STATISTICAL ANALYSIS PLAN
ALN-TTR02-011

Protocol Title:	APOLLO-B: A Phase 3, Randomized, Double-blind, Placebo-controlled Multicenter Study to Evaluate the Efficacy and Safety of Patisiran in Patients with Transthyretin Amyloidosis with Cardiomyopathy (ATTR Amyloidosis with Cardiomyopathy)
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LIST OF ABBREVIATIONS

Abbreviation	Definition
6-MWT	6-minute walk test
12m-DB	12-month double-blind placebo-controlled
^{99m}Tc-PYP	Technetium pyrophosphate
ADA	Antidrug antibody
AE	Adverse event
ALN-18328	siRNA targeting TTR
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANCOVA	Analysis of covariance
AST	Aspartate aminotransferase
ATC	Anatomical Therapeutic Class
ATTR	Amyloid transthyretin
BLQ	Below the lower limit of quantification
BMI	Body mass index
CI	Confidence interval
C_{max}	Maximum plasma concentration at end of infusion
$C_{max,ss}$	Steady-state C_{max}
C_{min}	Minimum pre-infusion concentration
$C_{min,ss}$	Steady-state C_{min}
CEC	Clinical Events Committee
CMH	Cochran-Mantel-Haenszel
CMR	Cardiac magnetic resonance
COVID-19	Coronavirus disease 2019
$C_{p(30min)}$	30-minute post-infusion concentration
$C_{p,ss(30min)}$	Steady-state $C_{p(30min)}$
CR	Copy reference
CTCAE	Common Terminology Criteria for Adverse Events
CV	Cardiovascular
DLin-MC3-DMA	1,2-Dilinoleyloxy-N,N-dimethylpropylamine
DMC	Data monitoring committee
ECG	Electrocardiogram

Abbreviation	Definition
eCRF	Electronic case report form
EDC	Electronic data capture
eGFR	Estimated glomerular filtration rate
FAS	Full Analysis Set
FSH	Follicle-stimulating hormone
H/CL	Heart to contralateral lung
hATTR	Hereditary ATTR
HF	Heart failure
HL	Hodges-Lehmann
HLT	High level term
HR	Hazard ratio
IV	Intravenous
ICH	International Council for Harmonisation
IRR	Infusion-related reaction
IRS	Interactive response system
KCCQ	Kansas City Cardiomyopathy Questionnaire
KCCQ-OS	Kansas City Cardiomyopathy Questionnaire-Overall Summary
LLOQ	Lower limit of quantification
LNP	Lipid nanoparticle
$\log_e$	Natural log transformation
LV	Left ventricular
MAR	Missing at random
mBMI	Modified body mass index
MCMC	Markov Chain Monte Carlo
MedDRA	Medical Dictionary for Regulatory Activities
MI	Multiple imputation
MMRM	Mixed effect model repeated measures
MNAR	Missing not at random
Norfolk QoL-DN	Norfolk Quality of Life - Diabetic Neuropathy
NT-proBNP	N-terminal prohormone B-type natriuretic peptide
NYHA	New York Heart Classification
OLE	Open label extension

Abbreviation	Definition
PD	Pharmacodynamic
PEG_{2000}-C-DMG	3-N-[(ω-methoxy poly(ethylene glycol)2000) carbamoyl]-1,2-dimyristyloxy-propylamine
PK	Pharmacokinetic
PMM	Pattern mixture model
PND	Polyneuropathy disability
PT	Preferred term
Q1	First quartile
Q3	Third quartile
q3W	Once every 3 weeks
QTc	Corrected QT
QTcF	Fridericia's cube-root corrected QT
SAE	Serious adverse event
SAP	Statistical analysis plan
SD	Standard deviation
SEM	Standard error of the mean
SI	International System of Units
siRNA	Small interfering RNA
SMQ	Standardized MedDRA Query
SOC	System organ class
TTR	Transthyretin
SUSAR	Suspected Unexpected Serious Adverse Reaction
ULN	Upper limit of normal
WHO	World Health Organization
WR	Win ratio
wt	Wild type
wtATTR	Wild type ATTR

1. INTRODUCTION

Transthyretin (TTR)-mediated amyloidosis (ATTR amyloidosis) is a rare, serious, life-threatening, multisystemic disease encompassing hereditary ATTR (hATTR) amyloidosis and wild type ATTR (wtATTR) amyloidosis, which result from either hereditary (genetic mutation) or nonhereditary (ageing) causes, respectively. In ATTR amyloidosis, deposition of TTR in various organs results in progressive, chronically debilitating morbidity and mortality. The most common manifestations of ATTR amyloidosis are polyneuropathy and cardiomyopathy (ie, ATTR amyloidosis with cardiomyopathy).

Patisiran is a small interfering RNA (siRNA) specific for TTR, which is formulated in a hepatotropic lipid nanoparticle (LNP) for intravenous (IV) administration.[Akinc 2010] The patisiran drug product (ALN-TTR02; patisiran-LNP, hereafter referred to as "patisiran") is designed to significantly suppress liver production of both wild type (wt) and all mutant forms of TTR, thereby having the potential to reduce amyloid formation and provide clinical benefit to patients with ATTR amyloidosis.

The APOLLO-B study (ALN-TTR02-011) is a Phase 3, randomized, double-blind, placebo-controlled, multicenter study designed to evaluate the efficacy and safety of patisiran in adult patients with ATTR amyloidosis with cardiomyopathy.

This statistical analysis plan (SAP) details comprehensive specifications of the efficacy, safety, pharmacodynamic (PD), and pharmacokinetic (PK) data summaries and statistical analyses in support of the clinical study report for Study ALN-TTR02-011. This SAP includes summaries and analyses specified and/or outlined in the protocol Amendment 3, dated 30 June 2021. Additional supportive analyses and changes to planned analyses are also included; notable changes are documented in Section 7. Changes to planned analyses made after database lock will be documented with justification in the clinical study report.

Table, figure, and listing specifications are contained in a separate document.

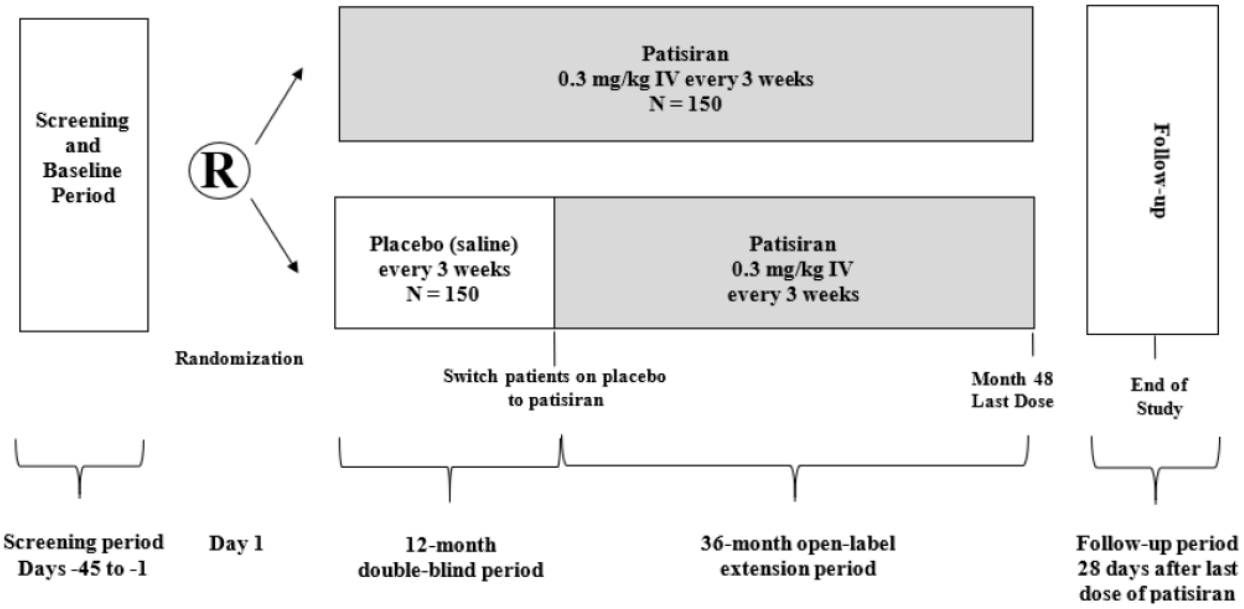
2. STUDY OVERVIEW

2.1. Synopsis of Study Design

The APOLLO-B study is a multicenter, multinational Phase 3 study designed to evaluate the efficacy and safety of patisiran in approximately 300 patients with ATTR amyloidosis (hereditary or wt) with cardiomyopathy; the study is comprised of a 1:1 randomized, double-blind, placebo-controlled period of 12 months followed by an open-label extension (OLE) period of 36 months to evaluate the long-term safety and efficacy of patisiran.

The study design schema is presented in Figure 1.

Figure 1: Study Design



2.2. Randomization Methodology

Using the interactive response system (IRS), patients will be randomized 1:1 to the patisiran or placebo arm. Randomization will be stratified by:

1. Baseline tafamidis (yes vs. no)
2. Type of amyloidosis (hATTR vs. wtATTR amyloidosis with cardiomyopathy)
3. New York Heart Classification (NYHA) Class I or II **and** age < 75 years vs. all other

Patients in the baseline tafamidis use category are defined as patients who are currently on tafamidis (for ≥6 months) with disease progression in the opinion of the investigator at baseline.

2.3. Blinding

Treatment assignments will be maintained by the IRS which has controlled access limited to unblinded team members and the unblinded pharmacist/designee preparing the infusion. Any unplanned unblinding occurring during the 12-month double-blind placebo-controlled treatment period (referred to as the 12m-DB period hereafter) will be documented and reported in the clinical study report.

Unblinding is only to occur in the case of patient emergencies or when necessary from a regulatory reporting perspective (eg, Suspected Unexpected Serious Adverse Reaction [SUSAR]), and after all patients have completed the 12m-DB period and the unblinded authorization has been executed. Details about the specifics of the blinding aspects for the study are outlined in the Randomization and Blinding Plan.

2.4. Study Procedures

The schedule of assessments is described in the study protocol (Table 1 and Table 2).

3. OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
Primary	
To evaluate the efficacy of patisiran compared with placebo treatment on functional capacity (6-minute walk test [6-MWT]) in patients with ATTR amyloidosis with cardiomyopathy	Change from baseline at Month 12 in 6-MWT
Secondary	
To evaluate the efficacy of patisiran compared with placebo treatment on: • Health status and health-related quality of life • Patient mortality, hospitalizations, and urgent heart failure (HF) visits	• Change from baseline at Month 12 in Kansas City Cardiomyopathy Questionnaire Overall Summary (KCCQ-OS) score • Composite endpoint of all-cause mortality, frequency of cardiovascular (CV) events (CV hospitalizations and urgent HF visits) and change from baseline in 6-MWT over the 12-month double-blind period • Composite endpoint of all-cause mortality and frequency of all-cause hospitalizations and urgent HF visits over the 12-month double-blind period
Exploratory	
To evaluate the efficacy of patisiran compared with placebo treatment on: • All-cause mortality and CV events • Cardiac biomarkers and biomarker-based risk assessment • Manifestations of cardiac amyloid involvement	• Composite endpoint of all-cause mortality and frequency of CV events (CV hospitalizations and urgent HF visits) over the 12-month double-blind period • Change from baseline at Month 12 in: – N-terminal prohormone B-type natriuretic peptide (NT-proBNP) – ATTR amyloidosis disease stage • Change from baseline at Month 12 in: – New York Heart Association (NYHA) Class – Echocardiographic parameters – Modified body mass index (mBMI) – Cardiac magnetic resonance (CMR) parameters – Technetium scintigraphy parameters – Troponin I levels

Objectives	Endpoints
	– Norfolk Quality of Life-Diabetic Neuropathy (Norfolk QoL-DN)
Pharmacodynamics (PD) and Pharmacokinetics (PK)	
• To evaluate the PD effect of patisiran on transthyretin (TTR) reduction • To determine the plasma concentration of patisiran and 2 lipid excipients • To assess presence of anti-drug antibodies (ADA)	• Change from baseline in serum TTR levels through Month 12 • Plasma PK exposure parameters (maximum plasma concentration at end of infusion [C_{max}], 30-minute post-infusion concentration [$C_{p(30min)}$], and pre-infusion concentration [C_{min}] • Frequency and titer of ADA
Safety	
• To evaluate the safety and tolerability of patisiran in patients with ATTR amyloidosis with cardiomyopathy	• Frequency of adverse events (AEs)

Scoring algorithms for the Kansas City Cardiomyopathy Questionnaire (KCCQ) and Norfolk Quality of Life-Diabetic Neuropathy (Norfolk QoL-DN) questionnaire are included in Appendix 9.3.1 and Appendix 9.3.2, respectively.

4. PATIENT POPULATION

The populations (analysis sets) are defined as follows:

- Full Analysis Set (FAS): All randomized patients who received any amount of study drug. Patients will be analyzed according to the treatment to which they were randomized.
- Safety Analysis Set: All randomized patients who received any amount of study drug. Patients will be summarized according to the treatment actually received.
- PK Analysis Set: All randomized patients who received at least one complete dose of study drug (see Section 6.4) and have at least 1 postdose blood sample for PK parameters and have evaluable PK data.
- PD Analysis Set: All randomized patients who received at least one complete dose of study drug (see Section 6.4) and who have an evaluable baseline and at least one evaluable post-baseline TTR sample.
- All Patisiran Treated Set: All randomized patients who received any amount of patisiran, including patients who took patisiran during the 12m-DB period and patients who first took placebo during the 12m-DB period and switched to patisiran during the OLE period.

The FAS will be used to evaluate efficacy endpoints. Safety during the 12m-DB period will be analyzed using the Safety Analysis Set. The PK and PD Analysis Sets will be used to conduct PK and PD analyses, respectively. The All Patisiran Treated Set will be used to summarize long-

term efficacy and safety data during patisiran treatment (see Section 5.11 for details). The number of patients included in all analysis sets will be provided.

5. GENERAL STATISTICAL METHODS

5.1. Determination of Sample Size

The planned enrollment for this study is 300 patients. For the change from baseline at Month 12 in the 6-MWT, assuming a treatment difference of 33 meters between patisiran and placebo in the treatment-naïve group and 20 meters in patients with baseline tafamidis, the weighted average treatment difference between patisiran and placebo in the overall population is approximately 29 meters (standard deviation [SD] = 75 meters), assuming 70% are in the treatment-naïve group and 30% are in the baseline tafamidis group. A sample size of 300 patients provides >90% power for a 2-sided test to detect a mean difference between treatment arms at a 2-sided alpha = 0.05.

5.2. General Considerations

Categorical variables will be summarized using counts and percentages.

Continuous variables will be summarized using the following descriptive summary statistics: number of patients (n), mean, standard deviation (SD), standard error of the mean (SEM), median, first quartile (Q1), third quartile (Q3), minimum, and maximum. The precision of the measurement for each continuous variable will be used to determine the number of decimal places to present in tables, figures and derived listings. Minimum and maximum values will be reported with the same precision as the units of measure. The mean, SD, SEM, median, Q1 and Q3 will be reported to one greater decimal place. Any values that require transformation to standard units (metric or International System of Units (SI) units) will be converted with the appropriate corresponding precision.

The day of the first dose of study drug administered is defined as Day 1. Study Day is defined as the number of days between the day of the first dose of study drug (Day 1) and the specific time point. The Study Day of a time point of interest is calculated as follows.

If after Day 1, Study Day = date of interest – date of the first dose of study drug + 1

If prior to Day 1, Study Day = date of interest – date of the first dose of study drug

Study days are negative when the time point of interest is prior to Day 1, positive when time of interest is after Day 1. There is no Day 0. For example, the day before the first study drug dose is defined as Day -1.

For the analysis of 6-MWT, only assessments confirmed as valid by the Colorado Prevention Center (6-MWT site training and oversight vendor) will be included. In addition, assessments where the timer was stopped after ≤4 minutes will be excluded from analysis.

For safety laboratory parameters, assessments collected and recorded as lower than the lower limit of quantification/detection will be replaced by the lower limit of quantification/detection. Any assessment collected and recorded as greater than the upper limit of quantification will be replaced by the upper limit of quantification.

For all analysis sets except for the All Patisiran Treated Set, summaries will be presented by treatment arm (patisiran and placebo).

For the All Patisiran Treated Set, summaries will be presented by the following groups:

- Patisiran/Patisiran: all patients who received patisiran during the 12m-DB including patients who continued to receive patisiran during the OLE period and patients who discontinued treatment during the 12m-DB period;
- Placebo/Patisiran: all patients who received placebo during the 12m-DB period and switched to patisiran in the OLE period;
- All Patisiran: all patients who received at least one dose of patisiran during either the 12m-DB or OLE periods.

5.3. Computing Environment

All statistical analyses will be conducted using SAS Version 9.4 or newer or R Version 3.4 or newer, unless otherwise noted.

5.4. Baseline Definitions

For 6-MWT, baseline will be defined as the last non-missing value available prior to the first dose of study drug.

For other efficacy parameters, baseline will be defined as the last non-missing value available on or before the date of first dose of study drug, unless otherwise specified.

For TTR, baseline will be defined as the average of all records collected on study, including those from any unscheduled visits, prior to the date and time of first dose.

For each parameter of the 12-lead electrocardiogram (ECG), baseline will be defined as the average of all available readings from the last visit prior to the first dose of study drug.

For all other parameters, baseline will be defined as the last non-missing value available prior to the first dose of study drug, unless otherwise specified.

For the All Patisiran Treated Set, baseline for patients who switched from placebo to patisiran will be redefined as:

- For TTR, the redefined baseline will be calculated as the mean of all TTR assessments performed on or after Month 9 in the 12m-DB period and prior to the first dose in the OLE period.
- For all the other endpoints, the redefined baseline will be defined as the last non-missing value available on or after Month 9 in the 12m-DB period and prior to the first dose in the OLE period, unless otherwise specified. For longitudinal efficacy endpoints, if a patient does not have a Month 12 assessment prior to the first dose in the OLE period, the redefined baseline will be defined as the last non-missing value available on or after Month 9 in the 12m-DB period and up to 3 weeks after the first dose in the OLE period, unless otherwise specified.

5.5. Randomization Stratification Factors

Stratification factors are recorded in both the IRS and the clinical database. In statistical analyses that use randomization stratification factors as covariates, the stratum assignment will reflect the values as recorded in the clinical database. In the presence of stratification errors, the stratification used in analysis may not match that in the IRS.

5.6. Visit Windows

Scheduled visits are expected to follow the protocol schedule. All data will be tabulated and analyzed per the evaluation visit as recorded on the electronic case report form (eCRF) even if the assessment is outside of the visit window.

For 6-MWT and KCCQ, assessments will be conducted at baseline and at Months 6, 9, and 12 in the 12m-DB period and at Months 18, 21, 24, 30, 33, 36, 42 and 48 in the OLE period. For all longitudinal efficacy assessments, if a scheduled post-baseline assessment is not performed, assessments performed within ±1.5 months of the scheduled assessment will be grouped with the scheduled assessments for that timepoint for analysis, with the following exception:

- For patients randomized to placebo who received patisiran in the OLE period, assessments performed after the first OLE dose will not be grouped with the scheduled Month 12 visits even if they are within ±1.5 months.

The derived visits will be used for all analyses.

Unless otherwise specified above, data collected at unscheduled visits will be included in by-patient data listings and figures but no assignment to a study visit will be made for the purpose of by-visit summary tabulations. However, unscheduled visits may be used in the calculation of baseline values (as discussed in Section 5.4) and for inclusion in any categorical shift summaries (eg, shift from baseline to "worst" post-baseline value).

5.7. Multiple Comparisons/Multiplicity

Formal statistical hypothesis testing will be performed on the primary and secondary efficacy endpoints with all tests conducted at the nominal 2-sided 0.05 significance level. The overall familywise error rate will be controlled at the 2-sided 0.05 significance level for the primary and secondary endpoints by a hierarchical ordering procedure. Endpoints will be tested in the following pre-specified hierarchy:

1. 6-minute walk test (6-MWT) change from baseline at Month 12
2. Kansas City Cardiomyopathy Questionnaire Overall Summary (KCCQ-OS) score change from baseline at Month 12
3. Composite endpoint of all-cause mortality, frequency of CV events (CV hospitalizations and urgent HF visits), and change from baseline in 6-MWT over the 12-month double-blind period
4. Composite endpoint of all-cause mortality and frequency of all-cause hospitalizations and urgent HF visits over the 12-month double-blind period in patients not on tafamidis at baseline

5. Composite endpoint of all-cause mortality and frequency of all-cause hospitalizations and urgent HF visits over the 12-month double-blind period in the overall population

There will be no multiplicity adjustment for exploratory endpoints.

5.8. Missing Data with Efficacy Endpoints

5.8.1. Summary of Missing Data

For the 6-MWT and KCCQ-OS efficacy endpoints, the number and percentage of patients with missing data, including due to COVID-19, at each scheduled visit will be summarized by treatment arm.

Time to treatment discontinuation in the 12m-DB period will be estimated descriptively using the Kaplan-Meier method by treatment arm. Patients who receive at least 1 dose of patisiran in the OLE period will be censored at the date of first dose in the OLE period.

5.8.2. Handling of Missing Data

For 6-MWT, the primary analysis will be based on the stratified Wilcoxon Rank Sum test. For patients with missing data at Month 12 who die during the 12m-DB period (not due to COVID-19) or who have become unable to walk due to progression of ATTR amyloidosis by Month 12, the missing Month 12 6-MWT change from baseline will be imputed as the worst 10^{th} percentile change observed across all patients in the 12m-DB period, capped by the worst possible change for the patient (ie, 0 – baseline 6-MWT). Missing data due to other reasons will be multiply imputed assuming data are missing at random (MAR). Data handling strategies for intercurrent events in the primary analysis of 6-MWT are detailed in Table 1. Sensitivity analyses for 6-MWT will be conducted to assess the impact of missing data as discussed in Section 6.6.1.3.

For KCCQ-OS, the primary analysis will be based on the mixed-effects model repeated measures (MMRM) method, which makes use of fully and partially observed data sequences from individual patients by estimating the covariance between data from different time points. The MMRM will be implemented using an unstructured approach to modeling both the treatment-by-time means and the (co)variances, leading to what is essentially a multivariate normal model wherein treatment arm means at the primary time point are adjusted to reflect both the actually observed data and the projected outcomes from the patients with missing data.[Mallinckrodt 2008] Data handling strategies for intercurrent events in the primary analysis of KCCQ-OS are detailed in Table 3. In this primary analysis, missing data will not be explicitly imputed and are assumed to be MAR. Sensitivity analyses for KCCQ-OS will be conducted to assess the impact of missing data as discussed in Section 6.6.2.1.3.

5.9. Categorization of Hospitalizations and Urgent HF Visits

For endpoints that include hospitalizations or urgent HF visits, the primary analysis will be based on events adjudicated by the Clinical Events Committee (CEC). The definitions for these events and the process of adjudication were prespecified in the CEC Charter. Specifically, the CEC adjudicates all non-elective CV and non-CV hospitalizations that result in at least 24-hour stay (or a change in calendar date if the hospital admission or discharge times are not available), as well as all urgent, unscheduled office or outpatient visits for heart failure (urgent HF visits).

Investigators also record their assessment of each inpatient admission or urgent/unscheduled healthcare visit on the eCRF. Hospitalizations and urgent HF visits based on the Investigator's assessment are defined as follows:

- Hospitalizations are defined as non-elective hospitalizations or visits requiring overnight stay (ie, discharge date at least one day after admission date). The exact times of admission/discharge are not collected in the eCRF; therefore, the 24-hour stay criteria cannot be applied. For each hospitalization, the investigator provides their classification of CV versus non-CV.
- Urgent HF visits are defined as events that are not classified as hospitalizations based on the above definition but do meet the following criteria:
  - Heart failure as the primary reason for the visit
  - IV diuretics administered during the visit
  - If the primary location for the visit is "Inpatient Admission", the visit must be marked as non-elective per the Investigator.

Hospitalizations and urgent HF visits based on the Investigator's assessment will be summarized. In addition, the concordance between the CEC adjudication outcome and the Investigator's assessment will be summarized.

5.10. Analysis Cutoff and Database Lock

For the final 12m-DB analysis, as this study will be ongoing, the study database will be locked with all data up to a prespecified cutoff date quality controlled, ie, data in the electronic data capture (EDC) system will be frozen and external data such as laboratory and PK data will be quality controlled (and quality assured, where appropriate) and cleaned. Additional details regarding the database lock process are located in the study Data Management Plan.

The final 12m-DB analysis will include data on or prior to this prespecified cutoff date. Survival follow-up data after the cutoff date will be kept in the dataset in order to determine the survival status of patients at the time of cutoff. For assessments with starting/ending dates (eg, AEs, medications, medical history), the starting date will be compared with the pre-specified cutoff date.

After the study is completed, ie, all patients either discontinue or complete the study, the database will be hard-locked and all data collected will be used for analysis.

5.11. Analyses for the Entire Study

The study design includes a 12m-DB period and an OLE period. The primary objective is to evaluate the efficacy and safety of patisiran compared with placebo during the 12m-DB period. In addition, the long-term efficacy and safety of patisiran during the entire patisiran treatment period (beyond 12m-DB) will be characterized for the All Patisiran Treated Set.

The detailed definitions for different treatment periods are as follows.

- **12m-DB Period**

The treatment comparison of patisiran versus placebo will focus on the 12m-DB period, defined as below:

1. For patients who received at least one dose of patisiran during the OLE period, all assessments collected prior to the first dose of patisiran in the OLE period will be included in the 12m-DB period. For patients randomized to patisiran, longitudinal efficacy assessments after the first OLE dose may be included in the 12m-DB period analyses as described in Section 5.6.
2. For patients who discontinued treatment and did not receive any patisiran doses in the OLE period, all assessments will be included in the 12m-DB period. Assessments collected within given time windows (details listed in below table) will be included in summary tables/figures and those outside the time windows will be listed only, unless otherwise specified (eg, AEs in Section 6.9).

Endpoint	**Windowing rule for patients who did not enter OLE**
Hospitalization/urgent HF visit/death	Events occurring on or before Day 417
Other efficacy endpoints	Assessments collected on or before Day 417
PK/PD endpoints	Assessments collected within 28 days of the last dose
Safety endpoints	Assessments with onset date within 28 days of the last dose[a]

[a] In the rare situation where a patient did not discontinue treatment in the 12m-DB period and did not enter the OLE period, all data will be included in the summary tables/figures. For AE summaries, related AEs will be considered treatment-emergent and included in summary tables regardless of time window.

- **OLE Period**

  The start day of the OLE period is defined as the day when the first dose of the OLE period is administered. Unless otherwise specified, the assessments collected or AEs with onset date after the administration of the first dose in the OLE period will be included in the OLE period. When assessments or AE onset dates are exactly the date of first dose in the OLE period and the assessment or AE onset time is missing, the records will be included in the OLE period.

- **During Patisiran Treatment**

  For all patients who received at least one dose of patisiran, data will be summarized for the "during patisiran treatment" period, defined as below.

  1. For patients who received patisiran in the 12m-DB period, all assessments collected after the first dose of patisiran during the entire study, including both the 12m-DB and OLE periods (if available), will be included in the "during patisiran treatment" period.
  2. For patients who received placebo in the 12m-DB period and switched to patisiran in the OLE period, all assessments collected after the first dose of patisiran in the OLE period will be included in the "during patisiran treatment" period.
  3. For patients who discontinued patisiran treatment during the 12m-DB period, the data handling will follow the same rules as discussed above for "12m-DB period". For patients who stopped participation in the study during the OLE period, assessments

collected within given time windows (details listed in below table) will be included in summary tables/figures and those outside the time windows will be listed only, unless otherwise specified (eg, AEs in Section 6.9). For ongoing patients, all data will be included.

Endpoint	Windowing rule for patients who discontinued patisiran in the OLE
Efficacy endpoints	Assessments collected within 90 days of the last dose
PD endpoint	Assessments collected within 24 days of the last dose
Safety endpoints	Assessments with onset date within 28 days of the last dose[a]

[a] For AE summaries, related AEs will be considered treatment-emergent and included in summary tables regardless of time window.

Longitudinal efficacy parameters will be summarized over the entire study, including the 12m-DB and OLE periods, for all patients to show the long-term efficacy of patisiran (for patients randomized to patisiran) as well as to show the trajectory changes comparing the placebo experience versus the patisiran experience (for patients randomized to placebo). In these summaries, patients will be analyzed according to the treatment to which they were randomized during the 12m-DB period.

6. STATISTICAL ANALYSIS

6.1. Patient Disposition

The number and percentage of patients in the following categories will be summarized by randomized treatment arm and overall, as appropriate:

- Randomized
- Treated
- Completed Month 12 visit
- Discontinued treatment in the 12m-DB period and primary reason for treatment discontinuation and discontinuation due to COVID-19
- Stopped participation in the study in the 12m-DB period and primary reason for stopping participation and stopping participation due to COVID-19
- Entered the OLE period
- Discontinued treatment in the OLE period and primary reason for treatment discontinuation and discontinuation due to COVID-19
- Stopped participation in the study in the OLE period and primary reason for stopping participation and stopping participation due to COVID-19.

The number and percentage of patients enrolled by country and site will be summarized by randomized treatment arm and overall. The number and percentage of patients in each level of each randomization stratification factor as recorded in IRS and in the clinical database, and a

comparison of the number and percentage of patients in each randomization stratification factor in IRS versus the clinical database will be summarized by randomized treatment arm and overall.

6.2. Demographics and Baseline Characteristics

Demographic and baseline characteristics, baseline disease characteristics, baseline efficacy parameters, and medical history information will be summarized by treatment arm and overall. No formal statistical comparisons will be performed.

Age at randomization, height, weight, body mass index (BMI), and mBMI will be summarized using descriptive statistics. Age group, sex, race, ethnicity, region, and country will be summarized by presenting the frequencies and percentages of patients in each category.

The following baseline disease characteristics will be summarized by presenting the numbers and percentages of patients in each category:

- Type of ATTR amyloidosis [hATTR; wtATTR]
- Genotype for hATTR patients
- Baseline tafamidis use [Yes; No]
- NYHA class [I; II; III]
- NYHA class I/II and age at randomization < 75 years [Yes; No]
- ATTR amyloidosis disease stage [1; 2; 3]
- Polyneuropathy disability (PND) score [0; I; II]
- Previous heart failure hospitalization [Yes; No]

The age at symptom onset and the time in years since diagnosis will be summarized, overall and by type of ATTR amyloidosis, using descriptive statistics. For patients who had at least 1 previous heart failure hospitalization, the age at first hospitalization for heart failure and the number of hospitalizations for heart failure in the previous 12 months will be summarized using descriptive statistics. For those who previously used tetramer stabilizers, the time from discontinuation of tetramer stabilizer to the start of study drug will be summarized using descriptive statistics. For patients in the baseline tafamidis group (per the clinical database), the time from the start of tafamidis therapy to the start of study drug will be summarized using descriptive statistics.

The number and percent of patients with each type of ATTR amyloidosis and with each genotype (for hATTR patients) will be summarized by country and treatment arm.

Medical history will be summarized by system organ class (SOC), high level term (HLT), and preferred term (PT). A patient contributes only once to the count for a given condition (overall, by SOC, by HLT, by PT).

All demographic and baseline data for each patient will be provided in data listings. Medical history data including general medical history, cardiac medical history, neuropathy history, historical inpatient admissions or urgent healthcare visits in the past 12 months, and ophthalmic medical history will be presented in data listings. Screening test results will also be presented in data listings.

6.3. Protocol Deviations

A protocol deviation is any change, divergence, or departure from the study design or procedures defined in the clinical protocol. Protocol deviations will be classified by medical review prior to the final 12m-DB analysis, and major protocol deviations will be identified. A major protocol deviation is a deviation that may significantly impact the completeness, accuracy, and/or reliability of the trial data; that may significantly affect a subject's rights, safety, or well-being. (ICH. E3 R1 Structure and Contents of Clinical Study Reports Guidance for Industry. 2013) All major deviations related to trial inclusion or exclusion criteria, conduct of the trial, patient management or patient assessment will be described in the clinical study report.

The Sponsor or designee will be responsible for producing the protocol deviation file (formatted as a Microsoft Excel file). This file will include a description of each protocol deviation and whether or not this deviation is classified as a major protocol deviation. Since the study will be ongoing at the time of the primary analysis, there will be continuing review of protocol deviations. The file with all protocol deviations through the prespecified cutoff date for the final 12m-DB analysis will be finalized prior to the interim lock of the database and study unblinding. After the study is completed, the file with all protocol deviations for the study will be finalized prior to the hard-lock of the database.

All protocol deviations, COVID-19-related protocol deviations, and major protocol deviations will be summarized.

6.4. Drug Exposure

Exposure to study drug in months and the number of doses of study drug received will be summarized by treatment arm. Summaries of the numbers and percentages of patients with no missing infusions, and the number of missing infusions per patient will also be provided. The total drug exposure will also be summarized.

The last date of exposure to study drug is defined as the earliest day of the following dates:

- Last dose date + 20 days
- Analysis cutoff date
- End of study date

Duration of exposure is defined as (the last date of exposure to study drug – date of the first dose +1)/30.4375. The exposure during the 12m-DB period is right censored by the date of the first OLE dose, ie, the last exposure day in the 12m-DB period is no later than the day before the first OLE dose. Similarly, the exposure during the OLE is left censored by the date of the first OLE dose, ie, the Day 1 of the OLE is the day of the first OLE dose. Dose interruptions and compliance are not taken into account for duration of exposure.

Study drug exposure data collected in the eCRFs of study drug administration will also be summarized for each infusion. The numbers and percentages of patients with complete, partial, and missing dose administrations will be summarized. Complete and partial administration is defined as follows:

- Complete: ≥80% (≥160 mL) of the planned infusion volume (200 mL)

- Partial: >0% to <80% (>0 to <160 mL) of the planned infusion volume (200 mL).

The number of patients who experienced interruptions of infusions for any reason will be tabulated, as well as the number of patients with infusion interruptions due to an infusion-related reaction (IRR).

Dosing information for each patient will be presented in a data listing.

6.5. Premedications

All patients will receive premedications in order to reduce the potential of an IRR. Premedications will be coded using the WHO Drug Dictionary (March 2019 or later). Results will be tabulated by anatomical therapeutic class (ATC) and preferred term.

Premedication data will be presented in a data listing.

6.6. Efficacy Analyses

Efficacy endpoints will be analyzed using the FAS.

6.6.1. Primary Endpoint

The primary efficacy endpoint is to compare the change in 6-MWT from baseline to Month 12 between treatment arms.

6.6.1.1. Definition of Estimand

For the objective of evaluating the efficacy of patisiran compared with placebo on 6-MWT, the estimand is defined as follows:

- **Target patient population:** patients with hATTR or wtATTR amyloidosis with cardiomyopathy. Patients may or may not be taking tafamidis at study entry.
- **Treatment condition:** Patisiran 0.3 mg/kg or placebo administered as an IV infusion q3W (once every 3 weeks) with or without concomitant use of tafamidis.
- **Endpoint:** Change from baseline in 6-MWT at Month 12.
- **Population-level summary:** The stratified Hodges-Lehmann (HL) estimate of the median difference in the change from baseline in 6-MWT at Month 12 between the patisiran and placebo arms. The *p* value for the treatment difference will be estimated using the stratified Wilcoxon Rank Sum test.

Intercurrent events and strategies: Intercurrent events include treatment discontinuation, serious COVID-19 AE, tafamidis drop-in, missing dose(s) due to COVID-19, inability to walk due to progression of ATTR amyloidosis, and death. They require different handling strategies, which are described in Table 1 below.

Table 1: Intercurrent Event Strategies for the Primary Analysis of 6-MWT

Intercurrent Event	Handling Strategy
Treatment discontinuation (not due to death)	Treatment policy strategy: Intercurrent event is ignored; 6-MWT assessments obtained after treatment discontinuation will be included in analysis.
Serious COVID-19 AE	Hypothetical strategy: Assessments obtained on or after the onset of a serious COVID-19 AE will be treated as missing and will be assumed as MAR. The goal is to estimate the scheduled visit values as if the patient had not been infected with COVID-19.
Tafamidis drop-in (ie, taking tafamidis for more than 28 days after randomization) in the subgroup of patients who were not on tafamidis at baseline	Treatment policy strategy: Intercurrent event is ignored; 6-MWT assessments obtained after tafamidis drop-in will be included in analysis.
Missing dose(s) due to COVID-19	Treatment policy strategy: Intercurrent event is ignored; 6-MWT assessments obtained after missing dose(s) due to COVID-19 will be included in analysis.
Inability to walk due to progression of ATTR amyloidosis (including patients who are unable to walk due to hospitalization related to progression of ATTR amyloidosis)[a]	Composite variable strategy: For patients who are unable to walk due to progression of ATTR amyloidosis, the missing Month 12 6-MWT change from baseline will be imputed as the worst 10^{th} percentile change observed across all patients in the 12m-DB period, capped by the worst possible change for the patient (ie, 0 – baseline 6-MWT).
Death (including heart transplant and ventricular assist device placement) not due to COVID-19[a]	Composite variable strategy: For patients who die (not due to COVID-19), the missing Month 12 6-MWT change from baseline will be imputed as the worst 10^{th} percentile change observed across all patients in the 12m-DB period, capped by the worst possible change for the patient (ie, 0 – baseline 6-MWT).
Death due to COVID-19	Hypothetical strategy: Missing data due to COVID-19 death will be assumed as MAR.

[a] The imputed outcome will be used in the stratified Wilcoxon Rank Sum test and stratified HL estimate but will not be used in the descriptive summaries.

6.6.1.2. Primary Analysis using the Stratified Wilcoxon Rank Sum Test and Hodges-Lehmann Estimate

The primary analysis will be performed using the stratified Wilcoxon Rank Sum test for the FAS, stratified by baseline tafamidis use (yes vs. no). The analysis will be based on the following assumptions for missing data at Month 12:

1. Patients who die during the 12m-DB period (not due to COVID-19) or who have become unable to walk due to progression of ATTR amyloidosis by Month 12 and have missing data: Assuming that deaths observed in the study will likely be related to worsening of disease, the missing Month 12 6-MWT change from baseline will be imputed as the

worst 10th percentile change, capped by the worst possible change for the patient (ie, 0 – baseline 6-MWT).

2. Patients who have missing data due to other reasons will be imputed assuming data are MAR. Since the pattern of missing data within patients may be non-monotone, multiple imputation will be conducted separately by treatment arm and baseline tafamidis use group using the Markov Chain Monte Carlo (MCMC) method. For each treatment arm/baseline tafamidis use group, the imputation model will include type of amyloidosis (hATTR vs. wtATTR), NYHA class (I/II vs. III), age at randomization (<75 vs. ≥75), baseline NT-proBNP (≤3000 ng/L vs >3000 ng/L), baseline 6-MWT, and all calculated values of change from baseline in 6-MWT at pre-specified visits.

Missing values will be imputed 100 times to generate 100 complete datasets using the procedures described above. The stratified Wilcoxon Rank Sum test will be applied to each imputed dataset for the change from baseline in 6-MWT at Month 12. The Z scores estimated from the stratified Wilcoxon Rank Sum test fit to each imputed dataset will be combined by applying Rubin's rules,[Rubin 1996; Rubin 1987] using SAS PROC MIANALYZE to produce inferential results for the *p* value.

The effect size comparing treatment groups will be estimated using the stratified HL estimate (stratified by baseline tafamidis use) of the median difference in the change from baseline between patisiran and placebo, together with its 95% confidence interval (CI). The stratified HL is an estimate of the median value of all paired differences between observations in the patisiran versus placebo groups accounting for baseline tafamidis use, calculated using the imputed datasets. The calculation will be repeated for each imputed dataset, and the results will be combined by applying Rubin's rules,[Rubin 1996; Rubin 1987] using SAS PROC MIANALYZE to obtain the stratified HL estimate and corresponding 95% CI.

6.6.1.3. Sensitivity Analyses

Sensitivity analyses will be conducted using the following methods to assess the impact of missing data and the robustness of the primary analysis.

Sensitivity Analysis 1: Stratified Wilcoxon Rank Sum Test Including All Censored Data

A sensitivity analysis including all 6-MWT assessments (ie, not treating assessments that occur on or after the onset of a serious COVID-19 AE as missing) will be conducted using the same analyses as specified in Section 6.6.1.2.

Sensitivity Analysis 2: Stratified Wilcoxon Rank Sum Test with Control-based Imputation for Missing Data in Off-treatment Patisiran Patients

A sensitivity analysis will be performed using the stratified Wilcoxon Rank Sum test where missing data from off-treatment patisiran patients are imputed based on data from the placebo group. This accommodates situations where the missingness mechanism may be missing not at random (MNAR). The model will be based on the following assumptions for missing data at Month 12:

1. Patients who have missing data due to COVID-19, including patients who have missing assessments due to COVID-19, or who have a serious COVID-19 AE or who die due to COVID-19 prior to the Month 12 assessment will be imputed assuming data are MAR.

Under the hypothetical estimand of interest where the COVID-19 pandemic did not occur, these assessments should have been obtained with no COVID-19 impact. Missing data meeting these criteria will be imputed separately for each treatment arm and baseline tafamidis use group using multiple imputation (MI) estimated from all non-missing data collected within each group.

2. For patients who have missing data unrelated to COVID-19, who are alive through the 12m-DB period and who have not become unable to walk due to progression of ATTR amyloidosis by Month 12, the imputation will be performed by baseline tafamidis use (yes/no) separately based on different patterns described as below:
    a. Placebo patients who have missing data: The missing data are considered MAR and will be imputed using multiple imputation (MI) estimated from placebo patients. The imputation is done regardless of whether a patient was on-treatment or discontinued treatment before the scheduled efficacy assessment.
    b. Patisiran patients who have missing data while on treatment: Patients are expected to continue to show benefit from treatment similar to that observed at the scheduled time point. Therefore, missing data during the on-treatment period (within 60 days of their last dose) are considered MAR and will be imputed using MI estimated from all non-missing data collected on treatment from the patisiran arm.
    c. Patisiran patients who have missing data after stopping their study treatment: Patients will no longer benefit from treatment in the future and will have trajectory similar to placebo patients after discontinuing treatment. Therefore, missing data after treatment discontinuation (more than 60 days after last dose of study drug) will be imputed using the data from placebo patients using the copy reference (CR) approach.
3. Patients who die during the 12m-DB period (not due to COVID-19) or who have become unable to walk due to progression of ATTR amyloidosis by Month 12 and have missing data: Assuming that deaths observed in the study will likely be related to worsening of disease, the missing Month 12 6-MWT change from baseline will be imputed as the worst 10^{th} percentile change, capped by the worst possible change for the patient (ie, 0 – baseline 6-MWT). The imputation will be done for patients from both the patisiran and placebo arms.

Missing values will be imputed 100 times to generate 100 complete datasets using the procedures described above. The stratified Wilcoxon Rank Sum test (stratified by baseline tafamidis use) will be applied to each imputed dataset for the change from baseline in 6-MWT at Month 12. The Z scores estimated from the stratified Wilcoxon Rank Sum test fit to each imputed dataset will be combined by applying Rubin's rules,[Rubin 1996; Rubin 1987] using SAS PROC MIANALYZE to produce inferential results for the *p* value.

The effect size comparing treatment groups will be estimated using the stratified HL estimate (stratified by baseline tafamidis use) of the median difference in the change from baseline between patisiran and placebo, together with its 95% CI. The calculation will be repeated for each imputed dataset, and the results will be combined by applying Rubin's rules,[Rubin 1996;

Rubin 1987] using SAS PROC MIANALYZE to obtain the stratified HL estimate and corresponding 95% CI.

More details on the implementation of the control-based imputation for missing data are discussed in Appendix 9.1.

Sensitivity Analysis 3: MMRM Model

A sensitivity analysis will be performed using a REML-based MMRM approach. The outcome variable is the change from baseline in 6-MWT; the model includes baseline 6-MWT as a continuous covariate and treatment arm, visit (Month 6, Month 9 or Month 12), baseline tafamidis use (yes vs. no), type of amyloidosis (hATTR vs. wtATTR), age at randomization (<75 vs. ≥75 years), the treatment-by-visit interaction, the treatment-by-baseline tafamidis interaction, the visit-by-baseline tafamidis interaction, and the treatment-by-visit-by-baseline tafamidis interaction as fixed factors. Assessments obtained on or after the onset of a serious COVID-19 AE will be treated as missing and will be assumed as MAR.

An unstructured covariance structure will be used to model the within-patient errors. If the model fails to converge, the following covariance structures will be specified in sequence and the first to converge will be used:

1. Toeplitz
2. Autoregressive (1)
3. Compound symmetry

The Satterthwaite approximation will be used to estimate the degrees of freedom.

The primary comparison is the contrast (difference in LS means) between the patisiran and placebo arms at Month 12. The LS mean coefficients will be computed using the observed proportions of the categorical covariates (baseline tafamidis use, type of ATTR amyloidosis and age) in the FAS. The analysis will be implemented with SAS PROC MIXED.

The MMRM analysis assumes multivariate normality for the error term in the analysis model. This normality assumption will be assessed by inspection of residual plots along with a formal test for normality using the Shapiro-Wilk test.

6.6.1.4. Binary Analyses

Binary analysis for 6-MWT will be conducted on the observed 6-MWT data without imputation. The number and percentage of patients with a ≥0 meter increase in 6-MWT from baseline to Month 12 will be calculated for each treatment arm and compared between 2 arms using the Cochran-Mantel-Haenszel (CMH) test, stratified by baseline tafamidis use (yes vs. no). All patients with missing Month 12 data will be counted in the denominator with two exceptions: patients who are missing Month 12 data due to COVID-19 and patients with a 6-MWT assessment collected on or after the onset of a serious COVID-19 AE will be excluded.

6.6.1.5. Overview of Primary Endpoint Analyses

The planned analyses of the primary endpoint 6-MWT are summarized in Table 2.

Table 2: Analysis of 6-MWT

Statistical Method
Primary analysis: Stratified Wilcoxon Rank Sum test
Sensitivity analysis 1: Stratified Wilcoxon Rank Sum test – including all available data without censoring
Sensitivity analysis 2: Stratified Wilcoxon Rank Sum test – implementing control-based imputation for missing data in off-treatment patisiran patients
Sensitivity analysis 3: MMRM
Other analysis: Binary analysis using stratified CMH

6.6.2. Secondary Endpoints

The secondary efficacy endpoints are specified in Section 3. To control overall type I error, the secondary endpoints will be tested in a hierarchical order as described in Section 5.7.

CV events will include all emergency/unplanned/non-elective hospitalizations after randomization adjudicated as being cardiovascular or being indeterminate, and all emergency/unplanned/non-elective visits after randomization that are adjudicated as being urgent HF visits. Deaths will include heart transplant and ventricular assist device placement, unless otherwise specified.

6.6.2.1. Change from Baseline at Month 12 in KCCQ-OS Score

6.6.2.1.1. Definition of Estimand

For the objective of evaluating the efficacy of patisiran compared with placebo on KCCQ-OS, the estimand is defined as follows:

- **Target patient population:** patients with hATTR or wtATTR amyloidosis with cardiomyopathy. Patients may or may not be taking tafamidis at study entry.
- **Treatment condition:** Patisiran 0.3 mg/kg or placebo administered as an IV infusion q3W (once every 3 weeks) with or without concomitant use of tafamidis.
- **Endpoint:** Change from baseline in KCCQ-OS at Month 12.
- **Population-level summary:** The least squares (LS) mean difference in the change from baseline in KCCQ-OS at Month 12 between the patisiran and placebo arms.

Intercurrent events and strategies: Intercurrent events include treatment discontinuation, serious COVID-19 AE, tafamidis drop-in, missing dose(s) due to COVID-19, and death. They require different handling strategies, which are described in Table 3 below.

Table 3: Intercurrent Event Strategies for the Primary Analysis of KCCQ-OS

Intercurrent Event	Handling Strategy
Treatment discontinuation (not due to death)	Treatment policy strategy: Intercurrent event is ignored; assessments obtained after treatment discontinuation will be included in analysis.

Intercurrent Event	Handling Strategy
Serious COVID-19 AE	Hypothetical strategy: Assessments obtained on or after the onset of a serious COVID-19 AE will be treated as missing and will be assumed as MAR. The goal is to estimate the scheduled visit values as if the patient had not been infected with COVID-19.
Tafamidis drop-in (ie, taking tafamidis for more than 28 days after randomization) in the subgroup of patients who were not on tafamidis at baseline	Treatment policy strategy: Intercurrent event is ignored; assessments obtained after tafamidis drop-in will be included in analysis.
Missing dose(s) due to COVID-19	Treatment policy strategy: Intercurrent event is ignored; assessments obtained after missing dose(s) due to COVID-19 will be included in analysis.
Death	Hypothetical strategy: Missing data due to death will be assumed as MAR.

6.6.2.1.2. Primary Analysis using MMRM for the Full Analysis Set

The change from baseline at Month 12 in KCCQ-OS will be analyzed using an MMRM model similar to the MMRM model for 6-MWT described in Section 6.6.1.3 while adjusting for baseline KCCQ-OS as a continuous covariate.

6.6.2.1.3. Sensitivity Analyses

Sensitivity analyses will be conducted using the following methods to assess the impact of missing data and the robustness of the primary analysis.

MMRM Model Including All Censored Data

A sensitivity analysis including all KCCQ-OS assessments, ie, not treating assessments that occur on or after the onset of a serious COVID-19 AE as missing, will be conducted using the same MMRM model as used for the primary analysis.

PMM Model

A sensitivity analysis using pattern mixture model (PMM) will be performed to assess the robustness of the primary MMRM results to the possible violation of the MAR assumption. The PMM accommodates situations where the missingness mechanism may be MNAR. The assumptions for missing data at Month 12 are the same as those specified in Section 6.6.1.3 with the exception of bullet 3, the imputation for patients who die during the 12m-DB period (not due to COVID-19). The revised bullet 3 is as follows:

3. Patients who die during the 12m-DB period (not due to COVID-19) and have missing data: Assuming that deaths observed in the study will likely be related to worsening of disease, the missing Month 12 KCCQ-OS change from baseline will be imputed by taking random samples from the worst 10% KCCQ-OS changes observed during the 12m-DB period in the entire population, capped by the worst possible change for the

patient (ie, 0 – baseline KCCQ-OS). The imputation will be done for patients from both the patisiran and placebo arms.

