Non-Interventional Study Protocol B3461099

Real-World Effectiveness of Tafamidis 80 mg or 61 mg on Neurologic Disease Progression in Patients with Mixed-Phenotype Variant Transthyretin Amyloid Cardiomyopathy (ATTRv-CM)

Statistical Analysis Plan (SAP)

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1. AMENDMENTS FROM PREVIOUS VERSION(S)

Not applicable.

2. INTRODUCTION

ATTR amyloidosis is a progressive, life-threatening, and heterogeneous rare disease caused by the deposition of transthyretin-derived amyloid fibrils in the peripheral nerves, heart, and other organs.[1,2] ATTR amyloidosis may arise from mutations in the transthyretin (TTR) gene (ATTRv amyloidosis), with more than 140 ATTRv genotypes having been identified,[3] or from the aggregation of non-mutated wild-type TTR (ATTRwt amyloidosis). Amyloid deposition in the peripheral nerves results in transthyretin amyloid polyneuropathy (ATTR-PN). Multiple symptoms and system involvement are hallmarks of ATTR-PN.[2,4,5] Amyloid fibril deposition in the extracellular matrix of the heart[2,6] results in ATTR-CM, and is characterized by arrhythmias and heart failure.[7,8] ATTRwt amyloidosis predominantly manifests as ATTR-CM. ATTRv amyloidosis is now widely recognized as a spectrum of disease that can manifest as polyneuropathy, cardiomyopathy, or a mixed phenotype, depending on the particular TTR variant, amyloid deposition pattern, and multisystem involvement.

Tafamidis meglumine is a first-in-class highly specific and selective stabilizer for both wild-type and amyloidogenic variants of transthyretin (TTR)[9] developed for the treatment of ATTR amyloidosis.[10]

Tafamidis meglumine 20 mg was the first disease-modifying therapy approved for the treatment of ATTR-PN and is currently labeled and approved for once-daily dosing for treatment of transthyretin amyloidosis in adult patients with stage 1 symptomatic polyneuropathy to delay peripheral neurologic impairment. It is available in over 40 countries worldwide (excluding the United States) and marketed as VYNDAQEL, tafamidis meglumine 20 mg for treating ATTR-PN.

Likewise, tafamidis delivered as either VYNDAQEL 80 mg (four 20-mg tafamidis meglumine capsules) orally once daily or VYNDAMAX 61 mg (one 61-mg tafamidis capsule) orally once daily[11] was the first disease-modifying therapy approved for the treatment of cardiomyopathy of wild-type or hereditary transthyretin-mediated amyloidosis in adults to reduce cardiovascular mortality and cardiovascular-related hospitalization. Tafamidis is currently approved for the treatment of ATTR-CM in approximately 46 countries worldwide (including the United States).

While the safety and efficacy of VYNDAQEL has been demonstrated by clinical studies for AATR-PN and ATTR-CN, neurologic endpoints were not evaluated in the ATTR-CM development program. Therefore, there is a need to assess the potential benefit of tafamidis, as either VYNDAQEL 80 mg (four 20-mg tafamidis meglumine capsules) orally once daily or VYNDAMAX 61 mg (one 61-mg tafamidis capsule) orally once daily for delaying neurologic disease progression in ATTR amyloidosis. To address this knowledge gap, this

observational study will use secondary data to examine neurologic disease progression among patients with ATTRv-CM treated in the real-world setting.

2.1. Study Design

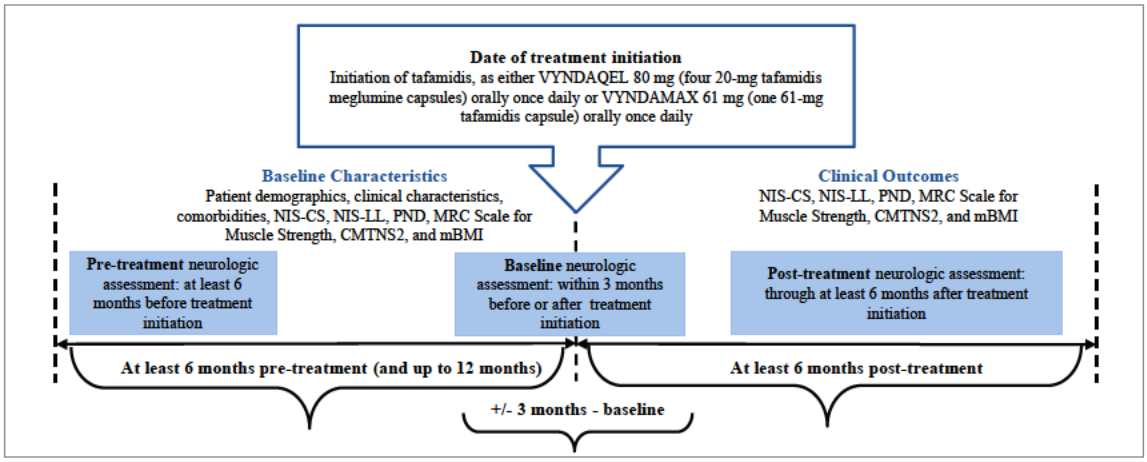
This will be an observational, retrospective cohort study using structured secondary anonymized data. Patients with mixed-phenotype ATTRv-CM receiving tafamidis, as either VYNDAQEL 80 mg (four 20-mg tafamidis meglumine capsules) orally once daily or VYNDAMAX 61 mg (one 61-mg tafamidis capsule) orally once daily for at least 6 months, will be identified. Relevant data will be extracted through at least 6 months following the initiation of tafamidis treatment (with a ±3-month window).

