, and secondary endpoints evaluating prevalence will be analyzed using "Evaluable Analysis Set". This analysis set excludes subjects with non-evaluable scintigraphy grade. • All endpoints other than those estimating prevalence will be analyzed using "All Enrolled Analysis Set". • Use ATTR-CM diagnosis as defined in Section 6.1.1.1 for the secondary, CCI and safety analysis. • Participants with non-evaluable scintigraphy grade will be summarized as a separate group.

Table 2. Summary of Major Changes in SAP Amendment 1

Section of the SAP	Summary of change	Rationale
Section 2.1 Study Objectives, Endpoints, and Estimands	CCI [redacted] • Collection of data if available for estimated glomerular filtration rate (eGFR), modified body mass index (mBMI) and Cardiac Troponin-T and I performed ±6 months of Screening Visit. CCI [redacted] • The following assessments were deleted: ECG, TTR concentration, banked biospecimens.	Study simplified to improve study feasibility and re-focus plan to achieve study objectives.
Section 2.2 Study Design	Sample size was decreased to 2000.	Sample size was decreased to 2000 which is adequate to

Section of the SAP	Summary of change	Rationale
		maintain the precision.
Section 3.1 Primary Endpoint	Added "during the study period".	Added clarity for the global prevalence estimation.
CCI [redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]
Section 3.4 Baseline Variables	Removed endomyocardial biopsy evaluation at Day 30.	Study simplified to improve study feasibility and re-focus plan to achieve study objectives.
Section 6.1 Primary Endpoint	Added: • "during the study period" in prevalence wording. Scintigraphy Grade 1 participants that are lost to follow-up will be assumed as not diagnosed with ATTR-CM.	Added clarity on the global prevalence estimation.
Section 6.1.1 Sensitivity/Supplementary Analysis	A sensitivity analysis, of the primary prevalence estimate was added.	A sensitivity analysis, of the primary prevalence estimate was added.
Section 6.1.1.1 Sensitivity/Supplementary Analysis	Participants with grade 1 scintigraphy that were diagnosed with ATTR-CM using a criteria other than cardiac biopsy will be summarized as "Indeterminate".	The sensitivity analysis for the prevalence was added.

Section of the SAP	Summary of change	Rationale
Section 6.2 Secondary Endpoint(s)	CCI [redacted]	Study simplified to better address site feasibility concerns.
CCI [redacted]	[redacted]	[redacted]
6.4 Safety summary	Visit 2 will be deleted from Safety Analysis.	Study simplified to better address site feasibility concerns.
Appendix 1	Include sensitivity analysis and to remove ECG, TTR concentration, banked biospecimens details.	Provide details on the Eficacy and safety statistical analyses.
Appendix 2	Removed ECG from Abbreviations	Correct inconsistency thru the statistical analysis plan (SAP).

Table 3. Summary of Major Changes in SAP Amendment 2

Section of the SAP	Summary of change	Rationale
Sections 2.1, 3, 3.3, 5.2.1, 5.2.2, 5.2.3, 6.1, 6.2, 6.4.1	Use ATTR-CM diagnosis as defined in Section 6.1.1.1.	Clarify and redefine the ATTR-CM diagnosis by using the sensitivity definition.
Section 3, 3.2, 5.2.1, 6.1, 6.2	Use the evaluable analysis set to analyze endpoints specified in the sections identified rather than All enrolled analysis set.	Clarify the population for analysis.
Section 4 Analysis Sets (populations for analysis)	Add table to display the "All Enrolled Analysis Set" and the "Evaluable Analysis Set".	Clarify the population for analysis.
Section 6.2	Removed Rest of the World from the list of regions.	No countries under this category are planned.
Section 6.1.1.1	Include analysis "The proportion of Scintigraphy Grade 1 participants that are diagnosed with ATTR-CM will be estimated." Remove Subgroup analysis.	The proportion of Scintigraphy Grade 1 analysis was added. After redefining the ATTR-CM diagnosis by using the sensitivity definition the subgroup analysis in Section 6.1.1.1 was redundant.
Section 6.4.1	Include "Participants with non-evaluable scintigraphy grade will be summarized in a separate group."	The participants with non-evaluable scintigraphy grade will be summarized in a separate group.

2. INTRODUCTION

This statistical analysis plan (SAP) provides the detailed methodology for summary and statistical analyses of the data collected in Study B3461087. This document may modify the plans outlined in the protocol; however, any major modifications of the primary endpoint definition or its analysis will also be reflected in a protocol amendment.