Note that the PMM imputation rules for KCCQ-OS will not consider being unable to walk due to progression of ATTR amyloidosis in defining the missing data patterns since patients are still able to complete the KCCQ assessment in this scenario.

Missing values will be imputed 100 times to generate 100 complete datasets using the procedures described above. An analysis of covariance (ANCOVA) model will be fit to each imputed dataset for the change from baseline in KCCQ-OS at Month 12. The ANCOVA model will include baseline KCCQ-OS as a continuous covariate and treatment arm, baseline tafamidis use (yes vs. no), type of ATTR amyloidosis (hATTR vs. wtATTR), age at randomization (<75 vs. ≥75), and the treatment-by-baseline tafamidis interaction as covariates. The LS mean coefficients will be computed using the observed proportions of the categorical covariates (baseline tafamidis use, type of ATTR amyloidosis and age) in the FAS. The LS mean and standard error of the mean (SEM) estimated from the ANCOVA model fit to each imputed dataset will be combined by applying Rubin's rules,[Rubin 1996; Rubin 1987] using SAS PROC MIANALYZE to produce inferential results including the treatment difference in LS means, 95% CI for the treatment difference, and the *p* value.

6.6.2.1.4. Binary Analyses

A binary analysis of KCCQ-OS, similar to the analysis described for 6-MWT in Section 6.6.1.4, will be conducted using a threshold of a ≥0 point increase in KCCQ-OS from baseline to Month 12.

6.6.2.2. Composite All-cause Mortality, Frequency of CV Events (CV hospitalizations and urgent HF visits), and Change from Baseline in 6-MWT over the 12-month Double-blind Period

6.6.2.2.1. Definition of Estimand

For the objective of evaluating the efficacy of patisiran compared with placebo on patient mortality, hospitalizations and urgent HF visits, and change from baseline in 6-MWT, the estimand is defined as follows:

- **Target patient population:** Patients with hATTR or wtATTR amyloidosis with cardiomyopathy. Patients may or may not be taking tafamidis at study entry.
- **Treatment condition:** Patisiran 0.3 mg/kg or placebo administered as an IV infusion q3W with or without concomitant use of tafamidis.
- **Endpoint:** Hierarchical comparison of all-cause mortality, frequency of CV events, and the change from baseline in 6-MWT over the 12m-DB period.
- **Population-level summary:** Stratified win ratio for the composite outcome between the patisiran and placebo arms.

Intercurrent events and strategies: Intercurrent events include treatment discontinuation, serious COVID-19 AE, COVID-19 related deaths and CV events, tafamidis drop-in, missing dose(s) due to COVID-19, inability to walk due to progression of ATTR amyloidosis, and heart

transplantation and ventricular assist device placement. They require different handling strategies, which are described in Table 4 below.

Table 4: Intercurrent Event Strategies for the Analysis of the Composite Endpoint

Intercurrent Event	Handling Strategy
Treatment discontinuation	Treatment policy strategy: Intercurrent event is ignored; deaths, CV events, and 6-MWT assessments that occur after treatment discontinuation will be included in analysis.
COVID-19 related death and CV events	Hypothetical strategy: Deaths and CV events due to COVID-19 will be excluded from analysis. The goal is to estimate the treatment effect in a hypothetical setting where the COVID-19 pandemic is not present.
Serious COVID-19 AE	Hypothetical strategy: 6-MWT assessments obtained on or after the onset of a serious COVID-19 AE will be treated as missing.
Tafamidis drop-in (ie, taking tafamidis for more than 28 days after randomization) in the subgroup of patients who were not on tafamidis at baseline	Treatment policy strategy: Intercurrent event is ignored; deaths, CV events, and 6-MWT assessments that occur after tafamidis drop-in will be included in analysis.
Missing dose(s) due to COVID-19	Treatment policy strategy: Intercurrent event is ignored; deaths, CV events, and 6-MWT assessments that occur after missing dose(s) due to COVID-19 will be included in analysis.
Heart transplantation and ventricular assist device placement	Composite variable strategy: Heart transplantation and ventricular assist device placement will be treated in the same manner as death.

6.6.2.2.2. Primary Analysis

The composite endpoint of all-cause mortality, frequency of CV events (CV hospitalizations and urgent HF visits) and change from baseline in 6-MWT will be analyzed using the stratified win ratio method,[Dong 2018] stratified by baseline tafamidis use (yes vs. no). This method makes within-stratum pairwise comparisons (for all possible patisiran/placebo patient pairs) of the 3 components in the hierarchical order specified above. Within a stratum, each patisiran patient will be compared with all placebo patients in a stepwise fashion, with the "winner" assigned a score of +1 and the "loser" assigned a score of "-1". The detailed steps are described as follows. All comparisons will be based on data collected during the 12m-DB period.

Step 1: compare all-cause mortality

The comparison of the two patients will be performed at the shorter of their survival follow-up times.

- If both patients are deceased, then the patient with a longer survival time is assigned a +1 and the other is assigned a -1
- If one patient is alive and the other is deceased, the alive patient is assigned a +1 and the deceased patient is assigned a -1

- Otherwise, proceed to step 2

Step 2: compare frequency of CV events

The comparison of the two patients will be performed at the shorter of their follow-up times while patients remain on the study.

- The patient with fewer CV events is assigned a +1 and the other is assigned a -1
- If the numbers of CV events are the same, go to step 3

Step 3: compare change from baseline in 6-MWT

- The patient who has less decline in 6-MWT at Month 12 is assigned a +1 and the other is assigned a -1. A patient who is unable to walk will be assigned as -1 when compared with another patient who is able to walk.
- If one or both patients have missing data at Month 12, compare change from baseline in 6-MWT at the latest common study visit where both patients had a 6-MWT assessment. The patient who has less decline in 6-MWT is assigned a +1 and the other is assigned a -1. A patient who is unable to walk will be assigned as -1 when compared with another patient who is able to walk.
- If tied or undetermined, assign score 0 to each patient

The point estimate of the stratified win ratio (WR) is defined as

$$WR = \frac{\sum_{m=1}^{2} n_t^{(m)}/N^{(m)}}{\sum_{m=1}^{2} n_c^{(m)}/N^{(m)}}$$

where $n_t^{(m)}$and $n_c^{(m)}$ are the number of patisiran/placebo pairs in stratum *m* in which the patisiran patient was the winner and in which the placebo patient was the winner, respectively, and $N^{(m)}$ is the total number of patients in stratum *m*.

A 95% CI and *p* value for the stratified win ratio will be estimated (see Section 9.2 for details).

6.6.2.3. Composite All-cause Mortality and Frequency of All-cause Hospitalizations and urgent HF visits over the 12-month Double-blind Period in Patients Not on Tafamidis at Baseline

6.6.2.3.1. Definition of Estimand

For the objective of evaluating the efficacy of patisiran compared with placebo on the composite outcome of mortality, hospitalizations and urgent HF visits in patients not on tafamidis at baseline, the estimand is defined as follows:

- **Target patient population:** Patients with hATTR or wtATTR amyloidosis with cardiomyopathy who are not taking tafamidis at study entry.
- **Treatment condition:** Patisiran 0.3 mg/kg or placebo administered as an IV infusion q3W with or without concomitant use of tafamidis.
- **Endpoint:** Timing and frequency of all-cause deaths, all-cause hospitalizations and urgent HF visits over the 12m-DB period.

- **Population-level summary:** Hazard ratio (HR) for the composite outcome between the patisiran and placebo arms.

Intercurrent events and strategies: Intercurrent events include treatment discontinuation, COVID-19 infection, tafamidis drop-in, missing dose(s) due to COVID-19, and heart transplantation and ventricular assist device placement. They require different handling strategies, which are described in Table 5 below.

Table 5: Intercurrent Event Strategies for the Analysis of the Composite Endpoint

Intercurrent Event	Handling Strategy
Treatment discontinuation	Treatment policy strategy: Intercurrent event is ignored; deaths, all-cause hospitalizations and urgent HF visits that occur after treatment discontinuation will be included in analysis.
COVID-19 related deaths, hospitalizations, and urgent HF visits	Hypothetical strategy: Deaths, all-cause hospitalizations and urgent HF visits due to COVID-19 will be excluded from analysis. The goal is to estimate the HR in a hypothetical setting where the COVID-19 pandemic is not present.
Tafamidis drop-in (ie, taking tafamidis for more than 28 days after randomization) in the subgroup of patients who were not on tafamidis at baseline	Treatment policy strategy: Intercurrent event is ignored; deaths, all-cause hospitalizations and urgent HF visits that occur after tafamidis drop-in will be included in analysis.
Missing dose(s) due to COVID-19	Treatment policy strategy: Intercurrent event is ignored; deaths, all-cause hospitalizations and urgent HF visits that occur after missing dose(s) due to COVID-19 will be included in analysis.
Heart transplantation and ventricular assist device placement	Composite variable strategy: Heart transplantation and ventricular assist device placement will be treated in the same manner as death.

6.6.2.3.2. Primary Analysis

The composite endpoint of all-cause mortality and frequency of all-cause hospitalizations and urgent HF visits in patients who are not on tafamidis at baseline will be analyzed using an Andersen-Gill model, including treatment, type of amyloidosis (hATTR vs. wtATTR), baseline NYHA class (I/II vs. III), and age at randomization (<75 vs. ≥75 years) as covariates.

6.6.2.3.3. Component Analysis

The components of the composite endpoint in patients not on tafamidis at baseline will be analyzed as follows. For all-cause mortality, Kaplan-Meier survival curves for each treatment group will be presented. The HR and corresponding 95% CI will be estimated from a Cox proportional hazards model including treatment as a covariate. Frequency of all-cause hospitalizations and urgent HF visits will be analyzed using Poisson regression with treatment, type of amyloidosis (hATTR vs. wtATTR), baseline NYHA class (I/II vs. III), and age at

randomization (<75 vs. ≥75 years) as covariates, adjusting for duration of follow-up (ie, include duration of follow-up as an offset).

6.6.2.4. Composite All-cause Mortality and Frequency of All-cause Hospitalizations and urgent HF visits over the 12-month Double-blind Period in the Overall Population

6.6.2.4.1. Definition of Estimand

The estimand is the same as that specified in Section 6.6.2.3.1, except the target patient population includes all patients with hATTR or wtATTR amyloidosis with cardiomyopathy (ie, patients may or may not be taking tafamidis at study entry).

6.6.2.4.2. Primary Analysis

The composite endpoint of all-cause mortality and frequency of all-cause hospitalizations and urgent HF visits will be analyzed using a modified Andersen-Gill model stratified by baseline tafamidis use (yes vs. no), including treatment, type of amyloidosis (hATTR vs. wtATTR), baseline NYHA class (I/II vs. III), and age at randomization (<75 vs. ≥75 years) as covariates.

6.6.2.4.3. Component Analysis

The all-cause mortality component of the composite endpoint will be evaluated using analyses similar to those described in Section 6.6.2.3.3. Frequency of all-cause hospitalizations and urgent HF visits will be analyzed using Poisson regression with treatment, baseline tafamidis use (yes vs. no), type of amyloidosis (hATTR vs. wtATTR), baseline NYHA class (I/II vs. III), age at randomization (<75 vs. ≥75 years), and the treatment-by-baseline tafamidis use interaction as covariates, adjusting for duration of follow-up (ie, include duration of follow-up as an offset).

6.6.3. Exploratory Endpoints

All analyses of exploratory endpoints will be conducted on the FAS. For continuous endpoints, descriptive statistics will be provided for actual value and change from baseline by treatment arm; descriptive statistics for percentage change from baseline by treatment arm may also be provided, as appropriate.

The composite endpoint of all-cause mortality and frequency of CV events (CV hospitalizations and urgent HF visits) over the 12m-DB period will be analyzed using a modified Andersen-Gill model similar to that for the composite endpoint of all-cause mortality ad frequency of all-cause hospitalizations and urgent HF visits (see Section 6.6.2.4). The frequency of CV events component will also be analyzed using Poisson regression with treatment, baseline tafamidis (yes vs. no), type of amyloidosis (hATTR vs. wtATTR), baseline NYHA class (I/II vs. III), age at randomization (<75 vs. ≥75 years), and the treatment-by-baseline tafamidis use interaction as covariates, adjusting for duration of follow-up.

The change from baseline at Month 12 in select echocardiographic parameters (mean left ventricular [LV] wall thickness, LV relative wall thickness, LV mass, LV end-diastolic volume, global LV longitudinal strain, and cardiac output) and Norfolk QoL-DN Total Score (see Section 9.3.2) will be analyzed using an ANCOVA model since they are only measured in the 12m-DB period at Month 12. The ANCOVA model will include the baseline value for the

parameter as a continuous covariate and treatment arm, baseline tafamidis use (yes vs. no), type of amyloidosis (hATTR vs. wtATTR), age at randomization (<75 vs. ≥75), and the treatment-by-baseline tafamidis use interaction as covariates. The Norfolk QoL-DN questionnaire is a patient-reported outcomes measure that is sensitive to the different features of diabetic neuropathy; since patients enrolled in this study are not required to have a history of neuropathy, the ANCOVA model for Norfolk QoL-DN Total Score may be repeated on the subset of patients with a history of neuropathy.

In the 12m-DB period, NT-proBNP and troponin I are measured at baseline, Month 3, Month 6, Month 9, and Month 12, and mBMI is measured at baseline, Month 6, and Month 12. NT-proBNP, troponin I, and mBMI will be analyzed using MMRM models similar to the model described for the primary analysis of KCCQ-OS while adjusting for the baseline value of the endpoint being modeled (see Section 6.6.2.1). Assessments obtained on or after the onset of a serious COVID-19 AE will not be treated as missing.

NT-proBNP has been shown to be highly skewed in the literature; a Q-Q plot of the residuals of the NT-proBNP MMRM model specified above will be used to assess the normality assumption. If the normality assumption is violated, a natural log transformation ($\log_e$) will be applied to NT-proBNP to normalize the distribution. Following the transformation, an MMRM model similar to the model described for the primary analysis of KCCQ-OS will be used; the outcome variable will be $\log_e$(post-baseline) – $\log_e$(baseline), and the model will include $\log_e$(baseline) as a continuous covariate. Adjusted geometric mean fold-changes from baseline with 95% CIs will be constructed by exponentially back-transforming the LS means, differences in LS means, and the limits of the corresponding 95% CIs.

CMR and technetium scintigraphy will be performed in a subset of patients at select sites to assess cardiac amyloid involvement. The analyses for these imaging assessments are detailed in the separate APOLLO-B Imaging Statistical Analysis Plan.

Categorical exploratory parameters, including ATTR amyloidosis disease stage and NYHA class will be descriptively summarized by presenting the number and percentage of patients in each category for each visit. The number and percentage of patients with improving, no change, and worsening in these parameters at each visit will also be summarized.

Data collected during the OLE period will be summarized descriptively.

6.6.4. Subgroup Analysis

Subgroup analyses will be conducted to assess the consistency of treatment effect within various subgroups defined by the following baseline characteristics:

- Age [<75; ≥75 at randomization]
- Baseline tafamidis use [Yes; No]
- Type of amyloidosis [hATTR; wtATTR]
- NYHA class [I/II; III]

For subgroup analyses of 6-MWT, the effect size comparing treatment groups within each subgroup will be estimated using the HL estimate of the median difference between patisiran and placebo, together with its 95% CI. For each of the 100 complete datasets described in

Section 6.6.1.2, the HL estimate of the median difference between patisiran and placebo will be calculated within each subgroup, and the results will be combined by applying Rubin's rules,[Rubin 1996; Rubin 1987] using SAS PROC MIANALYZE to obtain the HL estimate and corresponding 95% CI for the subgroup. A forest plot will be generated to illustrate the estimated treatment effect along with 95% CI within each subgroup.

Subgroup analyses will be performed for KCCQ-OS using MMRM models. The baseline tafamidis use subgroup analysis will use the same MMRM model as specified in Section 6.6.2.1.2. For the other subgroup analyses, the outcome variable is the change from baseline in KCCQ-OS, and the model includes baseline KCCQ-OS as a continuous covariate and treatment arm, visit, baseline tafamidis use (yes vs. no), subgroup, the treatment-by-visit interaction, the treatment-by-subgroup interaction, the visit-by-subgroup interaction, and the treatment-by-visit-by-subgroup interaction as fixed factors. If the number of patients in either treatment arm of a subgroup category is less than 30, descriptive statistics may be presented. A forest plot will be generated to illustrate the estimated treatment effect along with 95% CI within each subgroup.

Other subgroups may be examined, if deemed appropriate. The subgroup analyses may also be performed for other efficacy endpoints.

6.7. Pharmacodynamic Analysis

The PD parameter for this study is serum TTR. For all analyses of post-baseline TTR data, only post-baseline TTR assessments collected within 24 days (inclusive) after receiving a full dose of study drug (ie, the amount infused was ≥80% of the planned infusion volume) will be summarized.

Summary tables will be provided for observed values, changes and percentage changes from baseline for each scheduled time point by treatment arm.

The maximum percentage reduction and mean percentage reduction over 12 months will be summarized using descriptive statistics. Subgroup analysis will be provided for age (<65, 65 to <75, ≥75), sex (male vs. female), type of amyloidosis (hATTR vs. wtATTR), and baseline tafamidis use (yes vs. no). Other subgroups may be examined, if deemed appropriate.

All PD data will be displayed in data listings.

6.8. Pharmacokinetic Analysis

6.8.1. Study Variables

6.8.1.1. Concentration Data

Plasma concentrations of the 3 PK analytes (ALN-18328, DLin-MC3-DMA and PEG_{2000}-C-DMG) will be obtained. Concentration values that are below the limit of quantification (LLOQ or BLQ) will be set to zero for analysis.

6.8.1.2. Pharmacokinetic Parameters

The following plasma concentrations of ALN-18328 and the two lipids will be summarized by study visit:

- Observed post-infusion peak concentration (C_{max})
- Observed 30 min post-infusion concentration ($C_{p(30min)}$)
- Observed pre-infusion trough concentration (C_{min})

In addition, steady-state C_{max} (C_{max_ss}), steady-state C_{min} (C_{min_ss}) and steady-state $C_{p(30min)}$ ($C_{p\_ss(30min)}$) will be calculated as the average of the C_{max}, C_{min}, and $C_{p(30min)}$ values, respectively, at Week 24, Week 36, and Week 51.

6.8.2. Statistical Methods

Descriptive statistics for PK parameters will include the number of patients, mean, SD, SEM, coefficient of variation, median, minimum, maximum, geometric mean and geometric coefficient of variation.

The C_{max}, $C_{p(30min)}$ and C_{min} of the 3 analytes will be summarized by nominal sampling day. Mean concentrations (+/- SD) will be plotted versus nominal sampling time.

Steady-state PK parameters for the 3 analytes will be summarized overall and by subgroup, including age (<65, 65 to <75, ≥75), sex (male vs. female), and anti-drug antibody status (positive vs. negative). Other subgroups may be examined, if deemed appropriate.

Plasma concentration data will be presented in by-patient listings.

The PK-PD relationship between the plasma concentration of ALN-18328 and the percent change from baseline in serum TTR may be explored.

Mean and maximum percent TTR reduction from baseline will be summarized by quartiles of the steady state PK parameters for the 3 analytes. Change from baseline at Month 12 in clinical efficacy parameters may also be summarized by quartiles of the steady-state PK parameters for the 3 analytes.

The incidence of AEs and serious AEs (SAEs) will be summarized by quartiles of the steady-state PK parameters for the 3 analytes.

Population PK, PK/PD, and disease progression modeling analyses may be performed, if appropriate. If performed, the analyses will be described in a separate analysis plan and reported separately.

6.9. Safety Analysis

An adverse event is any untoward medical event associated with the use of a study drug, whether or not it is considered related to the study drug. The primary safety parameter is the frequency of AEs. Safety parameters also include vital signs, ECGs, clinical laboratory assessments, and physical exams. Analyses for safety parameters will be conducted using the Safety Analysis Set.

Time windows for safety data to be analyzed for the 12m-DB period and the entire study including both 12m-DB and OLE periods as well as safety follow-up are described in Section 5.11. All safety data, regardless of time windows, will be listed and summarized for selected endpoints, ie, AEs by SOC and PT, SAEs by SOC and PT, and selected laboratory parameters.

Subgroup analysis for safety variables may be conducted if deemed appropriate and necessary.

No inferential safety analysis is planned.

6.9.1. Adverse Events

AEs will be classified by the MedDRA coding system (version 23.0 or later) and displayed in tables and data listings using SOC and PT.

Analyses of AEs will be performed for those events that are considered treatment-emergent, where treatment-emergent is defined as any AE with onset during or after the administration of study drug through 28 days following the last dose of study drug. In addition, any event that was present at baseline but worsened in intensity or was subsequently considered drug-related by the Investigator through the end of the study will be considered treatment-emergent. Events with a fully or partially missing onset date will be assumed to be treatment emergent unless it can be unequivocally determined (from the partial onset date and/or a partial or complete stop date) that the event occurred prior to the first administration of study drug.

Adverse events will be summarized by the numbers and percentages of patients reporting a given AE. A patient contributes only once to the count for a given AE (overall, by SOC, by PT). Overall event counts and frequencies may also be summarized.

An overall summary of AEs will include the number and percentage of patients with any AE, any AE assessed by the Investigator as related to treatment, any severe AE, any severe AE related to treatment, any SAE, any SAE related to treatment, any AE leading to treatment discontinuation, any study drug related AE leading to treatment discontinuation, and any deaths.

Tabulations by SOC and PT will be produced for the following. The SOC and PT within each SOC will be presented alphabetically.

- All AEs;
- Severe AEs;
- All SAEs;
- AEs related to treatment;
- SAEs related to treatment;
- AEs leading to infusion interruption;
- AEs leading to treatment discontinuation;
- SAEs leading to treatment discontinuation;
- AEs related to pre-medication;
- AEs over time.

Tabulations by PT in decreasing order in frequency in the patisiran arm will be produced for the following:

- All AEs;
- All SAEs;
- AEs related to treatment;

- SAEs related to treatment.

AEs will also be summarized by maximum severity and by maximum relationship; patients who report multiple occurrences of the same AE (preferred term) will be classified according to the most severe occurrence or the most related occurrence, respectively. Similarly, AEs related to treatment will be summarized by maximum severity.

Infusion-related reaction signs and symptoms will be summarized by SOC and PT. The incidence and frequency of IRR signs and symptoms over time will also be summarized by SOC and PT.

AEs mapping to the Drug Related Hepatic Disorder standardized MedDRA query (SMQ) will be summarized by SOC and PT. AEs mapping to the Anaphylactic Reaction SMQ will be summarized by PT. AEs mapping to the SMQ Malignant or Unspecified Tumors will be summarized by HLT and PT. Other SMQs or AE groupings may be evaluated.

All AEs will be presented in patient data listings. Separate listings will be provided for death, SAEs, AEs leading to treatment discontinuation, IRR signs and symptoms, AEs with missing severity, AEs with missing relationship to study drug, AEs related to pre-medications, and AEs mapping to the SMQs as described above.

Additional AE considerations regarding COVID-19 are detailed in Section 6.11.3.

Ophthalmological assessments may be performed if a patient develops ocular symptoms suggestive of vitamin A deficiency. The ophthalmological assessment results will be presented in a listing.

Patients who underwent heart transplant and/or ventricular assist device placement will be presented in a listing.

6.9.2. Laboratory Data

Clinical laboratory values will be expressed in SI units. Missing laboratory data will not be imputed.

Summary data for each laboratory parameter will be presented for each continuous clinical laboratory parameter (including hematology, serum chemistry, liver function tests and coagulation studies). Descriptive statistics will be presented for the actual values, change from baseline, and percent change from baseline by visit.

Shift tables will be employed to summarize the baseline category versus the "worst" post-baseline category for selected parameters.

A listing for all patients with abnormal liver function tests, defined as an ALT >3×ULN, AST >3×ULN, or total bilirubin >2×ULN at any time point, will also be provided.

A table will be produced to summarize the number and percentage of patients in each of the below categories at any post-baseline time point.

- ALT >1 & ≤3, >3 & ≤5, >5 & ≤10, >10 & ≤20, >20×ULN;
- AST >1 & ≤3, >3 & ≤5, >5 & ≤10, >10 & ≤20, >20×ULN;
- ALT or AST >1 & ≤3, >3 & ≤5, >5 & ≤10, >10 & ≤20, >20×ULN;

- ALP > 1.5×ULN;
- Total Bilirubin >1.5 & ≤2, >2 & ≤3, >3 & ≤5 and >5×ULN;
- Total Bilirubin > 2×ULN concurrent with ALT or AST > 3×ULN.

In separate figures, the peak total bilirubin (at any time post-baseline) will be plotted against the peak AST, the peak ALT, and the peak AST or ALT levels at any time post-baseline.

For hematology and serum chemistry, summary tables of potentially clinically significant abnormalities will be provided. The results may also be graded according to the National Cancer Institute CTCAE Version 5.0 or above. A shift summary of baseline to maximum post-baseline CTCAE grade may be presented, as appropriate.

The estimated glomerular filtration rate (eGFR, mL/min/1.73 m^2) will be categorized as below: ≥ 90; 60-89; 45-59; 30-44; 15-29 and < 15. A shift summary of baseline to worst post-baseline eGFR category will be presented.

All laboratory data, including pregnancy and follicle stimulating hormone (FSH) test results, will be provided in data listings. Out-of-range laboratory results will be identified in the listings.

6.9.3. Vital Signs and Physical Examination

For vital signs, descriptive statistics by visit and treatment arm will be provided for each variable. Vital sign measurements will be presented for each patient in a data listing, with abnormal vital signs flagged.

For the physical examination, clinically significant findings prior to first dose of study drug will be recorded and summarized under Medical History, unless there is an SAE in which case the event will be recorded and summarized under Adverse Events. Physical examination findings that are new or worsened after first dose of study drug will be recorded and summarized under Adverse Events.

6.9.4. Electrocardiogram

Electrocardiogram (ECG) findings will include rhythm, ventricular rate, RR interval, PR interval, QRS duration, QT interval, and corrected QT (QTc) interval. QTc interval will be calculated using Fridericia's correction formula.

Fridericia's cube-root corrected QT: QTcF (ms) = QT (ms) × $\sqrt[3]{\frac{HR(bpm)}{60}}$.

RR, PR, QRS, QT, and QTcF intervals and their change from pre-dose baseline will be summarized for each treatment arm by scheduled visit. The number and percentage of patients with abnormal results (clinically significant abnormal or not clinically significant abnormal) will also be summarized by treatment arm and scheduled visit.

Patients will be categorized into ≤ 450, > 450 - 480, > 480 - 500, or > 500 ms per their maximum post-baseline absolute QTcF interval and ≤ 30, > 30 - 60, or > 60 ms per their maximum change from baseline QTcF interval. The number and percentage of subjects in each category will be summarized for each treatment arm.

All ECG data for each patient will be provided in a data listing.

6.9.5. Prior and Concomitant Medications

Prior and concomitant medications will be coded using the WHO Drug Dictionary (March 2020 or later). Prior medications include medications taken ≥ 1 time before the first dose of study drug, regardless of medication end date. Concomitant medications include medications taken ≥ 1 time on or after the first dose of study drug, regardless of medication start date. Results will be tabulated by ATC and preferred term.

When there are partial or missing dates, imputed dates will be used to determine 1) if a medication is prior or concomitant, and 2) duration of exposure to select medications, as needed. Imputed dates will not be presented in the listings.

For medications with partial start or stop dates: the first day/month will be imputed for start date, and the last day/month will be imputed for stop date. For medications with a completely missing start date, the medications will be considered as started prior to the first dose of study drug; medications will be classified as prior, concomitant or both depending on the medication stop dates. For medications with a completely missing stop date, the end of study date will be imputed.

Prior and concomitant medications will be presented in data listings. Previous tetramer stabilizer use will be listed separately.

6.10. Anti-Drug Antibody

The number and percentage of patients with confirmed positive ADA assay results at any time during study as well as at each scheduled visit will be summarized. The titer results for patients with confirmed positive ADA results will also be summarized using descriptive statistics.

ADA data and patients with confirmed positive ADA results will be presented in data listings.

6.11. COVID-19 Pandemic Impact Analyses

Additional data were collected to characterize the impact of the COVID-19 pandemic on general study conduct and disposition, and subsequently, additional analyses and summaries will be provided in acknowledgement of multiple regulatory guidances (FDA Guidance on Conduct of Clinical Trials of Medical Products during COVID-19 Pandemic: Guidance for Industry, Investigators, and Institutional Review Boards, US Food and Drug Administration, 2020; Guidance on the Management of Clinical Trials during the COVID-19 (Coronavirus) Pandemic, European Medicines Agency, 2020; Points to consider on implications of Coronavirus disease (COVID-19) on methodological aspects of ongoing clinical trials, European Medicines Agency, 2020; Statistical Considerations for Clinical Trials During the COVID-19 Public Health Emergency, US Food and Drug Administration, 2020).

Few patients were enrolled on APOLLO-B prior to the beginning of the COVID-19 global pandemic.

6.11.1. General Impact

Patients who discontinue treatment or stop study participation due to COVID-19 will be included in patient disposition summaries as described in Section 6.1.

Impact on study participation due to COVID-19, including visit completion, visit location changes, and study drug dosing changes, will be summarized overall on the patient level with both continuous and categorical descriptives, and overall and by visit on the event level with categorical descriptives. Patient- and event-level summaries of the impact on study participation due to COVID-19 may also be generated by site and/or region.

Impact on study participation due to COVID-19 will be presented in data listings by patient and by visit within patient.

6.11.2. Impact on Efficacy

Summaries of missing efficacy data due to the COVID-19 pandemic will be included in missing efficacy data summaries as described in Section 5.8.1. Patient data listings will flag assessments censored due to COVID-19.

The number and percentage of patients who died, were hospitalized or had an urgent HF visit due to COVID-19 will be summarized by treatment arm and overall. The total number of hospitalizations and urgent HF visits due to COVID-19 and the number of hospitalizations and urgent HF visits per patient due to COVID-19 will also be summarized using descriptive statistics by treatment arm and overall. Patient data listings will be presented for deaths, hospitalizations and urgent HF visits due to COVID-19.

Given the measures specified in the protocol designed to ensure data integrity, analyses excluding patients with COVID-19 related protocol deviations will not be prespecified, but may be considered post hoc, if warranted.

6.11.3. Impact on Adverse Events

An overall summary of AEs mapping to a COVID-19 custom query will include the number and percentage of patients, as well as events and event rates, with any AE, any AE assessed by the Investigator as related to treatment, any severe AE, any severe AE related to treatment, any SAE, any SAE related to treatment, any AE leading to treatment discontinuation, any study drug related AE leading to treatment discontinuation, and any deaths. AEs mapping to the COVID-19 custom query will be summarized by HLT and PT. Due to the evolving nature of COVID-19-related MedDRA terminology, the COVID-19 custom query will be based on the latest information available at the specified analysis timepoint.

AEs mapping to the COVID-19 custom query will also be presented in a data listing.

7. CHANGES TO PLANNED ANALYSES

7.1. Changes from Protocol in Original SAP

Change from Protocol	Detailed Description/Rationale
In the PK and PD Analysis Set definitions, "any amount of study drug" (as specified in the protocol) was updated to "at least one complete dose of study drug".	PK and PD data from patients who have not received at least one complete dose of study drug would not be representative of the planned dose level.

Change from Protocol	Detailed Description/Rationale
The model specified to analyze the composite endpoint of all-cause mortality and frequency of all-cause hospitalizations was updated from an Andersen-Gill model stratified by baseline tafamidis (as specified in the protocol) to an Andersen-Gill model including baseline tafamidis in the list of covariates.	Given the cap on baseline tafamidis for the study, a stratified Andersen-Gill model including several covariates may encounter convergence issues. Therefore, the model now includes baseline tafamidis use in the list of covariates.

7.2. Changes in SAP Amendment 1

The SAP Amendment 1 includes more details previously not discussed in the original SAP. Some changes to analysis methods were made following regulatory feedback. The details of the changes and rationales are listed in the table below.

Summary of Changes	Detailed Description/Rationale
Updated Figure 1	To align with protocol Amendment 3
In the protocol and original SAP, an interim assessment of the tafamidis drop-in rate was planned to assess the impact of the drop-in rate on the power of the study and the potential need to increase the sample size. This interim assessment was not conducted during the study.	Due to the low rate of tafamidis drop-in on APOLLO-B, the interim sample size reassessment was determined to be unnecessary.
Added details on estimand and handling of intercurrent events for primary and secondary endpoints. The primary analyses for efficacy endpoints were also updated to not censor observations after tafamidis drop-in or after missing doses due to COVID-19.	Updates made following regulatory feedback
Updated primary MMRM model specification	To align with protocol Amendment 3
Changed imputation approach in PMM model for patients who die (not due to COVID-19) or who become unable to walk due to progression of ATTR amyloidosis by Month 12	Update was made since such patients are not expected to behave in the same way as those who remain in the study on treatment and therefore require a different imputation approach.
Updated PMM appendix	To clarify the imputation procedure for the missing data patterns, including imputation for death/inability to walk as noted above
Updated data handling approach for tafamidis drop-in and missing doses due to COVID-19 in MMRM and binary sensitivity analyses	To align with updates made to the intercurrent event handling strategies for the estimand
Updated specification for the Andersen-Gill model to be stratified by baseline tafamidis use	To align with the protocol
The Poisson model for analysis of the frequency of hospitalizations and urgent HF visits was	This update was made to allow for potentially different treatment effects between the baseline tafamidis use (yes/no) subgroups.

Summary of Changes	Detailed Description/Rationale
updated to add the treatment-by-baseline tafamidis use interaction.	
Updated the subgroup analysis MMRM model specification	This update was made to allow for potentially different treatment effects between the baseline tafamidis use (yes/no) subgroups.
Removed statement that ECG parameters will be treated as missing if QRS duration is >120 ms	To maintain consistency with analyses conducted in other studies in the patisiran clinical development program
Removed description of efficacy and safety summaries by pandemic phase	Summaries by pandemic phase will not be performed since limited data are available prior to the start of the pandemic and limited post-pandemic data are anticipated at the time of the primary analysis.

7.3. Changes in SAP Amendment 2

The SAP Amendment 2 includes changes to the primary and sensitivity analyses of 6-MWT as well as additional updates. The details of the changes and rationales are listed in the table below.

Summary of Changes	Detailed Description/Rationale
Added composite endpoint for all-cause mortality and frequency of all-cause hospitalizations and urgent HF visits in patients not on tafamidis at baseline as a secondary endpoint	The APOLLO-B study enrolls patients who are tafamidis-naïve as well as patients who are on tafamidis and have demonstrated disease progression in the opinion of the Investigator. The subgroup of tafamidis-naïve patients is a more homogeneous subpopulation while patients on baseline tafamidis are expected to be more heterogeneous (eg, in terms of duration of prior tafamidis treatment). It's anticipated that a larger treatment effect could be observed in tafamidis-naïve patients compared to patients on baseline tafamidis. The composite outcome endpoint defined in all patients in the Full Analysis Set is the only secondary endpoint that is not sufficiently powered to detect a treatment difference. Since the majority of patients (75%) are tafamidis-naïve, defining the composite outcome endpoint in this more homogeneous, enriched subgroup could improve the power and precision of the estimated treatment effect.
Changed primary analysis of 6-MWT from MMRM to the stratified Wilcoxon Rank Sum test with HL estimate of the median treatment difference. The MMRM model for 6-MWT was moved to be a sensitivity analysis.	Parametric methods, such as MMRM or ANCOVA, may be sensitive to violations of the normality assumption (eg, skew, outliers) whereas nonparametric methods, such as Wilcoxon Rank Sum test or rank ANCOVA, are more robust to such violations. Based on review of blinded 6-MWT data in APOLLO-B, the normality assumption does not appear to hold. In addition, BridgeBio recently reported 6-MWT data in ATTR cardiomyopathy which showed an apparent deviation from normality with the median differing from the mean for the change from baseline in 6-MWT at Month 12. A literature search of past Phase 3 studies with 6-MWT endpoint also showed that normal assumptions often did not hold for this endpoint, and a non-parametric method was commonly used as the primary analysis. Given these considerations, the primary analysis method was therefore updated to the stratified Wilcoxon Rank Sum test.
Intercurrent event handling strategy updated for 6-MWT	For the primary analysis of 6-MWT, patients who die or who lose the ability to walk due to progression of ATTR amyloidosis will now be imputed as the worst possible change for the patient (ie, 0 – baseline 6-MWT).
Updated Section 6.6.2.1	Updated the text in the KCCQ-OS section since the prior version referenced the planned 6-MWT analyses, which have since been changed. The planned KCCQ-OS analyses in these sections are unchanged from the SAP Amendment 1.
Added binary analysis for KCCQ-OS	To quantify and compare the proportion of patients in each treatment group who maintain or improve in their KCCQ-OS score in the 12m-DB period.

Summary of Changes	Detailed Description/Rationale
Updated planned subgroup analyses	The HL estimate of the median difference between patisiran and placebo will be used for subgroup analyses of 6-MWT to align with the primary analysis. Clarified that the MMRM model used for the baseline tafamidis use subgroup analysis for KCCQ-OS is the same as the primary MMRM model for KCCQ-OS since these subgroup estimates can be obtained directly from the primary model.
Removed summaries for SAEs by maximum severity and by maximum relationship	Table summaries determined to be unnecessary as this can be assessed by reviewing the SAE listing.
Removed summaries for AEs/SAEs leading to study discontinuation	Patients who discontinue the study due to a TEAE are accounted for in the disposition summary. Given the overlap between TEAEs that led to treatment discontinuation and those that led to study withdrawal, it is not considered necessary to list/tabulate the AEs that led to study withdrawal.
Removed NYHA from list of covariates for ANCOVA models of echo parameters and Norfolk QoL-DN Total Score	To create a more parsimonious model
Added LV relative wall thickness, LV end-diastolic volume, and cardiac output to list of selected echo parameters to be analyzed by ANCOVA	To conduct a more comprehensive echocardiographic evaluation of differences between treatment groups in cardiac structure and function
Removed summary of PK exposure by mortality status.	Sparse PK samples are collected in APOLLO-B and relating these exposures to mortality may not provide meaningful interpretation of results. A separate safety exposure analysis using population PK-PD modeling may be conducted, if deemed necessary.

7.4. Changes in SAP Amendment 2.1

SAP Amendment 2.1 addresses regulatory feedback received from the FDA on SAP Amendment 2. The main changes in SAP Amendment 2.1 include an update to the population-level summary estimate for 6-MWT and a change to the imputed 6-MWT distance for patients who die or who lose the ability to walk due to progression of ATTR amyloidosis. The details of the main changes, including other additional updates, and rationales for the changes are listed in the table below.

Summary of Changes	**Detailed Description/Rationale**
Updated population-level summary estimate from unstratified Hodges-Lehmann estimate to stratified Hodges-Lehmann estimate	Update made following regulatory feedback to use HL estimate stratified by baseline tafamidis use, which is consistent with the planned stratified Wilcoxon rank sum test.
Updated imputed values for patients who die or who lose the ability to walk due to progression of ATTR amyloidosis	Update made following regulatory feedback. For the primary analysis of 6-MWT, patients who die or who lose the ability to walk due to progression of ATTR amyloidosis will now be imputed as the worst 10^{th} percentile change observed across all patients in the 12m-DB period, capped by the worst possible change for the patient (ie, 0 – baseline 6-MWT). This is expected to be less influential on the estimated treatment effect than simply imputing the worst possible change for these patients.
Added condition that 6-MWT assessments where the timer was stopped after ≤4 minutes will be excluded from analyses	Exclude assessments that were improperly conducted
Updated windowing rule for longitudinal efficacy assessments for patients randomized to patisiran	To allow assessments collected shortly after first OLE dose to be summarized with Month 12 assessments in order to reduce the amount of missing data. Efficacy endpoints are not expected to be meaningfully impacted within a short time after the first dose in the OLE period.
Added treatment-by-baseline tafamidis use interaction term to echo and Norfolk ANCOVA models	To allow for potentially different treatment effects between the baseline tafamidis use (yes/no) subgroups.
Added Section 5.9	To clarify that the primary analysis for endpoints related to hospitalizations and urgent HF visits will be based on CEC adjudication results

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9. APPENDICES

9.1. Detailed Statistical Methodology for the Control-based Imputation for Missing Data

Patients are classified into five patterns for missing data at Month 12:

1. Patients who have missing data due to COVID-19: missing data will be assumed to be MAR and imputed by using MI estimated from the treatment arm and baseline tafamidis use group to which the patient was randomized. This pattern includes:
    a. Patients who miss the Month 12 assessment due to COVID-19
    b. Patients who have a serious COVID-19 AE or who die due to COVID-19 before Month 12
2. Placebo patients who do not have missing data due to COVID-19 and are alive through the 12m-DB period and have not become unable to walk due to progression of ATTR amyloidosis by Month 12: missing data will be assumed to be MAR and imputed by using MI estimated from placebo patients in the baseline tafamidis use group to which the patient belongs.
3. Patisiran patients who do not have missing data due to COVID-19 and are alive through the 12m-DB period, have not become unable to walk due to progression of ATTR amyloidosis by Month 12, and have missing data with the last dose of study drug received within 60 days of the scheduled time point: missing data will be assumed to be MAR and imputed by using MI estimated from on-treatment patisiran patients in the baseline tafamidis use group to which the patient belongs.
4. Patisiran patients who do not have missing data due to COVID-19 and are alive through the 12m-DB period, have not become unable to walk due to progression of ATTR amyloidosis by Month 12, and the last dose of study drug was received more than 60 days prior to the scheduled time point: missing data will be assumed to be MNAR and imputed (using data from placebo patients in the baseline tafamidis use group to which the patient belongs) using the copy reference (CR) approach.
5. Patients who die (including heart transplant and ventricular assist device placement) during the 12m-DB period (not due to COVID-19) or who have become unable to walk due to progression of ATTR amyloidosis by Month 12: missing data will be assumed to be MNAR and imputed as the worst 10^{th} percentile change observed across all patients in the 12m-DB period, capped by the worst possible change for the patient (ie, 0 – baseline 6-MWT).

For patients who are alive through the 12m-DB period and who have not become unable to walk due to progression of ATTR amyloidosis, all missing data for the placebo arm and missing data for the patisiran arm during the on-treatment period (ie, assessments within 60 days of the last dose of study drug) will be imputed using MI under the MAR assumption. Since the pattern of missing data within patients may be non-monotone, multiple imputation will be conducted separately by treatment arm and baseline tafamidis use group using the Markov Chain Monte Carlo (MCMC) method. For each treatment arm/baseline tafamidis use group, the imputation model will include type of amyloidosis (hATTR vs. wtATTR), NYHA class (I/II vs. III), age at randomization (<75 vs. ≥75), NT-proBNP (≤3000 ng/L vs >3000 ng/L), baseline 6-MWT, and

all calculated values of change from baseline in 6-MWT at pre-specified visits. The input dataset DATAIN will exclude data from patients in pattern 5 above and exclude data from patisiran patients that were collected after treatment discontinuation (ie, more than 60 days after the last dose of study drug). Below is a sample SAS code for the multiple imputation:

```
proc mi data=DATAIN out=DATA_STEP1 seed=234 nimpute=100;
   by treatment bltaf;
   em maxiter=300 converge=1e-4 itprint outem=outem;
   var baseline_variables 6MWT_base 6MWT_chg_m6 6MWT_chg_m9 6MWT_chg_m12;
   mcmc chain=multiple initial=em;
run;
```

The MI procedure generates imputed values for all missing values. For patisiran patients, the imputed data from the on-treatment period will be kept while the imputed data after treatment discontinuation will be discarded and replaced by either the observed non-missing values (if available) or the imputed values using the CR approach described below. The imputation model will include type of amyloidosis (hATTR vs. wtATTR), NYHA class (I/II vs. III), age at randomization (<75 vs. ≥75), NT-proBNP (≤3000 ng/L vs >3000 ng/L), baseline 6-MWT, and all calculated values of change from baseline in 6-MWT at pre-specified visits.

```
proc mi data=DATA_STEP1_2 out=DATA_STEP2 nimpute=1 seed=xxx;
   by _imputation_ bltaf;
   class treatment;
   fcs nbiter=30 reg (6MWT_chg_m12 = baseline_variables 6MWT_base
      6MWT_chg_m6 6MWT_chg_m9);
   mnar model (6MWT_chg_m12 / modelobs=(treatment='placebo'));
   var baseline_variables 6MWT_base 6MWT_chg_m6 6MWT_chg_m9 6MWT_chg_m12;
run;
```

Finally, for patients in pattern 5, missing Month 12 6-MWT change from baseline values will be imputed as the worst 10th percentile change observed across all patients in the 12m-DB period, capped by the worst possible change for the patient (ie, 0 – baseline 6-MWT).

9.2. Calculation of Stratified Win Ratio *p* value and Confidence Interval

Dong et al (2018) [Dong 2018] note that the logarithm of the stratified win ratio is asymptotically normally distributed with mean $\upsilon_{\log(WR)}$ and variance $\sigma^2_{\log(WR)}$, with these terms defined in equations 7b and 8 in their paper, respectively. The point estimate of the mean, $\hat{\upsilon}_{\log(WR)}$, is the logarithm of the stratified win ratio statistic defined in Section 6.6.2.2. The variance estimate, $\hat{\sigma}^2_{\log(WR)}$, is calculated under the null hypothesis of the same treatment effect in the patisiran and placebo groups.

Then $\hat{z} = \frac{\hat{\upsilon}_{\log(WR)}}{\sqrt{\hat{\sigma}^2_{\log(WR)}}}$ is a standard normal deviate from which the *p* value is readily obtained.

The 95% confidence interval for the logarithm of the stratified win ratio is constructed as

$$\hat{\upsilon}_{\log(WR)} \pm 1.96 * \sqrt{\hat{\sigma}^2_{\log(WR)}}$$

and the limits of this confidence interval will then be exponentiated to construct the 95% confidence interval for the stratified win ratio.

9.3. Questionnaire/Scoring

In questionnaires, if multiple responses are provided to a single-response question, the question is deemed as missing.

9.3.1. Kansas City Cardiomyopathy Questionnaire (KCCQ)

The Kansas City Cardiomyopathy Questionnaire is a 23-item self-administered questionnaire developed to independently measure the patient's perception of their health status, which includes heart failure symptoms, impact on physical and social limitation, and how their heart failure impacts their quality of life within a 2-week recall period.

There are 10 summary scores for the KCCQ tool, which are calculated as follows:

1. Physical Limitation

Code responses to each of Questions 1a-1f as follows:

- Extremely limited = 1
- Quite a bit limited = 2
- Moderately limited = 3
- Slightly limited = 4
- Not at all limited = 5
- Limited for other reasons or did not do the activity = <missing value>

If at least 3 of Questions 1a-1f are not missing, then compute

- Physical limitation score = [(mean of the non-missing Questions 1a-1f) – 1]/4 * 100

2. Symptom Stability

Code the response to Question 2 as follows:

- Much worse = 1
- Slightly worse = 2
- Not changed = 3
- Slightly better = 4
- Much better = 5
- I've had no symptoms over the last 2 weeks = 3

The symptom stability score = [(Question 2) – 1]/4 * 100.

3. Symptom Frequency

Code responses to Questions 3, 5, 7 and 9 as follows:

- Questions 3 and 9
  - Every morning/night = 1

  - 3 or more times a week but not every day = 2
  - 1-2 times a week = 3
  - Less than once a week = 4
  - Never over the past 2 weeks = 5
- Questions 5 and 7
  - All of the time = 1
  - Several times a day = 2
  - At least once a day = 3
  - 3 or more times a week but not every day = 4
  - 1-2 times a week = 5
  - Less than once a week = 6
  - Never over the past 2 weeks = 7

If at least two of Questions 3, 5, 7 and 9 are not missing, then compute

- S3 = [(Question 3) – 1]/4
- S5 = [(Question 5) – 1]/6
- S7 = [(Question 7) – 1]/6
- S9 = [(Question 9) – 1]/4

The symptom frequency score = (mean of S3, S5, S7, S9)*100.

4. Symptom Burden

Code responses to Questions 4, 6 and 8 as follows:

- Extremely bothersome = 1
- Quite a bit bothersome = 2
- Moderately bothersome = 3
- Slightly bothersome = 4
- Not at all bothersome = 5
- I've had no swelling/fatigue/shortness of breath = 5

If at least one of Questions 4, 6 and 8 is not missing, then compute

- Symptom burden score = [(mean of the non-missing Questions 4, 6 and 8) – 1]/4 * 100

5. Total Symptom Score

Total symptom score = mean of the following available summary scores

- Symptom Frequency Score
- Symptom Burden Score

6. Self-efficacy

Code responses to Questions 10 and 11 as follows:

- Question 10
  - Not at all sure = 1
  - Not very sure = 2
  - Somewhat sure = 3
  - Mostly sure = 4
  - Completely sure = 5
- Question 11
  - Do not understand at all = 1
  - Do not understand very well = 2
  - Somewhat understand = 3
  - Mostly understand = 4
  - Completely understand = 5

If at least one of Questions 10 and 11 is not missing, then compute

- Self-efficacy score = [(mean of the non-missing Questions 10 and 11) – 1]/4 * 100

7. Quality of Life

Code responses to Questions 12, 13 and 14 as follows:

- Question 12
  - It has extremely limited my enjoyment of life = 1
  - It has limited my enjoyment of life quite a bit = 2
  - It has moderately limited my enjoyment of life = 3
  - It has slightly limited my enjoyment of life = 4
  - It has not limited my enjoyment of life at all = 5
- Question 13
  - Not at all satisfied = 1
  - Mostly dissatisfied = 2
  - Somewhat satisfied = 3
  - Mostly satisfied = 4

  - Completely satisfied = 5
- Question 14
  - I felt that way all of the time = 1
  - I felt that way most of the time = 2
  - I occasionally felt that way = 3
  - I rarely felt that way = 4
  - I never felt that way = 5

If at least one of Questions 12, 13 and 14 is not missing, then compute

- Quality of life score = [(mean of the non-missing Questions 12, 13 and 14) – 1]/4 * 100

8. Social Limitation

Code responses to each of Questions 15a-15d as follows:

- Severely limited = 1
- Limited quite a bit= 2
- Moderately limited = 3
- Slightly limited = 4
- Did not limit at all = 5
- Does not apply or did not do for other reasons = <missing value>

If at least 2 of Questions 15a-15d are not missing, then compute

- Social limitation score = [(mean of the non-missing Questions 15a-15d) – 1]/4 * 100

9. Overall Summary Score

Overall summary score = mean of the following available summary scores

- Physical Limitation Score
- Total Symptom Score
- Quality of Life Score
- Social Limitation Score

10. Clinical Summary Score

Clinical summary score = mean of the following available summary scores

- Physical Limitation Score
- Total Symptom Score

9.3.2. Norfolk Quality of Life-Diabetic Neuropathy (QOL-DN)

Norfolk QOL-DN is a tool for assessing patients' perception of the effects of diabetes and diabetic neuropathy. There are 35 questions divided into 5 domains. The range of possible total scores is -4 to 136.