2.1. Study Objectives, Endpoints, and Estimands

Study B3461087 has no study treatment intervention and therefore estimands do not apply to this study.

Primary Objective(s):	**Primary Endpoint(s):**
To assess the prevalence of transthyretin amyloid cardiomyopathy (ATTR-CM) among participants with heart failure with preserved ejection fraction (HFpEF) within a clinically at-risk population.	Estimate of global prevalence of ATTR-CM in HFpEF participants. Diagnosis of ATTR-CM is defined as: • Cardiac Scintigraphy Grade 1, with confirmation of transthyretin amyloid (ATTR) by cardiac biopsy, or: • Cardiac Scintigraphy Grade 2 or above.
Secondary Objective(s):	**Secondary Endpoint(s):**

To assess variations in prevalence of ATTR-CM as defined in Section 6.1.1.1 (wild-type [WT] and hereditary) among participants with HFpEF at a country, regional, and global level.	• Estimate of prevalence of ATTR-CM as defined in Section 6.1.1.1 in participants with HFpEF based on:
	  • Geographical or regional distribution;
	  • Age categories;
	  • Gender.
	• Proportion of participants with WT and hereditary forms among participants diagnosed with ATTR-CM as defined in Section 6.1.1.1.
	• Baseline characteristics of HFpEF participants with and without ATTR-CM as defined in Section 6.1.1.1:
	  • New York Heart Association (NYHA) classification;
	  • N terminal prohormone B type natriuretic peptide (NT-proBNP).

CCI [redacted]	[redacted]
[redacted]	[redacted]

2.2. Study Design

This study is a global, multi-center epidemiology study designed to estimate the global prevalence of ATTR-CM in participants with HFpEF within a clinically at risk population. The study will enroll approximately 2000 participants and is expected to detect 200 participants with ATTR-CM.

3. ENDPOINTS AND BASELINE VARIABLES: DEFINITIONS AND CONVENTIONS

3.1. Primary Endpoint(s)

The primary endpoint is an estimate of the global prevalence of ATTR-CM in HFpEF participants. Diagnosis of ATTR-CM is defined as:

- Cardiac Scintigraphy Grade 1, with confirmation of ATTR by cardiac biopsy during the study period, or:
- Cardiac Scintigraphy Grade 2 or above.

Prevalence is defined as the number of participants meeting the diagnosis criteria above divided by the number of evaluable participants in the study.

3.2. Secondary Endpoint(s)

The secondary endpoints are listed below will consider a participant as diagnosed with ATTR-CM based on the definition in Section 6.1.1.1.

- Estimate of prevalence of ATTR-CM in participants with HFpEF within the following subgroups, where prevalence within each subgroup is defined as the number of participants meeting the diagnosis criteria in Section 6.1.1.1 divided by the number of evaluable participants in the study in the given subgroup category:
  - Regions (North America, Europe, Asia);
  - Age categories (60-64, 65-69, 70-74, 75-79, 80-85, >85);
  - Gender (male, female).
- Participants diagnosed with ATTR-CM will be evaluated for TTR genotype, ie, wild-type or hereditary form. The proportions of these two genotypes will be evaluated as a secondary endpoint
- Baseline characteristics of HFpEF participants with and without ATTR-CM:
  - NYHA classification: Participants will be evaluated using the New York Heart Association classification at baseline visit (day 1). Detailed description of each class (Class I, Class II, Class III, Class IV) is available in Section 8.2.12 of the protocol.
  - NT-proBNP: A biomarker that will be assessed during visit 1.

CCI

CCI

[redacted]

CCI [redacted]

3.4. Baseline Variables

This study does not have a study treatment and all assessment for the primary, secondary, and CCI endpoints, are collected either during the Screening Visit or Visit 1 (Day 1).
CCI [redacted]

3.5. Safety Endpoints

Adverse events will be captured at screening, on day 1 and through the follow-up Visit 1. As there is no study treatment administered as part of this protocol, a definition of treatment emergence is not defined for adverse events. All adverse events documented in the study database will be reported and summarized.

4. ANALYSIS SETS (POPULATIONS FOR ANALYSIS)

All primary, sensitivity, and secondary endpoints estimating prevalence will be based on "Evaluable Analysis Set" defined below. All other endpoints, including CCI [redacted] and safety endpoints will be evaluated using "All Enrolled Analysis Set".