Part I: Symptoms

Items 1-7 (Part I) are a simple inventory of symptoms of neuropathy. The presence of the symptom is checked in whichever box applies, and an absence of a symptom is checked under "none." Positive responses are scored as 1; and negative responses, as 0.

Part II: Activities of Daily Life

Items 8-35 (Part II) pertain to Activities of Daily Life, and most of these are scaled on a 5-point Likert scale ranging from 0 ("Not a problem") to 4 ("Severe problem"). However, Questions 31 and 32 are scored differently. In Question 31, "Good", the middle item, is scored as0. "Very Good" is scored as –1, "Excellent" is scored as –2. "Fair" is scored as 1, and "Poor" is scored as 2. In Question 32, "About the Same," the middle item, is scored as 0. "Somewhat better" is scored as –1, "Much better" is scored as -2. "Somewhat worse" is scored as 1, and "Much worse" is scored as 2.

Subscales and Scoring Algorithm

The Total QOL and 5 domains should be summed as follows:

- Total QOL (35 items) $\Sigma(1\text{-}7, 8\text{-}35)$
- Physical Functioning/Large Fiber (15 items) $\Sigma(8, 11, 13\text{-}15, 24, 27\text{-}35)$
- Activities of Daily Living (ADLs) (5 items) $\Sigma(12, 22, 23, 25, 26)$
- Symptoms (8 items) $\Sigma(1\text{-}7, 9)$
- Small Fiber (4 items) $\Sigma(10, 16, 17, 18)$
- Autonomic (3 items) $\Sigma(19, 20, 21)$

Domain scores are calculated as the rounded integer value of the average scores of non-missing included items multiplied by the number of items if at least 50% of the items are non-missing. A domain score is missing if more than 50% of the included items are missing.

If the scores for all 5 domains are non-missing, then Total QOL is the sum of scores of the 5 domains; however, if at least 1 of the domains is missing and at least 50% of the items (18 items) are non-missing, then Total QOL is calculated as 35 times the mean of the non-missing items, rounded to the nearest integer. Otherwise, Total QOL is deemed as missing.

Signature Page for VV-CLIN-006731 v2.0
ALN-TTR02-011 SAP Amend2.1

Task: Approval Verdict: Approve	[redacted]T+0000
Task: Approval Verdict: Approve	[redacted]T+0000
Task: Approval Verdict: Approve	[redacted]9:20 GMT+0000

Signature Page for VV-CLIN-006731 v2.0
ALN-TTR02-011 SAP Amend2.1

STATISTICAL ANALYSIS PLAN
ALN-TTR02-011

Protocol Title:	APOLLO-B: A Phase 3, Randomized, Double-blind, Placebo-controlled Multicenter Study to Evaluate the Efficacy and Safety of Patisiran in Patients with Transthyretin Amyloidosis with Cardiomyopathy (ATTR Amyloidosis with Cardiomyopathy)
Short Title:	APOLLO-B: A Study to Evaluate Patisiran in Patients with Transthyretin Amyloidosis with Cardiomyopathy (ATTR Amyloidosis with Cardiomyopathy)
Study Treatment:	Patisiran (ALN-TTR02)
EudraCT Number:	2019-001458-24
IND Number:	141240
Protocol Date:	Original Protocol, 18 April 2019 Amendment 1: 20 December 2019 Amendment 2: 22 May 2020 Amendment 3: 30 June 2021
SAP Date:	Original SAP: 05 August 2020 Amendment 1: 12 October 2021 Amendment 2: 11 April 2022
Sponsor:	Alnylam Pharmaceuticals, Inc. 300 Third Street Cambridge, MA 02142 USA Telephone: [redacted]
Sponsor Contact:	[redacted]

APPROVAL SIGNATURE PAGE

Protocol Number:	ALN-TTR02-011
Protocol Title:	APOLLO-B: A Phase 3, Randomized, Double blind, Placebo-controlled Multicenter Study to Evaluate the Efficacy and Safety of Patisiran in Patients with Transthyretin Amyloidosis with Cardiomyopathy (ATTR Amyloidosis with Cardiomyopathy)
Analysis Plan Version and Date:	Amendment 2: 11 April 2022

This document has been authored and signed electronically on the final page by the following:

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LIST OF ABBREVIATIONS

Abbreviation	Definition
6-MWT	6-minute walk test
12m-DB	12-month double-blind placebo-controlled
^{99m}Tc-PYP	Technetium pyrophosphate
ADA	Antidrug antibody
AE	Adverse event
ALN-18328	siRNA targeting TTR
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANCOVA	Analysis of covariance
AST	Aspartate aminotransferase
ATC	Anatomical Therapeutic Class
ATTR	Amyloid transthyretin
BLQ	Below the lower limit of quantification
BMI	Body mass index
CI	Confidence interval
C_{max}	Maximum plasma concentration at end of infusion
$C_{max,ss}$	Steady-state C_{max}
C_{min}	Minimum pre-infusion concentration
$C_{min,ss}$	Steady-state C_{min}
CMH	Cochran-Mantel-Haenszel
CMR	Cardiac magnetic resonance
COVID-19	Coronavirus disease 2019
$C_{p(30min)}$	30-minute post-infusion concentration
$C_{p,ss(30min)}$	Steady-state $C_{p(30min)}$
CR	Copy reference
CTCAE	Common Terminology Criteria for Adverse Events
CV	Cardiovascular
DLin-MC3-DMA	1,2-Dilinoleyloxy-N,N-dimethylpropylamine
DMC	Data monitoring committee
ECG	Electrocardiogram
eCRF	Electronic case report form

Abbreviation	Definition
EDC	Electronic data capture
eGFR	Estimated glomerular filtration rate
FAS	Full Analysis Set
FSH	Follicle-stimulating hormone
H/CL	Heart to contralateral lung
hATTR	Hereditary ATTR
HF	Heart failure
HL	Hodges-Lehmann
HLT	High level term
HR	Hazard ratio
IV	Intravenous
ICH	International Council for Harmonisation
IRR	Infusion-related reaction
IRS	Interactive response system
KCCQ	Kansas City Cardiomyopathy Questionnaire
KCCQ-OS	Kansas City Cardiomyopathy Questionnaire-Overall Summary
LLOQ	Lower limit of quantification
LNP	Lipid nanoparticle
$\log_e$	Natural log transformation
LV	Left ventricular
MAR	Missing at random
mBMI	Modified body mass index
MCMC	Markov Chain Monte Carlo
MedDRA	Medical Dictionary for Regulatory Activities
MI	Multiple imputation
MMRM	Mixed effect model repeated measures
MNAR	Missing not at random
Norfolk QoL-DN	Norfolk Quality of Life - Diabetic Neuropathy
NT-proBNP	N-terminal prohormone B-type natriuretic peptide
NYHA	New York Heart Classification
OLE	Open label extension
PD	Pharmacodynamic

Abbreviation	Definition
PEG_{2000}-C-DMG	3-N-[(ω-methoxy poly(ethylene glycol)2000) carbamoyl]-1,2-dimyristyloxy-propylamine
PK	Pharmacokinetic
PMM	Pattern mixture model
PND	Polyneuropathy disability
PT	Preferred term
Q1	First quartile
Q3	Third quartile
q3W	Once every 3 weeks
QTc	Corrected QT
QTcF	Fridericia's cube-root corrected QT
SAE	Serious adverse event
SAP	Statistical analysis plan
SD	Standard deviation
SEM	Standard error of the mean
SI	International System of Units
siRNA	Small interfering RNA
SMQ	Standardized MedDRA Query
SOC	System organ class
TTR	Transthyretin
SUSAR	Suspected Unexpected Serious Adverse Reaction
ULN	Upper limit of normal
WHO	World Health Organization
WR	Win ratio
wt	Wild type
wtATTR	Wild type ATTR

1. INTRODUCTION

Transthyretin (TTR)-mediated amyloidosis (ATTR amyloidosis) is a rare, serious, life-threatening, multisystemic disease encompassing hereditary ATTR (hATTR) amyloidosis and wild type ATTR (wtATTR) amyloidosis, which result from either hereditary (genetic mutation) or nonhereditary (ageing) causes, respectively. In ATTR amyloidosis, deposition of TTR in various organs results in progressive, chronically debilitating morbidity and mortality. The most common manifestations of ATTR amyloidosis are polyneuropathy and cardiomyopathy (ie, ATTR amyloidosis with cardiomyopathy).

Patisiran is a small interfering RNA (siRNA) specific for TTR, which is formulated in a hepatotropic lipid nanoparticle (LNP) for intravenous (IV) administration.[Akinc 2010] The patisiran drug product (ALN-TTR02; patisiran-LNP, hereafter referred to as "patisiran") is designed to significantly suppress liver production of both wild type (wt) and all mutant forms of TTR, thereby having the potential to reduce amyloid formation and provide clinical benefit to patients with ATTR amyloidosis.

The APOLLO-B study (ALN-TTR02-011) is a Phase 3, randomized, double-blind, placebo-controlled, multicenter study designed to evaluate the efficacy and safety of patisiran in adult patients with ATTR amyloidosis with cardiomyopathy.

This statistical analysis plan (SAP) details comprehensive specifications of the efficacy, safety, pharmacodynamic (PD), and pharmacokinetic (PK) data summaries and statistical analyses in support of the clinical study report for Study ALN-TTR02-011. This SAP includes summaries and analyses specified and/or outlined in the protocol Amendment 3, dated 30 June 2021. Additional supportive analyses and changes to planned analyses are also included; notable changes are documented in Section 7. Changes to planned analyses made after database lock will be documented with justification in the clinical study report.

Table, figure, and listing specifications are contained in a separate document.

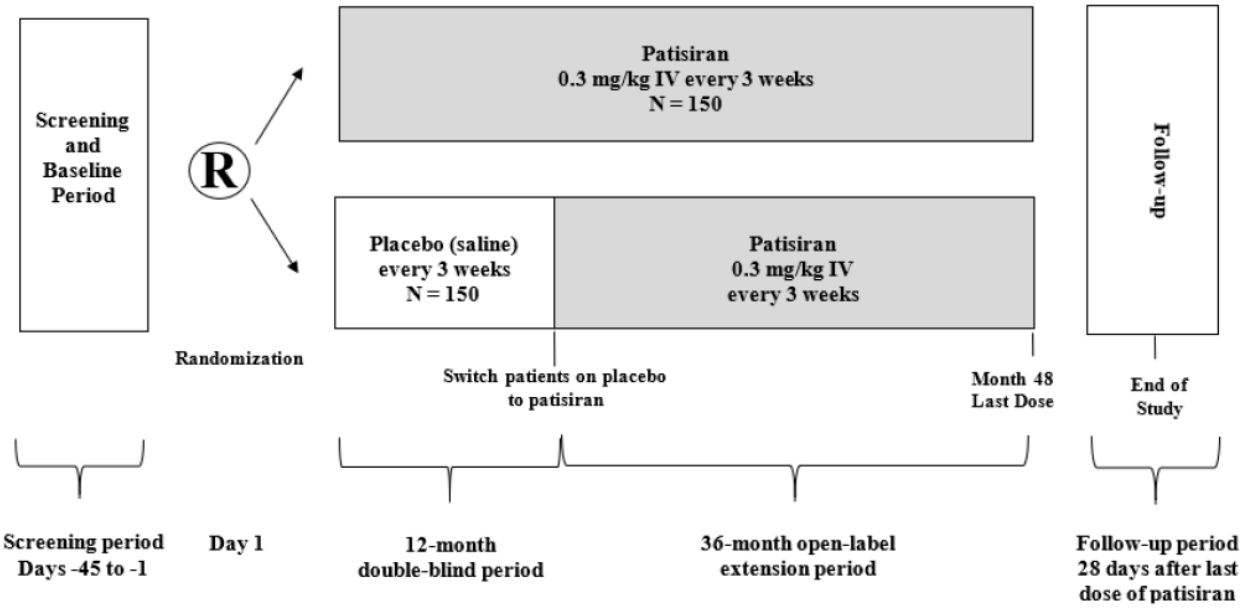
2. STUDY OVERVIEW

2.1. Synopsis of Study Design

The APOLLO-B study is a multicenter, multinational Phase 3 study designed to evaluate the efficacy and safety of patisiran in approximately 300 patients with ATTR amyloidosis (hereditary or wt) with cardiomyopathy; the study is comprised of a 1:1 randomized, double-blind, placebo-controlled period of 12 months followed by an open-label extension (OLE) period of 36 months to evaluate the long-term safety and efficacy of patisiran.

The study design schema is presented in Figure 1.

Figure 1: Study Design



2.2. Randomization Methodology

Using the interactive response system (IRS), patients will be randomized 1:1 to the patisiran or placebo arm. Randomization will be stratified by:

1. Baseline tafamidis (yes vs. no)
2. Type of amyloidosis (hATTR vs. wtATTR amyloidosis with cardiomyopathy)
3. New York Heart Classification (NYHA) Class I or II **and** age < 75 years vs. all other

Patients in the baseline tafamidis use category are defined as patients who are currently on tafamidis (for ≥6 months) with disease progression in the opinion of the investigator at baseline.

2.3. Blinding

Treatment assignments will be maintained by the IRS which has controlled access limited to unblinded team members and the unblinded pharmacist/designee preparing the infusion. Any unplanned unblinding occurring during the 12-month double-blind placebo-controlled treatment period (referred to as the 12m-DB period hereafter) will be documented and reported in the clinical study report.

Unblinding is only to occur in the case of patient emergencies or when necessary from a regulatory reporting perspective (eg, Suspected Unexpected Serious Adverse Reaction [SUSAR]), and after all patients have completed the 12m-DB period and the unblinded authorization has been executed. Details about the specifics of the blinding aspects for the study are outlined in the Randomization and Blinding Plan.

2.4. Study Procedures

The schedule of assessments is described in the study protocol (Table 1 and Table 2).

3. OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
Primary	
To evaluate the efficacy of patisiran compared with placebo treatment on functional capacity (6-minute walk test [6-MWT]) in patients with ATTR amyloidosis with cardiomyopathy	Change from baseline at Month 12 in 6-MWT
Secondary	
To evaluate the efficacy of patisiran compared with placebo treatment on: • Health status and health-related quality of life • Patient mortality, hospitalizations, and urgent heart failure (HF) visits	• Change from baseline at Month 12 in Kansas City Cardiomyopathy Questionnaire Overall Summary (KCCQ-OS) score • Composite endpoint of all-cause mortality, frequency of cardiovascular (CV) events (CV hospitalizations and urgent HF visits) and change from baseline in 6-MWT over the 12-month double-blind period • Composite endpoint of all-cause mortality and frequency of all-cause hospitalizations and urgent HF visits over the 12-month double-blind period
Exploratory	
To evaluate the efficacy of patisiran compared with placebo treatment on: • All-cause mortality and CV events • Cardiac biomarkers and biomarker-based risk assessment • Manifestations of cardiac amyloid involvement	• Composite endpoint of all-cause mortality and frequency of CV events (CV hospitalizations and urgent HF visits) over the 12-month double-blind period • Change from baseline at Month 12 in: – N-terminal prohormone B-type natriuretic peptide (NT-proBNP) – ATTR amyloidosis disease stage • Change from baseline at Month 12 in: – New York Heart Association (NYHA) Class – Echocardiographic parameters – Modified body mass index (mBMI) – Cardiac magnetic resonance (CMR) parameters – Technetium scintigraphy parameters – Troponin I levels

Objectives	Endpoints
	– Norfolk Quality of Life-Diabetic Neuropathy (Norfolk QoL-DN)
Pharmacodynamics (PD) and Pharmacokinetics (PK)	
• To evaluate the PD effect of patisiran on transthyretin (TTR) reduction • To determine the plasma concentration of patisiran and 2 lipid excipients • To assess presence of anti-drug antibodies (ADA)	• Change from baseline in serum TTR levels through Month 12 • Plasma PK exposure parameters (maximum plasma concentration at end of infusion [C_{max}], 30-minute post-infusion concentration [$C_{p(30min)}$], and pre-infusion concentration [C_{min}] • Frequency and titer of ADA
Safety	
• To evaluate the safety and tolerability of patisiran in patients with ATTR amyloidosis with cardiomyopathy	• Frequency of adverse events (AEs)

Scoring algorithms for the Kansas City Cardiomyopathy Questionnaire (KCCQ) and Norfolk Quality of Life-Diabetic Neuropathy (Norfolk QoL-DN) questionnaire are included in Appendix 9.3.1 and Appendix 9.3.2, respectively.

4. PATIENT POPULATION

The populations (analysis sets) are defined as follows:

- Full Analysis Set (FAS): All randomized patients who received any amount of study drug. Patients will be analyzed according to the treatment to which they were randomized.
- Safety Analysis Set: All randomized patients who received any amount of study drug. Patients will be summarized according to the treatment actually received.
- PK Analysis Set: All randomized patients who received at least one complete dose of study drug (see Section 6.4) and have at least 1 postdose blood sample for PK parameters and have evaluable PK data.
- PD Analysis Set: All randomized patients who received at least one complete dose of study drug (see Section 6.4) and who have an evaluable baseline and at least one evaluable post-baseline TTR sample.
- All Patisiran Treated Set: All randomized patients who received any amount of patisiran, including patients who took patisiran during the 12m-DB period and patients who first took placebo during the 12m-DB period and switched to patisiran during the OLE period.

The FAS will be used to evaluate efficacy endpoints. Safety during the 12m-DB period will be analyzed using the Safety Analysis Set. The PK and PD Analysis Sets will be used to conduct PK and PD analyses, respectively. The All Patisiran Treated Set will be used to summarize long-

term efficacy and safety data during patisiran treatment (see Section 5.10 for details). The number of patients included in all analysis sets will be provided.

5. GENERAL STATISTICAL METHODS

5.1. Determination of Sample Size

The planned enrollment for this study is 300 patients. For the change from baseline at Month 12 in the 6-MWT, assuming a treatment difference of 33 meters between patisiran and placebo in the treatment-naïve group and 20 meters in patients with baseline tafamidis, the weighted average treatment difference between patisiran and placebo in the overall population is approximately 29 meters (standard deviation [SD] = 75 meters), assuming 70% are in the treatment-naïve group and 30% are in the baseline tafamidis group. A sample size of 300 patients provides >90% power for a 2-sided test to detect a mean difference between treatment arms at a 2-sided alpha = 0.05.

5.2. General Considerations

Categorical variables will be summarized using counts and percentages.

Continuous variables will be summarized using the following descriptive summary statistics: number of patients (n), mean, standard deviation (SD), standard error of the mean (SEM), median, first quartile (Q1), third quartile (Q3), minimum, and maximum. The precision of the measurement for each continuous variable will be used to determine the number of decimal places to present in tables, figures and derived listings. Minimum and maximum values will be reported with the same precision as the units of measure. The mean, SD, SEM, median, Q1 and Q3 will be reported to one greater decimal place. Any values that require transformation to standard units (metric or International System of Units (SI) units) will be converted with the appropriate corresponding precision.

The day of the first dose of study drug administered is defined as Day 1. Study Day is defined as the number of days between the day of the first dose of study drug (Day 1) and the specific time point. The Study Day of a time point of interest is calculated as follows.

If after Day 1, Study Day = date of interest – date of the first dose of study drug + 1

If prior to Day 1, Study Day = date of interest – date of the first dose of study drug

Study days are negative when the time point of interest is prior to Day 1, positive when time of interest is after Day 1. There is no Day 0. For example, the day before the first study drug dose is defined as Day -1.

For safety laboratory parameters, assessments collected and recorded as lower than the lower limit of quantification/detection will be replaced by the lower limit of quantification/detection. Any assessment collected and recorded as greater than the upper limit of quantification will be replaced by the upper limit of quantification.

For all analysis sets except for the All Patisiran Treated Set, summaries will be presented by treatment arm (patisiran and placebo).

For the All Patisiran Treated Set, summaries will be presented by the following groups:

- Patisiran/Patisiran: all patients who received patisiran during the 12m-DB including patients who continued to receive patisiran during the OLE period and patients who discontinued treatment during the 12m-DB period;
- Placebo/Patisiran: all patients who received placebo during the 12m-DB period and switched to patisiran in the OLE period;
- All Patisiran: all patients who received at least one dose of patisiran during either the 12m-DB or OLE periods.

5.3. Computing Environment

All statistical analyses will be conducted using SAS Version 9.4 or newer or R Version 3.4 or newer, unless otherwise noted.

5.4. Baseline Definitions

For 6-MWT, baseline will be defined as the last non-missing value available prior to the first dose of study drug. Only 6-MWT assessments confirmed as valid by the Colorado Prevention Center (6-MWT site training and oversight vendor) will be included.

For other efficacy parameters, baseline will be defined as the last non-missing value available on or before the date of first dose of study drug, unless otherwise specified.

For TTR, baseline will be defined as the average of all records collected on study, including those from any unscheduled visits, prior to the date and time of first dose.

For each parameter of the 12-lead electrocardiogram (ECG), baseline will be defined as the average of all available readings from the last visit prior to the first dose of study drug.

For all other parameters, baseline will be defined as the last non-missing value available prior to the first dose of study drug, unless otherwise specified.

For the All Patisiran Treated Set, baseline for patients who switched from placebo to patisiran will be redefined as the values prior to the first dose of patisiran:

- For TTR, the redefined baseline will be calculated as the mean of all TTR assessments performed on or after Month 9 in the 12m-DB period and prior to the first dose in the OLE period.
- For all the other endpoints, the redefined baseline will be defined as the last non-missing value available prior to the first dose in the OLE period, unless otherwise specified.

5.5. Randomization Stratification Factors

Stratification factors are recorded in both the IRS and the clinical database. In statistical analyses that use randomization stratification factors as covariates, the stratum assignment will reflect the values as recorded in the clinical database. In the presence of stratification errors, the stratification used in analysis may not match that in the IRS.

5.6. Visit Windows

Scheduled visits are expected to follow the protocol schedule. All data will be tabulated and analyzed per the evaluation visit as recorded on the electronic case report form (eCRF) even if the assessment is outside of the visit window.

For 6-MWT and KCCQ, assessments will be conducted at baseline and at Months 6, 9, and 12 in the 12m-DB period and at Months 18, 21, 24, 30, 33, 36, 42 and 48 in the OLE period. If the Month 12 assessment is not performed, assessments performed within ±1.5 months of the scheduled assessment (eg, from an unscheduled, pre-tafamidis drop-in, or early treatment discontinuation visit) but prior to the first dose of patisiran in the OLE period will be grouped with the scheduled Month 12 assessments for analysis. For all other post-baseline visits, if a scheduled post-baseline assessment is not performed, assessments performed within ±1.5 months of the scheduled assessment will be grouped with the scheduled assessments for that timepoint for analysis. The derived visits will be used for all analyses.

For other efficacy assessments, if the scheduled post-baseline assessment is not performed, assessments performed within ±1.5 months of the scheduled assessment (eg, from an unscheduled, pre-tafamidis drop-in, or early treatment discontinuation visit, as applicable) will be grouped with the scheduled assessments for analysis. The derived visits will be used for all analyses.

Unless otherwise specified above, data collected at unscheduled visits will be included in by-patient data listings and figures but no assignment to a study visit will be made for the purpose of by-visit summary tabulations. However, unscheduled visits may be used in the calculation of baseline values (as discussed in Section 5.4) and for inclusion in any categorical shift summaries (eg, shift from baseline to "worst" post-baseline value).

5.7. Multiple Comparisons/Multiplicity

Formal statistical hypothesis testing will be performed on the primary and secondary efficacy endpoints with all tests conducted at the nominal 2-sided 0.05 significance level. The overall familywise error rate will be controlled at the 2-sided 0.05 significance level for the primary and secondary endpoints by a hierarchical ordering procedure. Endpoints will be tested in the following pre-specified hierarchy:

1. 6-minute walk test (6-MWT) change from baseline at Month 12
2. Kansas City Cardiomyopathy Questionnaire Overall Summary (KCCQ-OS) score change from baseline at Month 12
3. Composite endpoint of all-cause mortality, frequency of CV events (CV hospitalizations and urgent HF visits), and change from baseline in 6-MWT over the 12-month double-blind period
4. Composite endpoint of all-cause mortality and frequency of all-cause hospitalizations and urgent HF visits over the 12-month double-blind period in patients not on tafamidis at baseline
5. Composite endpoint of all-cause mortality and frequency of all-cause hospitalizations and urgent HF visits over the 12-month double-blind period in the overall population

There will be no multiplicity adjustment for exploratory endpoints.

5.8. Missing Data with Efficacy Endpoints

5.8.1. Summary of Missing Data

For the 6-MWT and KCCQ-OS efficacy endpoints, the number and percentage of patients with missing data, including due to COVID-19, at each scheduled visit will be summarized by treatment arm.

Time to treatment discontinuation in the 12m-DB period will be estimated descriptively using the Kaplan-Meier method by treatment arm. Patients who receive at least 1 dose of patisiran in the OLE period will be censored at the date of first dose in the OLE period.

5.8.2. Handling of Missing Data

For 6-MWT, the primary analysis will be based on the stratified Wilcoxon Rank Sum test. For patients with missing data at Month 12 who die during the 12m-DB period (not due to COVID-19) or who have become unable to walk due to progression of ATTR amyloidosis by Month 12, the missing Month 12 6-MWT change from baseline will be imputed as the worst possible change for the patient (ie, 0 – baseline 6-MWT). Missing data due to other reasons will be multiply imputed assuming data are missing at random (MAR). Data handling strategies for intercurrent events in the primary analysis of 6-MWT are detailed in Table 1. Sensitivity analyses for 6-MWT will be conducted to assess the impact of missing data as discussed in Section 6.6.1.3.

For KCCQ-OS, the primary analysis will be based on the mixed-effects model repeated measures (MMRM) method, which makes use of fully and partially observed data sequences from individual patients by estimating the covariance between data from different time points. The MMRM will be implemented using an unstructured approach to modeling both the treatment-by-time means and the (co)variances, leading to what is essentially a multivariate normal model wherein treatment arm means at the primary time point are adjusted to reflect both the actually observed data and the projected outcomes from the patients with missing data.[Mallinckrodt 2008] Data handling strategies for intercurrent events in the primary analysis of KCCQ-OS are detailed in Table 3. In this primary analysis, missing data will not be explicitly imputed and are assumed to be MAR. Sensitivity analyses for KCCQ-OS will be conducted to assess the impact of missing data as discussed in Section 6.6.2.1.3.

5.9. Analysis Cutoff and Database Lock

For the final 12m-DB analysis, as this study will be ongoing, the study database will be locked with all data up to a prespecified cutoff date quality controlled, ie, data in the electronic data capture (EDC) system will be frozen and external data such as laboratory and PK data will be quality controlled (and quality assured, where appropriate) and cleaned. Additional details regarding the database lock process are located in the study Data Management Plan.

The final 12m-DB analysis will include data on or prior to this prespecified cutoff date. Survival follow-up data after the cutoff date will be kept in the dataset in order to determine the survival status of patients at the time of cutoff. For assessments with starting/ending dates (eg, AEs,

medications, medical history), the starting date will be compared with the pre-specified cutoff date.

After the study is completed, ie, all patients either discontinue or complete the study, the database will be hard-locked and all data collected will be used for analysis.

5.10. Analyses for the Entire Study

The study design includes a 12m-DB period and an OLE period. The primary objective is to evaluate the efficacy and safety of patisiran compared with placebo during the 12m-DB period. In addition, the long-term efficacy and safety of patisiran during the entire patisiran treatment period (beyond 12m-DB) will be characterized for the All Patisiran Treated Set.

The detailed definitions for different treatment periods are as follows.

- **12m-DB Period**

  The treatment comparison of patisiran versus placebo will focus on the 12m-DB period, defined as below:

  1. For patients who received at least one dose of patisiran during the OLE period, all assessments collected prior to the first dose of patisiran in the OLE period will be included in the 12m-DB period.
  2. For patients who discontinued treatment and did not receive any patisiran doses in the OLE period, all assessments will be included in the 12m-DB period. Assessments collected within given time windows (details listed in below table) will be included in summary tables/figures and those outside the time windows will be listed only, unless otherwise specified (eg, AEs in Section 6.9).

Endpoint	**Windowing rule for patients who did not enter OLE**
Hospitalization/urgent HF visit/death	Events occurring on or before Day 417
Other efficacy endpoints	Assessments collected on or before Day 417
PK/PD endpoints	Assessments collected within 28 days of the last dose
Safety endpoints	Assessments with onset date within 28 days of the last dose[a]

[a] In the rare situation where a patient did not discontinue treatment in the 12m-DB period and did not enter the OLE period, all data will be included in the summary tables/figures. For AE summaries, related AEs will be considered treatment-emergent and included in summary tables regardless of time window.

- **OLE Period**

  The start day of the OLE period is defined as the day when the first dose of the OLE period is administered. The assessments collected or AEs with onset date after the administration of the first dose in the OLE period will be included in the OLE period. When assessments or AE onset dates are exactly the date of first dose in the OLE period and the assessment or AE onset time is missing, the records will be included in the OLE period.

- **During Patisiran Treatment**

  For all patients who received at least one dose of patisiran, data will be summarized for the "during patisiran treatment" period, defined as below.

  1. For patients who received patisiran in the 12m-DB period, all assessments collected after the first dose of patisiran during the entire study, including both the 12m-DB and OLE periods (if available), will be included in the "during patisiran treatment" period.
  2. For patients who received placebo in the 12m-DB period and switched to patisiran in the OLE period, all assessments collected after the first dose of patisiran in the OLE period will be included in the "during patisiran treatment" period.
  3. For patients who discontinued patisiran treatment during the 12m-DB period, the data handling will follow the same rules as discussed above for "12m-DB period". For patients who stopped participation in the study during the OLE period, assessments collected within given time windows (details listed in below table) will be included in summary tables/figures and those outside the time windows will be listed only, unless otherwise specified (eg, AEs in Section 6.9). For ongoing patients, all data will be included.

Endpoint	**Windowing rule for patients who discontinued patisiran in the OLE**
Efficacy endpoints	Assessments collected within 90 days of the last dose
PD endpoint	Assessments collected within 24 days of the last dose
Safety endpoints	Assessments with onset date within 28 days of the last dose[a]

[a] For AE summaries, related AEs will be considered treatment-emergent and included in summary tables regardless of time window.

Longitudinal efficacy parameters will be summarized over the entire study, including the 12m-DB and OLE periods, for all patients to show the long-term efficacy of patisiran (for patients randomized to patisiran) as well as to show the trajectory changes comparing the placebo experience versus the patisiran experience (for patients randomized to placebo). In these summaries, patients will be analyzed according to the treatment to which they were randomized during the 12m-DB period.

6. STATISTICAL ANALYSIS

6.1. Patient Disposition

The number and percentage of patients in the following categories will be summarized by randomized treatment arm and overall, as appropriate:

- Randomized
- Treated
- Completed Month 12 visit

- Discontinued treatment in the 12m-DB period and primary reason for treatment discontinuation and discontinuation due to COVID-19
- Stopped participation in the study in the 12m-DB period and primary reason for stopping participation and stopping participation due to COVID-19
- Entered the OLE period
- Discontinued treatment in the OLE period and primary reason for treatment discontinuation and discontinuation due to COVID-19
- Stopped participation in the study in the OLE period and primary reason for stopping participation and stopping participation due to COVID-19.

The number and percentage of patients enrolled by country and site will be summarized by randomized treatment arm and overall. The number and percentage of patients in each level of each randomization stratification factor as recorded in IRS and in the clinical database, and a comparison of the number and percentage of patients in each randomization stratification factor in IRS versus the clinical database will be summarized by randomized treatment arm and overall.

6.2. Demographics and Baseline Characteristics

Demographic and baseline characteristics, baseline disease characteristics, baseline efficacy parameters, and medical history information will be summarized by treatment arm and overall. No formal statistical comparisons will be performed.

Age at randomization, height, weight, body mass index (BMI), and mBMI will be summarized using descriptive statistics. Age group, sex, race, ethnicity, region, and country will be summarized by presenting the frequencies and percentages of patients in each category.

The following baseline disease characteristics will be summarized by presenting the numbers and percentages of patients in each category:

- Type of ATTR amyloidosis [hATTR; wtATTR]
- Genotype for hATTR patients
- Baseline tafamidis use [Yes; No]
- NYHA class [I; II; III]
- NYHA class I/II and age at randomization < 75 years [Yes; No]
- ATTR amyloidosis disease stage [1; 2; 3]
- Polyneuropathy disability (PND) score [0; I; II]
- Previous heart failure hospitalization [Yes; No]

The age at symptom onset and the time in years since diagnosis will be summarized, overall and by type of ATTR amyloidosis, using descriptive statistics. For patients who had at least 1 previous heart failure hospitalization, the age at first hospitalization for heart failure and the number of hospitalizations for heart failure in the previous 12 months will be summarized using descriptive statistics. For those who previously used tetramer stabilizers, the time from discontinuation of tetramer stabilizer to the start of study drug will be summarized using

descriptive statistics. For patients in the baseline tafamidis group (per the clinical database), the time from the start of tafamidis therapy to the start of study drug will be summarized using descriptive statistics.

The number and percent of patients with each type of ATTR amyloidosis and with each genotype (for hATTR patients) will be summarized by country and treatment arm.

Medical history will be summarized by system organ class (SOC), high level term (HLT), and preferred term (PT). A patient contributes only once to the count for a given condition (overall, by SOC, by HLT, by PT).

All demographic and baseline data for each patient will be provided in data listings. Medical history data including general medical history, cardiac medical history, neuropathy history, historical inpatient admissions or urgent healthcare visits in the past 12 months, and ophthalmic medical history will be presented in data listings. Screening test results will also be presented in data listings.

6.3. Protocol Deviations

A protocol deviation is any change, divergence, or departure from the study design or procedures defined in the clinical protocol. Protocol deviations will be classified by medical review prior to the final 12m-DB analysis, and major protocol deviations will be identified. A major protocol deviation is a deviation that may significantly impact the completeness, accuracy, and/or reliability of the trial data; that may significantly affect a subject's rights, safety, or well-being. (ICH. E3 R1 Structure and Contents of Clinical Study Reports Guidance for Industry. 2013) All major deviations related to trial inclusion or exclusion criteria, conduct of the trial, patient management or patient assessment will be described in the clinical study report.

The Sponsor or designee will be responsible for producing the protocol deviation file (formatted as a Microsoft Excel file). This file will include a description of each protocol deviation and whether or not this deviation is classified as a major protocol deviation. Since the study will be ongoing at the time of the primary analysis, there will be continuing review of protocol deviations. The file with all protocol deviations through the prespecified cutoff date for the final 12m-DB analysis will be finalized prior to the interim lock of the database and study unblinding. After the study is completed, the file with all protocol deviations for the study will be finalized prior to the hard-lock of the database.

All protocol deviations, COVID-19-related protocol deviations, and major protocol deviations will be summarized.

6.4. Drug Exposure

Exposure to study drug in months and the number of doses of study drug received will be summarized by treatment arm. Summaries of the numbers and percentages of patients with no missing infusions, and the number of missing infusions per patient will also be provided. The total volume infused will also be summarized.

The last date of exposure to study drug is defined as the earliest day of the following dates:

- Last dose date + 20 days

- Analysis cutoff date
- End of study date

Duration of exposure is defined as (the last date of exposure to study drug – date of the first dose +1)/30.4375. The exposure during the 12m-DB period is right censored by the date of the first OLE dose, ie, the last exposure day in the 12m-DB period is no later than the day before the first OLE dose. Similarly, the exposure during the OLE is left censored by the date of the first OLE dose, ie, the Day 1 of the OLE is the day of the first OLE dose. Dose interruptions and compliance are not taken into account for duration of exposure.

Study drug exposure data collected in the CRFs of study drug administration will also be summarized for each infusion. The numbers and percentages of patients with complete, partial, and missing dose administrations will be summarized. Complete and partial administration is defined as follows:

- Complete: ≥80% (≥160 mL) of the planned infusion volume (200 mL)
- Partial: >0% to <80% (>0 to <160 mL) of the planned infusion volume (200 mL).

The number of patients who experienced interruptions of infusions for any reason will be tabulated, as well as the number of patients with infusion interruptions due to an infusion-related reaction (IRR).

Dosing information for each patient will be presented in a data listing.

6.5. Premedications

All patients will receive premedications in order to reduce the potential of an IRR. Premedications will be coded using the WHO Drug Dictionary (March 2019 or later). Results will be tabulated by anatomical therapeutic class (ATC) and preferred term.

Premedication data will be presented in a data listing.

6.6. Efficacy Analyses

Efficacy endpoints will be analyzed using the FAS.

6.6.1. Primary Endpoint

The primary efficacy endpoint is to compare the change in 6-MWT from baseline to Month 12 between treatment arms. Only 6-MWT assessments confirmed as valid by the Colorado Prevention Center (6-MWT site training and oversight vendor) will be included in analyses.

6.6.1.1. Definition of Estimand

For the objective of evaluating the efficacy of patisiran compared with placebo on 6-MWT, the estimand is defined as follows:

- **Target patient population:** patients with hATTR or wtATTR amyloidosis with cardiomyopathy. Patients may or may not be taking tafamidis at study entry.
- **Treatment condition:** Patisiran 0.3 mg/kg or placebo administered as an IV infusion q3W (once every 3 weeks) with or without concomitant use of tafamidis.

- **Endpoint:** Change from baseline in 6-MWT at Month 12.
- **Population-level summary:** The Hodges-Lehmann (HL) estimate of the median difference in the change from baseline in 6-MWT at Month 12 between the patisiran and placebo arms. The *p* value for the treatment difference will be estimated using the stratified Wilcoxon Rank Sum test.

Intercurrent events and strategies: Intercurrent events include treatment discontinuation, serious COVID-19 AE, tafamidis drop-in, missing dose(s) due to COVID-19, inability to walk due to progression of ATTR amyloidosis, and death. They require different handling strategies, which are described in Table 1 below.

Table 1: Intercurrent Event Strategies for the Primary Analysis of 6-MWT

Intercurrent Event	Handling Strategy
Treatment discontinuation (not due to death)	Treatment policy strategy: Intercurrent event is ignored; 6-MWT assessments obtained after treatment discontinuation will be included in analysis.
Serious COVID-19 AE	Hypothetical strategy: Assessments obtained on or after the onset of a serious COVID-19 AE will be treated as missing and will be assumed as MAR. The goal is to estimate the scheduled visit values as if the patient had not been infected with COVID-19.
Tafamidis drop-in (ie, taking tafamidis for more than 28 days after randomization) in the subgroup of patients who were not on tafamidis at baseline	Treatment policy strategy: Intercurrent event is ignored; 6-MWT assessments obtained after tafamidis drop-in will be included in analysis.
Missing dose(s) due to COVID-19	Treatment policy strategy: Intercurrent event is ignored; 6-MWT assessments obtained after missing dose(s) due to COVID-19 will be included in analysis.
Inability to walk due to progression of ATTR amyloidosis (including patients who are unable to walk due to hospitalization related to progression of ATTR amyloidosis)	Composite variable strategy: For patients who are unable to walk due to progression of ATTR amyloidosis, the missing Month 12 6-MWT change from baseline will be imputed as the worst possible change for the patient (ie, 0 – baseline 6-MWT).
Death (including heart transplant and ventricular assist device placement) not due to COVID-19	Composite variable strategy: For patients who die (not due to COVID-19), the missing Month 12 6-MWT change from baseline will be imputed as the worst possible change for the patient (ie, 0 – baseline 6-MWT).
Death due to COVID-19	Hypothetical strategy: Missing data due to COVID-19 death will be assumed as MAR.

For patients who die or are unable to walk due to progression of ATTR amyloidosis, imputing the change at Month 12 with 0 minus baseline can significantly impact the descriptive summaries. Therefore, such imputation will only be done for the stratified Wilcoxon Rank Sum

test and HL estimate. The imputed values will not be used in the descriptive summaries or other analyses.

6.6.1.2. Primary Analysis using the Stratified Wilcoxon Rank Sum Test and Hodges-Lehmann Estimate

The primary analysis will be performed using the stratified Wilcoxon Rank Sum test for the FAS, stratified by baseline tafamidis use (yes vs. no). The analysis will be based on the following assumptions for missing data at Month 12:

1. Patients who die during the 12m-DB period (not due to COVID-19) or who have become unable to walk due to progression of ATTR amyloidosis by Month 12 and have missing data: Assuming that deaths observed in the study will likely be related to worsening of disease, the missing Month 12 6-MWT change from baseline will be imputed as the worst possible change for the patient (ie, 0 – baseline 6-MWT).
2. Patients who have missing data due to other reasons will be imputed assuming data are MAR. Since the pattern of missing data within patients may be non-monotone, multiple imputation will be conducted separately by treatment arm and baseline tafamidis use group using the Markov Chain Monte Carlo (MCMC) method. For each treatment arm/baseline tafamidis use group, the imputation model will include type of amyloidosis (hATTR vs. wtATTR), NYHA class (I/II vs. III), age at randomization (<75 vs. ≥75), baseline NT-proBNP (≤3000 ng/L vs >3000 ng/L), baseline 6-MWT, and all calculated values of change from baseline in 6-MWT at pre-specified visits.

Missing values will be imputed 100 times to generate 100 complete datasets using the procedures described above. The stratified Wilcoxon Rank Sum test (stratified by baseline tafamidis use) will be applied to each imputed dataset for the change from baseline in 6-MWT at Month 12. The Z scores estimated from the stratified Wilcoxon Rank Sum test fit to each imputed dataset will be combined by applying Rubin's rules,[Rubin 1996; Rubin 1987] using SAS PROC MIANALYZE to produce inferential results for the *p* value.

The effect size comparing treatment groups will be estimated using the HL estimate of the median difference in the change from baseline between patisiran and placebo, together with its 95% confidence interval (CI). The HL estimate is the median value of all paired differences between observations in the patisiran versus placebo groups, calculated using the imputed datasets. The calculation will be repeated for each imputed dataset, and the results will be combined by applying Rubin's rules,[Rubin 1996; Rubin 1987] using SAS PROC MIANALYZE to obtain the HL estimate and corresponding 95% CI.

6.6.1.3. Sensitivity Analyses

Sensitivity analyses will be conducted using the following methods to assess the impact of missing data and the robustness of the primary analysis.

Sensitivity Analysis 1: Stratified Wilcoxon Rank Sum Test Including All Censored Data

A sensitivity analysis including all 6-MWT assessments (ie, not treating assessments that occur on or after the onset of a serious COVID-19 AE as missing) will be conducted using the same analyses as specified in Section 6.6.1.2.

Sensitivity Analysis 2: Stratified Wilcoxon Rank Sum Test with Control-based Imputation for Missing Data in Off-treatment Patisiran Patients

A sensitivity analysis will be performed using the stratified Wilcoxon Rank Sum test where missing data from off-treatment patisiran patients are imputed based on data from the placebo group. This accommodates situations where the missingness mechanism may be missing not at random (MNAR). The model will be based on the following assumptions for missing data at Month 12:

1. Patients who have missing data due to COVID-19, including patients who have missing assessments due to COVID-19, or who have a serious COVID-19 AE or who die due to COVID-19 prior to the Month 12 assessment will be imputed assuming data are MAR. Under the hypothetical estimand of interest where the COVID-19 pandemic did not occur, these assessments should have been obtained with no COVID-19 impact. Missing data meeting these criteria will be imputed separately for each treatment arm and baseline tafamidis use group using multiple imputation (MI) estimated from all non-missing data collected within each group.
2. For patients who have missing data unrelated to COVID-19, who are alive through the 12m-DB period and who have not become unable to walk due to progression of ATTR amyloidosis by Month 12, the imputation will be performed by baseline tafamidis use (yes/no) separately based on different patterns described as below:
    a. Placebo patients who have missing data: The missing data are considered MAR and will be imputed using multiple imputation (MI) estimated from placebo patients. The imputation is done regardless of whether a patient was on-treatment or discontinued treatment before the scheduled efficacy assessment.
    b. Patisiran patients who have missing data while on treatment: Patients are expected to continue to show benefit from treatment similar to that observed at the scheduled time point. Therefore, missing data during the on-treatment period (within 60 days of their last dose) are considered MAR and will be imputed using MI estimated from all non-missing data collected on treatment from the patisiran arm.
    c. Patisiran patients who have missing data after stopping their study treatment: Patients will no longer benefit from treatment in the future and will have trajectory similar to placebo patients after discontinuing treatment. Therefore, missing data after treatment discontinuation (more than 60 days after last dose of study drug) will be imputed using the data from placebo patients using the copy reference (CR) approach.
3. Patients who die during the 12m-DB period (not due to COVID-19) or who have become unable to walk due to progression of ATTR amyloidosis by Month 12 and have missing data: Assuming that deaths observed in the study will likely be related to worsening of disease, the missing Month 12 6-MWT change from baseline will be imputed as the worst possible change for the patient (ie, 0 – baseline 6-MWT). The imputation will be done for patients from both the patisiran and placebo arms.

Missing values will be imputed 100 times to generate 100 complete datasets using the procedures described above. The stratified Wilcoxon Rank Sum test (stratified by baseline tafamidis use) will be applied to each imputed dataset for the change from baseline in 6-MWT at Month 12. The Z scores estimated from the stratified Wilcoxon Rank Sum test fit to each imputed dataset will be combined by applying Rubin's rules,[Rubin 1996; Rubin 1987] using SAS PROC MIANALYZE to produce inferential results for the *p* value.

The effect size comparing treatment groups will be estimated using the HL estimate of the median difference in the change from baseline between patisiran and placebo, together with its 95% CI. The calculation will be repeated for each imputed dataset, and the results will be combined by applying Rubin's rules,[Rubin 1996; Rubin 1987] using SAS PROC MIANALYZE to obtain the HL estimate and corresponding 95% CI.

More details on the implementation of the control-based imputation for missing data are discussed in Appendix 9.1.

Sensitivity Analysis 3: MMRM Model

A sensitivity analysis will be performed using a REML-based MMRM approach. The outcome variable is the change from baseline in 6-MWT; the model includes baseline 6-MWT as a continuous covariate and treatment arm, visit (Month 6, Month 9 or Month 12), baseline tafamidis use (yes vs. no), type of amyloidosis (hATTR vs. wtATTR), age at randomization (<75 vs. ≥75 years), the treatment-by-visit interaction, the treatment-by-baseline tafamidis interaction, the visit-by-baseline tafamidis interaction, and the treatment-by-visit-by-baseline tafamidis interaction as fixed factors. Assessments obtained on or after the onset of a serious COVID-19 AE will be treated as missing and will be assumed as MAR.

An unstructured covariance structure will be used to model the within-patient errors. If the model fails to converge, the following covariance structures will be specified in sequence and the first to converge will be used:

1. Toeplitz
2. Autoregressive (1)
3. Compound symmetry

The Satterthwaite approximation will be used to estimate the degrees of freedom.

The primary comparison is the contrast (difference in LS means) between the patisiran and placebo arms at Month 12. The LS mean coefficients will be computed using the observed proportions of the categorical covariates (baseline tafamidis use, type of ATTR amyloidosis and age) in the FAS. The analysis will be implemented with SAS PROC MIXED.

The MMRM analysis assumes multivariate normality for the error term in the analysis model. This normality assumption will be assessed by inspection of residual plots along with a formal teste for normality using the Shapiro-Wilk test.

6.6.1.4. Binary Analyses

Binary analysis for 6-MWT will be conducted on the observed 6-MWT data without imputation. The number and percentage of patients with a ≥0 meter increase in 6-MWT from baseline to Month 12 will be calculated for each treatment arm and compared between 2 arms using the

Cochran-Mantel-Haenszel (CMH) test, stratified by baseline tafamidis use (yes vs. no). All patients with missing Month 12 data will be counted in the denominator with two exceptions: patients who are missing Month 12 data due to COVID-19 and patients with a 6-MWT assessment collected on or after the onset of a serious COVID-19 AE will be excluded.

6.6.1.5. Overview of Primary Endpoint Analyses

The planned analyses of the primary endpoint 6-MWT are summarized in Table 2.

Table 2: Analysis of 6-MWT

Statistical Method
Primary analysis: Stratified Wilcoxon Rank Sum test
Sensitivity analysis 1: Stratified Wilcoxon Rank Sum test – including all available data without censoring
Sensitivity analysis 2: Stratified Wilcoxon Rank Sum test – implementing control-based imputation for missing data in off-treatment patisiran patients
Sensitivity analysis 3: MMRM
Other analysis: Binary analysis using stratified CMH

6.6.2. Secondary Endpoints

The secondary efficacy endpoints are specified in Section 3. To control overall type I error, the secondary endpoints will be tested in a hierarchical order as described in Section 5.7.

CV events will include all emergency/unplanned/non-elective hospitalizations after randomization adjudicated as being cardiovascular or being indeterminate, and all emergency/unplanned/non-elective visits after randomization that are adjudicated as being urgent HF visits. Deaths will include heart transplant and ventricular assist device placement, unless otherwise specified.

6.6.2.1. Change from Baseline at Month 12 in KCCQ-OS Score

6.6.2.1.1. Definition of Estimand

For the objective of evaluating the efficacy of patisiran compared with placebo on KCCQ-OS, the estimand is defined as follows:

- **Target patient population:** patients with hATTR or wtATTR amyloidosis with cardiomyopathy. Patients may or may not be taking tafamidis at study entry.
- **Treatment condition:** Patisiran 0.3 mg/kg or placebo administered as an IV infusion q3W (once every 3 weeks) with or without concomitant use of tafamidis.
- **Endpoint:** Change from baseline in KCCQ-OS at Month 12.
- **Population-level summary:** The least squares (LS) mean difference in the change from baseline in KCCQ-OS at Month 12 between the patisiran and placebo arms.

Intercurrent events and strategies: Intercurrent events include treatment discontinuation, serious COVID-19 AE, tafamidis drop-in, missing dose(s) due to COVID-19, and death. They require different handling strategies, which are described in Table 3 below.

Table 3: Intercurrent Event Strategies for the Primary Analysis of KCCQ-OS

Intercurrent Event	Handling Strategy
Treatment discontinuation (not due to death)	Treatment policy strategy: Intercurrent event is ignored; assessments obtained after treatment discontinuation will be included in analysis.
Serious COVID-19 AE	Hypothetical strategy: Assessments obtained on or after the onset of a serious COVID-19 AE will be treated as missing and will be assumed as MAR. The goal is to estimate the scheduled visit values as if the patient had not been infected with COVID-19.
Tafamidis drop-in (ie, taking tafamidis for more than 28 days after randomization) in the subgroup of patients who were not on tafamidis at baseline	Treatment policy strategy: Intercurrent event is ignored; assessments obtained after tafamidis drop-in will be included in analysis.
Missing dose(s) due to COVID-19	Treatment policy strategy: Intercurrent event is ignored; assessments obtained after missing dose(s) due to COVID-19 will be included in analysis.
Death	Hypothetical strategy: Missing data due to death will be assumed as MAR.

6.6.2.1.2. Primary Analysis using MMRM for the Full Analysis Set

The change from baseline at Month 12 in KCCQ-OS will be analyzed using an MMRM model similar to the MMRM model for 6-MWT described in Section 6.6.1.3 while adjusting for baseline KCCQ-OS as a continuous covariate.

6.6.2.1.3. Sensitivity Analyses

Sensitivity analyses will be conducted using the following methods to assess the impact of missing data and the robustness of the primary analysis.

MMRM Model Including All Censored Data

A sensitivity analysis including all KCCQ-OS assessments, ie, not treating assessments that occur on or after the onset of a serious COVID-19 AE as missing, will be conducted using the same MMRM model as used for the primary analysis.

PMM Model

A sensitivity analysis using pattern mixture model (PMM) will be performed to assess the robustness of the primary MMRM results to the possible violation of the MAR assumption. The PMM accommodates situations where the missingness mechanism may be MNAR. The assumptions for missing data at Month 12 are the same as those specified in Section 6.6.1.3 with

the exception of bullet 3, the imputation for patients who die during the 12m-DB period (not due to COVID-19). The revised bullet 3 is as follows:

3. Patients who die during the 12m-DB period (not due to COVID-19) and have missing data: Assuming that deaths observed in the study will likely be related to worsening of disease, the missing Month 12 KCCQ-OS change from baseline will be imputed by taking random samples from the worst 10% KCCQ-OS changes observed during the 12m-DB period in the entire population, capped by the worst possible change for the patient (ie, 0 – baseline KCCQ-OS). The imputation will be done for patients from both the patisiran and placebo arms.

Note that the PMM imputation rules for KCCQ-OS will not consider being unable to walk due to progression of ATTR amyloidosis in defining the missing data patterns since patients are still able to complete the KCCQ assessment in this scenario.

Missing values will be imputed 100 times to generate 100 complete datasets using the procedures described above. An analysis of covariance (ANCOVA) model will be fit to each imputed dataset for the change from baseline in KCCQ-OS at Month 12. The ANCOVA model will include baseline KCCQ-OS as a continuous covariate and treatment arm, baseline tafamidis use (yes vs. no), type of ATTR amyloidosis (hATTR vs. wtATTR), age at randomization (<75 vs. ≥75), and the treatment-by-baseline tafamidis interaction as covariates. The LS mean coefficients will be computed using the observed proportions of the categorical covariates (baseline tafamidis use, type of ATTR amyloidosis and age) in the FAS. The LS mean and standard error of the mean (SEM) estimated from the ANCOVA model fit to each imputed dataset will be combined by applying Rubin's rules,[Rubin 1996; Rubin 1987] using SAS PROC MIANALYZE to produce inferential results including the treatment difference in LS means, 95% CI for the treatment difference, and the *p* value.

6.6.2.1.4. Binary Analyses

A binary analysis of KCCQ-OS, similar to the analysis described for 6-MWT in Section 6.6.1.4, will be conducted using a threshold of a ≥0 point increase in KCCQ-OS from baseline to Month 12.

6.6.2.2. Composite All-cause Mortality, Frequency of CV Events (CV hospitalizations and urgent HF visits), and Change from Baseline in 6-MWT over the 12-month Double-blind Period

6.6.2.2.1. Definition of Estimand

For the objective of evaluating the efficacy of patisiran compared with placebo on patient mortality, hospitalizations and urgent HF visits, and change from baseline in 6-MWT, the estimand is defined as follows:

- **Target patient population:** Patients with hATTR or wtATTR amyloidosis with cardiomyopathy. Patients may or may not be taking tafamidis at study entry.
- **Treatment condition:** Patisiran 0.3 mg/kg or placebo administered as an IV infusion q3W with or without concomitant use of tafamidis.

- **Endpoint:** Hierarchical comparison of all-cause mortality, frequency of CV events, and the change from baseline in 6-MWT over the 12m-DB period.
- **Population-level summary:** Stratified win ratio for the composite outcome between the patisiran and placebo arms.

Intercurrent events and strategies: Intercurrent events include treatment discontinuation, serious COVID-19 AE, COVID-19 related deaths and CV events, tafamidis drop-in, missing dose(s) due to COVID-19, inability to walk due to progression of ATTR amyloidosis, and heart transplantation and ventricular assist device placement. They require different handling strategies, which are described in Table 4 below.

Table 4: Intercurrent Event Strategies for the Analysis of the Composite Endpoint

Intercurrent Event	Handling Strategy
Treatment discontinuation	Treatment policy strategy: Intercurrent event is ignored; deaths, CV events, and 6-MWT assessments that occur after treatment discontinuation will be included in analysis.
COVID-19 related death and CV events	Hypothetical strategy: Deaths and CV events due to COVID-19 will be excluded from analysis. The goal is to estimate the treatment effect in a hypothetical setting where the COVID-19 pandemic is not present.
Serious COVID-19 AE	Hypothetical strategy: 6-MWT assessments obtained on or after the onset of a serious COVID-19 AE will be treated as missing.
Tafamidis drop-in (ie, taking tafamidis for more than 28 days after randomization) in the subgroup of patients who were not on tafamidis at baseline	Treatment policy strategy: Intercurrent event is ignored; deaths, CV events, and 6-MWT assessments that occur after tafamidis drop-in will be included in analysis.
Missing dose(s) due to COVID-19	Treatment policy strategy: Intercurrent event is ignored; deaths, CV events, and 6-MWT assessments that occur after missing dose(s) due to COVID-19 will be included in analysis.
Inability to walk due to progression of ATTR amyloidosis (including patients who are unable to walk due to hospitalization related to progression of ATTR amyloidosis)	Composite variable strategy: For patients who are unable to walk due to progression of ATTR amyloidosis, the missing Month 12 6-MWT change from baseline will be imputed as the worst possible change for the patient (ie, 0 – baseline 6-MWT).
Heart transplantation and ventricular assist device placement	Composite variable strategy: Heart transplantation and ventricular assist device placement will be treated in the same manner as death.

6.6.2.2.2. Primary Analysis

The composite endpoint of all-cause mortality, frequency of CV events (CV hospitalizations and urgent HF visits) and change from baseline in 6-MWT will be analyzed using the stratified win ratio method,[Dong 2018] stratified by baseline tafamidis use (yes vs. no). This method makes

within-stratum pairwise comparisons (for all possible patisiran/placebo patient pairs) of the 3 components in the hierarchical order specified above. Within a stratum, each patisiran patient will be compared with all placebo patients in a stepwise fashion, with the "winner" assigned a score of +1 and the "loser" assigned a score of "-1". The detailed steps are described as follows. All comparisons will be based on data collected during the 12m-DB period.

Step 1: compare all-cause mortality

The comparison of the two patients will be performed at the shorter of their survival follow-up times.

- If both patients are deceased, then the patient with a longer survival time is assigned a +1 and the other is assigned a -1
- If one patient is alive and the other is deceased, the alive patient is assigned a +1 and the deceased patient is assigned a -1
- Otherwise, proceed to step 2

Step 2: compare frequency of CV events

The comparison of the two patients will be performed at the shorter of their follow-up times while patients remain on the study.

- The patient with fewer CV events is assigned a +1 and the other is assigned a -1
- If the numbers of CV events are the same, go to step 3

Step 3: compare change from baseline in 6-MWT

- The patient who has less decline in 6-MWT at Month 12 is assigned a +1 and the other is assigned a -1
- If one or both patients have missing data at Month 12, compare change from baseline in 6-MWT at the latest common study visit where both patients had a 6-MWT measurement. The patient who has less decline in 6-MWT is assigned a +1 and the other is assigned a -1
- If tied or undetermined, assign score 0 to each patient

The point estimate of the stratified win ratio (WR) is defined as

$$WR = \frac{\sum_{m=1}^{2} n_t^{(m)}/N^{(m)}}{\sum_{m=1}^{2} n_c^{(m)}/N^{(m)}}$$

where $n_t^{(m)}$ and $n_c^{(m)}$ are the number of patisiran/placebo pairs in stratum *m* in which the patisiran patient was the winner and in which the placebo patient was the winner, respectively, and $N^{(m)}$ is the total number of patients in stratum *m*.

A 95% CI and *p* value for the stratified win ratio will be estimated (see Section 9.2 for details).

6.6.2.3. Composite All-cause Mortality and Frequency of All-cause Hospitalizations and urgent HF visits over the 12-month Double-blind Period in Patients Not on Tafamidis at Baseline

6.6.2.3.1. Definition of Estimand

For the objective of evaluating the efficacy of patisiran compared with placebo on the composite outcome of mortality, hospitalizations and urgent HF visits in patients not on tafamidis at baseline, the estimand is defined as follows:

- **Target patient population:** Patients with hATTR or wtATTR amyloidosis with cardiomyopathy who are not taking tafamidis at study entry.
- **Treatment condition:** Patisiran 0.3 mg/kg or placebo administered as an IV infusion q3W with or without concomitant use of tafamidis.
- **Endpoint:** Timing and frequency of all-cause deaths, all-cause hospitalizations and urgent HF visits over the 12m-DB period.
- **Population-level summary:** Hazard ratio (HR) for the composite outcome between the patisiran and placebo arms.

Intercurrent events and strategies: Intercurrent events include treatment discontinuation, COVID-19 infection, tafamidis drop-in, missing dose(s) due to COVID-19, and heart transplantation and ventricular assist device placement. They require different handling strategies, which are described in Table 5 below.

Table 5: Intercurrent Event Strategies for the Analysis of the Composite Endpoint

Intercurrent Event	Handling Strategy
Treatment discontinuation	Treatment policy strategy: Intercurrent event is ignored; deaths, all-cause hospitalizations and urgent HF visits that occur after treatment discontinuation will be included in analysis.
COVID-19 related deaths, hospitalizations, and urgent HF visits	Hypothetical strategy: Deaths, all-cause hospitalizations and urgent HF visits due to COVID-19 will be excluded from analysis. The goal is to estimate the HR in a hypothetical setting where the COVID-19 pandemic is not present.
Tafamidis drop-in (ie, taking tafamidis for more than 28 days after randomization) in the subgroup of patients who were not on tafamidis at baseline	Treatment policy strategy: Intercurrent event is ignored; deaths, all-cause hospitalizations and urgent HF visits that occur after tafamidis drop-in will be included in analysis.
Missing dose(s) due to COVID-19	Treatment policy strategy: Intercurrent event is ignored; deaths, all-cause hospitalizations and urgent HF visits that occur after missing dose(s) due to COVID-19 will be included in analysis.

Intercurrent Event	Handling Strategy
Heart transplantation and ventricular assist device placement	Composite variable strategy: Heart transplantation and ventricular assist device placement will be treated in the same manner as death.

6.6.2.3.2. Primary Analysis

The composite endpoint of all-cause mortality and frequency of all-cause hospitalizations and urgent HF visits in patients who are not on tafamidis at baseline will be analyzed using an Andersen-Gill model, including treatment, type of amyloidosis (hATTR vs. wtATTR), baseline NYHA class (I/II vs. III), and age at randomization (<75 vs. ≥75 years) as covariates.

6.6.2.3.3. Component Analysis

The components of the composite endpoint in patients not on tafamidis at baseline will be analyzed as follows. For all-cause mortality, Kaplan-Meier survival curves for each treatment group will be presented. The HR and corresponding 95% CI will be estimated from a Cox proportional hazards model including treatment as a covariate. Frequency of all-cause hospitalizations and urgent HF visits in patients not on tafamidis at baseline will be analyzed using Poisson regression with treatment, type of amyloidosis (hATTR vs. wtATTR), baseline NYHA class (I/II vs. III), and age at randomization (<75 vs. ≥75 years) as covariates, adjusting for duration of follow-up (ie, include duration of follow-up as an offset).

6.6.2.4. Composite All-cause Mortality and Frequency of All-cause Hospitalizations and urgent HF visits over the 12-month Double-blind Period in the Overall Population

6.6.2.4.1. Definition of Estimand

The estimand is the same as that specified in Section 6.6.2.3.1, except the target patient population includes all patients with hATTR or wtATTR amyloidosis with cardiomyopathy (ie, patients may or may not be taking tafamidis at study entry).

6.6.2.4.2. Primary Analysis

The composite endpoint of all-cause mortality and frequency of all-cause hospitalizations and urgent HF visits will be analyzed using a modified Andersen-Gill model stratified by baseline tafamidis use (yes vs. no), including treatment, type of amyloidosis (hATTR vs. wtATTR), baseline NYHA class (I/II vs. III), and age at randomization (<75 vs. ≥75 years) as covariates.

6.6.2.4.3. Component Analysis

The all-cause mortality component of the composite endpoint will be evaluated using analyses similar to those described in Section 6.6.2.3.3. Frequency of all-cause hospitalizations and urgent HF visits will be analyzed using Poisson regression with treatment, baseline tafamidis use (yes vs. no), type of amyloidosis (hATTR vs. wtATTR), baseline NYHA class (I/II vs. III), age at randomization (<75 vs. ≥75 years), and the treatment-by-baseline tafamidis use interaction as covariates, adjusting for duration of follow-up (ie, include duration of follow-up as an offset).

6.6.3. Exploratory Endpoints

All analyses of exploratory endpoints will be conducted on the FAS. For continuous endpoints, descriptive statistics will be provided for actual value and change from baseline by treatment arm; descriptive statistics for percentage change from baseline by treatment arm may also be provided, as appropriate.

The composite endpoint of all-cause mortality and frequency of CV events (CV hospitalizations and urgent HF visits) over the 12m-DB period will be analyzed using a modified Andersen-Gill model similar to that for the composite endpoint of all-cause mortality ad frequency of all-cause hospitalizations and urgent HF visits (see Section 6.6.2.3). The frequency of CV events component will also be analyzed using Poisson regression with treatment, baseline tafamidis (yes vs. no), type of amyloidosis (hATTR vs. wtATTR), baseline NYHA class (I/II vs. III), age at randomization (<75 vs. ≥75 years), and the treatment-by-baseline tafamidis use interaction as covariates, adjusting for duration of follow-up.

The change from baseline at Month 12 in select echocardiographic parameters (mean left ventricular [LV] wall thickness, LV relative wall thickness, LV mass, LV end-diastolic volume, global LV longitudinal strain, and cardiac output) and Norfolk QoL-DN Total Score (see Section 9.3.2) will be analyzed using an ANCOVA model since they are only measured in the 12m-DB period at Month 12. The ANCOVA model will include the baseline value for the parameter as a continuous covariate and treatment arm, baseline tafamidis use (yes vs. no), type of amyloidosis (hATTR vs. wtATTR), and age at randomization (<75 vs. ≥75) as covariates. The Norfolk QoL-DN questionnaire is a patient-reported outcomes measure that is sensitive to the different features of diabetic neuropathy; since patients enrolled in this study are not required to have a history of neuropathy, the ANCOVA model for Norfolk QoL-DN Total Score may be repeated on the subset of patients with a history of neuropathy.

In the 12m-DB period, NT-proBNP and troponin I are measured at baseline, Month 3, Month 6, Month 9, and Month 12, and mBMI is measured at baseline, Month 6, and Month 12. NT-proBNP, troponin I, and mBMI will be analyzed using MMRM models similar to the model described for the primary analysis of KCCQ-OS while adjusting for the baseline value of the endpoint being modeled (see Section 6.6.2.1). Assessments obtained on or after the onset of a serious COVID-19 AE will not be treated as missing.

NT-proBNP has been shown to be highly skewed in the literature; a Q-Q plot of the residuals of the NT-proBNP MMRM model specified above will be used to assess the normality assumption. If the normality assumption is violated, a natural log transformation ($\log_e$) will be applied to NT-proBNP to normalize the distribution. Following the transformation, an MMRM model similar to the model described for the primary analysis of KCCQ-OS will be used; the outcome variable will be $\log_e$(post-baseline) – $\log_e$(baseline), and the model will include $\log_e$(baseline) as a continuous covariate. Adjusted geometric mean fold-changes from baseline with 95% CIs will be constructed by exponentially back-transforming the LS means, differences in LS means, and the limits of the corresponding 95% CIs.

CMR and technetium scintigraphy will be performed in a subset of patients at select sites to assess cardiac amyloid involvement. The analyses for these imaging assessments are detailed in the separate APOLLO-B Imaging Statistical Analysis Plan.

Categorical exploratory parameters, including ATTR amyloidosis disease stage and NYHA class will be descriptively summarized by presenting the number and percentage of patients in each category for each visit. The number and percentage of patients with improving, no change, and worsening in these parameters at each visit will also be summarized.

Data collected during the OLE period will be summarized descriptively.

6.6.4. Subgroup Analysis

Subgroup analyses will be conducted to assess the consistency of treatment effect within various subgroups defined by the following baseline characteristics:

- Age [<75; ≥75 at randomization]
- Baseline tafamidis use [Yes; No]
- Type of amyloidosis [hATTR; wtATTR]
- NYHA class [I/II; III]

For subgroup analyses of 6-MWT, the effect size comparing treatment groups within each subgroup will be estimated using the HL estimate of the median difference between patisiran and placebo, together with its 95% CI. For each of the 100 complete datasets described in Section 6.6.1.2, the HL estimate of the median difference between patisiran and placebo will be calculated within each subgroup, and the results will be combined by applying Rubin's rules,[Rubin 1996; Rubin 1987] using SAS PROC MIANALYZE to obtain the HL estimate and corresponding 95% CI for the subgroup. A forest plot will be generated to illustrate the estimated treatment effect along with 95% CI within each subgroup.

Subgroup analyses will be performed for KCCQ-OS using MMRM models. The baseline tafamidis use subgroup analysis will use the same MMRM model as specified in Section 6.6.2.1.2. For the other subgroup analyses, the outcome variable is the change from baseline in KCCQ-OS, and the model includes baseline KCCQ-OS as a continuous covariate and treatment arm, visit, baseline tafamidis use (yes vs. no), subgroup, the treatment-by-visit interaction, the treatment-by-subgroup interaction, the visit-by-subgroup interaction, and the treatment-by-visit-by-subgroup interaction as fixed factors. If the number of patients in either treatment arm of a subgroup category is less than 30, descriptive statistics may be presented. A forest plot will be generated to illustrate the estimated treatment effect along with 95% CI within each subgroup.

Other subgroups may be examined, if deemed appropriate. The subgroup analyses may also be performed for other efficacy endpoints.

6.7. Pharmacodynamic Analysis

The PD parameter for this study is serum TTR. For all analyses of post-baseline TTR data, only post-baseline TTR assessments collected within 24 days (inclusive) after receiving a full dose of study drug (ie, the amount infused was ≥80% of the planned infusion volume) will be summarized.

Summary tables will be provided for observed values, changes and percentage changes from baseline for each scheduled time point by treatment arm.

The maximum percentage reduction and mean percentage reduction over 12 months will be summarized using descriptive statistics. Subgroup analysis will be provided for age (<65, 65 to <75, ≥75), sex (male vs. female), type of amyloidosis (hATTR vs. wtATTR), and baseline tafamidis use (yes vs. no). Other subgroups may be examined, if deemed appropriate.

All PD data will be displayed in data listings.

6.8. Pharmacokinetic Analysis

6.8.1. Study Variables

6.8.1.1. Concentration Data

Plasma concentrations of the 3 PK analytes (ALN-18328, DLin-MC3-DMA and PEG_{2000}-C-DMG) will be obtained. Concentration values that are below the limit of quantification (LLOQ or BLQ) will be set to zero for analysis.

6.8.1.2. Pharmacokinetic Parameters

The following plasma concentrations of ALN-18328 and the two lipids will be summarized by study visit:

- Observed post-infusion peak concentration (C_{max})
- Observed 30 min post-infusion concentration ($C_{p(30min)}$)
- Observed pre-infusion trough concentration (C_{min})

In addition, steady-state C_{max} (C_{max_ss}), steady-state C_{min} (C_{min_ss}) and steady-state $C_{p(30min)}$ ($C_{p\_ss(30min)}$) will be calculated as the average of the C_{max}, C_{min}, and $C_{p(30min)}$ values, respectively, at Week 24, Week 36, and Week 51.

6.8.2. Statistical Methods

Descriptive statistics for PK parameters will include the number of patients, mean, SD, SEM, coefficient of variation, median, minimum, maximum, geometric mean and geometric coefficient of variation.

The C_{max}, $C_{p(30min)}$ and C_{min} of the 3 analytes will be summarized by nominal sampling day. Mean concentrations (+/- SD) will be plotted versus nominal sampling time.

Steady-state PK parameters for the 3 analytes will be summarized overall and by subgroup, including age (<65, 65 to <75, ≥75), sex (male vs. female), and anti-drug antibody status (positive vs. negative). Other subgroups may be examined, if deemed appropriate.

Plasma concentration data will be presented in by-patient listings.

The PK-PD relationship between the plasma concentration of ALN-18328 and the percent change from baseline in serum TTR may be explored.

Mean and maximum percent TTR reduction from baseline will be summarized by quartiles of the steady state PK parameters for the 3 analytes. Change from baseline at Month 12 in clinical efficacy parameters may also be summarized by quartiles of the steady-state PK parameters for the 3 analytes.

The incidence of AEs and serious AEs (SAEs) will be summarized by quartiles of the steady-state PK parameters for the 3 analytes.

Population PK, PK/PD, and disease progression modeling analyses may be performed, if appropriate. If performed, the analyses will be described in a separate analysis plan and reported separately.

6.9. Safety Analysis

An adverse event is any untoward medical event associated with the use of a study drug, whether or not it is considered related to the study drug. The primary safety parameter is the frequency of AEs. Safety parameters also include vital signs, ECGs, clinical laboratory assessments, and physical exams. Analyses for safety parameters will be conducted using the Safety Analysis Set.

Time windows for safety data to be analyzed for the 12m-DB period and the entire study including both 12m-DB and OLE periods as well as safety follow-up are described in Section 5.10. All safety data, regardless of time windows, will be listed and summarized for selected endpoints, ie, AEs by SOC and PT, SAEs by SOC and PT, and selected laboratory parameters.

Subgroup analysis for safety variables may be conducted if deemed appropriate and necessary.

No inferential safety analysis is planned.

6.9.1. Adverse Events

AEs will be classified by the MedDRA coding system (version 23.0 or later) and displayed in tables and data listings using SOC and PT.

Analyses of AEs will be performed for those events that are considered treatment-emergent, where treatment-emergent is defined as any AE with onset during or after the administration of study drug through 28 days following the last dose of study drug. In addition, any event that was present at baseline but worsened in intensity or was subsequently considered drug-related by the Investigator through the end of the study will be considered treatment-emergent. Events with a fully or partially missing onset date will be assumed to be treatment emergent unless it can be unequivocally determined (from the partial onset date and/or a partial or complete stop date) that the event occurred prior to the first administration of study drug.

Adverse events will be summarized by the numbers and percentages of patients reporting a given AE. A patient contributes only once to the count for a given AE (overall, by SOC, by PT). Overall event counts and frequencies may also be summarized.

An overall summary of AEs will include the number and percentage of patients with any AE, any AE assessed by the Investigator as related to treatment, any severe AE, any severe AE related to treatment, any SAE, any SAE related to treatment, any AE leading to treatment discontinuation, any study drug related AE leading to treatment discontinuation, and any deaths.

Tabulations by SOC and PT will be produced for the following. The SOC and PT within each SOC will be presented alphabetically.

- All AEs;
- Severe AEs;

- All SAEs;
- AEs related to treatment;
- SAEs related to treatment;
- AEs leading to infusion interruption;
- AEs leading to treatment discontinuation;
- SAEs leading to treatment discontinuation;
- AEs related to pre-medication;
- AEs over time.

Tabulations by PT in decreasing order in frequency in the patisiran arm will be produced for the following:

- All AEs;
- All SAEs;
- AEs related to treatment;
- SAEs related to treatment.

AEs will also be summarized by maximum severity and by maximum relationship; patients who report multiple occurrences of the same AE (preferred term) will be classified according to the most severe occurrence or the most related occurrence, respectively. Similarly, AEs related to treatment will be summarized by maximum severity.

Infusion-related reaction signs and symptoms will be summarized by SOC and PT. The incidence and frequency of IRR signs and symptoms over time will also be summarized by SOC and PT.

AEs mapping to the Drug Related Hepatic Disorder standardized MedDRA query (SMQ) will be summarized by SOC and PT. AEs mapping to the Anaphylactic Reaction SMQ will be summarized by PT. AEs mapping to the SMQ Malignant or Unspecified Tumors will be summarized by HLT and PT. Other SMQs or AE groupings may be evaluated.

All AEs will be presented in patient data listings. Separate listings will be provided for death, SAEs, AEs leading to treatment discontinuation, IRR signs and symptoms, AEs with missing severity, AEs with missing relationship to study drug, AEs related to pre-medications, and AEs mapping to the SMQs as described above.

Additional AE considerations regarding COVID-19 are detailed in Section 6.11.3.

Ophthalmological assessments may be performed if a patient develops ocular symptoms suggestive of vitamin A deficiency. The ophthalmological assessment results will be presented in a listing.

Patients who underwent heart transplant and/or ventricular assist device placement will be presented in a listing.

6.9.2. Laboratory Data

Clinical laboratory values will be expressed in SI units. Missing laboratory data will not be imputed.

Summary data for each laboratory parameter will be presented for each continuous clinical laboratory parameter (including hematology, serum chemistry, liver function tests and coagulation studies). Descriptive statistics will be presented for the actual values, change from baseline, and percent change from baseline by visit.

Shift tables will be employed to summarize the baseline category versus the "worst" post-baseline category for selected parameters.

A listing for all patients with abnormal liver function tests, defined as an ALT >3×ULN, AST >3×ULN, or total bilirubin >2×ULN at any time point, will also be provided.

A table will be produced to summarize the number and percentage of patients in each of the below categories at any post-baseline time point.

- ALT >1 & ≤3, >3 & ≤5, >5 & ≤10, >10 & ≤20, >20×ULN;
- AST >1 & ≤3, >3 & ≤5, >5 & ≤10, >10 & ≤20, >20×ULN;
- ALT or AST >1 & ≤3, >3 & ≤5, >5 & ≤10, >10 & ≤20, >20×ULN;
- ALP > 1.5×ULN;
- Total Bilirubin >1.5 & ≤2, >2 & ≤3, >3 & ≤5 and >5×ULN;
- Total Bilirubin > 2×ULN concurrent with ALT or AST > 3×ULN.

In separate figures, the peak total bilirubin (at any time post-baseline) will be plotted against the peak AST, the peak ALT, and the peak AST or ALT levels at any time post-baseline.

For hematology and serum chemistry, summary tables of potentially clinically significant abnormalities will be provided. The results may also be graded according to the National Cancer Institute CTCAE Version 5.0 or above. A shift summary of baseline to maximum post-baseline CTCAE grade may be presented, as appropriate.

The estimated glomerular filtration rate (eGFR, mL/min/1.73 m^2) will be categorized as below: ≥ 90; 60-89; 45-59; 30-44; 15-29 and < 15. A shift summary of baseline to worst post-baseline eGFR category will be presented.

All laboratory data, including pregnancy and follicle stimulating hormone (FSH) test results, will be provided in data listings. Out-of-range laboratory results will be identified in the listings.

6.9.3. Vital Signs and Physical Examination

For vital signs, descriptive statistics by visit and treatment arm will be provided for each variable. Vital sign measurements will be presented for each patient in a data listing, with abnormal vital signs flagged.

For the physical examination, clinically significant findings prior to first dose of study drug will be recorded and summarized under Medical History, unless there is an SAE in which case the event will be recorded and summarized under Adverse Events. Physical examination findings

that are new or worsened after first dose of study drug will be recorded and summarized under Adverse Events.

6.9.4. Electrocardiogram

Electrocardiogram (ECG) findings will include rhythm, ventricular rate, RR interval, PR interval, QRS duration, QT interval, and corrected QT (QTc) interval. QTc interval will be calculated using Fridericia's correction formula.

Fridericia's cube-root corrected QT: QTcF (ms) = QT (ms) $\times \sqrt[3]{\frac{HR(bpm)}{60}}$.

RR, PR, QRS, QT, and QTcF intervals and their change from pre-dose baseline will be summarized for each treatment arm by scheduled visit. The number and percentage of patients with abnormal results (clinically significant abnormal or not clinically significant abnormal) will also be summarized by treatment arm and scheduled visit.

Patients will be categorized into ≤ 450, > 450 - 480, > 480 - 500, or > 500 ms per their maximum post-baseline absolute QTcF interval and ≤ 30, > 30 - 60, or > 60 ms per their maximum change from baseline QTcF interval. The number and percentage of subjects in each category will be summarized for each treatment arm.

All ECG data for each patient will be provided in a data listing.

6.9.5. Prior and Concomitant Medications

Prior and concomitant medications will be coded using the WHO Drug Dictionary (March 2020 or later). Prior medications include medications taken ≥ 1 time before the first dose of study drug, regardless of medication end date. Concomitant medications include medications taken ≥ 1 time on or after the first dose of study drug, regardless of medication start date. Results will be tabulated by ATC and preferred term.

When there are partial or missing dates, imputed dates will be used to determine 1) if a medication is prior or concomitant, and 2) duration of exposure to select medications, as needed. Imputed dates will not be presented in the listings.

For medications with partial start or stop dates: the first day/month will be imputed for start date, and the last day/month will be imputed for stop date. For medications with a completely missing start date, the medications will be considered as started prior to the first dose of study drug; medications will be classified as prior, concomitant or both depending on the medication stop dates. For medications with a completely missing stop date, the end of study date will be imputed.

Prior and concomitant medications will be presented in data listings. Previous tetramer stabilizer use will be listed separately.

6.10. Anti-Drug Antibody

The number and percentage of patients with confirmed positive ADA assay results at any time during study as well as at each scheduled visit will be summarized. The titer results for patients with confirmed positive ADA results will also be summarized using descriptive statistics.

ADA data and patients with confirmed positive ADA results will be presented in data listings.

6.11. COVID-19 Pandemic Impact Analyses

Additional data were collected to characterize the impact of the COVID-19 pandemic on general study conduct and disposition, and subsequently, additional analyses and summaries will be provided in acknowledgement of multiple regulatory guidances (FDA Guidance on Conduct of Clinical Trials of Medical Products during COVID-19 Pandemic: Guidance for Industry, Investigators, and Institutional Review Boards, US Food and Drug Administration, 2020; Guidance on the Management of Clinical Trials during the COVID-19 (Coronavirus) Pandemic, European Medicines Agency, 2020; Points to consider on implications of Coronavirus disease (COVID-19) on methodological aspects of ongoing clinical trials, European Medicines Agency, 2020; Statistical Considerations for Clinical Trials During the COVID-19 Public Health Emergency, US Food and Drug Administration, 2020).

Few patients were enrolled on APOLLO-B prior to the beginning of the COVID-19 global pandemic.

6.11.1. General Impact

Patients who discontinue treatment or stop study participation due to COVID-19 will be included in patient disposition summaries as described in Section 6.1.

Impact on study participation due to COVID-19, including visit completion, visit location changes, and study drug dosing changes, will be summarized overall on the patient level with both continuous and categorical descriptives, and overall and by visit on the event level with categorical descriptives. Patient- and event-level summaries of the impact on study participation due to COVID-19 may also be generated by site and/or region.

Impact on study participation due to COVID-19 will be presented in data listings by patient and by visit within patient.

6.11.2. Impact on Efficacy

Summaries of missing efficacy data due to the COVID-19 pandemic will be included in missing efficacy data summaries as described in Section 5.8.1. Patient data listings will flag assessments censored due to COVID-19.

The number and percentage of patients who died, were hospitalized or had an urgent HF visit due to COVID-19 will be summarized by treatment arm and overall. The total number of hospitalizations and urgent HF visits due to COVID-19 and the number of hospitalizations and urgent HF visits per patient due to COVID-19 will also be summarized using descriptive statistics by treatment arm and overall. Patient data listings will be presented for deaths, hospitalizations and urgent HF visits due to COVID-19.

Given the measures specified in the protocol designed to ensure data integrity, analyses excluding patients with COVID-19 related protocol deviations will not be prespecified, but may be considered post hoc, if warranted.

6.11.3. Impact on Adverse Events

An overall summary of AEs mapping to a COVID-19 custom query will include the number and percentage of patients, as well as events and event rates, with any AE, any AE assessed by the Investigator as related to treatment, any severe AE, any severe AE related to treatment, any SAE, any SAE related to treatment, any AE leading to treatment discontinuation, any study drug related AE leading to treatment discontinuation, and any deaths. AEs mapping to the COVID-19 custom query will be summarized by HLT and PT. Due to the evolving nature of COVID-19-related MedDRA terminology, the COVID-19 custom query will be based on the latest information available at the specified analysis timepoint.

AEs mapping to the COVID-19 custom query will also be presented in a data listing.

7. CHANGES TO PLANNED ANALYSES

7.1. Changes from Protocol in Original SAP

Change from Protocol	Detailed Description/Rationale
In the PK and PD Analysis Set definitions, "any amount of study drug" (as specified in the protocol) was updated to "at least one complete dose of study drug".	PK and PD data from patients who have not received at least one complete dose of study drug would not be representative of the planned dose level.
The model specified to analyze the composite endpoint of all-cause mortality and frequency of all-cause hospitalizations was updated from an Andersen-Gill model stratified by baseline tafamidis (as specified in the protocol) to an Andersen-Gill model including baseline tafamidis in the list of covariates.	Given the cap on baseline tafamidis for the study, a stratified Andersen-Gill model including several covariates may encounter convergence issues. Therefore, the model now includes baseline tafamidis use in the list of covariates.

7.2. Changes in SAP Amendment 1

The SAP Amendment 1 includes more details previously not discussed in the original SAP. Some changes to analysis methods were made following regulatory feedback. The details of the changes and rationales are listed in the table below.

Summary of Changes	Detailed Description/Rationale
Updated Figure 1	To align with protocol Amendment 3
In the protocol and original SAP, an interim assessment of the tafamidis drop-in rate was planned to assess the impact of the drop-in rate on the power of the study and the potential need to increase the sample size. This interim assessment was not conducted during the study.	Due to the low rate of tafamidis drop-in on APOLLO-B, the interim sample size reassessment was determined to be unnecessary.

Summary of Changes	Detailed Description/Rationale
Added details on estimand and handling of intercurrent events for primary and secondary endpoints. The primary analyses for efficacy endpoints were also updated to not censor observations after tafamidis drop-in or after missing doses due to COVID-19.	Updates made following regulatory feedback
Updated primary MMRM model specification	To align with protocol Amendment 3
Changed imputation approach in PMM model for patients who die (not due to COVID-19) or who become unable to walk due to progression of ATTR amyloidosis by Month 12	Update was made since such patients are not expected to behave in the same way as those who remain in the study on treatment and therefore require a different imputation approach.
Updated PMM appendix	To clarify the imputation procedure for the missing data patterns, including imputation for death/inability to walk as noted above
Updated data handling approach for tafamidis drop-in and missing doses due to COVID-19 in MMRM and binary sensitivity analyses	To align with updates made to the intercurrent event handling strategies for the estimand
Updated specification for the Andersen-Gill model to be stratified by baseline tafamidis use	To align with the protocol
The Poisson model for analysis of the frequency of hospitalizations and urgent HF visits was updated to add the treatment-by-baseline tafamidis use interaction.	This update was made to allow for potentially different treatment effects between the baseline tafamidis use (yes/no) subgroups.
Updated the subgroup analysis MMRM model specification	This update was made to allow for potentially different treatment effects between the baseline tafamidis use (yes/no) subgroups.
Removed statement that ECG parameters will be treated as missing if QRS duration is >120 ms	To maintain consistency with analyses conducted in other studies in the patisiran clinical development program
Removed description of efficacy and safety summaries by pandemic phase	Summaries by pandemic phase will not be performed since limited data are available prior to the start of the pandemic and limited post-pandemic data are anticipated at the time of the primary analysis.

7.3. Changes in SAP Amendment 2

The SAP Amendment 2 includes changes to the primary and sensitivity analyses of 6-MWT as well as additional updates. The details of the changes and rationales are listed in the table below.

Summary of Changes	Detailed Description/Rationale
Added composite endpoint for all-cause mortality and frequency of all-cause hospitalizations and urgent HF visits in patients not on tafamidis at baseline as a secondary endpoint	The APOLLO-B study enrolls patients who are tafamidis-naïve as well as patients who are on tafamidis and have demonstrated disease progression in the opinion of the Investigator. The subgroup of tafamidis-naïve patients is a more homogeneous subpopulation while patients on baseline tafamidis are expected to be more heterogeneous (eg, in terms of duration of prior tafamidis treatment). It's anticipated that a larger treatment effect could be observed in tafamidis-naïve patients compared to patients on baseline tafamidis. The composite outcome endpoint defined in all patients in the Full Analysis Set is the only secondary endpoint that is not sufficiently powered to detect a treatment difference. Since the majority of patients (75%) are tafamidis-naïve, defining the composite outcome endpoint in this more homogeneous, enriched subgroup could improve the power and precision of the estimated treatment effect.
Changed primary analysis of 6-MWT from MMRM to the stratified Wilcoxon Rank Sum test with HL estimate of the median treatment difference. The MMRM model for 6-MWT was moved to be a sensitivity analysis.	Parametric methods, such as MMRM or ANCOVA, may be sensitive to violations of the normality assumption (eg, skew, outliers) whereas nonparametric methods, such as Wilcoxon Rank Sum test or rank ANCOVA, are more robust to such violations. Based on review of blinded 6-MWT data in APOLLO-B, the normality assumption does not appear to hold. In addition, BridgeBio recently reported 6-MWT data in ATTR cardiomyopathy which showed an apparent deviation from normality with the median differing from the mean for the change from baseline in 6-MWT at Month 12. A literature search of past Phase 3 studies with 6-MWT endpoint also showed that normal assumptions often did not hold for this endpoint, and a non-parametric method was commonly used as the primary analysis. Given these considerations, the primary analysis method was therefore updated to the stratified Wilcoxon Rank Sum test.
Intercurrent event handling strategy updated for 6-MWT	For the primary analysis of 6-MWT, patients who die or who lose the ability to walk due to progression of ATTR amyloidosis will now be imputed as the worst possible change for the patient (ie, 0 – baseline 6-MWT).
Updated Section 6.6.2.1	Updated the text in the KCCQ-OS section since the prior version referenced the planned 6-MWT analyses, which have since been changed. The planned KCCQ-OS analyses in these sections are unchanged from the SAP Amendment 1.
Added binary analysis for KCCQ-OS	To quantify and compare the proportion of patients in each treatment group who maintain or improve in their KCCQ-OS score in the 12m-DB period.

Summary of Changes	Detailed Description/Rationale
Updated planned subgroup analyses	The HL estimate of the median difference between patisiran and placebo will be used for subgroup analyses of 6-MWT to align with the primary analysis. Clarified that the MMRM model used for the baseline tafamidis use subgroup analysis for KCCQ-OS is the same as the primary MMRM model for KCCQ-OS since these subgroup estimates can be obtained directly from the primary model.
Removed summaries for SAEs by maximum severity and by maximum relationship	Table summaries determined to be unnecessary as this can be assessed by reviewing the SAE listing.
Removed summaries for AEs/SAEs leading to study discontinuation	Patients who discontinue the study due to a TEAE are accounted for in the disposition summary. Given the overlap between TEAEs that led to treatment discontinuation and those that led to study withdrawal, it is not considered necessary to list/tabulate the AEs that led to study withdrawal.
Removed NYHA from list of covariates for ANCOVA models of echo parameters and Norfolk QoL-DN Total Score	To create a more parsimonious model
Added LV relative wall thickness, LV end-diastolic volume, and cardiac output to list of selected echo parameters to be analyzed by ANCOVA	To conduct a more comprehensive echocardiographic evaluation of differences between treatment groups in cardiac structure and function
Removed summary of PK exposure by mortality status.	Sparse PK samples are collected in APOLLO-B and relating these exposures to mortality may not provide meaningful interpretation of results. A separate safety exposure analysis using population PK-PD modeling may be conducted, if deemed necessary.

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9. APPENDICES

9.1. Detailed Statistical Methodology for the Control-based Imputation for Missing Data

Patients are classified into five patterns for missing data at Month 12:

1. Patients who have missing data due to COVID-19: missing data will be assumed to be MAR and imputed by using MI estimated from the treatment arm and baseline tafamidis use group to which the patient was randomized. This pattern includes:
    a. Patients who miss the Month 12 assessment due to COVID-19
    b. Patients who have a serious COVID-19 AE or who die due to COVID-19 before Month 12
2. Placebo patients who do not have missing data due to COVID-19 and are alive through the 12m-DB period and have not become unable to walk due to progression of ATTR amyloidosis by Month 12: missing data will be assumed to be MAR and imputed by using MI estimated from placebo patients in the baseline tafamidis use group to which the patient belongs.
3. Patisiran patients who do not have missing data due to COVID-19 and are alive through the 12m-DB period, have not become unable to walk due to progression of ATTR amyloidosis by Month 12, and have missing data with the last dose of study drug received within 60 days of the scheduled time point: missing data will be assumed to be MAR and imputed by using MI estimated from on-treatment patisiran patients in the baseline tafamidis use group to which the patient belongs.
4. Patisiran patients who do not have missing data due to COVID-19 and are alive through the 12m-DB period, have not become unable to walk due to progression of ATTR amyloidosis by Month 12, and the last dose of study drug was received more than 60 days prior to the scheduled time point: missing data will be assumed to be MNAR and imputed (using data from placebo patients in the baseline tafamidis use group to which the patient belongs) using the copy reference (CR) approach.
5. Patients who die (including heart transplant and ventricular assist device placement) during the 12m-DB period (not due to COVID-19) or who have become unable to walk due to progression of ATTR amyloidosis by Month 12: missing data will be assumed to be MNAR and imputed as the worst possible change for the patient (0 – baseline 6-MWT).

For patients who are alive through the 12m-DB period and who have not become unable to walk due to progression of ATTR amyloidosis, all missing data for the placebo arm and missing data for the patisiran arm during the on-treatment period (ie, assessments within 60 days of the last dose of study drug) will be imputed using MI under the MAR assumption. Since the pattern of missing data within patients may be non-monotone, multiple imputation will be conducted separately by treatment arm and baseline tafamidis use group using the Markov Chain Monte Carlo (MCMC) method. For each treatment arm/baseline tafamidis use group, the imputation model will include type of amyloidosis (hATTR vs. wtATTR), NYHA class (I/II vs. III), age at randomization (<75 vs. ≥75), NT-proBNP (≤3000 ng/L vs >3000 ng/L), baseline 6-MWT, and all calculated values of change from baseline in 6-MWT at pre-specified visits. The input dataset

DATAIN will exclude data from patients in pattern 5 above and exclude data from patisiran patients that were collected after treatment discontinuation (ie, more than 60 days after the last dose of study drug). Below is a sample SAS code for the multiple imputation:

```
proc mi data=DATAIN out=DATA_STEP1 seed=234 nimpute=100;
   by treatment bltaf;
   em maxiter=300 converge=1e-4 itprint outem=outem;
   var baseline_variables 6MWT_base 6MWT_chg_m6 6MWT_chg_m9 6MWT_chg_m12;
   mcmc chain=multiple initial=em;
run;
```

The MI procedure generates imputed values for all missing values. For patisiran patients, the imputed data from the on-treatment period will be kept while the imputed data after treatment discontinuation will be discarded and replaced by either the observed non-missing values (if available) or the imputed values using the CR approach described below. The imputation model will include type of amyloidosis (hATTR vs. wtATTR), NYHA class (I/II vs. III), age at randomization (<75 vs. ≥75), NT-proBNP (≤3000 ng/L vs >3000 ng/L), baseline 6-MWT, and all calculated values of change from baseline in 6-MWT at pre-specified visits.

```
proc mi data=DATA_STEP1_2 out=DATA_STEP2 nimpute=1 seed=xxx;
   by _imputation_ bltaf;
   class treatment;
   fcs nbiter=30 reg (6MWT_chg_m12 = baseline_variables 6MWT_base
      6MWT_chg_m6 6MWT_chg_m9);
   mnar model (6MWT_chg_m12 / modelobs=(treatment='placebo'));
   var baseline_variables 6MWT_base 6MWT_chg_m6 6MWT_chg_m9 6MWT_chg_m12;
run;
```

Finally, for patients in pattern 5, missing Month 12 6-MWT change from baseline values will be imputed as the worst possible change for the patient (ie, 0 – baseline 6-MWT).

9.2. Calculation of Stratified Win Ratio *p* value and Confidence Interval

Dong et al (2018) [Dong 2018] note that the logarithm of the stratified win ratio is asymptotically normally distributed with mean $\upsilon_{\log(WR)}$ and variance $\sigma^2_{\log(WR)}$, with these terms defined in equations 7b and 8 in their paper, respectively. The point estimate of the mean, $\hat{\upsilon}_{\log(WR)}$, is the logarithm of the stratified win ratio statistic defined in Section 6.6.2.2. The variance estimate, $\hat{\sigma}^2_{\log(WR)}$, is calculated under the null hypothesis of the same treatment effect in the patisiran and placebo groups.

Then $\hat{z} = \frac{\hat{\upsilon}_{\log(WR)}}{\sqrt{\hat{\sigma}^2_{\log(WR)}}}$ is a standard normal deviate from which the *p* value is readily obtained.

The 95% confidence interval for the logarithm of the stratified win ratio is constructed as

$$\hat{\upsilon}_{\log(WR)} \pm 1.96 * \sqrt{\hat{\sigma}^2_{\log(WR)}}$$

and the limits of this confidence interval will then be exponentiated to construct the 95% confidence interval for the stratified win ratio.

9.3. Questionnaire/Scoring

In questionnaires, if multiple responses are provided to a single-response question, the question is deemed as missing.

9.3.1. Kansas City Cardiomyopathy Questionnaire (KCCQ)

The Kansas City Cardiomyopathy Questionnaire is a 23-item self-administered questionnaire developed to independently measure the patient's perception of their health status, which includes heart failure symptoms, impact on physical and social limitation, and how their heart failure impacts their quality of life within a 2-week recall period.

There are 10 summary scores for the KCCQ tool, which are calculated as follows:

1. Physical Limitation

Code responses to each of Questions 1a-1f as follows:

- Extremely limited = 1
- Quite a bit limited = 2
- Moderately limited = 3
- Slightly limited = 4
- Not at all limited = 5
- Limited for other reasons or did not do the activity = <missing value>

If at least 3 of Questions 1a-1f are not missing, then compute

- Physical limitation score = [(mean of the non-missing Questions 1a-1f) – 1]/4 * 100

2. Symptom Stability

Code the response to Question 2 as follows:

- Much worse = 1
- Slightly worse = 2
- Not changed = 3
- Slightly better = 4
- Much better = 5
- I've had no symptoms over the last 2 weeks = 3

The symptom stability score = [(Question 2) – 1]/4 * 100.

3. Symptom Frequency

Code responses to Questions 3, 5, 7 and 9 as follows:

- Questions 3 and 9
  - Every morning/night = 1

  - 3 or more times a week but not every day = 2
  - 1-2 times a week = 3
  - Less than once a week = 4
  - Never over the past 2 weeks = 5
- Questions 5 and 7
  - All of the time = 1
  - Several times a day = 2
  - At least once a day = 3
  - 3 or more times a week but not every day = 4
  - 1-2 times a week = 5
  - Less than once a week = 6
  - Never over the past 2 weeks = 7

If at least two of Questions 3, 5, 7 and 9 are not missing, then compute

- S3 = [(Question 3) – 1]/4
- S5 = [(Question 5) – 1]/6
- S7 = [(Question 7) – 1]/6
- S9 = [(Question 9) – 1]/4

The symptom frequency score = (mean of S3, S5, S7, S9)*100.