Population	Description
All Enrolled Analysis Set	All participants who meet the inclusion/exclusion criteria and completed Visit 1
Evaluable Analysis Set	All participants who meet the inclusion/exclusion criteria and completed Visit 1, excluding participants with Cardiac Scintigraphy assessed as non-evaluable

5. GENERAL METHODOLOGY AND CONVENTIONS

5.1. Hypotheses and Decision Rules

The primary objective of the study is to estimate global prevalence of ATTR-CM in HFpEF participants. No formal hypotheses testing will be performed.

5.2. General Methods

5.2.1. Analyses for Prevalence Endpoints

The primary endpoint estimating prevalence will be evaluated by dividing the number of participants who are classified as ATTR-CM positive by Cardiac Scintigraphy as defined in Section 3.1 by the total number of evaluable participants. The secondary endpoints estimating prevalence will be evaluated by dividing the number of participants who are classified as ATTR-CM positive by Cardiac Scintigraphy as defined in Section 6.1.1.1 by the total number of HFpEF evaluable participants in the study (primary) or the relevant subgroup (secondary). Exact 95% confidence intervals for the prevalence estimates will be calculated using the method of Clopper and Pearson.[1]

5.2.2. Analyses for Continuous Endpoints

All secondary CCI endpoints reported as numerical values will be analyzed descriptively using mean, standard deviation, median, IQR, minimum and maximum. Where applicable, secondary CCI endpoints of interest which specify comparisons between HFpEF participants diagnosed with ATTR-CM as defined in Section 6.1.1.1 and those that are not diagnosed with ATTR-CM that are normally distributed will be compared using a t-test. Endpoints that are not normally distributed will be compared using the Wilcoxon rank sum test.

5.2.3. Analyses for Categorical Endpoints

Secondary endpoints that are categorical will be summarized descriptively using frequency tables. The Chi-square test will be used to compare the proportions between HFpEF participants diagnosed with ATTR-CM as defined in Section 6.1.1.1 and those that are not diagnosed with ATTR-CM, where applicable. Fishers exact test will be used instead of the chi-square test if any one of the cells has ≤5 participants.

5.3. Methods to Manage Missing Data

No missing data imputation will be performed in the analyses described in Section 6.

6. ANALYSES AND SUMMARIES

6.1. Primary Endpoint(s)

The primary endpoint is an estimate of the global prevalence of ATTR-CM in HFpEF participants. Diagnosis of ATTR-CM is defined as:

- Cardiac Scintigraphy Grade 1, with confirmation of ATTR by cardiac biopsy during the study period, or:
- Cardiac Scintigraphy Grade 2 or above.

The primary endpoint will be evaluated by dividing the number of participants who are classified as ATTR-CM positive by Cardiac Scintigraphy (as noted above) by the total number of HFpEF evaluable participants in the study. The proportion of Scintigraphy Grade 1 participants that are diagnosed with ATTR-CM will be estimated. Scintigraphy

Grade 1 participants that are diagnosed with ATTR-CM using a criteria other than cardiac biopsy and participants lost to follow-up without a confirmation of cardiac biopsy will be assumed as not diagnosed with ATTR-CM in the primary analysis. Exact 95% confidence intervals for the prevalence will be calculated using the method of Clopper and Pearson.[1] The primary analysis will be generated using the "Evaluable Analysis Set".

6.1.1.1. Sensitivity/Supplementary Analysis

As a sensitivity analysis, the prevalence will be estimated using the diagnosis criteria described in the primary endpoint, but will include all participants with Scintigraphy Grade 1 diagnosed with ATTR-CM in the study (regardless of the cardiac biopsy). Specifically, participants with Scintigraphy Grade 1 that were diagnosed with ATTR-CM using a criteria other than cardiac biopsy will be included in the estimate of prevalence in this sensitivity analysis. Scintigraphy Grade 1 participants that are lost to follow-up prior to a diagnosis of ATTR-CM will be assumed as not diagnosed with ATTR-CM. The proportion of Scintigraphy Grade 1 participants that are diagnosed with ATTR-CM will be estimated.

6.2. Secondary Endpoint(s)

The Secondary endpoints listed below will be compared between HFpEF participants with and without ATTR-CM as defined in Section 6.1.1.1.

- The prevalence of ATTR-CM in HFpEF participants will be evaluated for subgroups including regions (North America, Europe, and Asia), age categories (60-64, 65-69, 70-74, 75-79, 80-85, >85), and gender (male, female). Exact 95% confidence intervals for the prevalence in subgroups will be calculated using the method of Clopper and Pearson.[1] Prevalence estimates along with 95% CIs will be generated for subgroups with at least 50 participants. For subgroups that enroll fewer than 50 participants, prevalence will not be estimated. These secondary analyses will be generated using the "Evaluable Analysis Set".