4. Symptom Burden

Code responses to Questions 4, 6 and 8 as follows:

- Extremely bothersome = 1
- Quite a bit bothersome = 2
- Moderately bothersome = 3
- Slightly bothersome = 4
- Not at all bothersome = 5
- I've had no swelling/fatigue/shortness of breath = 5

If at least one of Questions 4, 6 and 8 is not missing, then compute

- Symptom burden score = [(mean of the non-missing Questions 4, 6 and 8) – 1]/4 * 100

5. Total Symptom Score

Total symptom score = mean of the following available summary scores

- Symptom Frequency Score
- Symptom Burden Score

6. Self-efficacy

Code responses to Questions 10 and 11 as follows:

- Question 10
  - Not at all sure = 1
  - Not very sure = 2
  - Somewhat sure = 3
  - Mostly sure = 4
  - Completely sure = 5
- Question 11
  - Do not understand at all = 1
  - Do not understand very well = 2
  - Somewhat understand = 3
  - Mostly understand = 4
  - Completely understand = 5

If at least one of Questions 10 and 11 is not missing, then compute

- Self-efficacy score = [(mean of the non-missing Questions 10 and 11) – 1]/4 * 100

7. Quality of Life

Code responses to Questions 12, 13 and 14 as follows:

- Question 12
  - It has extremely limited my enjoyment of life = 1
  - It has limited my enjoyment of life quite a bit = 2
  - It has moderately limited my enjoyment of life = 3
  - It has slightly limited my enjoyment of life = 4
  - It has not limited my enjoyment of life at all = 5
- Question 13
  - Not at all satisfied = 1
  - Mostly dissatisfied = 2
  - Somewhat satisfied = 3
  - Mostly satisfied = 4

  - Completely satisfied = 5
- Question 14
  - I felt that way all of the time = 1
  - I felt that way most of the time = 2
  - I occasionally felt that way = 3
  - I rarely felt that way = 4
  - I never felt that way = 5

If at least one of Questions 12, 13 and 14 is not missing, then compute

- Quality of life score = [(mean of the non-missing Questions 12, 13 and 14) – 1]/4 * 100

8. Social Limitation

Code responses to each of Questions 15a-15d as follows:

- Severely limited = 1
- Limited quite a bit= 2
- Moderately limited = 3
- Slightly limited = 4
- Did not limit at all = 5
- Does not apply or did not do for other reasons = <missing value>

If at least 2 of Questions 15a-15d are not missing, then compute

- Social limitation score = [(mean of the non-missing Questions 15a-15d) – 1]/4 * 100

9. Overall Summary Score

Overall summary score = mean of the following available summary scores

- Physical Limitation Score
- Total Symptom Score
- Quality of Life Score
- Social Limitation Score

10. Clinical Summary Score

Clinical summary score = mean of the following available summary scores

- Physical Limitation Score
- Total Symptom Score

9.3.2. Norfolk Quality of Life-Diabetic Neuropathy (QOL-DN)

Norfolk QOL-DN is a tool for assessing patients' perception of the effects of diabetes and diabetic neuropathy. There are 35 questions divided into 5 domains. The range of possible total scores is -4 to 136.

Part I: Symptoms

Items 1-7 (Part I) are a simple inventory of symptoms of neuropathy. The presence of the symptom is checked in whichever box applies, and an absence of a symptom is checked under "none." Positive responses are scored as 1; and negative responses, as 0.

Part II: Activities of Daily Life

Items 8-35 (Part II) pertain to Activities of Daily Life, and most of these are scaled on a 5-point Likert scale ranging from 0 ("Not a problem") to 4 ("Severe problem"). However, Questions 31 and 32 are scored differently. In Question 31, "Good", the middle item, is scored as0. "Very Good" is scored as –1, "Excellent" is scored as –2. "Fair" is scored as 1, and "Poor" is scored as 2. In Question 32, "About the Same," the middle item, is scored as 0. "Somewhat better" is scored as –1, "Much better" is scored as -2. "Somewhat worse" is scored as 1, and "Much worse" is scored as 2.

Subscales and Scoring Algorithm

The Total QOL and 5 domains should be summed as follows:

- Total QOL (35 items) Σ(1-7, 8-35)
- Physical Functioning/Large Fiber (15 items) Σ(8, 11, 13-15, 24, 27-35)
- Activities of Daily Living (ADLs) (5 items) Σ(12, 22, 23, 25, 26)
- Symptoms (8 items) Σ(1-7, 9)
- Small Fiber (4 items) Σ(10, 16, 17, 18)
- Autonomic (3 items) Σ(19, 20, 21)

Domain scores are calculated as the rounded integer value of the average scores of non-missing included items multiplied by the number of items if at least 50% of the items are non-missing. A domain score is missing if more than 50% of the included items are missing.

If the scores for all 5 domains are non-missing, then Total QOL is the sum of scores of the 5 domains; however, if at least 1 of the domains is missing and at least 50% of the items (18 items) are non-missing, then Total QOL is calculated as 35 times the mean of the non-missing items, rounded to the nearest integer. Otherwise, Total QOL is deemed as missing.

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STATISTICAL ANALYSIS PLAN
ALN-TTR02-011

Protocol Title:	APOLLO-B: A Phase 3, Randomized, Double-blind, Placebo-controlled Multicenter Study to Evaluate the Efficacy and Safety of Patisiran in Patients with Transthyretin Amyloidosis with Cardiomyopathy (ATTR Amyloidosis with Cardiomyopathy)
Short Title:	APOLLO-B: A Study to Evaluate Patisiran in Patients with Transthyretin Amyloidosis with Cardiomyopathy (ATTR Amyloidosis with Cardiomyopathy)
Study Treatment:	Patisiran (ALN-TTR02)
EudraCT Number:	2019-001458-24
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Protocol Date:	Original Protocol, 18 April 2019 Amendment 1: 20 December 2019 Amendment 2: 22 May 2020 Amendment 3: 30 June 2021
SAP Date:	Original SAP: 05 August 2020 Amendment 1: 12 October 2021
Sponsor:	Alnylam Pharmaceuticals, Inc. 300 Third Street Cambridge, MA 02142 USA Telephone: [redacted]
Sponsor Contact:	[redacted]

APPROVAL SIGNATURE PAGE

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This document has been authored and approved by the following:

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Abbreviation	Definition
6-MWT	6-minute walk test
12m-DB	12-month double-blind placebo-controlled
^{99m}Tc-PYP	Technetium pyrophosphate
ADA	Antidrug antibody
AE	Adverse event
ALN-18328	siRNA targeting TTR
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANCOVA	Analysis of covariance
AST	Aspartate aminotransferase
ATC	Anatomical Therapeutic Class
ATTR	Amyloid transthyretin
BLQ	Below the lower limit of quantification
BMI	Body mass index
CI	Confidence interval
C_{max}	Maximum plasma concentration at end of infusion
$C_{max,ss}$	Steady-state C_{max}
C_{min}	Minimum pre-infusion concentration
$C_{min,ss}$	Steady-state C_{min}
CMH	Cochran-Mantel-Haenszel
CMR	Cardiac magnetic resonance
COVID-19	Coronavirus disease 2019
$C_{p(30min)}$	30-minute post-infusion concentration
$C_{p,ss(30min)}$	Steady-state $C_{p(30min)}$
CR	Copy reference
CTCAE	Common Terminology Criteria for Adverse Events
CV	Cardiovascular
DLin-MC3-DMA	1,2-Dilinoleyloxy-N,N-dimethylpropylamine
DMC	Data monitoring committee
ECG	Electrocardiogram
eCRF	Electronic case report form

Abbreviation	Definition
EDC	Electronic data capture
eGFR	Estimated glomerular filtration rate
FAS	Full Analysis Set
FSH	Follicle-stimulating hormone
H/CL	Heart to contralateral lung
hATTR	Hereditary ATTR
HF	Heart failure
HLT	High level term
HR	Hazard ratio
IV	Intravenous
ICH	International Council for Harmonisation
IRR	Infusion-related reaction
IRS	Interactive response system
KCCQ	Kansas City Cardiomyopathy Questionnaire
KCCQ-OS	Kansas City Cardiomyopathy Questionnaire-Overall Summary
LLOQ	Lower limit of quantification
LNP	Lipid nanoparticle
$\log_e$	Natural log transformation
LV	Left ventricular
MAR	Missing at random
mBMI	Modified body mass index
MCMC	Markov Chain Monte Carlo
MedDRA	Medical Dictionary for Regulatory Activities
MI	Multiple imputation
MMRM	Mixed effect model repeated measures
MNAR	Missing not at random
Norfolk QoL-DN	Norfolk Quality of Life - Diabetic Neuropathy
NT-proBNP	N-terminal prohormone B-type natriuretic peptide
NYHA	New York Heart Classification
OLE	Open label extension
PD	Pharmacodynamic
PEG_{2000}-C-DMG	3-N-[(ω-methoxy poly(ethylene glycol)2000) carbamoyl]-1,2-dimyristyloxy-propylamine

Abbreviation	Definition
PK	Pharmacokinetic
PMM	Pattern mixture model
PND	Polyneuropathy disability
PT	Preferred term
Q1	First quartile
Q3	Third quartile
q3W	Once every 3 weeks
QTc	Corrected QT
QTcF	Fridericia's cube-root corrected QT
SAE	Serious adverse event
SAP	Statistical analysis plan
SD	Standard deviation
SEM	Standard error of the mean
SI	International System of Units
siRNA	Small interfering RNA
SMQ	Standardized MedDRA Query
SOC	System organ class
TTR	Transthyretin
SUSAR	Suspected Unexpected Serious Adverse Reaction
ULN	Upper limit of normal
WHO	World Health Organization
WR	Win ratio
wt	Wild type
wtATTR	Wild type ATTR

1. INTRODUCTION

Transthyretin (TTR)-mediated amyloidosis (ATTR amyloidosis) is a rare, serious, life-threatening, multisystemic disease encompassing hereditary ATTR (hATTR) amyloidosis and wild type ATTR (wtATTR) amyloidosis, which result from either hereditary (genetic mutation) or nonhereditary (ageing) causes, respectively. In ATTR amyloidosis, deposition of TTR in various organs results in progressive, chronically debilitating morbidity and mortality. The most common manifestations of ATTR amyloidosis are polyneuropathy and cardiomyopathy (ie, ATTR amyloidosis with cardiomyopathy).

Patisiran is a small interfering RNA (siRNA) specific for TTR, which is formulated in a hepatotropic lipid nanoparticle (LNP) for intravenous (IV) administration.[Akinc 2010] The patisiran drug product (ALN-TTR02; patisiran-LNP, hereafter referred to as "patisiran") is designed to significantly suppress liver production of both wild type (wt) and all mutant forms of TTR, thereby having the potential to reduce amyloid formation and provide clinical benefit to patients with ATTR amyloidosis.

The APOLLO-B study (ALN-TTR02-011) is a Phase 3, randomized, double-blind, placebo-controlled, multicenter study designed to evaluate the efficacy and safety of patisiran in adult patients with ATTR amyloidosis with cardiomyopathy.

This statistical analysis plan (SAP) details comprehensive specifications of the efficacy, safety, pharmacodynamic (PD), and pharmacokinetic (PK) data summaries and statistical analyses in support of the clinical study report for Study ALN-TTR02-011. This SAP includes summaries and analyses specified and/or outlined in the protocol Amendment 3, dated 30 June 2021. Additional supportive analyses and changes to planned analyses are also included; notable changes are documented in Section 7. Changes to planned analyses made after database lock will be documented with justification in the clinical study report.

Table, figure, and listing specifications are contained in a separate document.

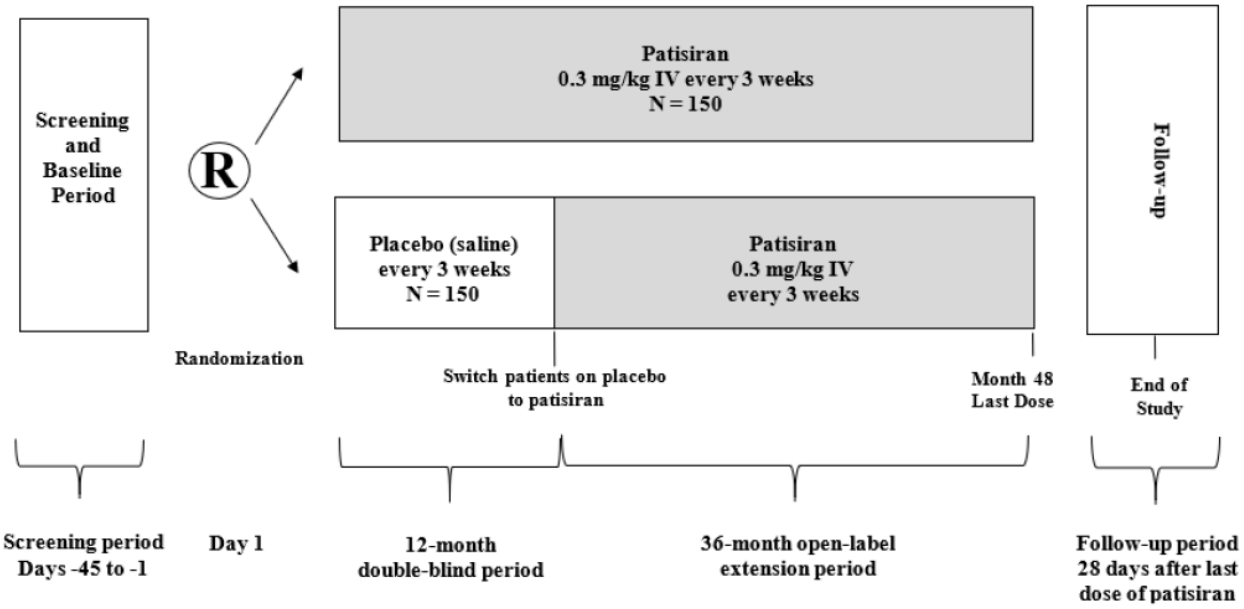
2. STUDY OVERVIEW

2.1. Synopsis of Study Design

The APOLLO-B study is a multicenter, multinational Phase 3 study designed to evaluate the efficacy and safety of patisiran in approximately 300 patients with ATTR amyloidosis (hereditary or wt) with cardiomyopathy; the study is comprised of a 1:1 randomized, double-blind, placebo-controlled period of 12 months followed by an open-label extension (OLE) period of 36 months to evaluate the long-term safety and efficacy of patisiran.

The study design schema is presented in Figure 1.

Figure 1: Study Design



2.2. Randomization Methodology

Using the interactive response system (IRS), patients will be randomized 1:1 to the patisiran or placebo arm. Randomization will be stratified by:

1. Baseline tafamidis (yes vs. no)
2. Type of amyloidosis (hATTR vs. wtATTR amyloidosis with cardiomyopathy)
3. New York Heart Classification (NYHA) Class I or II **and** age < 75 years vs. all other

Patients in the baseline tafamidis use category are defined as patients who are currently on tafamidis (for ≥6 months) with disease progression in the opinion of the investigator at baseline.

2.3. Blinding

Treatment assignments will be maintained by the IRS which has controlled access limited to unblinded team members and the unblinded pharmacist/designee preparing the infusion. Any unplanned unblinding occurring during the 12-month double-blind placebo-controlled treatment period (referred to as the 12m-DB period hereafter) will be documented and reported in the clinical study report.

Unblinding is only to occur in the case of patient emergencies or when necessary from a regulatory reporting perspective (eg, Suspected Unexpected Serious Adverse Reaction [SUSAR]), and after all patients have completed the 12m-DB period and the unblinded authorization has been executed. Details about the specifics of the blinding aspects for the study are outlined in the Randomization and Blinding Plan.

2.4. Study Procedures

The schedule of assessments is described in the study protocol (Table 1 and Table 2).

3. OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
Primary	
To evaluate the efficacy of patisiran compared with placebo treatment on functional capacity (6-minute walk test [6-MWT]) in patients with ATTR amyloidosis with cardiomyopathy	Change from baseline at Month 12 in 6-MWT
Secondary	
To evaluate the efficacy of patisiran compared with placebo treatment on: • Health status and health-related quality of life • Patient mortality, hospitalizations, and urgent heart failure (HF) visits	• Change from baseline at Month 12 in Kansas City Cardiomyopathy Questionnaire Overall Summary (KCCQ-OS) score • Composite endpoint of all-cause mortality, frequency of cardiovascular (CV) events (CV hospitalizations and urgent HF visits) and change from baseline in 6-MWT over the 12-month double-blind period • Composite endpoint of all-cause mortality and frequency of all-cause hospitalizations and urgent HF visits over the 12-month double-blind period
Exploratory	
To evaluate the efficacy of patisiran compared with placebo treatment on: • All-cause mortality and CV events • Cardiac biomarkers and biomarker-based risk assessment • Manifestations of cardiac amyloid involvement	• Composite endpoint of all-cause mortality and frequency of CV events (CV hospitalizations and urgent HF visits) over the 12-month double-blind period • Change from baseline at Month 12 in: – N-terminal prohormone B-type natriuretic peptide (NT-proBNP) – ATTR amyloidosis disease stage • Change from baseline at Month 12 in: – New York Heart Association (NYHA) Class – Echocardiographic parameters – Modified body mass index (mBMI) – Cardiac magnetic resonance (CMR) parameters – Technetium scintigraphy parameters – Troponin I levels

Objectives	Endpoints
	– Norfolk Quality of Life-Diabetic Neuropathy (Norfolk QoL-DN)
Pharmacodynamics (PD) and Pharmacokinetics (PK)	
• To evaluate the PD effect of patisiran on transthyretin (TTR) reduction • To determine the plasma concentration of patisiran and 2 lipid excipients • To assess presence of anti-drug antibodies (ADA)	• Change from baseline in serum TTR levels through Month 12 • Plasma PK exposure parameters (maximum plasma concentration at end of infusion [C_{max}], 30-minute post-infusion concentration [$C_{p(30min)}$], and pre-infusion concentration [C_{min}] • Frequency and titer of ADA
Safety	
• To evaluate the safety and tolerability of patisiran in patients with ATTR amyloidosis with cardiomyopathy	• Frequency of adverse events (AEs)

Scoring algorithms for the Kansas City Cardiomyopathy Questionnaire (KCCQ) and Norfolk Quality of Life-Diabetic Neuropathy (Norfolk QoL-DN) questionnaire are included in Appendix 9.3.1 and Appendix 9.3.2, respectively.

4. PATIENT POPULATION

The populations (analysis sets) are defined as follows:

- Full Analysis Set (FAS): All randomized patients who received any amount of study drug. Patients will be analyzed according to the treatment to which they were randomized.
- Safety Analysis Set: All randomized patients who received any amount of study drug. Patients will be summarized according to the treatment actually received.
- PK Analysis Set: All randomized patients who received at least one complete dose of study drug (see Section 6.4) and have at least 1 postdose blood sample for PK parameters and have evaluable PK data.
- PD Analysis Set: All randomized patients who received at least one complete dose of study drug (see Section 6.4) and who have an evaluable baseline and at least one evaluable post-baseline TTR sample.
- All Patisiran Treated Set: All randomized patients who received any amount of patisiran, including patients who took patisiran during the 12m-DB period and patients who first took placebo during the 12m-DB period and switched to patisiran during the OLE period.

The FAS will be used to evaluate efficacy endpoints. Safety during the 12m-DB period will be analyzed using the Safety Analysis Set. The PK and PD Analysis Sets will be used to conduct PK and PD analyses, respectively. The All Patisiran Treated Set will be used to summarize long-

term efficacy and safety data during patisiran treatment (see Section 5.10 for details). The number of patients included in all analysis sets will be provided.

5. GENERAL STATISTICAL METHODS

5.1. Determination of Sample Size

The planned enrollment for this study is 300 patients. For the change from baseline at Month 12 in the 6-MWT, assuming a treatment difference of 33 meters between patisiran and placebo in the treatment-naïve group and 20 meters in patients with baseline tafamidis, the weighted average treatment difference between patisiran and placebo in the overall population is approximately 29 meters (standard deviation [SD] = 75 meters), assuming 70% are in the treatment-naïve group and 30% are in the baseline tafamidis group. A sample size of 300 patients provides >90% power for a 2-sided test to detect a mean difference between treatment arms at a 2-sided alpha = 0.05.

5.2. General Considerations

Categorical variables will be summarized using counts and percentages.

Continuous variables will be summarized using the following descriptive summary statistics: number of patients (n), mean, standard deviation (SD), standard error of the mean (SEM), median, first quartile (Q1), third quartile (Q3), minimum, and maximum. The precision of the measurement for each continuous variable will be used to determine the number of decimal places to present in tables, figures and derived listings. Minimum and maximum values will be reported with the same precision as the units of measure. The mean, SD, SEM, median, Q1 and Q3 will be reported to one greater decimal place. Any values that require transformation to standard units (metric or International System of Units (SI) units) will be converted with the appropriate corresponding precision.

The day of the first dose of study drug administered is defined as Day 1. Study Day is defined as the number of days between the day of the first dose of study drug (Day 1) and the specific time point. The Study Day of a time point of interest is calculated as follows.

If after Day 1, Study Day = date of interest – date of the first dose of study drug + 1

If prior to Day 1, Study Day = date of interest – date of the first dose of study drug

Study days are negative when the time point of interest is prior to Day 1, positive when time of interest is after Day 1. There is no Day 0. For example, the day before the first study drug dose is defined as Day -1.

For safety laboratory parameters, assessments collected and recorded as lower than the lower limit of quantification/detection will be replaced by the lower limit of quantification/detection. Any assessment collected and recorded as greater than the upper limit of quantification will be replaced by the upper limit of quantification.

For all analysis sets except for the All Patisiran Treated Set, summaries will be presented by treatment arm (patisiran and placebo).

For the All Patisiran Treated Set, summaries will be presented by the following groups:

- Patisiran/Patisiran: all patients who received patisiran during the 12m-DB including patients who continued to receive patisiran during the OLE period and patients who discontinued treatment during the 12m-DB period;
- Placebo/Patisiran: all patients who received placebo during the 12m-DB period and switched to patisiran in the OLE period;
- All Patisiran: all patients who received at least one dose of patisiran during either the 12m-DB or OLE periods.

5.3. Computing Environment

All statistical analyses will be conducted using SAS Version 9.4 or newer or R Version 3.4 or newer, unless otherwise noted.

5.4. Baseline Definitions

For 6-MWT, baseline will be defined as the last non-missing value available prior to the first dose of study drug.

For TTR, baseline will be defined as the average of all records collected on study, including those from any unscheduled visits, prior to the date and time of first dose.

For each parameter of the 12-lead electrocardiogram (ECG), baseline will be defined as the average of all available readings from the last visit prior to the first dose of study drug.

For all other parameters, baseline will be defined as the last non-missing value available prior to the first dose of study drug, unless otherwise specified.

For the All Patisiran Treated Set, baseline for patients who switched from placebo to patisiran will be redefined as the values prior to the first dose of patisiran:

- For TTR, the redefined baseline will be calculated as the mean of all TTR assessments performed on or after Month 9 in the 12m-DB period and prior to the first dose in the OLE period.
- For all the other endpoints, the redefined baseline will be defined as the last non-missing value available prior to the first dose in the OLE period, unless otherwise specified.

5.5. Randomization Stratification Factors

Stratification factors are recorded in both the IRS and the clinical database. In statistical analyses that use randomization stratification factors as covariates, the stratum assignment will reflect the values as recorded in the clinical database. In the presence of stratification errors, the stratification used in analysis may not match that in the IRS.

5.6. Visit Windows

Scheduled visits are expected to follow the protocol schedule. All data will be tabulated and analyzed per the evaluation visit as recorded on the electronic case report form (eCRF) even if the assessment is outside of the visit window.

For 6-MWT and KCCQ, assessments will be conducted at baseline and at Months 6, 9, and 12 in the 12m-DB period and at Months 18, 21, 24, 30, 33, 36, 42 and 48 in the OLE period. If the Month 12 assessment is not performed, assessments performed within ±1.5 months of the scheduled assessment (eg, from an unscheduled, pre-tafamidis drop-in, or early treatment discontinuation visit) but prior to the first dose of patisiran in the OLE period will be grouped with the scheduled Month 12 assessments for analysis. For all other post-baseline visits, if a scheduled post-baseline assessment is not performed, assessments performed within ±1.5 months of the scheduled assessment will be grouped with the scheduled assessments for that timepoint for analysis. The derived visits will be used for all analyses.

For other efficacy assessments, if the scheduled post-baseline assessment is not performed, assessments performed within ±1.5 months of the scheduled assessment (eg, from an unscheduled, pre-tafamidis drop-in, or early treatment discontinuation visit, as applicable) will be grouped with the scheduled assessments for analysis. The derived visits will be used for all analyses.

Unless otherwise specified above, data collected at unscheduled visits will be included in by-patient data listings and figures but no assignment to a study visit will be made for the purpose of by-visit summary tabulations. However, unscheduled visits may be used in the calculation of baseline values (as discussed in Section 5.4) and for inclusion in any categorical shift summaries (eg, shift from baseline to "worst" post-baseline value).

5.7. Multiple Comparisons/Multiplicity

Formal statistical hypothesis testing will be performed on the primary and secondary efficacy endpoints with all tests conducted at the nominal 2-sided 0.05 significance level. The overall familywise error rate will be controlled at the 2-sided 0.05 significance level for the primary and secondary endpoints by a hierarchical ordering procedure. Endpoints will be tested in the following pre-specified hierarchy:

1. 6-minute walk test (6-MWT) change from baseline at Month 12
2. Kansas City Cardiomyopathy Questionnaire Overall Summary (KCCQ-OS) score change from baseline at Month 12
3. Composite endpoint of all-cause mortality, frequency of CV events (CV hospitalizations and urgent HF visits), and change from baseline in 6-MWT over the 12-month double-blind period
4. Composite endpoint of all-cause mortality and frequency of all-cause hospitalizations and urgent HF visits over the 12-month double-blind period

There will be no multiplicity adjustment for exploratory endpoints.

5.8. Missing Data with Efficacy Endpoints

5.8.1. Summary of Missing Data

For the 6-MWT and KCCQ-OS efficacy endpoints, the number and percentage of patients with missing data, including due to COVID-19, at each scheduled visit will be summarized by treatment arm.

Time to treatment discontinuation in the 12m-DB period will be estimated descriptively using the Kaplan-Meier method by treatment arm. Patients who receive at least 1 dose of patisiran in the OLE period will be censored at the date of first dose in the OLE period.

5.8.2. Handling of Missing Data

For the 6-MWT and KCCQ-OS efficacy endpoints, the primary analysis will be based on the mixed-effects model repeated measures (MMRM) method, which makes use of fully and partially observed data sequences from individual patients by estimating the covariance between data from different time points. The MMRM will be implemented using an unstructured approach to modeling both the treatment-by-time means and the (co)variances, leading to what is essentially a multivariate normal model wherein treatment arm means at the primary time point are adjusted to reflect both the actually observed data and the projected outcomes from the patients with missing data.[Mallinckrodt 2008] Data handling strategies for intercurrent events in the primary analysis of 6-MWT are detailed in Table 1. Sensitivity analyses for these endpoints will be conducted to assess the impact of missing data as discussed in Section 6.6.1.3.

5.9. Analysis Cutoff and Database Lock

For the final 12m-DB analysis, as this study will be ongoing, the study database will be locked with all data up to a prespecified cutoff date quality controlled, ie, data in the electronic data capture (EDC) system will be frozen and external data such as laboratory and PK data will be quality controlled (and quality assured, where appropriate) and cleaned. Additional details regarding the database lock process are located in the study Data Management Plan.

The final 12m-DB analysis will include data on or prior to this prespecified cutoff date. For assessments with starting/ending dates (eg, AEs, medications, medical history), the starting date will be compared with the pre-specified cutoff date.

After the study is completed, ie, all patients either discontinue or complete the study, the database will be hard-locked and all data collected will be used for analysis.

5.10. Analyses for the Entire Study

The study design includes a 12m-DB period and an OLE period. The primary objective is to evaluate the efficacy and safety of patisiran compared with placebo during the 12m-DB period. In addition, the long-term efficacy and safety of patisiran during the entire patisiran treatment period (beyond 12m-DB) will be characterized for the All Patisiran Treated Set.

The detailed definitions for different treatment periods are as follows.

- **12m-DB Period**

  The treatment comparison of patisiran versus placebo will focus on the 12m-DB period, defined as below:

  1. For patients who received at least one dose of patisiran during the OLE period, all assessments collected prior to the first dose of patisiran in the OLE period will be included in the 12m-DB period.
  2. For patients who discontinued treatment and did not receive any patisiran doses in the OLE period, all assessments will be included in the 12m-DB period. Assessments

collected within given time windows (details listed in below table) will be included in summary tables/figures and those outside the time windows will be listed only, unless otherwise specified (eg, AEs in Section 6.9).

Endpoint	**Windowing rule for patients who did not enter OLE**
Hospitalization/urgent HF visit/death	Events occurring on or before Day 417
Other efficacy endpoints	Assessments collected on or before Day 417
PK/PD endpoints	Assessments collected within 28 days of the last dose
Safety endpoints	Assessments with onset date within 28 days of the last dose[a]

[a] In the rare situation where a patient did not discontinue treatment in the 12m-DB period and did not enter the OLE period, all data will be included in the summary tables/figures. For AE summaries, related AEs will be considered treatment-emergent and included in summary tables regardless of time window.

- **OLE Period**

  The start day of the OLE period is defined as the day when the first dose of the OLE period is administered. The assessments collected or AEs with onset date after the administration of the first dose in the OLE period will be included in the OLE period. When assessments or AE onset dates are exactly the date of first dose in the OLE period and the assessment or AE onset time is missing, the records will be included in the OLE period.

- **During Patisiran Treatment**

  For all patients who received at least one dose of patisiran, data will be summarized for the "during patisiran treatment" period, defined as below.

  1. For patients who received patisiran in the 12m-DB period, all assessments collected after the first dose of patisiran during the entire study, including both the 12m-DB and OLE periods (if available), will be included in the "during patisiran treatment" period.
  2. For patients who received placebo in the 12m-DB period and switched to patisiran in the OLE period, all assessments collected after the first dose of patisiran in the OLE period will be included in the "during patisiran treatment" period.
  3. For patients who discontinued patisiran treatment during the 12m-DB period, the data handling will follow the same rules as discussed above for "12m-DB period". For patients who stopped participation in the study during the OLE period, assessments collected within given time windows (details listed in below table) will be included in summary tables/figures and those outside the time windows will be listed only, unless otherwise specified (eg, AEs in Section 6.9). For ongoing patients, all data will be included.

Endpoint	Windowing rule for patients who discontinued patisiran in the OLE
Efficacy endpoints	Assessments collected within 90 days of the last dose
PD endpoint	Assessments collected within 24 days of the last dose
Safety endpoints	Assessments with onset date within 28 days of the last dose[a]

[a] For AE summaries, related AEs will be considered treatment-emergent and included in summary tables regardless of time window.

Longitudinal efficacy parameters will be summarized over the entire study, including the 12m-DB and OLE periods, for all patients to show the long-term efficacy of patisiran (for patients randomized to patisiran) as well as to show the trajectory changes comparing the placebo experience versus the patisiran experience (for patients randomized to placebo). In these summaries, patients will be analyzed according to the treatment to which they were randomized during the 12m-DB period.

6. STATISTICAL ANALYSIS

6.1. Patient Disposition

The number and percentage of patients in the following categories will be summarized by randomized treatment arm and overall, as appropriate:

- Randomized
- Treated
- Completed Month 12 visit
- Discontinued treatment in the 12m-DB period and primary reason for treatment discontinuation and discontinuation due to COVID-19
- Stopped participation in the study in the 12m-DB period and primary reason for stopping participation and stopping participation due to COVID-19
- Entered the OLE period
- Discontinued treatment in the OLE period and primary reason for treatment discontinuation and discontinuation due to COVID-19
- Stopped participation in the study in the OLE period and primary reason for stopping participation and stopping participation due to COVID-19.

The number and percentage of patients enrolled by country and site will be summarized by randomized treatment arm and overall. The number and percentage of patients in each level of each randomization stratification factor as recorded in IRS and in the clinical database, and a comparison of the number and percentage of patients in each randomization stratification factor in IRS versus the clinical database will be summarized by randomized treatment arm and overall.

6.2. Demographics and Baseline Characteristics

Demographic and baseline characteristics, baseline disease characteristics, baseline efficacy parameters, and medical history information will be summarized by treatment arm and overall. No formal statistical comparisons will be performed.

Age at randomization, height, weight, body mass index (BMI), and mBMI will be summarized using descriptive statistics. Age group, sex, race, ethnicity, region, and country will be summarized by presenting the frequencies and percentages of patients in each category.

The following baseline disease characteristics will be summarized by presenting the numbers and percentages of patients in each category:

- Type of ATTR amyloidosis [hATTR; wtATTR]
- Genotype for hATTR patients
- Baseline tafamidis use [Yes; No]
- NYHA Class [I; II; III]
- NYHA Class I/II and age < 75 years [Yes; No]

- ATTR amyloidosis disease stage [1; 2; 3]
- Polyneuropathy disability (PND) score [0; I; II]
- Previous heart failure hospitalization [Yes; No]

The age at symptom onset and the time in years since diagnosis will be summarized, overall and by type of ATTR amyloidosis, using descriptive statistics. For patients who had at least 1 previous heart failure hospitalization, the age at first hospitalization for heart failure and the number of hospitalizations for heart failure in the previous 12 months will be summarized using descriptive statistics. For those who previously used tetramer stabilizers, the time from discontinuation of tetramer stabilizer to the start of study drug will be summarized using descriptive statistics. For patients in the baseline tafamidis group (per the clinical database), the time from the start of tafamidis therapy to the start of study drug will be summarized using descriptive statistics.

The number and percent of patients with each type of ATTR amyloidosis and with each genotype (for hATTR patients) will be summarized by country and treatment arm.

Medical history will be summarized by system organ class (SOC), high level term (HLT), and preferred term (PT). A patient contributes only once to the count for a given condition (overall, by SOC, by HLT, by PT).

All demographic and baseline data for each patient will be provided in data listings. Medical history data including general medical history, cardiac medical history, neuropathy history, historical inpatient admissions or urgent healthcare visits in the past 12 months, and ophthalmic medical history will be presented data listings. Screening test results will also be presented in data listings.

6.3. Protocol Deviations

A protocol deviation is any change, divergence, or departure from the study design or procedures defined in the clinical protocol. Protocol deviations will be classified by medical review prior to the final 12m-DB analysis, and major protocol deviations will be identified. A major protocol deviation is a deviation that may significantly impact the completeness, accuracy, and/or reliability of the trial data; that may significantly affect a subject's rights, safety, or well-being. (ICH. E3 R1 Structure and Contents of Clinical Study Reports Guidance for Industry. 2013) All major deviations related to trial inclusion or exclusion criteria, conduct of the trial, patient management or patient assessment will be described in the clinical study report.

The Sponsor or designee will be responsible for producing the protocol deviation file (formatted as a Microsoft Excel file). This file will include a description of each protocol deviation and whether or not this deviation is classified as a major protocol deviation. Since the study will be ongoing at the time of the primary analysis, there will be continuing review of protocol deviations. The file with all protocol deviations through the prespecified cutoff date for the final 12m-DB analysis will be finalized prior to the interim lock of the database and study unblinding. After the study is completed, the file with all protocol deviations for the study will be finalized prior to the hard-lock of the database.

All protocol deviations, COVID-19-related protocol deviations, and major protocol deviations will be summarized.

6.4. Drug Exposure

Exposure to study drug in months and the number of doses of study drug received will be summarized by treatment arm. Summaries of the numbers and percentages of patients with no missing infusions, and the number of missing infusions per patient will also be provided. The total volume infused will also be summarized.

The last date of exposure to study drug is defined as the earliest day of the following dates:

- Last dose date + 20 days
- Analysis cutoff date
- End of study date

Duration of exposure is defined as (the last date of exposure to study drug – date of the first dose +1)/30.4375. The exposure during the 12m-DB period is right censored by the date of the first OLE dose, ie, the last exposure day in the 12m-DB period is no later than the day before the first OLE dose. Similarly, the exposure during the OLE is left censored by the date of the first OLE dose, ie, the Day 1 of the OLE is the day of the first OLE dose. Dose interruptions and compliance are not taken into account for duration of exposure.

Study drug exposure data collected in the CRFs of study drug administration will also be summarized for each infusion. The numbers and percentages of patients with complete, partial, and missing dose administrations will be summarized. Complete and partial administration is defined as follows:

- Complete: ≥80% (≥160 mL) of the planned infusion volume (200 mL)
- Partial: >0% to <80% (>0 to <160 mL) of the planned infusion volume (200 mL).

The number of patients who experienced interruptions of infusions for any reason will be tabulated, as well as the number of patients with infusion interruptions due to an infusion-related reaction (IRR).

Dosing information for each patient will be presented in a data listing.

6.5. Premedications

All patients will receive premedications in order to reduce the potential of an IRR. Premedications will be coded using the WHO Drug Dictionary (March 2019 or later). Results will be tabulated by anatomical therapeutic class (ATC) and preferred term.

Premedication data will be presented in a data listing.

6.6. Efficacy Analyses

Efficacy endpoints will be analyzed using the FAS.

6.6.1. Primary Endpoint

The primary efficacy endpoint is to compare the change in 6-MWT from baseline to Month 12 between treatment arms. Only 6-MWT assessments confirmed as valid by the Colorado Prevention Center (6-MWT site training and oversight vendor) will be included in analyses.

6.6.1.1. Definition of Estimand

For the objective of evaluating the efficacy of patisiran compared with placebo on 6-MWT, the estimand is defined as follows:

- **Target patient population:** patients with hATTR or wtATTR amyloidosis with cardiomyopathy. Patients may or may not be taking tafamidis at study entry.
- **Treatment condition:** Patisiran 0.3 mg/kg or placebo administered as an IV infusion q3W (once every 3 weeks) with or without concomitant use of tafamidis.
- **Endpoint:** Change from baseline in 6-MWT at Month 12.
- **Population-level summary:** The least squares (LS) mean difference in the change from baseline in 6-MWT at Month 12 between the patisiran and placebo arms.

Intercurrent events and strategies: Intercurrent events include treatment discontinuation, serious COVID-19 AE, tafamidis drop-in, missing dose(s) due to COVID-19, inability to walk due to progression of ATTR amyloidosis, and death. They require different handling strategies, which are described in Table 1 below.

Table 1: Intercurrent Event Strategies for the Primary Analysis of 6-MWT

Intercurrent Event	Handling Strategy
Treatment discontinuation (not due to death)	Treatment policy strategy: Intercurrent event is ignored; 6-MWT assessments obtained after treatment discontinuation will be included in analysis.
Serious COVID-19 AE	Hypothetical strategy: Assessments obtained on or after the onset of a serious COVID-19 AE will be treated as missing and will be assumed as MAR. The goal is to estimate the scheduled visit values as if the patient had not been infected with COVID-19.
Tafamidis drop-in (ie, taking tafamidis for more than 28 days after randomization) in the subgroup of patients who were not on tafamidis at baseline	Treatment policy strategy: Intercurrent event is ignored; 6-MWT assessments obtained after tafamidis drop-in will be included in analysis.
Missing dose(s) due to COVID-19	Treatment policy strategy: Intercurrent event is ignored; 6-MWT assessments obtained after missing dose(s) due to COVID-19 will be included in analysis.
Inability to walk due to progression of ATTR amyloidosis (including patients who are unable to walk due to hospitalization related to progression of ATTR amyloidosis)	Composite variable strategy: For patients who are unable to walk due to progression of ATTR amyloidosis, the missing Month 12 6-MWT change from baseline will be imputed with the worst 10^{th} percentile change observed across all patients in the 12m-DB period, capped by the worst possible change for the patient (ie, 0 – baseline 6-MWT).
Death (including heart transplant and ventricular assist device placement)	Hypothetical strategy: Missing data due to death will be assumed as MAR. The goal is to estimate the scheduled visit values as if the patient were alive at these visits.

6.6.1.2. Primary Analysis using MMRM for the Full Analysis Set

The primary analysis will be performed using a REML-based MMRM approach for the FAS. The outcome variable is the change from baseline in 6-MWT; the model includes baseline 6-MWT as a continuous covariate and treatment arm, visit (Month 6, Month 9 or Month 12), baseline tafamidis use (yes vs. no), type of amyloidosis (hATTR vs. wtATTR), age (<75 vs. ≥75 years), the treatment-by-visit interaction, the treatment-by-baseline tafamidis interaction, the visit-by-baseline tafamidis interaction, and the treatment-by-visit-by-baseline tafamidis interaction as fixed factors.

An unstructured covariance structure will be used to model the within-patient errors. If the model fails to converge, the following covariance structures will be specified in sequence and the first to converge will be used:

1. Toeplitz
2. Autoregressive (1)
3. Compound symmetry

The Satterthwaite approximation will be used to estimate the degrees of freedom.

The primary comparison is the contrast (difference in LS means) between the patisiran and placebo arms at Month 12. The analysis will be implemented with SAS PROC MIXED.

6.6.1.3. Sensitivity Analyses

Sensitivity analyses will be conducted using the following methods to assess the impact of missing data and the robustness of the primary analysis. Data handling approaches for intercurrent events in the two sensitivity analyses are described in Table 2 below.

Table 2: Intercurrent Event Strategies for the Sensitivity Analyses of 6-MWT

Intercurrent Event	**Handling Strategy for Pattern Mixture Model (PMM)**	**Handling Strategy for MMRM Including All Censored Data**
Treatment discontinuation (not due to death)	Same as primary analysis (treatment policy strategy)	
Serious COVID-19 AE	Treatment policy strategy: Intercurrent event is ignored; 6-MWT assessments obtained on or after the onset of a serious COVID-19 AE will be included in analysis.	
Tafamidis drop-in (ie, taking tafamidis for more than 28 days after randomization) in the subgroup of patients who were not on tafamidis at baseline	Same as primary analysis (treatment policy strategy)	
Missing dose(s) due to COVID-19	Same as primary analysis (treatment policy strategy)	

Intercurrent Event	Handling Strategy for Pattern Mixture Model (PMM)	Handling Strategy for MMRM Including All Censored Data
Inability to walk due to progression of ATTR amyloidosis (including patients who are unable to walk due to hospitalization related to progression of ATTR amyloidosis)	Composite variable strategy: For patients who are unable to walk due to progression of ATTR amyloidosis, the missing Month 12 6-MWT change from baseline will be imputed by taking random samples from the worst 10% 6-MWT changes observed during the 12m-DB period[a].	Same as primary analysis (Composite variable strategy:
Death (including heart transplant and ventricular assist device placement) not due to COVID-19	Composite variable strategy: For patients who die during the 12m-DB period (not due to COVID-19), the missing Month 12 6-MWT change from baseline will be imputed by taking random samples from the worst 10% 6-MWT changes observed during the 12m-DB period[a].	Same as primary analysis (hypothetical strategy)
Death due to COVID-19	Same as primary analysis (hypothetical strategy)	

[a] For each patient, the 6-MWT change is capped by the worst possible change for the patient (ie, 0 – baseline 6-MWT).

Pattern Mixture Model (PMM)

A sensitivity analysis using pattern mixture model (PMM) will be performed to assess the robustness of the primary MMRM results to the possible violation of the missing at random (MAR) missingness assumption. The PMM accommodates situations where the missingness mechanism may be missing not at random (MNAR). The model will be based on the following assumptions for missing data at Month 12:

1. Patients who have missing data due to COVID-19, including patients who have missing assessments or who die due to COVID-19 prior to the Month 12 assessment will be imputed assuming data are MAR. Under the hypothetical estimand of interest where the COVID-19 pandemic did not occur, these assessments should have been obtained with no COVID-19 impact. Missing data meeting these criteria will be imputed separately for each treatment arm and baseline tafamidis use group using multiple imputation (MI) estimated from all non-missing data collected within each group.
2. For patients who have missing data unrelated to COVID-19, who are alive through the 12m-DB period and who have not become unable to walk due to progression of ATTR amyloidosis by Month 12, the imputation will be performed by baseline tafamidis use (yes/no) separately based on different patterns described as below:
    a. Placebo patients who have missing data: The missing data are considered MAR and will be imputed using multiple imputation (MI) estimated from placebo patients. The

imputation is done regardless of whether a patient was on-treatment or discontinued treatment before the scheduled efficacy assessment.

b. Patisiran patients who have missing data while on treatment: Patients are expected to continue to show benefit from treatment similar to that observed at the scheduled time point. Therefore, missing data during the on-treatment period (within 60 days of their last dose) are considered MAR and will be imputed using MI estimated from all non-missing data collected on treatment from the patisiran arm.
c. Patisiran patients who have missing data after stopping their study treatment: Patients will no longer benefit from treatment in the future and will have trajectory similar to placebo patients after discontinuing treatment. Therefore, missing data after treatment discontinuation (more than 60 days after last dose of study drug) will be imputed using the data from placebo patients using the copy reference (CR) approach.

3. Patients who die during the 12m-DB period (not due to COVID-19) or who have become unable to walk due to progression of ATTR amyloidosis by Month 12 and have missing data: Assuming that deaths observed in the study will likely be related to worsening of disease, the missing Month 12 6-MWT change from baseline will be imputed by taking random samples from the worst 10% 6-MWT changes observed during the 12m-DB period in the entire population, capped by the worst possible change for the patient (ie, 0 – baseline 6-MWT). The imputation will be done for patients from both the patisiran and placebo arms.

Missing values will be imputed 100 times to generate 100 complete datasets using the procedures described above. An analysis of covariance (ANCOVA) model will be fit to each imputed dataset for the change from baseline in 6-MWT at Month 12. The ANCOVA model will include baseline 6-MWT as a continuous covariate and treatment arm, baseline tafamidis use (yes vs. no), type of ATTR amyloidosis (hATTR vs. wtATTR), age (<75 vs. ≥75), and the treatment-by-baseline tafamidis interaction as covariates. The LS mean and standard error of the mean (SEM) estimated from the ANCOVA model fit to each imputed dataset will be combined by applying Rubin's rules,[Rubin 1996; Rubin 1987] using SAS PROC MIANALYZE to produce inferential results including the treatment difference in LS means, 95% confidence interval (CI) for the treatment difference, and the p-value. More details on the implementation of the PMM are discussed in Appendix 9.1.

MMRM Model Including All Censored Data

A sensitivity analysis including all 6-MWT assessments, ie, not censoring assessments that occur on or after the onset of a serious COVID-19 AE (see Table 2), will be conducted using the same MMRM model as used for the primary analysis.

6.6.1.4. Binary Analyses

The number and percentage of patients with a ≥0 meter increase in 6-MWT from baseline to Month 12 will be calculated for each treatment arm and compared between 2 arms using the Cochran-Mantel-Haenszel (CMH) test, stratified by baseline tafamidis use (yes vs. no); 6-MWT assessments collected on or after the onset of a serious COVID-19 AE will also be treated as missing (see Table 1). All patients with missing Month 12 data will be counted in the denominator with two exceptions: patients who are missing Month 12 data due to COVID-19

and patients with a 6-MWT assessment collected on or after the onset of a serious a COVID-19 AE will be excluded.

6.6.1.5. Overview of Primary Endpoint Analyses

The planned analyses of the primary endpoint 6-MWT are summarized in Table 3.

Table 3: Analysis of 6-MWT

Statistical Method
Primary analysis: MMRM
Sensitivity analysis: PMM
Sensitivity analysis: MMRM - including all available data without censoring
Other analysis: Binary analysis using stratified CMH

6.6.2. Secondary Endpoints

The secondary efficacy endpoints are specified in Section 3. To control overall type I error, the secondary endpoints will be tested in a hierarchical order as described in Section 5.7.

CV events will include all emergency/unplanned/non-elective hospitalizations after randomization adjudicated as being cardiovascular or being indeterminate, and all emergency/unplanned/non-elective visits after randomization that are adjudicated as being urgent HF visits.

6.6.2.1. Change from Baseline at Month 12 in KCCQ-OS Score

The change from baseline at Month 12 in KCCQ-OS score will be analyzed using an MMRM model similar to the model described for the primary analysis of 6-MWT while adjusting for baseline KCCQ-OS as a continuous covariate. The estimand for KCCQ-OS is similar to that for 6-MWT with one exception: the intercurrent event of being unable to walk due to progression of ATTR amyloidosis is not applicable since patients are still able to complete the KCCQ assessment in this scenario.

In addition, sensitivity analyses will be conducted using a PMM model and an MMRM model including data collected on or after the onset of a serious COVID-19 AE, similar to the models described for the sensitivity analyses of 6-MWT. Note that the PMM imputation rules for KCCQ-OS will not consider being unable to walk due to progression of ATTR amyloidosis in defining the missing data patterns since patients are still able to complete the KCCQ assessment in this scenario.

6.6.2.2. Composite All-cause Mortality, Frequency of CV Events (CV hospitalizations and urgent HF visits), and Change from Baseline in 6-MWT over the 12-month Double-blind Period

6.6.2.2.1. Definition of Estimand

For the objective of evaluating the efficacy of patisiran compared with placebo on patient mortality, hospitalizations and urgent HF visits, and change from baseline in 6-MWT, the estimand is defined as follows:

- **Target patient population:** Patients with hATTR or wtATTR amyloidosis with cardiomyopathy. Patients may or may not be taking tafamidis at study entry.
- **Treatment condition:** Patisiran 0.3 mg/kg or placebo administered as an IV infusion q3W with or without concomitant use of tafamidis.
- **Endpoint:** Hierarchical comparison of all-cause mortality, frequency of CV events, and the change from baseline in 6-MWT over the 12m-DB period.
- **Population-level summary:** Stratified win ratio for the composite outcome between the patisiran and placebo arms.

Intercurrent events and strategies: Intercurrent events include treatment discontinuation, serious COVID-19 AE, COVID-19 related deaths and CV events, tafamidis drop-in, missing dose(s) due to COVID-19, inability to walk due to progression of ATTR amyloidosis, and heart transplantation and ventricular assist device placement. They require different handling strategies, which are described in Table 4 below.

Table 4: Intercurrent Event Strategies for the Analysis of the Composite Endpoint

Intercurrent Event	Handling Strategy
Treatment discontinuation	Treatment policy strategy: Intercurrent event is ignored; deaths, CV events, and 6-MWT assessments that occur after treatment discontinuation will be included in analysis.
COVID-19 related death and CV events	Hypothetical strategy: Deaths and CV events due to COVID-19 will be excluded from analysis; The goal is to estimate the treatment effect in a hypothetical setting where the COVID-19 pandemic is not present.
Serious COVID-19 AE	Hypothetical strategy: 6-MWT assessments obtained on or after the onset of a serious COVID-19 AE will be treated as missing.
Tafamidis drop-in (ie, taking tafamidis for more than 28 days after randomization) in the subgroup of patients who were not on tafamidis at baseline	Treatment policy strategy: Intercurrent event is ignored; deaths, CV events, and 6-MWT assessments that occur after tafamidis drop-in will be included in analysis.
Missing dose(s) due to COVID-19	Treatment policy strategy: Intercurrent event is ignored; deaths, CV events, and 6-MWT assessments that occur after missing dose(s) due to COVID-19 will be included in analysis.

Intercurrent Event	Handling Strategy
Inability to walk due to progression of ATTR amyloidosis (including patients who are unable to walk due to hospitalization related to progression of ATTR amyloidosis)	Composite variable strategy: For patients who are unable to walk due to progression of ATTR amyloidosis, the missing 6-MWT will be imputed with the worst 10th percentile change observed across all patients in the 12m-DB period, capped by the worst possible change for the patient (ie, 0 – baseline 6-MWT).
Heart transplantation and ventricular assist device placement	Composite variable strategy: Heart transplantation and ventricular assist device placement will be treated in the same manner as death.

6.6.2.2.2. Primary Analysis

The composite endpoint of all-cause mortality, frequency of CV events (CV hospitalizations and urgent HF visits) and change from baseline in 6-MWT will be analyzed using the stratified win ratio method,[Dong 2018] stratified by baseline tafamidis use. This method makes within-stratum pairwise comparisons (for all possible patisiran/placebo patient pairs) of the 3 components in the hierarchical order specified above. Within a stratum, each patisiran patient will be compared with all placebo patients in a stepwise fashion, with the "winner" assigned a score of +1 and the "loser" assigned a score of "-1". The detailed steps are described as follows. All comparisons will be based on data collected during the 12m-DB period.

Step 1: compare all-cause mortality

The comparison of the two patients will be performed at the shorter of their survival follow-up times.

- If both patients are deceased, then the patient with a longer survival time is assigned a +1 and the other is assigned a -1
- If one patient is alive and the other is deceased, the alive patient is assigned a +1 and the deceased patient is assigned a -1
- Otherwise, proceed to step 2

Step 2: compare frequency of CV events

The comparison of the two patients will be performed at the shorter of their follow-up times while patients remain on the study.

- The patient with fewer CV events is assigned a +1 and the other is assigned a -1
- If the numbers of CV events are the same, go to step 3

Step 3: compare change from baseline in 6-MWT

- The patient who has less decline in 6-MWT at Month 12 is assigned a +1 and the other is assigned a -1
- If one or both patients have missing data at Month 12, compare change from baseline in 6-MWT at the latest common study visit where both patients had a 6-MWT measurement. The patient who has less decline in 6-MWT is assigned a +1 and the other is assigned a -1

- If tied or undetermined, assign score 0 to each patient

The point estimate of the stratified win ratio (WR) is defined as

$$WR = \frac{\sum_{m=1}^{2} n_t^{(m)} / N^{(m)}}{\sum_{m=1}^{2} n_c^{(m)} / N^{(m)}}$$

where $n_t^{(m)}$and $n_c^{(m)}$ are the number of patisiran/placebo pairs in stratum *m* in which the patisiran patient was the winner and in which the placebo patient was the winner, respectively, and $N^{(m)}$ is the total number of patients in stratum *m*.

A 95% CI and p-value for the stratified win ratio will be estimated (see Section 9.2 for details).

6.6.2.3. Composite All-cause Mortality and Frequency of All-cause Hospitalizations and urgent HF visits over the 12-month Double-blind Period

6.6.2.3.1. Definition of Estimand

For the objective of evaluating the efficacy of patisiran compared with placebo on patient mortality, hospitalizations and urgent HF visits, the estimand is defined as follows:

- **Target patient population:** Patients with hATTR or wtATTR amyloidosis with cardiomyopathy. Patients may or may not be taking tafamidis at study entry.
- **Treatment condition:** Patisiran 0.3 mg/kg or placebo administered as an IV infusion q3W with or without concomitant use of tafamidis.
- **Endpoint:** Timing and frequency of all-cause deaths, all-cause hospitalizations and urgent HF visits over the 12m-DB period.
- **Population-level summary:** Hazard ratio (HR) for the composite outcome between the patisiran and placebo arms.

Intercurrent events and strategies: Intercurrent events include treatment discontinuation, COVID-19 infection, tafamidis drop-in, missing dose(s) due to COVID-19, and heart transplantation and ventricular assist device placement. They require different handling strategies, which are described in Table 5 below.

Table 5: Intercurrent Event Strategies for the Analysis of the Composite Endpoint

Intercurrent Event	Handling Strategy
Treatment discontinuation	Treatment policy strategy: Intercurrent event is ignored; deaths, all-cause hospitalizations and urgent HF visits that occur after treatment discontinuation will be included in analysis.
COVID-19 related deaths, hospitalizations, and urgent HF visits	Hypothetical strategy: Deaths, all-cause hospitalizations and urgent HF visits due to COVID-19 will be excluded from analysis. The goal is to estimate the HR in a hypothetical setting where the COVID-19 pandemic is not present.

Intercurrent Event	Handling Strategy
Tafamidis drop-in (ie, taking tafamidis for more than 28 days after randomization) in the subgroup of patients who were not on tafamidis at baseline	Treatment policy strategy: Intercurrent event is ignored; deaths, all-cause hospitalizations and urgent HF visits that occur after tafamidis drop-in will be included in analysis.
Missing dose(s) due to COVID-19	Treatment policy strategy: Intercurrent event is ignored; deaths, all-cause hospitalizations and urgent HF visits that occur after missing dose(s) due to COVID-19 will be included in analysis.
Heart transplantation and ventricular assist device placement	Composite variable strategy: Heart transplantation and ventricular assist device placement will be treated in the same manner as death.

6.6.2.3.2. Primary Analysis

The composite endpoint of all-cause mortality and frequency of all-cause hospitalizations and urgent HF visits will be analyzed using a modified Andersen-Gill model stratified by baseline tafamidis use (yes vs. no), including treatment, type of amyloidosis (hATTR vs. wtATTR), NYHA Class (I/II vs. III), and age (<75 vs. ≥75 years) as covariates.

6.6.2.3.3. Component Analysis

The components of the composite endpoint will be analyzed as follows. For all-cause mortality, Kaplan-Meier survival curves for each treatment group will be presented. All-cause mortality will also be analyzed using a log-rank test. The HR and corresponding 95% CI will be estimated from a Cox proportional hazards model including treatment as a covariate. Frequency of all-cause hospitalizations and urgent HF visits will be analyzed using Poisson regression with treatment, baseline tafamidis (yes vs. no), type of amyloidosis (hATTR vs. wtATTR), NYHA Class (I/II vs. III), age (<75 vs. ≥75 years), and the treatment-by-baseline tafamidis use interaction as covariates, adjusting for duration of follow-up (ie, include duration of follow-up as an offset).

6.6.3. Exploratory Endpoints

All analyses of exploratory endpoints will be conducted on the FAS. For continuous endpoints, descriptive statistics will be provided for actual value and change from baseline by treatment arm; descriptive statistics for percentage change from baseline by treatment arm may also be provided, as appropriate.

The composite endpoint of all-cause mortality and frequency of CV events (CV hospitalizations and urgent HF visits) over the 12m-DB period will be analyzed using a modified Andersen-Gill model similar to that for the composite endpoint of all-cause mortality ad frequency of all-cause hospitalizations and urgent HF visits (see Section 6.6.2.3). The frequency of CV events component will also be analyzed using Poisson regression with treatment, baseline tafamidis (yes vs. no), type of amyloidosis (hATTR vs. wtATTR), NYHA Class (I/II vs. III), age (<75 vs. ≥75 years), and the treatment-by-baseline tafamidis use interaction as covariates, adjusting for duration of follow-up.

The change from baseline at Month 12 in select echocardiographic parameters (mean left ventricular [LV] wall thickness, LV mass, and global LV longitudinal strain) and Norfolk QoL-DN Total Score (see Section 9.3.2) will be analyzed using an ANCOVA model since they are only measured in the 12m-DB period at Month 12. The ANCOVA model will include the baseline value for the parameter as a continuous covariate and treatment arm, baseline tafamidis use (yes vs. no), type of amyloidosis (hATTR vs. wtATTR), NYHA class (I/II vs. III), and age (<75 vs. ≥75) as covariates. The Norfolk QoL-DN questionnaire is a patient-reported outcomes measure that is sensitive to the different features of diabetic neuropathy; since patients enrolled in this study are not required to have a history of neuropathy, the ANCOVA model for Norfolk QoL-DN Total Score may be repeated on the subset of patients with a history of neuropathy.

In the 12m-DB period, NT-proBNP and troponin I are measured at baseline, Month 3, Month 6, Month 9, and Month 12, and mBMI is measured at baseline, Month 6, and Month 12. NT-proBNP, troponin I, and mBMI will be analyzed using MMRM models similar to the model described for the primary analysis of KCCQ-OS while adjusting for the baseline value of the endpoint being modeled (see Section 6.6.2.1).

NT-proBNP has been shown to be highly skewed in the literature; a Q-Q plot of the residuals of the NT-proBNP MMRM model specified above will be used to assess the normality assumption. If the normality assumption is violated, a natural log transformation ($\log_e$) will be applied to NT-proBNP to normalize the distribution. Following the transformation, an MMRM model similar to the model described for the primary analysis of KCCQ-OS will be used; the outcome variable will be $\log_e$(post-baseline) – $\log_e$(baseline), and the model will include $\log_e$(baseline) as a continuous covariate. Assessments obtained on or after the onset of a serious COVID-19 AE will not be treated as missing. Adjusted geometric mean fold-changes from baseline with 95% CIs will be constructed by exponentially back-transforming the LS means, differences in LS means, and the limits of the corresponding 95% CIs.

CMR and technetium scintigraphy will be performed in a subset of patients at select sites to assess cardiac amyloid involvement. The analyses for these imaging assessments are detailed in the separate APOLLO-B Imaging Statistical Analysis Plan.

Categorical exploratory parameters, including ATTR amyloidosis disease stage and NYHA class will be descriptively summarized by presenting the number and percentage of patients in each category for each visit. The number and percentage of patients with improving, no change, and worsening in these parameters at each visit will also be summarized.

Data collected during the OLE period will be summarized descriptively.

6.6.4. Subgroup Analysis

Subgroup analyses will be conducted to assess the consistency of treatment effect within various subgroups defined by the following baseline characteristics:

- Age [<75; ≥75 at randomization]
- Baseline tafamidis use [Yes; No]
- Type of amyloidosis [hATTR; wtATTR]
- NYHA class [I/II; III]

Subgroup analyses will be performed for the primary endpoint 6-MWT and KCCQ-OS using MMRM models. The outcome variable is the change from baseline in the parameter, and the model includes the baseline value as a continuous covariate and treatment arm, visit, subgroup, the treatment-by-visit interaction, the treatment-by-subgroup interaction, the visit-by-subgroup interaction, and the treatment-by-visit-by-subgroup interaction as fixed factors. Subgroup analyses for the age, type of amyloidosis, and NYHA class baseline characteristics will also include baseline tafamidis use (yes vs. no) as a factor in the MMRM models. If the number of patients in either treatment arm of a subgroup category is less than 30, only descriptive statistics will be presented. A forest plot will be generated to illustrate the estimated treatment effect along with 95% CI within each subgroup.

Other subgroups may be examined, if deemed appropriate. The subgroup analyses may also be performed for other efficacy endpoints.

6.7. Pharmacodynamic Analysis

The PD parameter for this study is serum TTR. For all analyses of post-baseline TTR data, only post-baseline TTR assessments collected within 24 days (inclusive) after receiving a full dose of study drug (ie, the amount infused was ≥80% of the planned infusion volume) will be summarized.

Summary tables will be provided for observed values, changes and percentage changes from baseline for each scheduled time point by treatment arm.

The maximum percentage reduction and mean percentage reduction over 12 months will be summarized using descriptive statistics. Subgroup analysis will be provided for age (<75 vs. ≥75), sex (male vs. female), type of amyloidosis (hATTR vs. wtATTR), and baseline tafamidis use (yes vs. no).

All PD data will be displayed in data listings.

6.8. Pharmacokinetic Analysis

6.8.1. Study Variables

6.8.1.1. Concentration Data

Plasma concentrations of the 3 PK analytes (ALN-18328, DLin-MC3-DMA and PEG_{2000}-C-DMG) will be obtained. Concentration values that are below the limit of quantification (LLOQ or BLQ) will be set to zero for analysis.

6.8.1.2. Pharmacokinetic Parameters

The following plasma concentrations of ALN-18328 and the two lipids will be summarized by study visit:

- Observed post-infusion peak concentration (C_{max})
- Observed 30 min post-infusion concentration ($C_{p(30min)}$)
- Observed pre-infusion trough concentration (C_{min})

In addition, steady-state C_{max} (C_{max_ss}), steady-state C_{min} (C_{min_ss}) and steady-state $C_{p(30min)}$ ($C_{p\_ss(30min)}$) will be calculated as the average of the C_{max}, C_{min}, and $C_{p(30min)}$ values, respectively, at Week 24, Week 36, and Week 51.

6.8.2. Statistical Methods

Descriptive statistics for PK parameters will include the number of patients, mean, SD, SEM, coefficient of variation, median, minimum, maximum, geometric mean and geometric coefficient of variation.

The C_{max}, $C_{p(30min)}$ and C_{min} of the 3 analytes will be summarized by nominal sampling day. Mean concentrations (+/- SD) will be plotted versus nominal sampling time.

Steady-state PK parameters for the 3 analytes will be summarized overall and by subgroup, including age (<75 vs. ≥75), gender (male vs. female), and anti-drug antibody status (positive vs. negative).

Plasma concentration data will be presented in by-patient listings.

The PK-PD relationship between the plasma concentration of ALN-18328 and the percent change from baseline in serum TTR may be explored.

Mean and maximum percent TTR reduction from baseline will be summarized by quartiles of the steady state PK parameters for the 3 analytes. Change from baseline at Month 12 in clinical efficacy parameters may also be summarized by quartiles of the steady-state PK parameters for the 3 analytes.

PK exposure will be summarized by mortality status. In addition, the incidence of AEs and serious AEs (SAEs) will be summarized by quartiles of the steady-state PK parameters for the 3 analytes.

Population PK, PK/PD, and disease progression modeling analyses may be performed, if appropriate. If performed, the analyses will be described in a separate analysis plan and reported separately.

6.9. Safety Analysis

An adverse event is any untoward medical event associated with the use of a study drug, whether or not it is considered related to the study drug. The primary safety parameter is the frequency of AEs. Safety parameters also include vital signs, ECGs, clinical laboratory assessments, and physical exams. Analyses for safety parameters will be conducted using the Safety Analysis Set.

Time windows for safety data to be analyzed for the 12m-DB period and the entire study including both 12m-DB and OLE periods as well as safety follow-up are described in Section 5.10 . All safety data, regardless of time windows, will be listed and summarized for selected endpoints, ie, AEs by SOC and PT, SAEs by SOC and PT, and selected laboratory parameters.

Subgroup analysis for safety variables may be conducted if deemed appropriate and necessary.

No inferential safety analysis is planned.

6.9.1. Adverse Events

AEs will be classified by the MedDRA coding system (version 23.0 or later) and displayed in tables and data listings using SOC and PT.

Analyses of AEs will be performed for those events that are considered treatment-emergent, where treatment-emergent is defined as any AE with onset during or after the administration of study drug through 28 days following the last dose of study drug. In addition, any event that was present at baseline but worsened in intensity or was subsequently considered drug-related by the Investigator through the end of the study will be considered treatment-emergent. Events with a fully or partially missing onset date will be assumed to be treatment emergent unless it can be unequivocally determined (from the partial onset date and/or a partial or complete stop date) that the event occurred prior to the first administration of study drug.

Adverse events will be summarized by the numbers and percentages of patients reporting a given AE. A patient contributes only once to the count for a given AE (overall, by SOC, by PT). Overall event counts and frequencies may also be summarized.

An overall summary of AEs will include the number and percentage of patients with any AE, any AE assessed by the Investigator as related to treatment, any severe AE, any severe AE related to treatment, any SAE, any SAE related to treatment, any AE leading to treatment discontinuation, any study drug related AE leading to treatment discontinuation, any AE leading to study withdrawal, any study drug related AE leading to study withdrawal, and any deaths.

Tabulations by SOC and PT will be produced for the following. The SOC and PT within each SOC will be presented alphabetically.

- All AEs;
- Severe AEs;
- All SAEs;
- AEs related to treatment;
- SAEs related to treatment;
- AEs leading to infusion interruption;
- AEs leading to treatment discontinuation;
- SAEs leading to treatment discontinuation;
- AEs leading to study withdrawal;
- SAEs leading to study withdrawal;
- AEs related to pre-medication;
- AEs over time.

Tabulations by PT in decreasing order in frequency in the patisiran arm will be produced for the following:

- All AEs;
- All SAEs;

- AEs related to treatment;
- SAEs related to treatment.

AEs and SAEs will also be summarized by maximum severity and by maximum relationship; patients who report multiple occurrences of the same AE (preferred term) will be classified according to the most severe occurrence or the most related occurrence, respectively. Similarly, AEs related to treatment will be summarized by maximum severity.

Infusion-related reaction signs and symptoms will be summarized by SOC and PT. The incidence and frequency of IRR signs and symptoms over time will also be summarized by SOC and PT.

AEs mapping to the Drug Related Hepatic Disorder standardized MedDRA query (SMQ) will be summarized by SOC and PT. AEs mapping to the Anaphylactic Reaction SMQ will be summarized by PT. AEs mapping to the SMQ Malignant or Unspecified Tumors will be summarized by HLT and PT. Other SMQs or AE groupings may be evaluated.

All AEs will be presented in patient data listings. Separate listings will be provided for death, SAEs, AEs leading to treatment discontinuation, IRR signs and symptoms, AEs with missing severity, AEs with missing relationship to study drug, AEs related to pre-medications, and AEs mapping to the SMQs as described above.

Additional AE considerations regarding COVID-19 are detailed in Section 6.11.3.

Ophthalmological assessments may be performed if a patient develops ocular symptoms suggestive of vitamin A deficiency. The ophthalmological assessment results will be presented in a listing.

Patients who underwent heart transplant and/or ventricular assist device placement will be presented in a listing.

6.9.2. Laboratory Data

Clinical laboratory values will be expressed in SI units. Missing laboratory data will not be imputed.

Summary data for each laboratory parameter will be presented for each continuous clinical laboratory parameter (including hematology, serum chemistry, liver function tests and coagulation studies). Descriptive statistics will be presented for the actual values, change from baseline, and percent change from baseline by visit.

Shift tables will be employed to summarize the baseline category versus the "worst" post-baseline category for selected parameters.

A listing for all patients with abnormal liver function tests, defined as an ALT >3×ULN, AST >3×ULN, or total bilirubin >2×ULN at any time point, will also be provided.

A table will be produced to summarize the number and percentage of patients in each of the below categories at any post-baseline time point.

- ALT >1 & ≤3, >3 & ≤5, >5 & ≤10, >10 & ≤20, >20×ULN;
- AST >1 & ≤3, >3 & ≤5, >5 & ≤10, >10 & ≤20, >20×ULN;

- ALT or AST >1 & ≤3, >3 & ≤5, >5 & ≤10, >10 & ≤20, >20×ULN;
- ALP > 1.5×ULN;
- Total Bilirubin >1.5 & ≤2, >2 & ≤3, >3 & ≤5 and >5×ULN;
- Total Bilirubin > 2×ULN concurrent with ALT or AST > 3×ULN.

In separate figures, the peak total bilirubin (at any time post-baseline) will be plotted against the peak AST, the peak ALT, and the peak AST or ALT levels at any time post-baseline.

For hematology and serum chemistry, summary tables of potentially clinically significant abnormalities will be provided. The results may also be graded according to the National Cancer Institute CTCAE Version 5.0 or above. A shift summary of baseline to maximum post-baseline CTCAE grade may be presented, as appropriate.

The estimated glomerular filtration rate (eGFR, mL/min/1.73 m^2) will be categorized as below: ≥ 90; 60-89; 45-59; 30-44; 15-29 and < 15. A shift summary of baseline to worst post-baseline eGFR category will be presented.

All laboratory data, including pregnancy and follicle stimulating hormone (FSH) test results, will be provided in data listings. Out-of-range laboratory results will be identified in the listings.

6.9.3. Vital Signs and Physical Examination

For vital signs, descriptive statistics by visit and treatment arm will be provided for each variable. Vital sign measurements will be presented for each patient in a data listing, with abnormal vital signs flagged.

For the physical examination, clinically significant findings prior to first dose of study drug will be recorded and summarized under Medical History, unless there is an SAE in which case the event will be recorded and summarized under Adverse Events. Physical examination findings that are new or worsened after first dose of study drug will be recorded and summarized under Adverse Events.

6.9.4. Electrocardiogram

Electrocardiogram (ECG) findings will include rhythm, ventricular rate, RR interval, PR interval, QRS duration, QT interval, and corrected QT (QTc) interval. QTc interval will be calculated using Fridericia's correction formula.

Fridericia's cube-root corrected QT: QTcF (ms) = QT (ms) × $\sqrt[3]{\frac{HR(bpm)}{60}}$.

RR, PR, QRS, QT, and QTcF intervals and their change from pre-dose baseline will be summarized for each treatment arm by scheduled visit. The number and percentage of patients with abnormal results (clinically significant abnormal or not clinically significant abnormal) will also be summarized by treatment arm and scheduled visit.

Patients will be categorized into ≤ 450, > 450 - 480, > 480 - 500, or > 500 ms per their maximum post-baseline absolute QTcF interval and ≤ 30, > 30 - 60, or > 60 ms per their maximum change from baseline QTcF interval. The number and percentage of subjects in each category will be summarized for each treatment arm.

All ECG data for each patient will be provided in a data listing.

6.9.5. Prior and Concomitant Medications

Prior and concomitant medications will be coded using the WHO Drug Dictionary (March 2020 or later). Prior medications include medications taken ≥ 1 time before the first dose of study drug, regardless of medication end date. Concomitant medications include medications taken ≥ 1 time on or after the first dose of study drug, regardless of medication start date. Results will be tabulated by ATC and preferred term.

When there are partial or missing dates, imputed dates will be used to determine 1) if a medication is prior or concomitant, and 2) duration of exposure to select medications, as needed. Imputed dates will not be presented in the listings.

For medications with partial start or stop dates: the first day/month will be imputed for start date, and the last day/month will be imputed for stop date. For medications with a completely missing start date, the medications will be considered as started prior to the first dose of study drug; medications will be classified as prior, concomitant or both depending on the medication stop dates. For medications with a completely missing stop date, the end of study date will be imputed.

Prior and concomitant medications will be presented in data listings. Previous tetramer stabilizer use will be listed separately.

6.10. Anti-Drug Antibody

The number and percentage of patients with confirmed positive ADA assay results at any time during study as well as at each scheduled visit will be summarized. The titer results for patients with confirmed positive ADA results will also be summarized using descriptive statistics.

ADA data and patients with confirmed positive ADA results will be presented in data listings.

6.11. COVID-19 Pandemic Impact Analyses

Additional data were collected to characterize the impact of the COVID-19 pandemic on general study conduct and disposition, and subsequently, additional analyses and summaries will be provided in acknowledgement of multiple regulatory guidances (FDA Guidance on Conduct of Clinical Trials of Medical Products during COVID-19 Pandemic: Guidance for Industry, Investigators, and Institutional Review Boards, US Food and Drug Administration, 2020; Guidance on the Management of Clinical Trials during the COVID-19 (Coronavirus) Pandemic, European Medicines Agency, 2020; Points to consider on implications of Coronavirus disease (COVID-19) on methodological aspects of ongoing clinical trials, European Medicines Agency, 2020; Statistical Considerations for Clinical Trials During the COVID-19 Public Health Emergency, US Food and Drug Administration, 2020).

Few patients were enrolled on APOLLO-B prior to the beginning of the COVID-19 global pandemic.

6.11.1. General Impact

Patients who discontinue treatment or stop study participation due to COVID-19 will be included in patient disposition summaries as described in Section 6.1.

Impact on study participation due to COVID-19, including visit completion, visit location changes, and study drug dosing changes, will be summarized overall on the patient level with both continuous and categorical descriptives, and overall and by visit on the event level with categorical descriptives. Patient- and event-level summaries of the impact on study participation due to COVID-19 may also be generated by site and/or region.

Impact on study participation due to COVID-19 will be presented in data listings by patient and by visit within patient.

6.11.2. Impact on Efficacy

Summaries of missing efficacy data due to the COVID-19 pandemic will be included in missing efficacy data summaries as described in Section 5.8.1. Patient data listings will flag assessments censored due to COVID-19.

The number and percentage of patients who died, were hospitalized or had an urgent HF visit due to COVID-19 will be summarized by treatment arm and overall. The total number of hospitalizations and urgent HF visits due to COVID-19 and the number of hospitalizations and urgent HF visits per patient due to COVID-19 will also be summarized using descriptive statistics by treatment arm and overall. Patient data listings will be presented for deaths, hospitalizations and urgent HF visits due to COVID-19.

Given the measures specified in the protocol designed to ensure data integrity, analyses excluding patients with COVID-19 related protocol deviations will not be prespecified, but may be considered post hoc, if warranted.

6.11.3. Impact on Adverse Events

An overall summary of AEs mapping to a COVID-19 custom query will include the number and percentage of patients, as well as events and event rates, with any AE, any AE assessed by the Investigator as related to treatment, any severe AE, any severe AE related to treatment, any SAE, any SAE related to treatment, any AE leading to treatment discontinuation, any study drug related AE leading to treatment discontinuation, any AE leading to study discontinuation, any study drug related AE leading to study discontinuation, and any deaths. AEs mapping to the COVID-19 custom query will be summarized by HLT and PT. Due to the evolving nature of COVID-19-related MedDRA terminology, the COVID-19 custom query will be based on the latest information available at the specified analysis timepoint.

AEs mapping to the COVID-19 custom query will also be presented in a data listing.

7. CHANGES TO PLANNED ANALYSES

7.1. Changes from Protocol in Original SAP

Change from Protocol	Detailed Description/Rationale
In the PK and PD Analysis Set definitions, "any amount of study drug" (as specified in the protocol) was updated to "at least one complete dose of study drug".	PK and PD data from patients who have not received at least one complete dose of study drug would not be representative of the planned dose level.
The model specified to analyze the composite endpoint of all-cause mortality and frequency of all-cause hospitalizations was updated from an Andersen-Gill model stratified by baseline tafamidis (as specified in the protocol) to an Andersen-Gill model including baseline tafamidis in the list of covariates.	Given the cap on baseline tafamidis for the study, a stratified Andersen-Gill model including several covariates may encounter convergence issues. Therefore, the model now includes baseline tafamidis use in the list of covariates.

7.2. Changes in SAP Amendment 1

The SAP Amendment 1 includes more details previously not discussed in the original SAP. Some changes to analysis methods were made following regulatory feedback. The details of the changes and rationales are listed in the table below.

Summary of Changes	Detailed Description/Rationale
Updated Figure 1	To align with protocol Amendment 3
In the protocol and original SAP, an interim assessment of the tafamidis drop-in rate was planned to assess the impact of the drop-in rate on the power of the study and the potential need to increase the sample size. This interim assessment was not conducted during the study.	Due to the low rate of tafamidis drop-in on APOLLO-B, the interim sample size reassessment was determined to be unnecessary.
Added details on estimand and handling of intercurrent events for primary and secondary endpoints. The primary analyses for efficacy endpoints were also updated to not censor observations after tafamidis drop-in or after missing doses due to COVID-19.	Updates made following regulatory feedback
Updated primary MMRM model specification	To align with protocol Amendment 3

Summary of Changes	**Detailed Description/Rationale**
Changed imputation approach in PMM model for patients who die (not due to COVID-19) or who become unable to walk due to amyloidosis by Month 12	Update was made since such patients are not expected to behave in the same way as those who remain in the study on treatment and therefore require a different imputation approach.
Updated PMM appendix	To clarify the imputation procedure for the missing data patterns, including imputation for death/inability to walk as noted above
Updated data handling approach for tafamidis drop-in and missing doses due to COVID-19 in MMRM and binary sensitivity analyses	To align with updates made to the intercurrent event handling strategies for the estimand
Updated specification for the Andersen-Gill model to be stratified by baseline tafamidis use	To align with the protocol
The Poisson model for analysis of the frequency of hospitalizations and urgent HF visits was updated to add the treatment-by-baseline tafamidis use interaction.	This update was made to allow for potentially different treatment effects between the baseline tafamidis use (yes/no) subgroups.
Updated the subgroup analysis MMRM model specification	This update was made to allow for potentially different treatment effects between the baseline tafamidis use (yes/no) subgroups.
Removed statement that ECG parameters will be treated as missing if QRS duration is >120 ms	To maintain consistency with analyses conducted in other studies in the patisiran clinical development program
Removed description of efficacy and safety summaries by pandemic phase	Summaries by pandemic phase will not be performed since limited data are available prior to the start of the pandemic and limited post-pandemic data are anticipated at the time of the primary analysis.

8. REFERENCES

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9. APPENDICES

9.1. Detailed Statistical Methodology for the Pattern Mixture Model

Patients are classified into five patterns for missing data at Month 12:

1. Patients who have missing data due to COVID-19: missing data will be assumed to be MAR and imputed by using MI estimated from the treatment arm and baseline tafamidis use group to which the patient was randomized. This pattern includes:
   a. Patients who miss the Month 12 assessment due to COVID-19
   b. Patients who die due to COVID-19 before Month 12
2. Placebo patients who do not have missing data due to COVID-19 and are alive through the 12m-DB period and have not become unable to walk due to progression of ATTR amyloidosis by Month 12: missing data will be assumed to be MAR and imputed by using MI estimated from placebo patients in the baseline tafamidis use group to which the patient belongs.
3. Patisiran patients who do not have missing data due to COVID-19 and are alive through the 12m-DB period, have not become unable to walk due to progression of ATTR amyloidosis by Month 12, and have missing data with the last dose of study drug received within 60 days of the scheduled time point: missing data will be assumed to be MAR and imputed by using MI estimated from on-treatment patisiran patients in the baseline tafamidis use group to which the patient belongs.
4. Patisiran patients who do not have missing data due to COVID-19 and are alive through the 12m-DB period, have not become unable to walk due to progression of ATTR amyloidosis by Month 12, and the last dose of study drug was received more than 60 days prior to the scheduled time point: missing data will be assumed to be MNAR and imputed (using data from placebo patients in the baseline tafamidis use group to which the patient belongs) using the copy reference (CR) approach.
5. Patients who die (including heart transplant and ventricular assist device placement) during the 12m-DB period (not due to COVID-19) or who have become unable to walk due to progression of ATTR amyloidosis by Month 12: missing data will be assumed to be MNAR and imputed by taking random samples from the worst 10% change from baseline values observed during the 12m-DB period in the entire population, capped by the worst possible change for the patient (0 – baseline 6-MWT).

For patients who are alive through the 12m-DB period and who have not become unable to walk due to progression of ATTR amyloidosis, all missing data for the placebo arm and missing data for the patisiran arm during the on-treatment period (ie, assessments within 60 days of the last dose of study drug) will be imputed using MI under the MAR assumption. Since the pattern of missing data within patients may be non-monotone, multiple imputation will be conducted separately by treatment arm and baseline tafamidis use group using the Markov Chain Monte Carlo (MCMC) method. For each treatment arm/baseline tafamidis use group, the imputation model will include sex, type of amyloidosis, NYHA class, age at randomization, ATTR amyloidosis disease stage, baseline 6-MWT, and all calculated values of change from baseline in 6-MWT at pre-specified visits. The input dataset DATAIN will exclude data from patients in pattern 5 above and exclude data from patisiran patients that were collected after treatment

discontinuation (ie, more than 60 days after the last dose of study drug). Below is a sample SAS code for the multiple imputation:

```
proc mi data=DATAIN out=DATA_STEP1 seed=234 nimpute=100;
   by treatment bltaf;
   em maxiter=300 converge=1e-4 itprint outem=outem;
   var baseline_variables 6MWT_base 6MWT_chg_m6 6MWT_chg_m9 6MWT_chg_m12;
   mcmc chain=multiple initial=em;
run;
```

The MI procedure generates imputed values for all missing values. For patisiran patients, the imputed data from the on-treatment period will be kept while the imputed data after treatment discontinuation will be discarded and replaced by either the observed non-missing values (if available) or the imputed values using the CR approach described below. The imputation model will include sex, type of amyloidosis, NYHA class, age at randomization, ATTR amyloidosis disease stage, baseline 6-MWT, and all calculated values of change from baseline in 6-MWT at pre-specified visits.

```
proc mi data=DATA_STEP1_2 out=DATA_STEP2 nimpute=1 seed=xxx;
   by _imputation_ bltaf;
   class treatment;
   fcs nbiter=30 reg (6MWT_chg_m12 = baseline_variables 6MWT_base
      6MWT_chg_m6 6MWT_chg_m9);
   mnar model (6MWT_chg_m12 / modelobs=(treatment='placebo'));
   var baseline_variables 6MWT_base 6MWT_chg_m6 6MWT_chg_m9 6MWT_chg_m12;
run;
```

Finally, for patients in pattern 5, missing Month 12 6-MWT change from baseline valueswill be imputed 100 times using a random draw from the worst 10% 6-MWT change from baseline values observed during the 12m-DB period among all non-missing values in the dataset DATAIN, capped by the worst possible change for the patient (ie, 0 – baseline 6-MWT).

9.2. Calculation of Stratified Win Ratio p-value and Confidence Interval

Dong et al (2018) [Dong 2018] note that the logarithm of the stratified win ratio is asymptotically normally distributed with mean $\upsilon_{\log(WR)}$ and variance $\sigma^2_{\log(WR)}$, with these terms defined in equations 7b and 8 in their paper, respectively. The point estimate of the mean, $\hat{\upsilon}_{\log(WR)}$, is the logarithm of the stratified win ratio statistic defined in Section 6.6.2.2. The variance estimate, $\hat{\sigma}^2_{\log(WR)}$, is calculated under the null hypothesis of the same treatment effect in the patisiran and placebo groups.

Then $\hat{z} = \frac{\hat{\upsilon}_{\log(WR)}}{\sqrt{\hat{\sigma}^2_{\log(WR)}}}$ is a standard normal deviate from which the p-value is readily obtained.

The 95% confidence interval for the logarithm of the stratified win ratio is constructed as

$$\hat{\upsilon}_{\log(WR)} \pm 1.96 * \sqrt{\hat{\sigma}^2_{\log(WR)}}$$

and the limits of this confidence interval will then be exponentiated to construct the 95% confidence interval for the stratified win ratio.

9.3. Questionnaire/Scoring

In questionnaires, if multiple responses are provided to a single-response question, the question is deemed as missing.

9.3.1. Kansas City Cardiomyopathy Questionnaire (KCCQ)

The Kansas City Cardiomyopathy Questionnaire is a 23-item self-administered questionnaire developed to independently measure the patient's perception of their health status, which includes heart failure symptoms, impact on physical and social function, and how their heart failure impacts their quality of life within a 2-week recall period.

There are 10 summary scores for the KCCQ tool, which are calculated as follows:

1. Physical Function

Code responses to each of Questions 1a-1f as follows:

- Extremely limited = 1
- Quite a bit limited = 2
- Moderately limited = 3
- Slightly limited = 4
- Not at all limited = 5
- Limited for other reasons or did not do the activity = <missing value>

If at least 3 of Questions 1a-1f are not missing, then compute

- Physical function score = [(mean of the non-missing Questions 1a-1f) – 1]/4 * 100

2. Symptom Stability

Code the response to Question 2 as follows:

- Much worse = 1
- Slightly worse = 2
- Not changed = 3
- Slightly better = 4
- Much better = 5
- I've had no symptoms over the last 2 weeks = 3

The symptom stability score = [(Question 2) – 1]/4 * 100.

3. Symptom Frequency

Code responses to Questions 3, 5, 7 and 9 as follows:

- Questions 3 and 9
  - Every morning/night = 1

  - 3 or more times a week but not every day = 2
  - 1-2 times a week = 3
  - Less than once a week = 4
  - Never over the past 2 weeks = 5
- Questions 5 and 7
  - All of the time = 1
  - Several times a day = 2
  - At least once a day = 3
  - 3 or more times a week but not every day = 4
  - 1-2 times a week = 5
  - Less than once a week = 6
  - Never over the past 2 weeks = 7

If at least two of Questions 3, 5, 7 and 9 are not missing, then compute

- S3 = [(Question 3) – 1]/4
- S5 = [(Question 5) – 1]/6
- S7 = [(Question 7) – 1]/6
- S9 = [(Question 9) – 1]/4

The symptom frequency score = (mean of S3, S5, S7, S9)*100.

4. Symptom Burden

Code responses to Questions 4, 6 and 8 as follows:

- Extremely bothersome = 1
- Quite a bit bothersome = 2
- Moderately bothersome = 3
- Slightly bothersome = 4
- Not at all bothersome = 5
- I've had no swelling/fatigue/shortness of breath = 5

If at least one of Questions 4, 6 and 8 is not missing, then compute

- Symptom burden score = [(mean of the non-missing Questions 4, 6 and 8) – 1]/4 * 100

5. Total Symptom Score

Total symptom score = mean of the following available summary scores

- Symptom Frequency Score
- Symptom Burden Score

6. Self-efficacy

Code responses to Questions 10 and 11 as follows:

- Question 10
  - Not at all sure = 1
  - Not very sure = 2
  - Somewhat sure = 3
  - Mostly sure = 4
  - Completely sure = 5
- Question 11
  - Do not understand at all = 1
  - Do not understand very well = 2
  - Somewhat understand = 3
  - Mostly understand = 4
  - Completely understand = 5

If at least one of Questions 10 and 11 is not missing, then compute

- Self-efficacy score = [(mean of the non-missing Questions 10 and 11) – 1]/4 * 100

7. Quality of Life

Code responses to Questions 12, 13 and 14 as follows:

- Question 12
  - It has extremely limited my enjoyment of life = 1
  - It has limited my enjoyment of life quite a bit = 2
  - It has moderately limited my enjoyment of life = 3
  - It has slightly limited my enjoyment of life = 4
  - It has not limited my enjoyment of life at all = 5
- Question 13
  - Not at all satisfied = 1
  - Mostly dissatisfied = 2
  - Somewhat satisfied = 3
  - Mostly satisfied = 4

  - Completely satisfied = 5
- Question 14
  - I felt that way all of the time = 1
  - I felt that way most of the time = 2
  - I occasionally felt that way = 3
  - I rarely felt that way = 4
  - I never felt that way = 5

If at least one of Questions 12, 13 and 14 is not missing, then compute

- Quality of life score = [(mean of the non-missing Questions 12, 13 and 14) – 1]/4 * 100

8. Social Limitation

Code responses to each of Questions 15a-15d as follows:

- Severely limited = 1
- Limited quite a bit= 2
- Moderately limited = 3
- Slightly limited = 4
- Did not limit at all = 5
- Does not apply or did not do for other reasons = <missing value>

If at least 2 of Questions 15a-15d are not missing, then compute

- Social limitation score = [(mean of the non-missing Questions 15a-15d) – 1]/4 * 100

9. Overall Summary Score

Overall summary score = mean of the following available summary scores

- Physical Limitation Score
- Total Symptom Score
- Quality of Life Score
- Social Limitation Score

10. Clinical Summary Score

Clinical summary score = mean of the following available summary scores

- Physical Limitation Score
- Total Symptom Score

9.3.2. Norfolk Quality of Life-Diabetic Neuropathy (QOL-DN)

Norfolk QOL-DN is a tool for assessing patients' perception of the effects of diabetes and diabetic neuropathy. There are 35 questions divided into 5 domains. The range of possible total scores is -4 to 136.

Part I: Symptoms

Items 1-7 (Part I) are a simple inventory of symptoms of neuropathy. The presence of the symptom is checked in whichever box applies, and an absence of a symptom is checked under "none." Positive responses are scored as 1; and negative responses, as 0.

Part II: Activities of Daily Life

Items 8-35 (Part II) pertain to Activities of Daily Life, and most of these are scaled on a 5-point Likert scale ranging from 0 ("Not a problem") to 4 ("Severe problem"). However, Questions 31 and 32 are scored differently. In Question 31, "Good", the middle item, is scored as0. "Very Good" is scored as –1, "Excellent" is scored as –2. "Fair" is scored as 1, and "Poor" is scored as 2. In Question 32, "About the Same," the middle item, is scored as 0. "Somewhat better" is scored as –1, "Much better" is scored as -2. "Somewhat worse" is scored as 1, and "Much worse" is scored as 2.

Subscales and Scoring Algorithm

The Total QOL and 5 domains should be summed as follows:

- Total QOL (35 items) Σ(1-7, 8-35)
- Physical Functioning/Large Fiber (15 items) Σ(8, 11, 13-15, 24, 27-35)
- Activities of Daily Living (ADLs) (5 items) Σ(12, 22, 23, 25, 26)
- Symptoms (8 items) Σ(1-7, 9)
- Small Fiber (4 items) Σ(10, 16, 17, 18)
- Autonomic (3 items) Σ(19, 20, 21)

Domain scores are calculated as the rounded integer value of the average scores of non-missing included items multiplied by the number of items if at least 50% of the items are non-missing. A domain score is missing if more than 50% of the included items are missing.

If the scores for all 5 domains are non-missing, then Total QOL is the sum of scores of the 5 domains; however, if at least 1 of the domains is missing and at least 50% of the items (18 items) are non-missing, then Total QOL is calculated as 35 times the mean of the non-missing items, rounded to the nearest integer. Otherwise, Total QOL is deemed as missing.

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Task: Approval Verdict: Approve	[redacted]T+0000
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ALN-TTR02-011 SAP Amend1

STATISTICAL ANALYSIS PLAN
ALN-TTR02-011

Protocol Title:	APOLLO-B: A Phase 3, Randomized, Double-blind, Placebo-controlled Multicenter Study to Evaluate the Efficacy and Safety of Patisiran in Patients with Transthyretin Amyloidosis with Cardiomyopathy (ATTR Amyloidosis with Cardiomyopathy)
Short Title:	APOLLO-B: A Study to Evaluate Patisiran in Patients with Transthyretin Amyloidosis with Cardiomyopathy (ATTR Amyloidosis with Cardiomyopathy)
Study Treatment:	Patisiran (ALN-TTR02)
EudraCT Number:	2019-001458-24
IND Number:	141240
Protocol Date:	Original Protocol, 18 April 2019 Amendment 1: 20 December 2019 Amendment 2: 22 May 2020
SAP Date:	Original SAP: 05 August 2020
Sponsor:	Alnylam Pharmaceuticals, Inc. 300 Third Street Cambridge, MA 02142 USA Telephone: [redacted]
Sponsor Contact:	[redacted]

APPROVAL SIGNATURE PAGE

Protocol Number:	ALN-TTR02-011
Protocol Title:	APOLLO-B: A Phase 3, Randomized, Double blind, Placebo-controlled Multicenter Study to Evaluate the Efficacy and Safety of Patisiran in Patients with Transthyretin Amyloidosis with Cardiomyopathy (ATTR Amyloidosis with Cardiomyopathy)
Analysis Plan Version and Date:	Original: 05 August 2020

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Abbreviation	Definition
6-MWT	6-minute walk test
12m-DB	12-month double-blind placebo-controlled
^{99m}Tc-PYP	Technetium pyrophosphate
ADA	Antidrug antibody
AE	Adverse event
ALN-18328	siRNA targeting TTR
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANCOVA	Analysis of covariance
AST	Aspartate aminotransferase
ATC	Anatomical Therapeutic Class
ATTR	Amyloid transthyretin
BLQ	Below the lower limit of quantification
BMI	Body mass index
CI	Confidence interval
C_{max}	Maximum plasma concentration at end of infusion
$C_{max,ss}$	Steady-state C_{max}
C_{min}	Minimum pre-infusion concentration
$C_{min,ss}$	Steady-state C_{min}
CMH	Cochran-Mantel-Haenszel
CMR	Cardiac magnetic resonance
COVID-19	Coronavirus disease 2019
$C_{p(30min)}$	30-minute post-infusion concentration
$C_{p,ss(30min)}$	Steady-state $C_{p(30min)}$
CR	Copy reference
CTCAE	Common Terminology Criteria for Adverse Events
CV	Cardiovascular
DLin-MC3-DMA	1,2-Dilinoleyloxy-N,N-dimethylpropylamine
DMC	Data monitoring committee
ECG	Electrocardiogram
eCRF	Electronic case report form

Abbreviation	Definition
EDC	Electronic data capture
eGFR	Estimated glomerular filtration rate
FAS	Full Analysis Set
FSH	Follicle-stimulating hormone
H/CL	Heart to contralateral lung
hATTR	Hereditary ATTR
HF	Heart failure
HLT	High level term
IV	Intravenous
ICH	International Council for Harmonisation
IRR	Infusion-related reaction
IRS	Interactive response system
KCCQ	Kansas City Cardiomyopathy Questionnaire
KCCQ-OS	Kansas City Cardiomyopathy Questionnaire-Overall Summary
LLOQ	Lower limit of quantification
LNP	Lipid nanoparticle
$\log_e$	Natural log transformation
LV	Left ventricular
MAR	Missing at random
mBMI	Modified body mass index
MCMC	Markov Chain Monte Carlo
MedDRA	Medical Dictionary for Regulatory Activities
MI	Multiple imputation
MMRM	Mixed effect model repeated measures
MNAR	Missing not at random
Norfolk QoL-DN	Norfolk Quality of Life - Diabetic Neuropathy
NT-proBNP	N-terminal prohormone B-type natriuretic peptide
NYHA	New York Heart Classification
OLE	Open label extension
PD	Pharmacodynamic
PEG_{2000}-C-DMG	3-N-[(ω-methoxy poly(ethylene glycol)2000) carbamoyl]-1,2-dimyristyloxy-propylamine
PK	Pharmacokinetic

Abbreviation	Definition
PMM	Pattern mixture model
PND	Polyneuropathy disability
PT	Preferred term
Q1	First quartile
Q3	Third quartile
QTc	Corrected QT
QTcF	Fridericia's cube-root corrected QT
SAE	Serious adverse event
SAP	Statistical analysis plan
SD	Standard deviation
SEM	Standard error of the mean
SI	International System of Units
siRNA	Small interfering RNA
SMQ	Standardized MedDRA Query
SOC	System organ class
TTR	Transthyretin
SUSAR	Suspected Unexpected Serious Adverse Reaction
ULN	Upper limit of normal
WHO	World Health Organization
WR	Win ratio
wt	Wild type
wtATTR	Wild type ATTR

1. INTRODUCTION

Transthyretin (TTR)-mediated amyloidosis (ATTR amyloidosis) is a rare, serious, life-threatening, multisystemic disease encompassing hereditary ATTR (hATTR) amyloidosis and wild type ATTR (wtATTR) amyloidosis, which result from either hereditary (genetic mutation) or nonhereditary (ageing) causes, respectively. In ATTR amyloidosis, deposition of TTR in various organs results in progressive, chronically debilitating morbidity and mortality. The most common manifestations of ATTR amyloidosis are polyneuropathy and cardiomyopathy (ie, ATTR amyloidosis with cardiomyopathy).

Patisiran is a small interfering RNA (siRNA) specific for TTR, which is formulated in a hepatotropic lipid nanoparticle (LNP) for intravenous (IV) administration [Akinc 2010]. The patisiran drug product (ALN-TTR02; patisiran-LNP, hereafter referred to as "patisiran") is designed to significantly suppress liver production of both wild type (wt) and all mutant forms of TTR, thereby having the potential to reduce amyloid formation and provide clinical benefit to patients with ATTR amyloidosis.

The APOLLO-B study (ALN-TTR02-011) is a Phase 3, randomized, double-blind, placebo-controlled, multicenter study designed to evaluate the efficacy and safety of patisiran in adult patients with ATTR amyloidosis with cardiomyopathy.

This statistical analysis plan (SAP) details comprehensive specifications of the efficacy, safety, pharmacodynamic (PD), and pharmacokinetic (PK) data summaries and statistical analyses in support of the clinical study report for Study ALN-TTR02-011. This SAP includes summaries and analyses specified and/or outlined in the protocol Amendment 2, dated 22 May 2020. Additional supportive analyses and changes to planned analyses are also included; notable changes are documented in Section 7. Changes to planned analyses made after database lock will be documented with justification in the clinical study report.

Table, figure, and listing specifications are contained in a separate document.