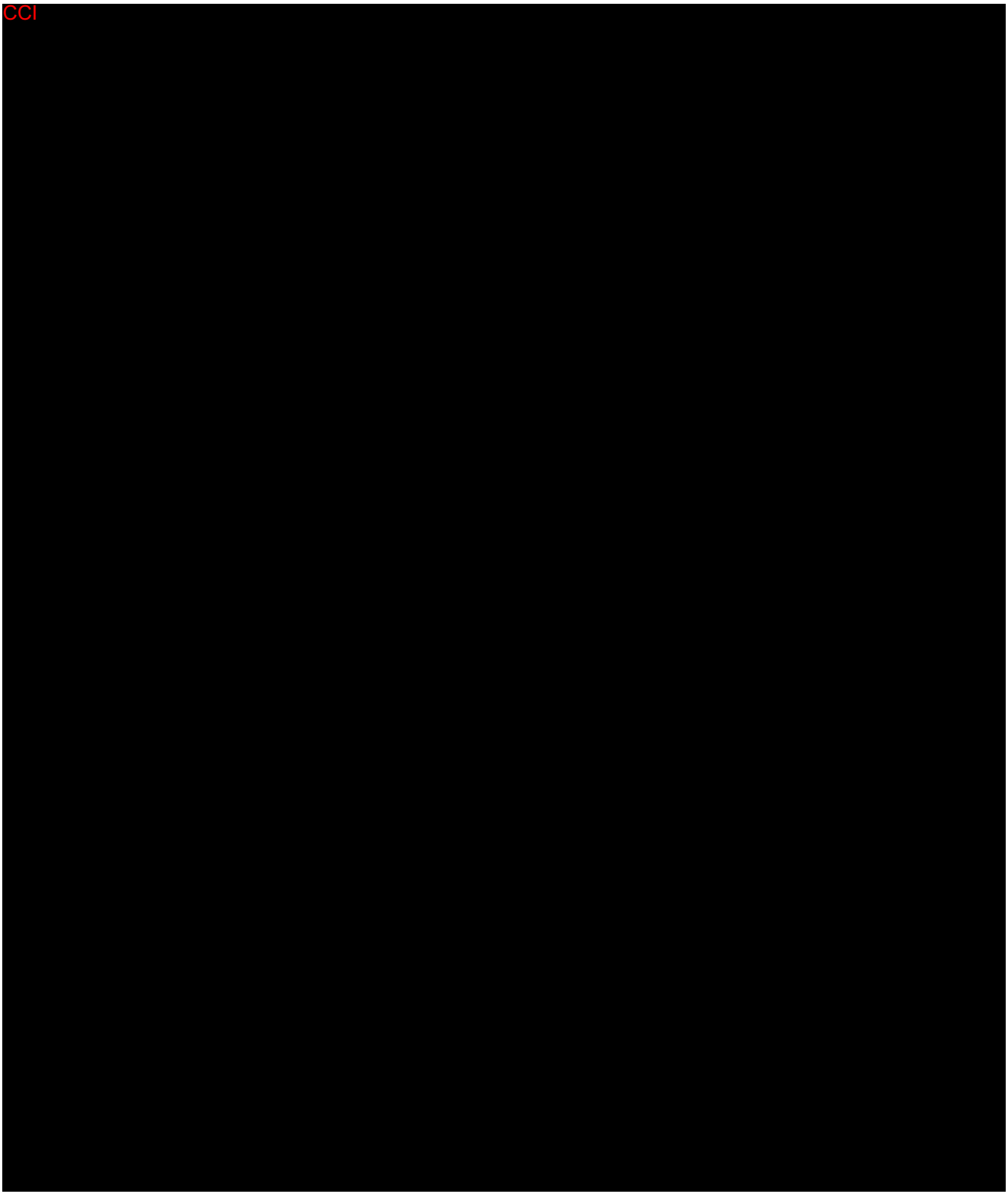
- Participants diagnosed with ATTR-CM will be evaluated for TTR genotype, ie, wild-type or hereditary form. The proportions of these two genotypes will be evaluated as a secondary endpoint using descriptive statistics only (ie, number and percentage).