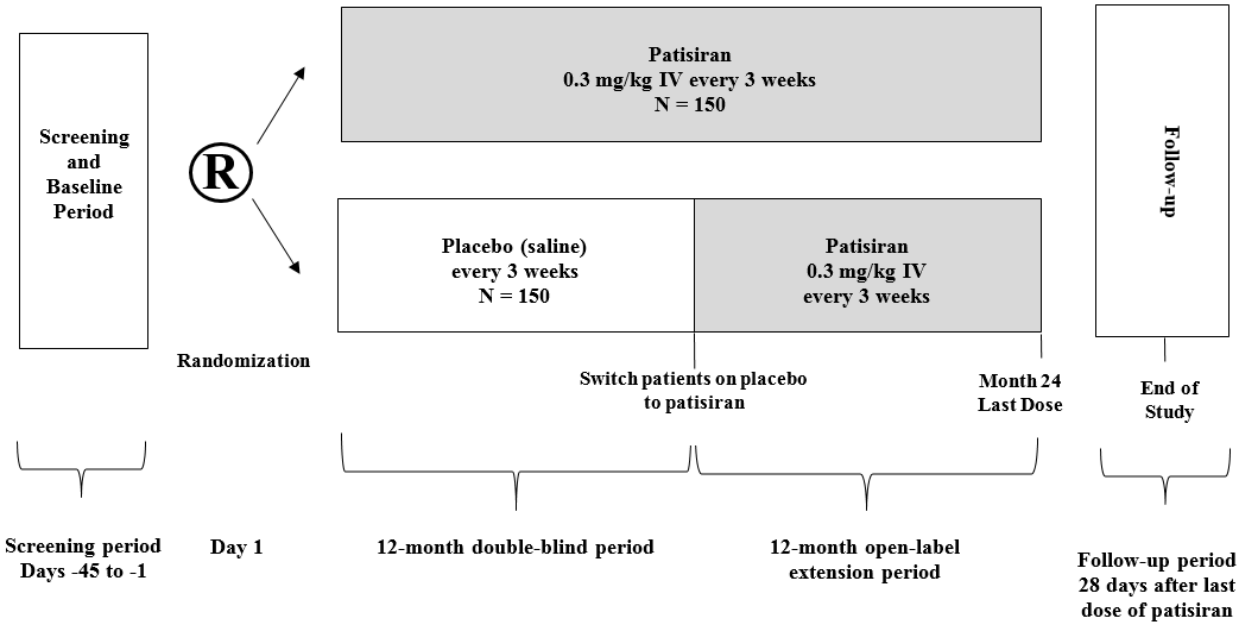
2. STUDY OVERVIEW

2.1. Synopsis of Study Design

The APOLLO-B study is a multicenter, multinational Phase 3 study designed to evaluate the efficacy and safety of patisiran in approximately 300 patients with ATTR amyloidosis (hereditary or wt) with cardiomyopathy; the study is comprised of a 1:1 randomized, double-blind, placebo-controlled period of 12 months followed by an open-label extension (OLE) period of 12 months to evaluate the long-term safety and efficacy of patisiran.

The study design schema is presented in Figure 1.

Figure 1: Study Design



2.2. Randomization Methodology

Using the interactive response system (IRS), patients will be randomized 1:1 to the patisiran or placebo arm. Randomization will be stratified by:

1. Baseline tafamidis (yes vs. no)
2. Type of amyloidosis (hATTR vs. wtATTR amyloidosis with cardiomyopathy)
3. New York Heart Classification (NYHA) Class I or II **and** age < 75 years vs. all other

Patients in the baseline tafamidis use category are defined as patients who are currently on tafamidis (for ≥6 months) with disease progression in the opinion of the investigator at baseline.

2.3. Blinding

Treatment assignments will be maintained by the IRS which has controlled access limited to unblinded team members and the unblinded pharmacist/designee preparing the infusion. Any unplanned unblinding occurring during the 12-month double-blind placebo-controlled treatment period (referred to as the 12m-DB period hereafter) will be documented and reported in the clinical study report.

Unblinding is only to occur in the case of patient emergencies or when necessary from a regulatory reporting perspective (eg, Suspected Unexpected Serious Adverse Reaction [SUSAR]), and after all patients have completed the 12m-DB period and the unblinded authorization has been executed. Details about the specifics of the blinding aspects for the study are outlined in the Randomization and Blinding Plan.

2.4. Study Procedures

The schedule of assessments is described in the study protocol (Table 1 and Table 2).

3. OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
Primary	
To evaluate the efficacy of patisiran compared with placebo treatment on functional capacity (6-minute walk test [6-MWT]) in patients with ATTR amyloidosis with cardiomyopathy	Change from baseline at Month 12 in 6-MWT
Secondary	
To evaluate the efficacy of patisiran compared with placebo treatment on: • Health status and health-related quality of life • Patient mortality, hospitalizations, and urgent heart failure (HF) visits	• Change from baseline at Month 12 in Kansas City Cardiomyopathy Questionnaire Overall Summary (KCCQ-OS) score • Composite endpoint of all-cause mortality, frequency of cardiovascular (CV) events (CV hospitalizations and urgent HF visits) and change from baseline in 6-MWT over the 12-month double-blind period • Composite endpoint of all-cause mortality and frequency of all-cause hospitalizations and urgent HF visits over the 12-month double-blind period
Exploratory	
To evaluate the efficacy of patisiran compared with placebo treatment on: • All-cause mortality and CV events • Cardiac biomarkers and biomarker-based risk assessment • Manifestations of cardiac amyloid involvement	• Composite endpoint of all-cause mortality and frequency of CV events (CV hospitalizations and urgent HF visits) over the 12-month double-blind period • Change from baseline at Month 12 in: – N-terminal prohormone B-type natriuretic peptide (NT-proBNP) – ATTR amyloidosis disease stage • Change from baseline at Month 12 in: – New York Heart Association (NYHA) Class – Echocardiographic parameters – Modified body mass index (mBMI) – Cardiac magnetic resonance (CMR) parameters – Technetium scintigraphy parameters – Troponin I levels

Objectives	Endpoints
	– Norfolk Quality of Life-Diabetic Neuropathy (Norfolk QoL-DN)
Pharmacodynamics (PD) and Pharmacokinetics (PK)	
• To evaluate the PD effect of patisiran on transthyretin (TTR) reduction • To determine the plasma concentration of patisiran and 2 lipid excipients • To assess presence of anti-drug antibodies (ADA)	• Change from baseline in serum TTR levels through Month 12 • Plasma PK exposure parameters (maximum plasma concentration at end of infusion [C_{max}], 30-minute post-infusion concentration [$C_{p(30min)}$], and pre-infusion concentration [C_{min}] • Frequency and titer of ADA
Safety	
• To evaluate the safety and tolerability of patisiran in patients with ATTR amyloidosis with cardiomyopathy	• Frequency of adverse events (AEs)

Scoring algorithms for the Kansas City Cardiomyopathy Questionnaire (KCCQ) and Norfolk Quality of Life-Diabetic Neuropathy (Norfolk QoL-DN) questionnaire are included in Appendix 9.3.1 and Appendix 9.3.2, respectively.

4. PATIENT POPULATION

The populations (analysis sets) are defined as follows:

- Full Analysis Set (FAS): All randomized patients who received any amount of study drug. Patients will be analyzed according to the treatment to which they were randomized.
- Safety Analysis Set: All randomized patients who received any amount of study drug. Patients will be summarized according to the treatment actually received.
- PK Analysis Set: All randomized patients who received at least one complete dose of study drug (see Section 6.4) and have at least 1 postdose blood sample for PK parameters and have evaluable PK data.
- PD Analysis Set: All randomized patients who received at least one complete dose of study drug (see Section 6.4) and who have an evaluable baseline and at least one evaluable post-baseline TTR sample.
- All Patisiran Treated Set: All randomized patients who received any amount of patisiran, including patients who took patisiran during the 12m-DB period and patients who first took placebo during the 12m-DB period and switched to patisiran during the OLE period.

The FAS will be used to evaluate efficacy endpoints. Safety during the 12m-DB period will be analyzed using the Safety Analysis Set. The PK and PD Analysis Sets will be used to conduct PK and PD analyses, respectively. The All Patisiran Treated Set will be used to summarize long-

term efficacy and safety data during patisiran treatment (see Section 5.12 for details). The number of patients included in all analysis sets will be provided.

5. GENERAL STATISTICAL METHODS

5.1. Determination of Sample Size

The planned enrollment for this study is 300 patients. For the change from baseline at Month 12 in the 6-MWT, assuming a treatment difference of 33 meters between patisiran and placebo in the treatment-naïve group and 20 meters in patients with baseline tafamidis, the weighted average treatment difference between patisiran and placebo in the overall population is approximately 29 meters (standard deviation [SD] = 75 meters), assuming 70% are in the treatment-naïve group and 30% are in the baseline tafamidis group. A sample size of 300 patients provides >90% power for a 2-sided test to detect a mean difference between treatment arms at a 2-sided alpha = 0.05. Additional patients may be enrolled based on a recommendation to increase the sample size in the interim sample size reassessment (see Section 5.11).

5.2. General Considerations

Categorical variables will be summarized using counts and percentages.

Continuous variables will be summarized using the following descriptive summary statistics: number of patients (n), mean, standard deviation (SD), standard error of the mean (SEM), median, first quartile (Q1), third quartile (Q3), minimum, and maximum. The precision of the measurement for each continuous variable will be used to determine the number of decimal places to present in tables, figures and derived listings. Minimum and maximum values will be reported with the same precision as the units of measure. The mean, SD, SEM, median, Q1 and Q3 will be reported to one greater decimal place. Any values that require transformation to standard units (metric or International System of Units (SI) units) will be converted with the appropriate corresponding precision.

The day of the first dose of study drug administered is defined as Day 1. Study Day is defined as the number of days between the day of the first dose of study drug (Day 1) and the specific time point. The Study Day of a time point of interest is calculated as follows.

If after Day 1, Study Day = date of interest – date of the first dose of study drug + 1

If prior to Day 1, Study Day = date of interest – date of the first dose of study drug

Study days are negative when the time point of interest is prior to Day 1, positive when time of interest is after Day 1. There is no Day 0. For example, the day before the first study drug dose is defined as Day -1.

For safety laboratory parameters, assessments collected and recorded as lower than the lower limit of quantification/detection will be replaced by the lower limit of quantification/detection. Any assessment collected and recorded as greater than the upper limit of quantification will be replaced by the upper limit of quantification.

For all analysis sets except for the All Patisiran Treated Set, summaries will be presented by treatment arm (patisiran and placebo).

For the All Patisiran Treated Set, summaries will be presented by the following groups:

- Patisiran/Patisiran: all patients who received patisiran during the 12m-DB including patients who continued to receive patisiran during the OLE period and patients who discontinued treatment during the 12m-DB period;
- Placebo/Patisiran: all patients who received placebo during the 12m-DB period and switched to patisiran in the OLE period;
- All Patisiran: all patients who received at least one dose of patisiran during either the 12m-DB or OLE periods.

5.3. Computing Environment

All statistical analyses will be conducted using SAS Version 9.4 or newer or R Version 3.4 or newer, unless otherwise noted.

5.4. Baseline Definitions

For 6-MWT, baseline will be defined as the last non-missing value available prior to the first dose of study drug.

For TTR, baseline will be defined as the average of all records collected on study, including those from any unscheduled visits, prior to the date and time of first dose.

For each parameter of the 12-lead electrocardiogram (ECG), baseline will be defined as the average of all available readings from the last visit prior to the first dose of study drug.

For all other parameters, baseline will be defined as the last non-missing value available prior to the first dose of study drug, unless otherwise specified.

For the All Patisiran Treated Set, baseline for patients who switched from placebo to patisiran will be redefined as the values prior to the first dose of patisiran:

- For TTR, the redefined baseline will be calculated as the mean of all TTR assessments performed on or after Month 9 in the 12m-DB period and prior to the first dose in the OLE period.
- For all the other endpoints, the redefined baseline will be defined as the last non-missing value available prior to the first dose in the OLE period, unless otherwise specified.

5.5. Randomization Stratification Factors

Stratification factors are recorded in both the IRS and the clinical database. In statistical analyses that use randomization stratification factors as covariates, the stratum assignment will reflect the values as recorded in the clinical database. In the presence of stratification errors, the stratification used in analysis may not match that in the IRS.

5.6. Efficacy Censoring Rules

5.6.1. Initiation of Tafamidis Treatment After Randomization (Tafamidis Drop-in)

Patients who are tafamidis-naïve at study entry may begin tafamidis treatment (ie, tafamidis drop-in) at any time during the study, which may confound the efficacy outcomes.

In the tafamidis ATTR-ACT study, the change from baseline in 6-MWT and KCCQ-OS started to diverge between tafamidis and placebo at Month 6; also, the survival curves for all-cause mortality and for CV-related hospitalizations did not begin to diverge between tafamidis and placebo until 18 months and 9 months, respectively [Maurer 2018].

As Month 6 was the first post-baseline assessment for 6-MWT and KCCQ-OS in the ATTR-ACT study, it is possible that the treatment effect was evident even earlier for these endpoints. Therefore, for the primary analysis of 6-MWT and KCCQ-OS, assessments collected from patients after taking tafamidis for more than 28 days will be treated as missing. These data will be included in listings with a footnote and will also be used in sensitivity analyses, as specified.

Given the 12-month period for the primary analysis of the composite endpoints, tafamidis drop-in is not expected to meaningfully alter a patient's time to death, hospitalization or urgent heart failure visit in that timeframe. Therefore, for the analysis of endpoints incorporating mortality, hospitalizations, and urgent HF visits through Month 12, events that occur after tafamidis drop-in will be included in analyses (ie, they will not be censored).

For cardiac biomarkers (NT-proBNP, troponin I), assessments collected from patients after taking tafamidis for more than 28 days will be treated as missing in the primary analysis. These data will be included in listings with a footnote and will also be used in sensitivity analyses, as specified. For other exploratory endpoints, data collected post tafamidis drop-in will be included in analyses.

A listing will be provided for patients who begin tafamidis treatment while on study.

5.6.2. Assessments and Events Impacted by Coronavirus Disease 2019 (COVID-19)

Patients who experience a serious COVID-19 AE may have worsening in general health and wellbeing that is not associated with the natural course of ATTR amyloidosis or with study drug. Assessments will be censored on or after the onset of a serious COVID-19 AE for the primary analyses of 6-MWT and KCCQ-OS.

Similarly, patients who miss multiple study drug infusions due to COVID-19 may not derive the full benefit of study drug. For the primary analyses of 6-MWT and KCCQ-OS, if >20% of the planned study drug infusions are missed due to COVID-19 prior to a planned assessment, the assessment will be treated as missing in the analysis (eg, missing ≥2 doses prior to the Month 6 assessment, missing ≥3 doses prior to the Month 9 assessment, and missing ≥4 doses prior to the Month 12 assessment).

For the analysis of the composite endpoint and component analyses related to mortality, hospitalizations and urgent HF visits, the events due to COVID-19 will be excluded from analyses. For patients who die due to COVID-19, their survival time will be censored at the date of death.

5.7. Visit Windows

Scheduled visits are expected to follow the protocol schedule. All data will be tabulated and analyzed per the evaluation visit as recorded on the electronic case report form (eCRF) even if the assessment is outside of the visit window.

For 6-MWT and KCCQ, assessments will be conducted at baseline, Month 6, Month 9, and Month 12 in the 12m-DB period and at Month 18, Month 21, and Month 24 in the OLE period. If the Month 12 assessment is not performed, assessments performed within ±1.5 months of the scheduled assessment (eg, from an unscheduled, pre-tafamidis drop-in, or early treatment discontinuation visit) but prior to the first dose of patisiran in the OLE period will be grouped with the scheduled Month 12 assessments for analysis. For all other post-baseline visits, if a scheduled post-baseline assessment is not performed, assessments performed within ±1.5 months of the scheduled assessment will be grouped with the scheduled assessments for that timepoint for analysis. If a patient begins tafamidis treatment (ie, tafamidis drop-in) after randomization but more than 1.5 months before Month 6, the pre-tafamidis drop-in assessment will be used to linearly interpolate the Month 6 value, unless otherwise specified. The derived visits will be used for all analyses.

For other efficacy assessments, if the scheduled post-baseline assessment is not performed, assessments performed within ±1.5 months of the scheduled assessment (eg, from an unscheduled, pre-tafamidis drop-in, or early treatment discontinuation visit, as applicable) will be grouped with the scheduled assessments for analysis. The derived visits will be used for all analyses.

Unless otherwise specified above, data collected at unscheduled visits will be included in by-patient data listings and figures, but no assignment to a study visit will be made for the purpose of by-visit summary tabulations. However, unscheduled visits may be used in the calculation of baseline values (as discussed in Section 5.4) and for inclusion in any categorical shift summaries (eg, shift from baseline to "worst" post-baseline value).

5.8. Multiple Comparisons/Multiplicity

Formal statistical hypothesis testing will be performed on the primary and secondary efficacy endpoints with all tests conducted at the nominal 2-sided 0.05 significance level. The overall familywise error rate will be controlled at the 2-sided 0.05 significance level for the primary and secondary endpoints by a hierarchical ordering procedure. Endpoints will be tested in the following pre-specified hierarchy:

1. 6-minute walk test (6-MWT) change from baseline at Month 12
2. Kansas City Cardiomyopathy Questionnaire Overall Summary (KCCQ-OS) score change from baseline at Month 12
3. Composite endpoint of all-cause mortality, frequency of CV events (CV hospitalizations and urgent HF visits), and change from baseline in 6-MWT over the 12-month double-blind period
4. Composite endpoint of all-cause mortality and frequency of all-cause hospitalizations and urgent HF visits over the 12-month double-blind period

There will be no multiplicity adjustment for exploratory endpoints.

5.9. Missing Data with Efficacy Endpoints

5.9.1. Summary of Missing Data

For the 6-MWT and KCCQ-OS efficacy endpoints, the number and percentage of patients with missing data, including due to COVID-19, at each scheduled visit will be summarized by treatment arm.

Time to treatment discontinuation in the 12m-DB period will be estimated descriptively using the Kaplan-Meier method by treatment arm. Patients who receive at least 1 dose of patisiran in the OLE period will be censored at the date of first dose in the OLE period.

5.9.2. Handling of Missing Data

For the 6-MWT and KCCQ-OS efficacy endpoints, the primary analysis will be based on the mixed-effects model repeated measures (MMRM) method, which makes use of fully and partially observed data sequences from individual patients by estimating the covariance between data from different time points. The MMRM will be implemented using an unstructured approach to modeling both the treatment-by-time means and the (co)variances, leading to what is essentially a multivariate normal model wherein treatment arm means at the primary time point are adjusted to reflect both the actually observed data and the projected outcomes from the patients with missing data [Mallinckrodt 2008]. In this primary analysis, missing data are assumed to be missing at random (MAR). Sensitivity analyses for these endpoints will be conducted to assess the impact of missing data as discussed in Section 6.6.1.2.

5.10. Analysis Cutoff and Database Lock

For the final 12m-DB analysis, as this study will be ongoing, the study database will be locked with all data up to a prespecified cutoff date quality controlled, ie, data in the electronic data capture (EDC) system will be frozen and external data such as laboratory and PK data will be quality controlled (and quality assured, where appropriate) and cleaned. Additional details regarding the database lock process are located in the study Data Management Plan.

The final 12m-DB analysis will include data on or prior to this prespecified cutoff date. For assessments with starting/ending dates (eg, AEs, medications, medical history), the starting date will be compared with the pre-specified cutoff date.

After the study is completed, ie, all patients either discontinue or complete the study, the database will be hard locked and all data collected will be used for analysis.

5.11. Interim Sample Size Reassessment

Patients who are tafamidis-naïve at baseline may opt to begin tafamidis treatment (ie, “tafamidis drop-in”) during the double-blind period, which could result in a loss of statistical power. An interim assessment may be performed to assess the impact of tafamidis drop-in rate on the power and the potential need to increase the sample size. The interim assessment, if conducted, would examine the tafamidis drop-in rate in a blinded manner (ie, without patients' real treatment assignments). The details of the interim assessment (if conducted) will be provided in a separate Interim Sample Size Reassessment Plan.

5.12. Analyses for the Entire Study

The study design includes a 12m-DB period and an OLE period. The primary objective is to evaluate the efficacy and safety of patisiran compared with placebo during the 12m-DB period. In addition, the long-term efficacy and safety of patisiran during the entire patisiran treatment period (beyond 12m-DB) will be characterized for the All Patisiran Treated Set.

The detailed definitions for different treatment periods are as follows.

- **12m-DB Period**

  The treatment comparison of patisiran versus placebo will focus on the 12m-DB period, defined as below:

  1. For patients who received at least one dose of patisiran during the OLE period, all assessments collected prior to the first dose of patisiran in the OLE period will be included in the 12m-DB period.
  2. For patients who discontinued treatment and did not receive any patisiran doses in the OLE period, all assessments will be included in the 12m-DB period. Assessments collected within given time windows (details listed in below table) will be included in summary tables/figures and those outside the time windows will be listed only, unless otherwise specified (eg, AEs in Section 6.9).

Endpoint	**Windowing rule for patients who did not enter OLE**
Hospitalization/urgent HF visit/death	Events occurring on or before Day 417
Other efficacy endpoints	Assessments collected on or before Day 417
PK/PD endpoints	Assessments collected within 28 days of the last dose
Safety endpoints	Assessments with onset date within 28 days of the last dose [1]

[1] In the rare situation where a patient did not discontinue treatment in the 12m-DB period and did not enter the OLE period, all data will be included in the summary tables/figures. For AE summaries, related AEs will be considered treatment-emergent and included in summary tables regardless of time window.

- **OLE Period**

  The start day of the OLE period is defined as the day when the first dose of the OLE period is administered. The assessments collected or AEs with onset date after the administration of the first dose in the OLE period will be included in the OLE period. When assessments or AE onset dates are exactly the date of first dose in the OLE period and the assessment or AE onset time is missing, the records will be included in the OLE period.

- **During Patisiran Treatment**

  For all patients who received at least one dose of patisiran, data will be summarized for the "during patisiran treatment" period, defined as below.

  1. For patients who received patisiran in the 12m-DB period, all assessments collected after the first dose of patisiran during the entire study, including both the 12m-DB

and OLE periods (if available), will be included in the "during patisiran treatment" period.

2. For patients who received placebo in the 12m-DB period and switched to patisiran in the OLE period, all assessments collected after the first dose of patisiran in the OLE period will be included in the "during patisiran treatment" period.
3. For patients who discontinued patisiran treatment during the 12m-DB period, the data handling will follow the same rules as discussed above for "12m-DB period". For patients who stopped participation in the study during the OLE period, assessments collected within given time windows (details listed in below table) will be included in summary tables/figures and those outside the time windows will be listed only, unless otherwise specified (eg, AEs in Section 6.9). For ongoing patients, all data will be included.

Endpoint	**Windowing rule for patients who discontinued patisiran in the OLE**
Hospitalization/urgent HF visit/death	Events occurring on or before Day 774 (end of follow-up window)
Other efficacy endpoints	Assessments collected within 28 days of the last dose
PD endpoint	Assessments collected within 24 days of the last dose
Safety endpoints	Assessments with onset date within 28 days of the last dose [1]

[1] For AE summaries, related AEs will be considered treatment-emergent and included in summary tables regardless of time window.

Longitudinal efficacy parameters will be summarized over the entire study, including the 12m-DB and OLE periods, for all patients to show the long-term efficacy of patisiran (for patients randomized to patisiran) as well as to show the trajectory changes comparing the placebo experience versus the patisiran experience (for patients randomized to placebo). In these summaries, patients will be analyzed according to the treatment to which they were randomized during the 12m-DB period.

6. STATISTICAL ANALYSIS

6.1. Patient Disposition

The number and percentage of patients in the following categories will be summarized by randomized treatment arm and overall as appropriate:

- Randomized
- Treated
- Completed Month 12 visit
- Discontinued treatment in the 12m-DB period and primary reason for treatment discontinuation and discontinuation due to COVID-19
- Stopped participation in the study in the 12m-DB period and primary reason for stopping participation and stopping participation due to COVID-19
- Entered the OLE period
- Discontinued treatment in the OLE period and primary reason for treatment discontinuation and discontinuation due to COVID-19
- Stopped participation in the study in the OLE period and primary reason for stopping participation and stopping participation due to COVID-19.

The number and percentage of patients enrolled by country and site will be summarized by randomized treatment arm and overall. The number and percentage of patients in each level of each randomization stratification factor as recorded in IRS and in the clinical database, and a comparison of the number and percentage of patients in each randomization stratification factor in IRS versus the clinical database will be summarized by randomized treatment arm and overall.

6.2. Demographics and Baseline Characteristics

Demographic and baseline characteristics, baseline disease characteristics, baseline efficacy parameters, and medical history information will be summarized by treatment arm and overall. No formal statistical comparisons will be performed.

Age at randomization, height, weight, body mass index (BMI), and mBMI will be summarized using descriptive statistics. Age group, sex, race, ethnicity, region, and country will be summarized by presenting the frequencies and percentages of patients in each category.

The following baseline disease characteristics will be summarized by presenting the numbers and percentages of patients in each category:

- Type of ATTR amyloidosis [hATTR; wtATTR]
- Genotype for hATTR patients
- Baseline tafamidis use [Yes; No]
- NYHA Class [I; II; III]
- NYHA Class I/II and age < 75 years [Yes; No]

- ATTR amyloidosis disease stage [1; 2; 3]
- Polyneuropathy disability (PND) score [0; I; II]
- Previous heart failure hospitalization [Yes; No]

The age at symptom onset and the time in years since diagnosis will be summarized, overall and by type of ATTR amyloidosis, using descriptive statistics. For patients who had at least 1 previous heart failure hospitalization, the age at first hospitalization for heart failure and the number of hospitalizations for heart failure in the previous 12 months will be summarized using descriptive statistics. For those who previously used tetramer stabilizers, the time from discontinuation of tetramer stabilizer to the start of study drug will be summarized using descriptive statistics. For patients in the baseline tafamidis group (per the clinical database), the time from the start of tafamidis therapy to the start of study drug will be summarized using descriptive statistics.

The number and percent of patients with each type of ATTR amyloidosis and with each genotype (for hATTR patients) will be summarized by country and treatment arm.

Medical history will be summarized by system organ class (SOC), high level term (HLT), and preferred term (PT). A patient contributes only once to the count for a given condition (overall, by SOC, by HLT, by PT).

All demographic and baseline data for each patient will be provided in data listings. Medical history data including general medical history, cardiac medical history, neuropathy history, historical inpatient admissions or urgent healthcare visits in the past 12 months, and ophthalmic medical history will be presented data listings. Screening test results will also be presented in data listings.

6.3. Protocol Deviations

A protocol deviation is any change, divergence, or departure from the study design or procedures defined in the clinical protocol. Protocol deviations will be classified by medical review prior to the final 12m-DB analysis, and major protocol deviations will be identified. A major protocol deviation is a deviation that may significantly impact the completeness, accuracy, and/or reliability of the trial data; that may significantly affect a subject's rights, safety, or well-being. (ICH. E3 R1 Structure and Contents of Clinical Study Reports Guidance for Industry. 2013) All major deviations related to trial inclusion or exclusion criteria, conduct of the trial, patient management or patient assessment will be described in the clinical study report.

The Sponsor or designee will be responsible for producing the protocol deviation file (formatted as a Microsoft Excel file). This file will include a description of each protocol deviation and whether or not this deviation is classified as a major protocol deviation. Since the study will be ongoing at the time of the primary analysis, there will be continuing review of protocol deviations. The file with all protocol deviations through the prespecified cutoff date for the final 12m-DB analysis will be finalized prior to the interim lock of the database and study unblinding. After the study is completed, the file with all protocol deviations for the study will be finalized prior to the hard-lock of the database.

All protocol deviations, COVID-19-related protocol deviations, and major protocol deviations will be summarized.

6.4. Drug Exposure

Exposure to study drug in months and the number of doses of study drug received will be summarized by treatment arm. Summaries of the numbers and percentages of patients with no missing infusions, and the number of missing infusions per patient will also be provided. The total volume infused will also be summarized.

The last date of exposure to study drug is defined as the earliest day of the following dates:

- Last dose date + 20 days
- Analysis cutoff date
- End of study date

Duration of exposure is defined as (the last date of exposure to study drug – date of the first dose +1)/30.4375. The exposure during the 12m-DB period is right censored by the date of the first OLE dose, ie, the last exposure day in the 12m-DB period is no later than the day before the first OLE dose. Similarly, the exposure during the OLE is left censored by the date of the first OLE dose, ie, the Day 1 of the OLE is the day of the first OLE dose. Dose interruptions and compliance are not taken into account for duration of exposure.

Study drug exposure data collected in the CRFs of study drug administration will also be summarized for each infusion. The numbers and percentages of patients with complete, partial, and missing dose administrations will be summarized. Complete and partial administration is defined as follows:

- Complete: ≥80% (≥160 mL) of the planned infusion volume (200 mL)
- Partial: >0% to <80% (>0 to <160 mL) of the planned infusion volume (200 mL).

The number of patients who experienced interruptions of infusions for any reason will be tabulated, as well as the number of patients with infusion interruptions due to an infusion-related reaction (IRR).

Dosing information for each patient will be presented in a data listing.

6.5. Premedications

All patients will receive premedications in order to reduce the potential of an IRR. Premedications will be coded using the WHO Drug Dictionary (March 2019 or later). Results will be tabulated by anatomical therapeutic class (ATC) and preferred term.

Premedication data will be presented in a data listing.

6.6. Efficacy Analyses

Efficacy endpoints will be analyzed using the FAS.

6.6.1. Primary Endpoint

The primary efficacy endpoint is to compare the change in 6-MWT from baseline to Month 12 between treatment arms. Only 6-MWT assessments confirmed as valid by the Colorado Prevention Center (6-MWT site training and oversight vendor) will be included in analyses. The

6-MWT value for patients unable to perform the walk due to progression of their amyloidosis will be imputed as 0. The change from baseline will then be calculated as 0 – baseline 6-MWT.

6.6.1.1. Primary Analysis using MMRM for the Full Analysis Set

The primary analysis will be performed using a REML-based MMRM approach for the FAS. The outcome variable is the change from baseline in 6-MWT; the model includes baseline 6-MWT as a continuous covariate, fixed effect terms including treatment arm, visit (Month 6, Month 9 or Month 12), treatment-by-visit interaction, baseline tafamidis use (yes vs. no), type of amyloidosis (hATTR vs. wtATTR), and age (<75 vs. ≥75), and patient as a random effect.

An unstructured covariance structure will be used to model the within-patient errors. If the model fails to converge, the following covariance structures will be specified in sequence and the first to converge will be used:

1. Toeplitz
2. Autoregressive (1)
3. Compound symmetry

The Satterthwaite approximation will be used to estimate the degrees of freedom.

The primary comparison is the contrast (difference in least squares means [LS means]) between the patisiran and placebo arms at Month 12. The analysis will be implemented with SAS PROC MIXED.

6.6.1.2. Sensitivity Analyses

Sensitivity analyses will be conducted using the following methods to assess the impact of missing data and the robustness of the primary analysis.

Pattern Mixture Model (PMM)

A sensitivity analysis using pattern mixture model (PMM) will be performed to assess the robustness of the primary MMRM results to the possible violation of the missing at random (MAR) missingness assumption. The PMM accommodates situations where the missingness mechanism may not be missing not at random (MNAR). The model will be based on the following assumptions for missing data at Month 12:

1. Patients who have missing data due to COVID-19, including patients who have missing assessments, who have data censored because of multiple missed doses due to COVID-19, who have data censored because a serious COVID-19 AE was reported before the assessment, or who die due to COVID-19 will be imputed assuming data are MAR. Under the hypothetical estimand of interest where the COVID-19 pandemic did not occur, these assessments should have been obtained with no COVID-19 impact. Missing data meeting these criteria will be imputed separately for each treatment arm using multiple imputation (MI) estimated from all non-missing data collected within each treatment arm.
2. Patients who have missing data unrelated to COVID-19:
    a. Placebo patients who have missing data: The missing data are considered MAR and will be imputed using multiple imputation (MI) estimated from placebo

patients. The imputation is done regardless of whether a patient was on-treatment or discontinued treatment before the scheduled efficacy assessment.

b. Patisiran patients who have missing data while on treatment: Patients are expected to continue to show benefit from treatment similar to that observed at the scheduled time point. Therefore, missing data during the on-treatment period (within 60 days of their last dose) are considered MAR and will be imputed using MI estimated from all non-missing data collected on treatment from the patisiran arm.
c. Patisiran patients who have missing data after stopping their study treatment: Patients will no longer benefit from treatment in the future and will have trajectory similar to placebo patients after discontinuing treatment. Therefore, missing data after treatment discontinuation (more than 60 days after last dose of study drug) will be imputed using the data from placebo patients using the copy reference (CR) approach.

Missing values will be imputed 100 times to generate 100 complete datasets using the procedures described above. An analysis of covariance (ANCOVA) model will be fit to each imputed dataset for the change from baseline in 6-MWT at Month 12. The ANCOVA model will include baseline 6-MWT as a continuous covariate and treatment arm, baseline tafamidis use (yes vs. no), type of ATTR amyloidosis (hATTR vs. wtATTR), and age (<75 vs. ≥75) as covariates. The LS mean and standard error of the mean (SEM) estimated from the ANCOVA model fit to each imputed dataset will be combined by applying Rubin's rules [Rubin 1987; Rubin 1996], using SAS PROC MIANALYZE to produce inferential results including the treatment difference in LS means, 95% confidence interval (CI) for the treatment difference, and the p-value. More details on the implementation of the PMM are discussed in Appendix 9.1.

MMRM Model Including All Censored Data

A sensitivity analysis including all 6-MWT assessments, ie, not censoring assessments after tafamidis drop-in (see Section 5.6.1) or assessments impacted by COVID-19 (see Section 5.6.2), will be conducted using the same MMRM model as used for the primary analysis.

6.6.1.3. Binary Analyses

The number and percentage of patients with a ≥0 meter increase in 6-MWT from baseline to Month 12 will be calculated for each treatment arm and compared between 2 arms using the Cochran-Mantel-Haenszel (CMH) test, stratified by baseline tafamidis use (yes vs. no); 6-MWT assessments collected from patients after taking tafamidis for more than 28 days will be treated as missing (see Section 5.6.1), and assessments impacted by COVID-19 will also be treated as missing (see Section 5.6.2). All patients with missing Month 12 data will be counted in the denominator with two exceptions: patients who are missing Month 12 due to tafamidis drop-in for reasons other than disease progression and patients who are missing Month 12 due to COVID-19 impact (see Section 5.6.2) will be excluded.

6.6.1.4. Overview of Primary Endpoint Analyses

The planned analyses of the primary endpoint 6-MWT are summarized in Table 1.

Table 1 Analysis of 6-MWT

Statistical Method
Primary analysis: MMRM
Sensitivity analysis: PMM
Sensitivity analysis: MMRM - including all censored data
Other analysis: Binary analysis using stratified CMH

6.6.2. Secondary Endpoints

The secondary efficacy endpoints are specified in Section 3. To control overall type I error, the secondary endpoints will be tested in a hierarchical order as described in Section 5.8.

Patients who undergo a heart transplantation and/or ventricular assist device placement will be handled in the same manner as death. CV-related events will include all hospitalizations adjudicated as being CV-related, all hospitalizations adjudicated as being indeterminate, and all emergency/unplanned/non-elective visits that are adjudicated as being urgent HF visits.

6.6.2.1. Change from Baseline at Month 12 in KCCQ-OS Score

The change from baseline at Month 12 in KCCQ-OS score will be analyzed using an MMRM model similar to the model described for the primary analysis of 6-MWT while adjusting for baseline KCCQ-OS as a continuous covariate. In addition, sensitivity analyses will be conducted using a PMM model and an MMRM model including data post tafamidis drop-in similar to the models described for the sensitivity analyses of 6-MWT.

6.6.2.2. Composite All-cause Mortality, Frequency of CV Events (CV hospitalizations and urgent HF visits), and Change from Baseline in 6-MWT over the 12-month Double-blind Period

The composite endpoint of all-cause mortality, frequency of CV events (CV hospitalizations and urgent HF visits) and change from baseline in 6-MWT will be analyzed using the stratified win ratio method [Dong 2018], stratified by baseline tafamidis use. This method makes within-stratum pairwise comparisons (for all possible patisiran/placebo patient pairs) of the 3 components in the hierarchical order specified above. Within a stratum, each patisiran patient will be compared with all placebo patients in a stepwise fashion, with the "winner" assigned a score of +1 and the "loser" assigned a score of "-1". See Section 5.6.2 for handling of deaths, hospitalizations, and urgent HF visits due to COVID-19 and 6-MWT assessments impacted by COVID-19.

Step 1: compare all-cause mortality at Month 12

- If both patients are deceased, then the patient with a longer survival time is assigned a +1 and the other is assigned a -1
- If one patient is alive and the other is deceased, the alive patient is assigned a +1 and the deceased patient is assigned a -1
- Proceed to step 2 if any of the following scenarios occur
  - Both patients are alive at Month 12

- Both patients have unknown vital status at Month 12
- One patient has unknown vital status and the other is deceased at Month 12

Step 2: compare frequency of CV events over the 12m-DB period

The comparison of the two patients will be performed at the shorter of their follow-up times.

- The patient with fewer CV events is assigned a +1 and the other is assigned a -1
- If the numbers of CV events are the same, go to step 3

Step 3: compare change from baseline in 6-MWT

- The patient who has less decline in 6-MWT at Month 12 is assigned a +1 and the other is assigned a -1
- If one or both patients have missing data at Month 12, compare change from baseline in 6-MWT at the latest common study visit where both patients had a 6-MWT measurement. The patient who has less decline in 6-MWT is assigned a +1 and the other is assigned a -1
- If tied or undetermined, assign score 0 to each patient

The point estimate of the stratified win ratio (WR) is defined as

$$WR = \frac{\sum_{m=1}^{2} n_t^{(m)} / N^{(m)}}{\sum_{m=1}^{2} n_c^{(m)} / N^{(m)}}$$

where $n_t^{(m)}$ and $n_c^{(m)}$ are the number of patisiran/placebo pairs in stratum m in which the patisiran patient was the winner and in which the placebo patient was the winner, respectively, and $N^{(m)}$ is the total number of patients in stratum m.

A 95% CI and p-value for the stratified win ratio will be estimated (see Section 9.2 for details).

6.6.2.3. Composite All-cause Mortality and Frequency of All-cause Hospitalizations and urgent HF visits over the 12-month Double-blind Period

The composite endpoint of all-cause mortality and frequency of all-cause hospitalizations and urgent HF visits will be analyzed using an Andersen-Gill model including treatment, baseline tafamidis use (yes vs. no), type of amyloidosis (hATTR vs. wtATTR), NYHA Class (I/II vs. III), and age (<75 vs. ≥75 years) as covariates. See Section 5.6.2 for handling of deaths, hospitalizations and urgent HF visits due to COVID-19.

The components of the composite endpoint will be analyzed as follows. For all-cause mortality, Kaplan-Meier survival curves for each treatment group will be presented. All-cause mortality will also be analyzed using a Cox proportional hazards model including treatment as a covariate. Frequency of all-cause hospitalizations and urgent HF visits will be analyzed using Poisson regression with treatment, baseline tafamidis (yes vs. no), type of amyloidosis (hATTR vs. wtATTR), NYHA Class (I/II vs. III), and age (<75 vs. ≥75 years) as covariates, adjusting for duration of follow-up (ie, include duration of follow-up as an offset).

6.6.3. Exploratory Endpoints

All analyses of exploratory endpoints will be conducted on the FAS. For continuous endpoints, descriptive statistics will be provided for actual value and change from baseline by treatment arm; descriptive statistics for percentage change from baseline by treatment arm may also be provided, as appropriate.

The composite endpoint of all-cause mortality and frequency of CV events (CV hospitalizations and urgent HF visits) over the 12m-DB period will be analyzed using an Andersen-Gill model, including treatment, baseline tafamidis use (yes vs. no), type of amyloidosis (hATTR vs. wtATTR), NYHA Class (I/II vs. III), and age (<75 vs. ≥75 years) as covariates. See Section 5.6.2 for handling of deaths, hospitalizations, and urgent HF visits due to COVID-19. The frequency of CV events component will also be analyzed using Poisson regression with treatment, baseline tafamidis (yes vs. no), type of amyloidosis (hATTR vs. wtATTR), NYHA Class (I/II vs. III), and age (<75 vs. ≥75 years) as covariates, adjusting for duration of follow-up.

The change from baseline at Month 12 in select echocardiographic parameters (mean left ventricular (LV) wall thickness, LV mass, and global LV longitudinal strain) and Norfolk QoL-DN Total Score (see Section 9.3.2) will be analyzed using an ANCOVA model since they are only measured in the 12m-DB period at Month 12. The ANCOVA model will include the baseline value for the parameter as a continuous covariate and treatment arm, baseline tafamidis use (yes vs. no), type of amyloidosis (hATTR vs. wtATTR), NYHA class (I/II vs. III), and age (<75 vs. ≥75) as covariates. The Norfolk QoL-DN questionnaire is a patient-reported outcomes measure that is sensitive to the different features of diabetic neuropathy; since patients enrolled in this study are not required to have a history of neuropathy, the ANCOVA model for Norfolk QoL-DN Total Score may be repeated on the subset of patients with a history of neuropathy.

In the 12m-DB period, NT-proBNP and troponin I are measured at baseline, Month 3, Month 6, Month 9, and Month 12, and mBMI is measured at baseline, Month 6, and Month 12. NT-proBNP, troponin I, and mBMI will be analyzed using MMRM models similar to the model described for the primary analysis of 6-MWT while adjusting for the baseline value of the endpoint being modeled. NT-proBNP and troponin I assessments collected from patients after taking tafamidis for more than 28 days will be treated as missing in the primary analysis (see Section 5.6.1). Modified BMI assessments will not be censored after tafamidis drop-in. Sensitivity analyses including all data, ie, not censoring assessments after tafamidis drop-in, will be conducted for NT-proBNP and troponin I using the same MMRM model as used for the primary analysis.

NT-proBNP has been shown to be highly skewed in the literature; a Q-Q plot of the residuals of the NT-proBNP MMRM model specified above will be used to assess the normality assumption. If the normality assumption is violated, a natural log transformation ($\log_e$) will be applied to NT-proBNP to normalize the distribution. Following the transformation, an MMRM model similar to the model described for the primary analysis of 6-MWT will be used; the outcome variable will be $\log_e$(post-baseline) – $\log_e$(baseline), and the model will include $\log_e$(baseline) as a continuous covariate. Adjusted geometric mean fold-changes from baseline with 95% CIs will be constructed by exponentially back-transforming the LS means, differences in LS means, and the limits of the corresponding 95% CIs.

CMR and technetium scintigraphy will be performed in a subset of patients at select sites to assess cardiac amyloid involvement. The analyses for these imaging assessments are detailed in the separate APOLLO-B Imaging Statistical Analysis Plan.

Categorical exploratory parameters, including ATTR amyloidosis disease stage and NYHA class will be descriptively summarized by presenting the number and percentage of patients in each category for each visit. The number and percentage of patients with improving, no change, and worsening in these parameters at each visit will also be summarized.

Data collected during the OLE period will be summarized descriptively.

6.6.4. Subgroup Analysis

Subgroup analyses will be conducted to assess the consistency of treatment effect within various subgroups defined by the following baseline characteristics:

- Age [<75; ≥75 at randomization]
- Baseline tafamidis use [Yes; No]
- Type of amyloidosis [hATTR; wtATTR]
- NYHA class [I/II; III]

Subgroup analyses will be performed for the primary endpoint 6-MWT and KCCQ-OS using MMRM models with baseline value score as a continuous covariate; subgroup analyses by age, type of amyloidosis, and NYHA class will also include baseline tafamidis use (yes vs. no) as a factor in the MMRM models. If the number of patients in either treatment arm of a subgroup is less than 10, only descriptive statistics will be presented. A forest plot will be generated to illustrate the estimated treatment effect along with 95% CI within each subgroup.

Other subgroups may be examined, if deemed appropriate. The subgroup analyses may also be performed for other efficacy endpoints.

6.7. Pharmacodynamic Analysis

The PD parameter for this study is serum TTR. For all analyses of post-baseline TTR data, only post-baseline TTR assessments collected within 24 days (inclusive) after receiving a full dose of study drug (ie, the amount infused was ≥80% of the planned infusion volume) will be summarized.

Summary tables will be provided for observed values, changes and percentage changes from baseline for each scheduled time point by treatment arm.

The maximum percentage reduction and mean percentage reduction over 12 months will be summarized using descriptive statistics. Subgroup analysis will be provided for age (<75 vs. ≥75), sex (male vs. female), type of amyloidosis (hATTR vs. wtATTR), and baseline tafamidis use (yes vs. no).

All PD data will be displayed in data listings.

6.8. Pharmacokinetic Analysis

6.8.1. Study Variables

6.8.1.1. Concentration Data

Plasma concentrations of the 3 PK analytes (ALN-18328, DLin-MC3-DMA and PEG_{2000}-C-DMG) will be obtained. Concentration values that are below the limit of quantification (LLOQ or BLQ) will be set to zero for analysis.

6.8.1.2. Pharmacokinetic Parameters

The following plasma concentrations of ALN-18328 and the two lipids will be summarized by study visit:

- Observed post-infusion peak concentration (C_{max})
- Observed 30 min post-infusion concentration ($C_{p(30min)}$)
- Observed pre-infusion trough concentration (C_{min})

In addition, steady-state C_{max} (C_{max_ss}), steady-state C_{min} (C_{min_ss}) and steady-state $C_{p(30min)}$ ($C_{p\_ss(30min)}$) will be calculated as the average of the C_{max}, C_{min}, and $C_{p(30min)}$ values, respectively, at Week 24, Week 36, and Week 51.

6.8.2. Statistical Methods

Descriptive statistics for PK parameters will include the number of patients, mean, SD, coefficient of variation, median, minimum, maximum, geometric mean and geometric coefficient of variation.

The C_{max}, $C_{p(30min)}$ and C_{min} of the 3 analytes will be summarized by nominal sampling day. Mean concentrations (+/- SD) will be plotted versus nominal sampling time.

Steady-state PK parameters for the 3 analytes will be summarized overall and by subgroup, including age (<75 vs. ≥75), gender (male vs. female), and anti-drug antibody status (positive vs. negative).

Plasma concentration data will be presented in by-patient listings.

The PK-PD relationship between the plasma concentration of ALN-18328 and the percent change from baseline in serum TTR may be explored.

Mean and maximum percent TTR reduction from baseline will be summarized by quartiles of the steady state PK parameters for the 3 analytes. Change from baseline at Month 12 in clinical efficacy parameters may also be summarized by quartiles of the steady-state PK parameters for the 3 analytes.

PK exposure will be summarized by mortality status. In addition, the incidence of AEs and serious AEs (SAEs) will be summarized by quartiles of the steady-state PK parameters for the 3 analytes.

Population PK, PK/PD, and disease progression modeling analyses may be performed, if appropriate. If performed, the analyses will be described in a separate analysis plan and reported separately.

6.9. Safety Analysis

An adverse event is any untoward medical event associated with the use of a study drug, whether or not it is considered related to the study drug. The primary safety parameter is the frequency of AEs. Safety parameters also include vital signs, ECGs, clinical laboratory assessments, and physical exams. Analyses for safety parameters will be conducted using the Safety Analysis Set.

Time windows for safety data to be analyzed for the 12m-DB period and the entire study including both 12m-DB and OLE periods as well as safety follow-up are described in Section 5.12 . All safety data, regardless of time windows, will be listed and summarized for selected endpoints, ie, AEs by SOC and PT, SAEs by SOC and PT, and selected laboratory parameters.

Subgroup analysis for safety variables may be conducted if deemed appropriate and necessary.

No inferential safety analysis is planned.

6.9.1. Adverse Events

AEs will be classified by the MedDRA coding system (version 23.0 or later) and displayed in tables and data listings using SOC and PT.

Analyses of AEs will be performed for those events that are considered treatment-emergent, where treatment-emergent is defined as any AE with onset during or after the administration of study drug through 28 days following the last dose of study drug. In addition, any event that was present at baseline but worsened in intensity or was subsequently considered drug-related by the Investigator through the end of the study will be considered treatment-emergent. Events with a fully or partially missing onset date will be assumed to be treatment emergent unless it can be unequivocally determined (from the partial onset date and/or a partial or complete stop date) that the event occurred prior to the first administration of study drug.

Adverse events will be summarized by the numbers and percentages of patients reporting a given AE. A patient contributes only once to the count for a given AE (overall, by SOC, by PT). Overall event counts and frequencies may also be summarized.

An overall summary of AEs will include the number and percentage of patients with any AE, any AE assessed by the Investigator as related to treatment, any severe AE, any severe AE related to treatment, any SAE, any SAE related to treatment, any AE leading to treatment discontinuation, any study drug related AE leading to treatment discontinuation, any AE leading to study withdrawal, any study drug related AE leading to study withdrawal, and any deaths.

Tabulations by SOC and PT will be produced for the following. The SOC and PT within each SOC will be presented alphabetically.

- All AEs;
- Severe AEs;
- All SAEs;
- AEs related to treatment;
- SAEs related to treatment;

- AEs leading to infusion interruption;
- AEs leading to treatment discontinuation;
- SAEs leading to treatment discontinuation;
- AEs leading to study withdrawal;
- SAEs leading to study withdrawal;
- AEs related to pre-medication;
- AEs over time.

Tabulations by PT in decreasing order in frequency in the patisiran arm will be produced for the following:

- All AEs;
- All SAEs;
- AEs related to treatment;
- SAEs related to treatment.

AEs and SAEs will also be summarized by maximum severity and by maximum relationship; patients who report multiple occurrences of the same AE (preferred term) will be classified according to the most severe occurrence or the most related occurrence, respectively. Similarly, AEs related to treatment will be summarized by maximum severity.

Infusion-related reaction signs and symptoms will be summarized by SOC and PT. The incidence and frequency of IRR signs and symptoms over time will also be summarized by SOC and PT.

AEs mapping to the Drug Related Hepatic Disorder standardized MedDRA query (SMQ) will be summarized by SOC and PT. AEs mapping to the Anaphylactic Reaction SMQ will be summarized by PT. AEs mapping to the SMQ Malignant or Unspecified Tumors will be summarized by HLT and PT. Other SMQs or AE groupings may be evaluated.

All AEs will be presented in patient data listings. Separate listings will be provided for death, SAEs, AEs leading to treatment discontinuation, IRR signs and symptoms, AEs with missing severity, AEs with missing relationship to study drug, AEs related to pre-medications, and AEs mapping to the SMQs as described above.

Additional AE considerations regarding COVID-19 are detailed in Section 6.11.3.

Ophthalmological assessments may be performed if a patient develops ocular symptoms suggestive of vitamin A deficiency. The ophthalmological assessment results will be presented in a listing.

Patients who underwent heart transplant and/or ventricular assist device placement will be presented in a listing.

6.9.2. Laboratory Data

Clinical laboratory values will be expressed in SI units. Missing laboratory data will not be imputed.

Summary data for each laboratory parameter will be presented for each continuous clinical laboratory parameter (including hematology, serum chemistry, liver function tests and coagulation studies). Descriptive statistics will be presented for the actual values, change from baseline, and percent change from baseline by visit.

Shift tables will be employed to summarize the baseline category versus the "worst" post-baseline category for selected parameters.

A listing for all patients with abnormal liver function tests, defined as an ALT >3×ULN, AST >3×ULN, or total bilirubin >2×ULN at any time point, will also be provided.

A table will be produced to summarize the number and percentage of patients in each of the below categories at any post-baseline time point.

- ALT >1 & ≤3, >3 & ≤5, >5 & ≤10, >10 & ≤20, >20×ULN;
- AST >1 & ≤3, >3 & ≤5, >5 & ≤10, >10 & ≤20, >20×ULN;
- ALT or AST >1 & ≤3, >3 & ≤5, >5 & ≤10, >10 & ≤20, >20×ULN;
- ALP > 1.5×ULN;
- Total Bilirubin >1.5 & ≤2, >2 & ≤3, >3 & ≤5 and >5×ULN;
- Total Bilirubin > 2×ULN concurrent with ALT or AST > 3×ULN.

In separate figures, the peak total bilirubin (at any time post-baseline) will be plotted against the peak AST, the peak ALT, and the peak AST or ALT levels at any time post-baseline.

For hematology and serum chemistry, summary tables of potentially clinically significant abnormalities will be provided. The results may also be graded according to the National Cancer Institute CTCAE Version 5.0 or above. A shift summary of baseline to maximum post-baseline CTCAE grade may be presented, as appropriate.

The estimated glomerular filtration rate (eGFR, mL/min/1.73 m^2) will be categorized as below: ≥ 90; 60-89; 45-59; 30-44; 15-29 and < 15. A shift summary of baseline to worst post-baseline eGFR category will be presented.

All laboratory data, including pregnancy and follicle stimulating hormone (FSH) test results, will be provided in data listings. Out-of-range laboratory results will be identified in the listings.

6.9.3. Vital Signs and Physical Examination

For vital signs, descriptive statistics by visit and treatment arm will be provided for each variable. Vital sign measurements will be presented for each patient in a data listing, with abnormal vital signs flagged.

For the physical examination, clinically significant findings prior to first dose of study drug will be recorded and summarized under Medical History, unless there is an SAE in which case the event will be recorded and summarized under Adverse Events. Physical examination findings that are new or worsened after first dose of study drug will be recorded and summarized under Adverse Events.

6.9.4. Electrocardiogram

Electrocardiogram (ECG) findings will include rhythm, ventricular rate, RR interval, PR interval, QRS duration, QT interval, and corrected QT (QTc) interval. QTc interval will be calculated using Fridericia's correction formula.

Fridericia's cube-root corrected QT: QTcF (ms) = QT (ms) $\times \sqrt[3]{\frac{HR(bpm)}{60}}$.

RR, PR, QRS, QT, and QTcF intervals and their change from pre-dose baseline will be summarized for each treatment arm by scheduled visit. The number and percentage of patients with normal, abnormal, and clinically significant abnormal results will also be summarized by treatment arm and scheduled visit.

Patients will be categorized into ≤ 450, > 450 - 480, > 480 - 500, or > 500 ms per their maximum post-baseline absolute QTcF interval and ≤ 30, > 30 - 60, or > 60 ms per their maximum change from baseline QTcF interval. Parameters from an ECG assessment will be treated as missing in the summaries if the QRS duration is >120 ms. The number and percentage of subjects in each category will be summarized for each treatment arm.

All ECG data for each patient will be provided in a data listing.

6.9.5. Prior and Concomitant Medications

Prior and concomitant medications will be coded using the WHO Drug Dictionary (March 2020 or later). Prior medications include medications taken ≥ 1 time before the first dose of study drug, regardless of medication end date. Concomitant medications include medications taken ≥ 1 time on or after the first dose of study drug, regardless of medication start date. Results will be tabulated by ATC and preferred term.

When there are partial or missing dates, imputed dates will be used to determine 1) if a medication is prior or concomitant, and 2) duration of exposure to select medications, as needed. Imputed dates will not be presented in the listings.

For medications with partial start or stop dates: the first day/month will be imputed for start date, and the last day/month will be imputed for stop date. For medications with a completely missing start date, the medications will be considered as started prior to the first dose of study drug; medications will be classified as prior, concomitant or both depending on the medication stop dates. For medications with a completely missing stop date, the end of study date will be imputed.

Prior and concomitant medications will be presented in data listings. Previous tetramer stabilizer use will be listed separately.

6.10. Anti-Drug Antibody

The number and percentage of patients with confirmed positive ADA assay results at any time during study as well as at each scheduled visit will be summarized. The titer results for patients with confirmed positive ADA results will also be summarized using descriptive statistics.

ADA data and patients with confirmed positive ADA results will be presented in data listings.

6.11. COVID-19 Pandemic Impact Analyses

Additional data were collected to characterize the impact of the COVID-19 pandemic on general study conduct and disposition, and subsequently, additional analyses and summaries will be provided in acknowledgement of multiple regulatory guidances (FDA Guidance on Conduct of Clinical Trials of Medical Products during COVID-19 Pandemic: Guidance for Industry, Investigators, and Institutional Review Boards, US Food and Drug Administration, 2020; Guidance on the Management of Clinical Trials during the COVID-19 (Coronavirus) Pandemic, European Medicines Agency, 2020; Points to consider on implications of Coronavirus disease (COVID-19) on methodological aspects of ongoing clinical trials, European Medicines Agency, 2020; Statistical Considerations for Clinical Trials During the COVID-19 Public Health Emergency, US Food and Drug Administration, 2020).

Few patients were enrolled on APOLLO-B prior to the beginning of the COVID-19 global pandemic.

6.11.1. General Impact

Patients who discontinue treatment or stop study participation due to COVID-19 will be included in patient disposition summaries as described in Section 6.1.

Impact on study participation due to COVID-19, including visit completion, visit location changes, and study drug dosing changes, will be summarized overall on the patient level with both continuous and categorical descriptives, and overall and by visit on the event level with categorical descriptives. Patient- and event-level summaries of the impact on study participation due to COVID-19 may also be generated by site and/or region.

Impact on study participation due to COVID-19 will be presented in data listings by patient and by visit within patient.

6.11.2. Impact on Efficacy

Summaries of missing efficacy data due to the COVID-19 pandemic will be included in missing efficacy data summaries as described in Section 5.9.1. Patient data listings will flag assessments censored due to COVID-19.

The occurrence of multiple missed study drug infusions due to COVID-19 or the occurrence of a serious COVID-19 AE on or prior to an assessment may confound the efficacy outcomes. Therefore, 6-MWT and KCCQ-OS assessments collected in these scenarios will be censored (treated as missing) in the primary analyses of 6-MWT and KCCQ-OS (see Section 5.6.2). For the analysis of the composite endpoint and component analyses related to mortality, hospitalizations and urgent HF visits, the events due to COVID-19 will be excluded from analyses (see Section 5.6.2). The number and percentage of patients who died, were hospitalized or had an urgent HF visit due to COVID-19 will be summarized by treatment arm and overall. The total number of hospitalizations and urgent HF visits due to COVID-19 and the number of hospitalizations and urgent HF visits per patient due to COVID-19 will also be summarized using descriptive statistics by treatment arm and overall. Patient data listings will be presented for deaths, hospitalizations and urgent HF visits due to COVID-19.

Given the measures specified in the protocol designed to ensure data integrity, analyses excluding patients with COVID-19 related protocol deviations will not be prespecified, but may be considered post hoc, if warranted.

6.11.3. Impact on Adverse Events

An overall summary of AEs mapping to a COVID-19 custom query will include the number and percentage of patients, as well as events and event rates, with any AE, any AE assessed by the Investigator as related to treatment, any severe AE, any severe AE related to treatment, any SAE, any SAE related to treatment, any AE leading to treatment discontinuation, any study drug related AE leading to treatment discontinuation, any AE leading to study discontinuation, any study drug related AE leading to study discontinuation, and any deaths. AEs mapping to the COVID-19 custom query will be summarized by HLT and PT. Due to the evolving nature of COVID-19-related MedDRA terminology, the COVID-19 custom query will be based on the latest information available at the specified analysis timepoint.

AEs mapping to the COVID-19 custom query will also be presented in a data listing.

6.11.4. Efficacy and Safety Summaries by Pandemic Phase

The scope of the COVID-19 pandemic is rapidly evolving. Efficacy and safety summaries by pandemic phase will be produced, as appropriate. Details of these summaries will be provided in a future SAP amendment.

7. CHANGES TO PLANNED ANALYSES

Change from Protocol	Detailed Description/Rationale
In the PK and PD Analysis Set definitions, "any amount of study drug" (as specified in the protocol) was updated to "at least one complete dose of study drug".	PK and PD data from patients who have not received at least one complete dose of study drug would not be representative of the planned dose level.
The model specified to analyze the composite endpoint of all-cause mortality and frequency of all-cause hospitalizations was updated from an Andersen-Gill model stratified by baseline tafamidis (as specified in the protocol) to an Andersen-Gill model including baseline tafamidis in the list of covariates.	Given the cap on baseline tafamidis for the study, a stratified Andersen-Gill model including several covariates may encounter convergence issues. Therefore, the model now includes baseline tafamidis use in the list of covariates.

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9. APPENDICES

9.1. Detailed Statistical Methodology for the Pattern Mixture Model

Patients are classified into three distinct patterns for missing data at Month 12:

1. Placebo patients: missing data will be assumed to be MAR and imputed by using MI estimated from placebo patients
2. Patisiran patients who have either missing data related to COVID-19 or missing data with the last dose of study drug received within 60 days of the scheduled time point: missing data will be assumed to be MAR and imputed by using MI estimated from on treatment patisiran patients
3. Patisiran patients who have missing data unrelated to COVID-19 and the last dose of study drug was received more than 60 days prior to the scheduled time point: missing data will be assumed to be MNAR and imputed using the copy reference (CR) approach

As a first step, patients with missing data at Month 12 will be assigned to a missing data pattern based on the criteria above.

Next, missing data for patients in patterns 1 and 2 above will be imputed using MI. Since the pattern of missing data within patients may be non-monotone, multiple imputation will be conducted among patisiran and placebo patients separately using the Markov Chain Monte Carlo (MCMC) method. For each treatment arm, the imputation model will include baseline tafamidis use, type of amyloidosis, NYHA class, age at randomization, ATTR amyloidosis disease stage, baseline 6-MWT, and all calculated values of change from baseline in 6-MWT at pre-specified visits. Below is a sample SAS code for the multiple imputation:

```
proc mi data=DATAIN out=DATA_STEP1 seed=234 nimpute=100;
   by treatment;
   em maxiter=300 converge=1e-4 itprint outem=outem;
   var baseline_variables 6MWT_base 6MWT_chg_m6 6MWT_chg_m9 6MWT_chg_m12;
   mcmc chain=multiple initial=em;
run;
```

For patisiran patients, the input dataset DATAIN will include only data collected while on treatment. Any data collected from patisiran patients after treatment discontinuation will be excluded from the input dataset. The MI procedure generates imputed values for all missing values. For patients in pattern 3, imputed scores will be discarded and replaced by imputed values using the CR approach. The imputation model will include baseline tafamidis use, type of amyloidosis, NYHA class, age at randomization, ATTR amyloidosis disease stage, baseline 6-MWT, and all calculated values of change from baseline in 6-MWT at pre-specified visits.

```
proc mi data=DATA_STEP1_2 out=DATA_STEP2 nimpute=1 seed=xxx;
   by _imputation_;
   class treatment;
   fcs nbiter=30 reg (6MWT_chg_m12 = baseline_variables 6MWT_base
      6MWT_chg_m6 6MWT_chg_m9);
   mnar model (6MWT_chg_m12 / modelobs=(treatment='placebo'));
   var baseline_variables 6MWT_base 6MWT_chg_m6 6MWT_chg_m9 6MWT_chg_m12;
run;
```

9.2. Calculation of Stratified Win Ratio p-value and Confidence Interval

Dong et al (2018) note that the logarithm of the stratified win ratio is asymptotically normally distributed with mean $\upsilon_{\log(WR)}$ and variance $\sigma^2_{\log(WR)}$, with these terms defined in equations 7b and 8 in their paper, respectively. The point estimate of the mean, $\hat{\upsilon}_{\log(WR)}$, is the logarithm of the stratified win ratio statistic defined in Section 6.6.2.2. The variance estimate, $\hat{\sigma}^2_{\log(WR)}$, is calculated under the null hypothesis of the same treatment effect in the patisiran and placebo groups.

Then $\hat{z} = \frac{\hat{\upsilon}_{\log(WR)}}{\sqrt{\hat{\sigma}^2_{\log(WR)}}}$ is a standard normal deviate from which the p-value is readily obtained.

The 95% confidence interval for the logarithm of the stratified win ratio is constructed as

$$\hat{\upsilon}_{\log(WR)} \pm 1.96 * \sqrt{\hat{\sigma}^2_{\log(WR)}}$$

and the limits of this confidence interval will then be exponentiated to construct the 95% confidence interval for the stratified win ratio.

9.3. Questionnaire/Scoring

In questionnaires, if multiple responses are provided to a single-response question, the question is deemed as missing.

9.3.1. Kansas City Cardiomyopathy Questionnaire (KCCQ)

The Kansas City Cardiomyopathy Questionnaire is a 23-item self-administered questionnaire developed to independently measure the patient's perception of their health status, which includes heart failure symptoms, impact on physical and social function, and how their heart failure impacts their quality of life within a 2-week recall period.

There are 10 summary scores for the KCCQ tool, which are calculated as follows:

1. Physical Function

Code responses to each of Questions 1a-1f as follows:

- Extremely limited = 1
- Quite a bit limited = 2
- Moderately limited = 3
- Slightly limited = 4
- Not at all limited = 5
- Limited for other reasons or did not do the activity = <missing value>

If at least 3 of Questions 1a-1f are not missing, then compute

- Physical function score = [(mean of the non-missing Questions 1a-1f) – 1]/4 * 100

2. Symptom Stability

Code the response to Question 2 as follows:

- Much worse = 1
- Slightly worse = 2
- Not changed = 3
- Slightly better = 4
- Much better = 5
- I've had no symptoms over the last 2 weeks = 3

The symptom stability score = [(Question 2) – 1]/4 * 100.

3. Symptom Frequency

Code responses to Questions 3, 5, 7 and 9 as follows:

- Questions 3 and 9
  - Every morning/night = 1
  - 3 or more times a week but not every day = 2
  - 1-2 times a week = 3
  - Less than once a week = 4
  - Never over the past 2 weeks = 5
- Questions 5 and 7
  - All of the time = 1
  - Several times a day = 2
  - At least once a day = 3
  - 3 or more times a week but not every day = 4
  - 1-2 times a week = 5
  - Less than once a week = 6
  - Never over the past 2 weeks = 7

If at least two of Questions 3, 5, 7 and 9 are not missing, then compute

- S3 = [(Question 3) – 1]/4
- S5 = [(Question 5) – 1]/6
- S7 = [(Question 7) – 1]/6
- S9 = [(Question 9) – 1]/4

The symptom frequency score = (mean of S3, S5, S7, S9)*100.

4. Symptom Burden

Code responses to Questions 4, 6 and 8 as follows:

- Extremely bothersome = 1
- Quite a bit bothersome = 2
- Moderately bothersome = 3
- Slightly bothersome = 4
- Not at all bothersome = 5
- I've had no swelling/fatigue/shortness of breath = 5

If at least one of Questions 4, 6 and 8 is not missing, then compute

- Symptom burden score = [(mean of the non-missing Questions 4, 6 and 8) – 1]/4 * 100

5. Total Symptom Score

Total symptom score = mean of the following available summary scores

- Symptom Frequency Score
- Symptom Burden Score

6. Self-efficacy

Code responses to Questions 10 and 11 as follows:

- Question 10
  - Not at all sure = 1
  - Not very sure = 2
  - Somewhat sure = 3
  - Mostly sure = 4
  - Completely sure = 5
- Question 11
  - Do not understand at all = 1
  - Do not understand very well = 2
  - Somewhat understand = 3
  - Mostly understand = 4
  - Completely understand = 5

If at least one of Questions 10 and 11 is not missing, then compute

- Self-efficacy score = [(mean of the non-missing Questions 10 and 11) – 1]/4 * 100

7. Quality of Life

Code responses to Questions 12, 13 and 14 as follows:

- Question 12
  - It has extremely limited my enjoyment of life = 1
  - It has limited my enjoyment of life quite a bit = 2
  - It has moderately limited my enjoyment of life = 3
  - It has slightly limited my enjoyment of life = 4
  - It has not limited my enjoyment of life at all = 5
- Question 13
  - Not at all satisfied = 1
  - Mostly dissatisfied = 2
  - Somewhat satisfied = 3
  - Mostly satisfied = 4
  - Completely satisfied = 5
- Question 14
  - I felt that way all of the time = 1
  - I felt that way most of the time = 2
  - I occasionally felt that way = 3
  - I rarely felt that way = 4
  - I never felt that way = 5

If at least one of Questions 12, 13 and 14 is not missing, then compute

- Quality of life score = [(mean of the non-missing Questions 12, 13 and 14) – 1]/4 * 100

8. Social Limitation

Code responses to each of Questions 15a-15d as follows:

- Severely limited = 1
- Limited quite a bit= 2
- Moderately limited = 3
- Slightly limited = 4
- Did not limit at all = 5
- Does not apply or did not do for other reasons = <missing value>

If at least 2 of Questions 15a-15d are not missing, then compute

- Social limitation score = [(mean of the non-missing Questions 15a-15d) – 1]/4 * 100

9. Overall Summary Score

Overall summary score = mean of the following available summary scores

- Physical Limitation Score
- Total Symptom Score
- Quality of Life Score
- Social Limitation Score

10. Clinical Summary Score

Clinical summary score = mean of the following available summary scores

- Physical Limitation Score
- Total Symptom Score

9.3.2. Norfolk Quality of Life-Diabetic Neuropathy (QOL-DN)

Norfolk QOL-DN is a tool for assessing patients' perception of the effects of diabetes and diabetic neuropathy. There are 35 questions divided into 5 domains. The range of possible total scores is -4 to 136.

Part I: Symptoms

Items 1-7 (Part I) are a simple inventory of symptoms of neuropathy. The presence of the symptom is checked in whichever box applies, and an absence of a symptom is checked under "none." Positive responses are scored as 1; and negative responses, as 0.

Part II: Activities of Daily Life

Items 8-35 (Part II) pertain to Activities of Daily Life, and most of these are scaled on a 5-point Likert scale ranging from 0 ("Not a problem") to 4 ("Severe problem"). However, Questions 31 and 32 are scored differently. In Question 31, "Good", the middle item, is scored as0. "Very Good" is scored as –1, "Excellent" is scored as –2. "Fair" is scored as 1, and "Poor" is scored as 2. In Question 32, "About the Same," the middle item, is scored as 0. "Somewhat better" is scored as –1, "Much better" is scored as -2. "Somewhat worse" is scored as 1, and "Much worse" is scored as 2.

Subscales and Scoring Algorithm

The Total QOL and 5 domains should be summed as follows:

- Total QOL (35 items) Σ(1-7, 8-35)
- Physical Functioning/Large Fiber (15 items) Σ(8, 11, 13-15, 24, 27-35)
- Activities of Daily Living (ADLs) (5 items) Σ(12, 22, 23, 25, 26)
- Symptoms (8 items) Σ(1-7, 9)
- Small Fiber (4 items) Σ(10, 16, 17, 18)
- Autonomic (3 items) Σ(19, 20, 21)

Domain scores are calculated as the rounded integer value of the average scores of non-missing included items multiplied by the number of items if at least 50% of the items are non-missing. A domain score is missing if more than 50% of the included items are missing.

If the scores for all 5 domains are non-missing, then Total QOL is the sum of scores of the 5 domains; however, if at least 1 of the domains is missing and at least 50% of the items (18 items) are non-missing, then Total QOL is calculated as 35 times the mean of the non-missing items, rounded to the nearest integer. Otherwise, Total QOL is deemed as missing.

Signature Page for VV-CLIN-004130 v1.0
ALN-TTR02-011 Statistical Analysis Plan

Approval	[redacted]T+0000

Approval	[redacted]46:22 GMT+0000

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ALN-TTR02-011 Statistical Analysis Plan

IMAGING STATISTICAL ANALYSIS PLAN
ALN-TTR02-011

Protocol Title:	APOLLO-B: A Phase 3, Randomized, Double-blind, Placebo-controlled Multicenter Study to Evaluate the Efficacy and Safety of Patisiran in Patients with Transthyretin Amyloidosis with Cardiomyopathy (ATTR Amyloidosis with Cardiomyopathy)
Short Title:	APOLLO-B: A Study to Evaluate Patisiran in Patients with Transthyretin Amyloidosis with Cardiomyopathy (ATTR Amyloidosis with Cardiomyopathy)
Study Treatment:	Patisiran (ALN-TTR02)
EudraCT Number:	2019-001458-24
IND Number:	141240
Protocol Date:	Original protocol, 18 April 2019 Amendment 1: 20 December 2019 Amendment 2: 22 May 2020 Amendment 3: 30 June 2021
SAP Date:	Original SAP: 05 August 2020 Amendment 1: 27 June 2022
Sponsor:	Alnylam Pharmaceuticals, Inc. 300 Third Street Cambridge, MA 02142 USA Telephone: [redacted]
Sponsor Contact:	[redacted]

APPROVAL SIGNATURE PAGE

Protocol Number:	ALN-TTR02-011
Protocol Title:	APOLLO-B: A Phase 3, Randomized, Double blind, Placebo-controlled Multicenter Study to Evaluate the Efficacy and Safety of Patisiran in Patients with Transthyretin Amyloidosis with Cardiomyopathy (ATTR Amyloidosis with Cardiomyopathy)
Analysis Plan Version and Date:	Amendment 1: 27 June 2022

This document has been authored and signed electronically on the final page by the following:

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Alnylam Pharmaceuticals, Inc.

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LIST OF ABBREVIATIONS

Abbreviation	Definition
12m-DB	12-month double blind
ANCOVA	Analysis of covariance
CMR	Cardiac magnetic resonance
ECV	Extracellular volume
H/CL	Heart to contralateral lung
LA	Left atrial
LGE	Late gadolinium enhancement
LV	Left ventricular
OLE	Open label extension
RV	Right ventricular
SAP	Statistical analysis plan

1. INTRODUCTION

The APOLLO-B study (ALN-TTR02-011) is a Phase 3, randomized, double-blind, placebo-controlled, multicenter study designed to evaluate the efficacy and safety of patisiran in adult patients with ATTR amyloidosis with cardiomyopathy. The cardiac magnetic resonance (CMR) and technetium scintigraphy imaging assessments will be used to evaluate imaging manifestations of cardiac amyloid involvement and are planned to be conducted for subsets of patients who opt-in to having assessments performed by one or both imaging modalities during the APOLLO-B study.

CMR imaging and technetium scintigraphy are non-invasive methods of characterizing transthyretin amyloid burden in the myocardium. Their utility in the diagnosis and prognosis of ATTR amyloidosis with cardiomyopathy are well established.[Fontana 2015; Gillmore 2016; Martinez-Naharro 2019] With CMR, the extent of late gadolinium enhancement (LGE), elevation in native T1, and extracellular volume (ECV) correlate with amyloid burden and death.[Fontana 2014; Fontana 2015; Martinez-Naharro 2019] The utility of these imaging methods to assess response to disease-modifying therapies are presently unknown. Anecdotal reports suggest that certain therapies may be associated with regression of cardiac amyloid, as assessed by CMR or technetium scintigraphy.[Florian 2020; Groothof 2020] Neither imaging modality, however, has been prospectively evaluated in a blinded, placebo-controlled trial for ATTR amyloidosis. The exploratory analyses here may hence serve to identify mechanisms and markers of treatment response or disease progression.

This imaging statistical analysis plan (SAP) details comprehensive specifications of the CMR and technetium scintigraphy data summaries and statistical analyses in support of the clinical study report for Study ALN-TTR02-011.

Table, figure, and listing specifications for this analysis are contained in a separate document.

2. STUDY OVERVIEW

Cardiac magnetic resonance will be assessed in a subset of up to 60 patients and technetium scintigraphy will be assessed in a subset of up to 100 patients; CMR and technetium scintigraphy will be performed at select sites as optional imaging assessments to assess cardiac amyloid involvement. The assessments will be performed at baseline, Month 12, Month 18, Month 24, Month 36, and Month 48 as specified in the Schedule of Assessments in the study protocol (Table 1, Table 2, Table 3, and Table 4).

3. OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
To evaluate the efficacy of patisiran compared with placebo treatment on manifestations of cardiac amyloid involvement	• Change from baseline at Month 12 in global average extracellular volume (ECV) (%) by CMR • Change from baseline at Month 12 in normalized left ventricle (LV) total uptake by technetium scintigraphy

4. PATIENT POPULATION

The populations (analysis sets) are defined as follows:

- CMR Analysis Set: All patients who were randomized and received any amount of study drug and have at least one CMR assessment at baseline or post-baseline. Patients will be analyzed according to the treatment to which they were randomized.
- Technetium Analysis Set: All patients who were randomized and received any amount of study drug and have at least one technetium scintigraphy assessment at baseline or post-baseline. Patients will be analyzed according to the treatment to which they were randomized.
- CMR All Patisiran Treated Analysis Set: All patients who were randomized and received any amount of patisiran and have at least one CMR assessment at baseline or post-baseline. Patients receiving at least one dose of patisiran includes patients who received patisiran during the 12-month double blind (12m-DB) period and patients who first received placebo during the 12m-DB period and switched to patisiran during the open label extension (OLE) period.
- Technetium All Patisiran Treated Analysis Set: All patients who were randomized and received any amount of patisiran and have at least one technetium scintigraphy assessment at baseline or post-baseline. Patients receiving at least one dose of patisiran includes patients who received patisiran during the 12m-DB period and patients who first received placebo during the 12m-DB period and switched to patisiran during the OLE period.

The CMR Analysis Set and Technetium Analysis Set will be used to analyze the CMR and technetium scintigraphy endpoints, respectively, during the 12m-DB period. The CMR All Patisiran Treated Analysis Set and Technetium All Patisiran Treated Analysis Set will be used to summarize long-term CMR and technetium scintigraphy data, respectively, during patisiran treatment (see Section 5.5 for details).

5. GENERAL STATISTICAL METHODS

Details of general considerations, computing environment, baseline definitions, randomization stratification factors, multiple comparisons/multiplicity, analysis cutoff and database lock are specified in the APOLLO-B SAP (Section 5).

5.1. Determination of Sample Size

CMR and technetium scintigraphy assessments are planned to be conducted in approximately 60 patients and up to 100 patients, respectively.

For the change from baseline at Month 12 in global average ECV (%), assuming a mean treatment difference of -4.8 between patisiran and placebo in the treatment-naïve group and -3.0 in patients with baseline tafamidis, the weighted average treatment difference between patisiran and placebo in the overall population is approximately -4.3 (standard deviation = 4.6), assuming 70% are in the treatment-naïve group and 30% are in the baseline tafamidis group. A sample

size of 60 patients provides >90% power for a 2-sided test to detect a mean difference between treatment arms at a 2-sided alpha = 0.05.

For the change from baseline at Month 12 in normalized LV total uptake, assuming a mean treatment difference of -15.0 between patisiran and placebo in the treatment-naïve group and -9.4 in patients with baseline tafamidis, the weighted average treatment difference between patisiran and placebo in the overall population is approximately -13.3 (standard deviation = 14.0), assuming 70% are in the treatment-naïve group and 30% are in the baseline tafamidis group. A sample size of 100 patients provides >90% power for a 2-sided test to detect a mean difference between treatment arms at a 2-sided alpha = 0.05.

5.2. Baseline Definitions

For CMR and technetium scintigraphy parameters, baseline will be defined as the last non-missing value up to 6 weeks after the first dose of study drug.

For the CMR All Patisiran Treated Set and the Technetium All Patisiran Treated Set, baseline for patients who switched from placebo to patisiran will be redefined as the last non-missing value available on or after Month 9 in the 12m-DB period and prior to the first dose in the OLE period, unless otherwise specified. If a patient does not have a Month 12 assessment prior to the first dose in the OLE period, the redefined baseline will be defined as the last non-missing value available on or after Month 9 in the 12m-DB period and up to 6 weeks after the first dose in the OLE period.

5.3. Visit Windows

CMR and technetium scintigraphy assessments will be conducted at baseline and Month 12 in the 12m-DB period and at Months 18, 24, 36, and 48 in the OLE period. For patients randomized to placebo, if the scheduled Month 12 assessment is not performed prior to the first dose of patisiran in the OLE period, assessments performed within ±3 months of the scheduled assessment (eg, from an unscheduled visit) and prior to the first dose of patisiran in the OLE period will be grouped with the scheduled Month 12 assessments for analysis. The same windowing for Month 12 will be applied for patients randomized to patisiran, with the exception that assessments performed up to 6 weeks after the first dose of patisiran in the OLE period will be grouped with the scheduled Month 12 assessments for analysis; this window was extended since these endpoints for patisiran patients are not expected to be meaningfully impacted within a short time after the first dose in the OLE period. For assessments in the OLE period, if a scheduled post-baseline assessment is not performed, assessments performed within ±3 months of the scheduled assessment will be grouped with the scheduled Month 18, 24, 36, or 48 assessments for analysis. The derived visits will be used for all analyses.

5.4. Missing Data

No data imputation will be performed for missing CMR and technetium scintigraphy endpoints.

5.5. Analyses for the Entire Study

The study design includes a 12m-DB period and an OLE period. The objective in this imaging analysis is to evaluate the efficacy of patisiran compared with placebo on manifestations of cardiac amyloid involvement during the 12m-DB period. In addition, the long-term efficacy of patisiran during the entire patisiran treatment period (beyond 12m-DB) will be characterized for the CMR All Patisiran Treated Set and the Technetium All Patisiran Treated Set. Longitudinal efficacy parameters will be summarized over the entire study, including the 12m-DB and OLE periods, for all patients to show the long-term efficacy of patisiran (for patients randomized to patisiran) as well as to show the trajectory changes comparing the placebo experience versus the patisiran experience (for patients randomized to placebo). In these summaries, patients will be analyzed according to the treatment to which they were randomized during the 12m-DB period.

Detailed definitions for different treatment periods and windowing rules are specified in the APOLLO-B SAP (Section 5.11). For CMR and technetium assessments, if a patient randomized to patisiran does not have a Month 12 assessment prior to the first dose of patisiran in the OLE period, assessments collected up to 6 weeks after the first OLE dose will be grouped with the Month 12 assessments for analysis.

6. STATISTICAL ANALYSES

6.1. Patient Disposition

For all analysis sets, the patient disposition will be summarized similarly as specified in the APOLLO-B SAP (Section 6.1).

6.2. Demographics and Baseline Characteristics

For all analysis sets, demographic and baseline characteristics, baseline disease characteristics, baseline efficacy parameters, and medical history information will be summarized and listed similarly as specified in the APOLLO-B SAP (Section 6.2).

6.3. Drug Exposure

For all analysis sets, the duration of study drug exposure and number of doses of study drug received will be summarized and listed similarly as specified in the APOLLO-B SAP (Section 6.4).

6.4. Efficacy Analyses

6.4.1. Global Average Extracellular Volume

The primary CMR endpoint is the change from baseline to Month 12 in global average ECV.

The primary analysis will be performed using an analysis of covariance (ANCOVA) model for the CMR Analysis Set. All available data will be included in the primary analysis. The outcome variable is the change from baseline in global average ECV, and the model includes baseline global average ECV as a continuous covariate and treatment arm as a fixed term. If the number of patients in either treatment arm is less than 15, descriptive statistics may be presented.

6.4.2. Normalized LV Total Uptake

The primary technetium scintigraphy endpoint is the change from baseline to Month 12 in normalized LV total uptake.

The primary analysis will be performed in the Technetium Analysis Set using an ANCOVA model similar to the model described for the primary analysis of global average ECV while adjusting for baseline normalized LV total uptake as a continuous covariate. If the number of patients in either treatment arm is less than 15, descriptive statistics may be presented.

6.4.3. Other CMR and Technetium Scintigraphy Parameters

All other CMR and technetium scintigraphy parameters are listed in Table 1.

Table 1 Other CMR and Technetium Scintigraphy Parameters

Cardiac Magnetic Resonance	
Global percent of LGE (%)	LGE by segment (%)
Global mass of LGE (g)	T1 mapping pre-contrast by segment (ms)
Left ventricular (LV) mass (g)	Right ventricular (RV) mass (g)
LV mass index (g/m^2) [1]	RV mass index (g/m^2) [1]
LV end-diastolic volume (mL)	RV end-diastolic volume (mL)
LV end-diastolic volume index (mL/m^2) [1]	RV end-diastolic volume index (mL/m^2) [1]
LV end-systolic volume (mL)	RV end-systolic volume (mL)
LV end-systolic volume index (mL/m^2) [1]	RV end-systolic volume index (mL/m^2) [1]
LV stroke volume (mL) [2]	RV stroke volume (mL) [2]
LV stroke volume index (mL/m^2) [1]	RV stroke volume index (mL/m^2) [1]
LV ejection fraction (%) [3]	RV ejection fraction (%) [3]
LV mass to end-diastolic volume ratio (g/mL) [4]	
Cardiac output (L/min) [5]	
Cardiac index (L/min/m^2) [1]	
Left atrial (LA) reservoir emptying volume (mL)	LA reservoir emptying percent (%)
LA contractile emptying volume (mL)	LA contractile emptying percent (%)
LA total emptying volume (mL)	LA total emptying percent (%)
LA volume maximum (mL)	LA volume minimum (mL)
ECV by segment (%)	
Technetium Scintigraphy	
Heart to contralateral lung (H/CL) ratio of uptake at 3 hours post injection	Perugini grade [6]

[1] Calculated by dividing the value of the parameter listed in the row above by body surface area
[2] Stroke volume calculated as the end-diastolic volume – end-systolic volume for the LV or RV, as indicated
[3] Ejection fraction calculated as (stroke volume / end-diastolic volume) * 100 for the LV or RV, as indicated
[4] Calculated as LV end-diastolic volume / LV end-diastolic volume
[5] Calculated as heart rate * LV stroke volume
[6] Scoring algorithm for Perugini grades is provided in Appendix 9.1

The CMR Analysis Set and Technetium Analysis Set will be used to summarize all other CMR and technetium parameters for the 12m-DB period, respectively.

For continuous parameters, descriptive statistics will be provided for actual value and change from baseline by treatment arm and visit; descriptive statistics for percentage change from baseline by treatment arm and visit may also be provided, as appropriate. Categorical parameters will be descriptively summarized by treatment arm by presenting the number and percentage of patients in each category for each visit.

All CMR and technetium scintigraphy data will be presented in by-patient listings.

7. CHANGES TO PLANNED ANALYSES

The SAP Amendment 1 includes more details previously not discussed in the original SAP. The details of the changes and rationales are listed in the table below.

Summary of Changes	**Detailed Description/Rationale**
Updated planned timepoints for CMR and technetium scintigraphy assessments	To align with protocol Amendment 3
Updated primary endpoint for technetium scintigraphy	Technetium uptake in the LV will be quantified as a unitless ratio of the activity in the LV wall to the activity of the injected dose. The normalized LV tracer uptake is a reasonable metric to assess changes in the same patient over time and may be more sensitive than the heart/lung ratio or visual scores.
Added baseline definition for imaging parameters as the last non-missing value up to 6 weeks after the first dose of study drug	To allow assessments collected shortly after start of study drug to be summarized with baseline assessment in order to reduce the amount of missing data. Imaging parameters are not expected to be meaningfully impacted within a short time after starting study drug.
Updated windowing rule for patisiran patients for Month 12 to include assessments taken up to 6 weeks after the first dose of patisiran in the OLE period	To allow assessments collected shortly after first OLE dose for patisiran patients to be summarized with Month 12 assessments in order to reduce the amount of missing data. Imaging parameters are not expected to be meaningfully impacted within a short time after the first dose in the OLE period.
Updated data handling to not treat data after tafamidis drop-in as missing	To align with handling of the tafamidis drop-in intercurrent event in the primary analysis of efficacy parameters in the main APOLLO-B SAP

8. REFERENCES

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9. APPENDICES

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IMAGING STATISTICAL ANALYSIS PLAN
ALN-TTR02-011

Protocol Title:	APOLLO-B: A Phase 3, Randomized, Double-blind, Placebo-controlled Multicenter Study to Evaluate the Efficacy and Safety of Patisiran in Patients with Transthyretin Amyloidosis with Cardiomyopathy (ATTR Amyloidosis with Cardiomyopathy)
Short Title:	APOLLO-B: A Study to Evaluate Patisiran in Patients with Transthyretin Amyloidosis with Cardiomyopathy (ATTR Amyloidosis with Cardiomyopathy)
Study Treatment:	Patisiran (ALN-TTR02)
EudraCT Number:	2019-001458-24
IND Number:	141240
Protocol Date:	Original protocol, 18 April 2019 Amendment 1: 20 December 2019 Amendment 2: 22 May 2020
SAP Date:	Original SAP: 05 August 2020
Sponsor:	Alnylam Pharmaceuticals, Inc. 300 Third Street Cambridge, MA 02142 USA Telephone: [redacted]
Sponsor Contact:	[redacted]

APPROVAL SIGNATURE PAGE

Protocol Number:	ALN-TTR02-011
Protocol Title:	APOLLO-B: A Phase 3, Randomized, Double blind, Placebo-controlled Multicenter Study to Evaluate the Efficacy and Safety of Patisiran in Patients with Transthyretin Amyloidosis with Cardiomyopathy (ATTR Amyloidosis with Cardiomyopathy)
Analysis Plan Version and Date:	Original: 05 August 2020

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LIST OF ABBREVIATIONS

Abbreviation	Definition
12m-DB	12-month double blind
ANCOVA	Analysis of covariance
CMR	Cardiac magnetic resonance
ECV	Extracellular volume
H/CL	Heart to contralateral lung
LA	Left atrial
LGE	Late gadolinium enhancement
LV	Left ventricular
OLE	Open label extension
RV	Right ventricular
SAP	Statistical analysis plan

1. INTRODUCTION

The APOLLO-B study (ALN-TTR02-011) is a Phase 3, randomized, double-blind, placebo-controlled, multicenter study designed to evaluate the efficacy and safety of patisiran in adult patients with ATTR amyloidosis with cardiomyopathy. The cardiac magnetic resonance (CMR) and technetium scintigraphy imaging assessments will be used to evaluate imaging manifestations of cardiac amyloid involvement and are planned to be conducted for subsets of patients who opt-in to having assessments performed by one or both imaging modalities during the APOLLO-B study.

CMR imaging and technetium scintigraphy are non-invasive methods of characterizing transthyretin amyloid burden in the myocardium. Their utility in the diagnosis and prognosis of ATTR amyloidosis with cardiomyopathy are well established.[Fontana 2015; Gillmore 2016; Martinez-Naharro 2019] With CMR, the extent of late gadolinium enhancement (LGE), elevation in native T1, and extracellular volume (ECV) correlate with amyloid burden and death.[Fontana 2014; Fontana 2015; Martinez-Naharro 2019] The utility of these imaging methods to assess response to disease-modifying therapies are presently unknown. Anecdotal reports suggest that certain therapies may be associated with regression of cardiac amyloid, as assessed by CMR or technetium scintigraphy.[Florian 2020; Groothof 2020] Neither imaging modality, however, has been prospectively evaluated in a blinded, placebo-controlled trial for ATTR amyloidosis. The exploratory analyses here may hence serve to identify mechanisms and markers of treatment response or disease progression.

This imaging statistical analysis plan (SAP) details comprehensive specifications of the CMR and technetium scintigraphy data summaries and statistical analyses in support of the clinical study report for Study ALN-TTR02-011.

Table, figure, and listing specifications for this analysis are contained in a separate document.

2. STUDY OVERVIEW

Cardiac magnetic resonance will be assessed in a subset of up to 60 patients and technetium scintigraphy will be assessed in a subset of up to 100 patients; CMR and technetium scintigraphy will be performed at select sites as optional imaging assessments to assess cardiac amyloid involvement. The assessments will be performed at baseline, Month 12, Month 18 and Month 24, as specified in the Schedule of Assessments in the study protocol (Table 1 and Table 2).

3. OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
To evaluate the efficacy of patisiran compared with placebo treatment on manifestations of cardiac amyloid involvement	• Change from baseline at Month 12 in global average extracellular volume (ECV) (%) by CMR • Change from baseline at Month 12 in cardiac uptake (%) by technetium scintigraphy

4. PATIENT POPULATION

The populations (analysis sets) are defined as follows:

- CMR Analysis Set: All patients who were randomized and received any amount of study drug and who have at least one CMR assessment. Patients will be analyzed according to the treatment to which they were randomized.
- Technetium Analysis Set: All patients who were randomized and received any amount of study drug and who have at least one technetium scintigraphy assessment. Patients will be analyzed according to the treatment to which they were randomized.
- CMR All Patisiran Treated Analysis Set: All patients who were randomized and received any amount of patisiran and who have at least one CMR assessment. Patients receiving at least one dose of patisiran includes patients who received patisiran during the 12-month double blind (12m-DB) period and patients who first received placebo during the 12m-DB period and switched to patisiran during the open label extension (OLE) period.
- Technetium All Patisiran Treated Analysis Set: All patients who were randomized and received any amount of patisiran and who have at least one technetium scintigraphy assessment. Patients receiving at least one dose of patisiran includes patients who received patisiran during the 12m-DB period and patients who first received placebo during the 12m-DB period and switched to patisiran during the OLE period.

The CMR Analysis Set and Technetium Analysis Set will be used to analyze the CMR and technetium scintigraphy endpoints, respectively, during the 12m-DB period. The CMR All Patisiran Treated Analysis Set and Technetium All Patisiran Treated Analysis Set will be used to summarize long-term CMR and technetium scintigraphy data, respectively, during patisiran treatment (see Section 5.4 for details).

5. GENERAL STATISTICAL METHODS

Details of general considerations, computing environment, baseline definitions, randomization stratification factors, multiple comparisons/multiplicity, analysis cutoff and database lock are specified in the APOLLO-B SAP (Section 5).

5.1. Determination of Sample Size

CMR and technetium scintigraphy assessments are planned to be conducted in approximately 60 patients and up to 100 patients, respectively.

For the change from baseline at Month 12 in global average ECV (%), assuming a mean treatment difference of -4.8 between patisiran and placebo in the treatment-naïve group and -3.0 in patients with baseline tafamidis, the weighted average treatment difference between patisiran and placebo in the overall population is approximately -4.3 (standard deviation = 4.6), assuming 70% are in the treatment-naïve group and 30% are in the baseline tafamidis group. A sample size of 60 patients provides >90% power for a 2-sided test to detect a mean difference between treatment arms at a 2-sided alpha = 0.05.

For the change from baseline at Month 12 in cardiac uptake (%), assuming a mean treatment difference of -15.0 between patisiran and placebo in the treatment-naïve group and -9.4 in patients with baseline tafamidis, the weighted average treatment difference between patisiran and placebo in the overall population is approximately -13.3 (standard deviation = 14.0), assuming 70% are in the treatment-naïve group and 30% are in the baseline tafamidis group. A sample size of 100 patients provides >90% power for a 2-sided test to detect a mean difference between treatment arms at a 2-sided alpha = 0.05.

5.2. Visit Windows

CMR and technetium scintigraphy assessments will be conducted at baseline and Month 12 in the 12m-DB period and at Month 18 and Month 24 in the OLE period. If the scheduled Month 12 assessment is not performed, assessments performed within ±3 months of the scheduled assessment (eg, from an unscheduled visit) but prior to the first dose of patisiran in the OLE period will be grouped with the scheduled Month 12 assessments for analysis. For assessments in the OLE period, if a scheduled post-baseline assessment is not performed, assessments performed within ±3 months of the scheduled assessment will be grouped with the scheduled Month 18 or Month 24 assessments for analysis. The derived visits will be used for all analyses.

5.3. Missing Data

No data imputation will be performed for missing CMR and technetium scintigraphy endpoints.

5.4. Analyses for the Entire Study

The study design includes a 12m-DB period and an OLE period. The objective in this imaging analysis is to evaluate the efficacy of patisiran compared with placebo on manifestations of cardiac amyloid involvement during the 12m-DB period. In addition, the long-term efficacy of patisiran during the entire patisiran treatment period (beyond 12m-DB) will be characterized for the CMR All Patisiran Treated Set and the Technetium All Patisiran Treated Set. Longitudinal efficacy parameters will be summarized over the entire study, including the 12m-DB and OLE periods, for all patients to show the long-term efficacy of patisiran (for patients randomized to patisiran) as well as to show the trajectory changes comparing the placebo experience versus the patisiran experience (for patients randomized to placebo). In these summaries, patients will be analyzed according to the treatment to which they were randomized during the 12m-DB period

Detailed definitions for different treatment periods and windowing rules are specified in the APOLLO-B SAP (Section 5.12).

6. STATISTICAL ANALYSES

6.1. Patient Disposition

For all analysis sets, the patient disposition will be summarized similarly as specified in the APOLLO-B SAP (Section 6.1).

6.2. Demographics and Baseline Characteristics

For all analysis sets, demographic and baseline characteristics, baseline disease characteristics, baseline efficacy parameters, and medical history information will be summarized and listed similarly as specified in the APOLLO-B SAP (Section 6.2).

6.3. Drug Exposure

For all analysis sets, the duration of study drug exposure and number of doses of study drug received will be summarized and listed similarly as specified in the APOLLO-B SAP (Section 6.4).

6.4. Efficacy Analyses

6.4.1. Global Average Extracellular Volume

The primary CMR endpoint is the change from baseline to Month 12 in global average ECV.

The primary analysis will be performed using an analysis of covariance (ANCOVA) model for the CMR Analysis Set. All available data will be included in the primary analysis, including data collected post tafamidis drop-in (see APOLLO-B SAP, Section 5.6.1). The outcome variable is the change from baseline in ECV, and the model includes baseline ECV as a continuous covariate and treatment arm and baseline tafamidis use (yes vs. no) as factors.

A sensitivity analysis excluding data collected from patients after taking tafamidis for more than 28 days (see APOLLO-B SAP, Section 5.6.1) will be conducted using the same ANCOVA model as used for the primary analysis.

6.4.2. Cardiac Uptake

The primary technetium scintigraphy endpoint is the change from baseline to Month 12 in cardiac uptake.

The primary analysis will be performed in the Technetium Analysis Set using an ANCOVA model similar to the model described for the primary analysis of ECV while adjusting for baseline cardiac uptake as a continuous covariate. In addition, a sensitivity analysis will be conducted using an ANCOVA model excluding data collected from patients after taking tafamidis for more than 28 days, similar to the model described for the sensitivity analysis of ECV.

6.4.3. Other CMR and Technetium Scintigraphy Parameters

All other CMR and technetium scintigraphy parameters are listed in Table 1.

Table 1 Other CMR and Technetium Scintigraphy Parameters

Cardiac Magnetic Resonance	
Global percent of LGE (%)	LGE by segment (%)
Global mass of LGE (g)	T1 mapping pre-contrast by segment (ms)
Left ventricular (LV) mass (g)	Right ventricular (RV) mass (g)
LV mass index (g/m^2) [1]	RV mass index (g/m^2) [1]
LV end-diastolic volume (mL)	RV end-diastolic volume (mL)
LV end-diastolic volume index (mL/m^2) [1]	RV end-diastolic volume index (mL/m^2) [1]
LV end-systolic volume (mL)	RV end-systolic volume (mL)
LV end-systolic volume index (mL/m^2) [1]	RV end-systolic volume index (mL/m^2) [1]
LV stroke volume (mL) [2]	RV stroke volume (mL) [2]
LV stroke volume index (mL/m^2) [1]	RV stroke volume index (mL/m^2) [1]
LV ejection fraction (%) [3]	RV ejection fraction (%) [3]
LV mass to end-diastolic volume ratio (g/mL) [4]	
Cardiac output (L/min) [5]	
Cardiac index (L/min/m^2) [1]	
Left atrial (LA) reservoir emptying volume (mL)	LA reservoir emptying percent (%)
LA contractile emptying volume (mL)	LA contractile emptying percent (%)
LA total emptying volume (mL)	LA total emptying percent (%)
LA volume maximum (mL)	LA volume minimum (mL)
ECV by segment (%)	
Technetium Scintigraphy	
Heart to contralateral lung (H/CL) ratio of uptake at 3 hours post injection	Perugini grade [6]

[1] Calculated by dividing the value of the parameter listed in the row above by body surface area
[2] Stroke volume calculated as the end-diastolic volume – end-systolic volume for the LV or RV, as indicated
[3] Ejection fraction calculated as (stroke volume / end-diastolic volume) * 100 for the LV or RV, as indicated
[4] Calculated as LV end-diastolic volume / LV end-diastolic volume
[5] Calculated as heart rate * LV stroke volume
[6] Scoring algorithm for Perugini grades is provided in Appendix 9.1

The CMR Analysis Set and Technetium Analysis Set will be used to summarize all other CMR and technetium parameters for the 12m-DB period, respectively.

For continuous parameters, descriptive statistics will be provided for actual value and change from baseline by treatment arm and visit; descriptive statistics for percentage change from baseline by treatment arm and visit may also be provided, as appropriate. Categorical parameters will be descriptively summarized by treatment arm by presenting the number and percentage of patients in each category for each visit.

All CMR and technetium scintigraphy data will be presented in by-patient listings.

7. CHANGES TO PLANNED ANALYSES

Not applicable.

8. REFERENCES

Florian A, Bietenbeck M, Chatzantonis G, Husing-Kabar A, Schmidt H,Yilmaz A. Regression of cardiac amyloid load documented by cardiovascular magnetic resonance in a patient with hereditary amyloidosis. Clin Res Cardiol. 2020 Jul;109(7):949-56.

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