- NYHA classification proportions will be compared between HFpEF participants diagnosed with ATTR-CM and those that are not diagnosed with ATTR-CM using the Chi-square test. Fisher's exact test will be used instead if any one of the cells has ≤5 participants. Proportion of NYHA classifications will also be descriptively summarized (frequency tables) by scintigraphy grade for participants who are diagnosed with ATTR-CM. Participants with non-evaluable scintigraphy grade will be summarized in a separate group.

- NT-proBNP will be compared between HFpEF participants diagnosed with ATTR-CM and those that are not diagnosed with ATTR-CM using a Wilcoxon rank sum test.

- NT-proBNP (mean, standard deviation, median, IQR, minimum and maximum) will be descriptively analyzed by scintigraphy grade and NYHA classification for participants who are diagnosed with ATTR-CM. Participants with non-evaluable scintigraphy grade will be summarized in a separate group.

CCI



CCI

[redacted]

6.4. Safety Summaries and Analyses

6.4.1. Adverse Events

All adverse events and serious adverse events collected at the Screening Visit, Visit 1 or during follow-up, will be coded according to the Medical Dictionary for Regulatory Activities (MedDRA) and will be summarized by system organ class and preferred terms overall and separately for HFpEF participants diagnosed with ATTR-CM and HFpEF participants not diagnosed with ATTR-CM as defined in Section 6.1.1.1. Participants with non-evaluable scintigraphy grade will be summarized in a separate group. All adverse events will be listed.

6.4.2. Physical Examination

Physical examination is performed at Screening Visit only. Any abnormal findings from physical examination will be summarized using a frequency table.

7. INTERIM ANALYSES

7.1. Interim Analyses and Summaries

Interim analyses will be performed during the course of the study to allow for the reporting of data to the scientific community.

8. REFERENCES

1. Clopper, C.; Pearson, E. S. [1934]. The Use Of Confidence Or Fiducial Limits Illustrated In The Case Of The Binomial. Biometrika 26: 404–413.

9. APPENDICES

Appendix 1. Summary of Primary, Secondary, CCI [redacted] Analyses

Endpoint	Analysis Set	ATTR-CM vs HFpEF Participants	Analysis of ATTR-CM Participants by		Analysis
			Scintigraphy Grade	NYHA Baseline Classification	
Primary					
Global prevalence of ATTR-CM among participants with HFpEF.	Evaluable	N/A	N/A	N/A	Prevalence
Sensitivity/Supplementary Analysis					
Global prevalence of ATTR-CM as defined in Section 6.1.1.1 among participants with HFpEF will be estimated using the diagnosis criteria described in the primary endpoint, but will include all participants with Scintigraphy Grade 1 diagnosed with ATTR CM in the study (regardless of the cardiac biopsy).	Evaluable	N/A	N/A	N/A	Prevalence
Prevalence of ATTR-CM as defined in Section 6.1.1.1 among participants with HFpEF (regardless of the cardiac biopsy) in sub-groups (regions, age categories, gender).	Evaluable	N/A	N/A	N/A	Prevalence
Secondary					
Prevalence of ATTR-CM as defined in Section 6.1.1.1 among participants with HFpEF in sub-groups (regions, age categories, gender).	Evaluable	N/A	N/A	N/A	Prevalence
Proportion of participants with WT and hereditary forms among participants diagnosed with ATTR-CM as defined in Section 6.1.1.1.	All Enrolled	✓	✓	✓	Proportion

New York Heart Association (NYHA) classification.	All Enrolled	✓	✓	N/A	Proportion
NTproBNP.	All Enrolled	✓	✓	✓	Descriptive Mean
CCI					

Appendix 2. List of Abbreviations

Abbreviation	Term
6-MWT	6-minute walk test
ATTR	transthyretin amyloid
ATTR-CM	transthyretin amyloid cardiomyopathy
ATTR-PN	transthyretin amyloid polyneuropathy
ECG	electrocardiogram
eGFR	estimated glomerular filtration rate
HCRU	healthcare resource utilization
HFpEF	heart failure with preserved ejection fraction
IVST	interventricular septal wall thickness
KCCQ	Kansas City Cardiomyopathy Questionnaire
KCCQ-OS	Kansas City Cardiomyopathy Questionnaire overall score
LVEF	left ventricular ejection fraction
mBMI	modified body mass index
MedDRA	Medical Dictionary for Regulatory Activities
MCR	myocardial contractile reserve
N/A	not applicable
NT-proBNP	N-terminal prohormone B-type natriuretic peptide
NYHA	New York Heart Association
SAP	statistical analysis plan
TTR	transthyretin
WT	wild-type