The index date of this study is defined as the date of tafamidis treatment initiation with at least 6-month (and up to 12 months) pre-index period as the study pre-treatment baseline. Patients will be followed from index date for at least 6 months post-treatment (with a ±3-month window).

This study also includes three assessment windows in which the study endpoints will be extracted and evaluated. The pre-treatment assessment will be extracted at least 6 (and up to 12 months) prior to treatment initiation. The tafamidis initiation assessment will be obtained 3 months before or 3 months after the start of tafamidis treatment. The post-treatment endpoints will be assessed at least 6 months after (with a ±3-month window) treatment initiation.

The final study design will be determined based on feasibility assessment.

Figure 1. Overview of Study Design



*Charcot-Marie-Tooth neuropathy score v2 (CMTNS2); mBMI: Modified body mass index; MRC: Medical Research Council; NIS-CS: Neuropathy impairment score–composite score; NIS-LL: Neuropathy impairment score–lower limbs; PND: Polyneuropathy disability.

2.1.1. Study Population

The study will include patients with mixed-phenotype ATTRv-CM who are treated with tafamidis, as either VYNDAQEL 80 mg (four 20-mg tafamidis meglumine capsules) orally once daily or VYNDAMAX 61 mg (one 61-mg tafamidis capsule) orally once daily. All patients meeting the study enrollment criteria will be included.

Inclusion criteria

- Age ≥18 years at diagnosis
- Diagnosed with hereditary ATTR-CM, mixed phenotype
- Treated with tafamidis (VYNDAQEL 80 mg [four 20-mg tafamidis meglumine capsules] orally once daily or VYNDAMAX 61 mg [one 61-mg tafamidis capsule] orally once daily) for ≥6 months (with a ± 3-month window)
- Have had ≥1 pre- and ≥2 post-treatment neurologic assessments*+

Exclusion criteria

- History of organ transplant
- Wild-type TTR genotype
- Individuals who are non-ambulatory
- Prior treatment with any disease-modifying therapy (investigational or approved) alone or in combination, except tafamidis, as either VYNDAQEL 80 mg (four 20-mg tafamidis meglumine capsules) orally once daily or VYNDAMAX 61 mg (one 61-mg tafamidis capsule) orally once daily
- Peripheral neuropathy attributed to causes other than ATTR amyloidosis (eg, diabetes mellitus, B12 deficiency, HIV infection)

2.1.2. Data Source

The source of data for this study will be de-identified secondary data from an anonymized research database.

2.1.3. Treatment/Cohort Labels

Not applicable.

2.2. Study Objectives

Primary objective:

- To assess neurologic function before and after initiation of tafamidis, as either VYNDAQEL 80 mg (four 20-mg tafamidis meglumine capsules) orally once daily or VYNDAMAX 61 mg (one 61-mg tafamidis capsule) orally once daily, in patients with mixed-phenotype ATTRv-CM in a real-world setting.

Secondary objective:

- To assess modified body mass index (mBMI) in patients with mixed-phenotype ATTRv-CM receiving tafamidis, as either VYNDAQEL 80 mg (four 20-mg tafamidis meglumine capsules) orally once daily or VYNDAMAX 61 mg (one 61-mg tafamidis capsule) orally once daily, in a real-world setting

3. HYPOTHESES AND DECISION RULES

3.1. Statistical Hypotheses

All analyses in this study are exploratory in nature and do not include formal hypothesis testing. This study will still report p-values at the 0.05 level.

3.2. Statistical Decision Rules

The alpha level will be 0.05, 2-sided. No adjustments for multiple comparisons will be made.

4. ANALYSIS SETS/POPULATIONS

4.1. Full Analysis Set

The study will include all patients with mixed-phenotype ATTRv-CM who meet the study protocol I/E criteria and are treated with tafamidis, as either VYNDAQEL 80 mg (four 20-mg tafamidis meglumine capsules) orally once daily or VYNDAMAX 61 mg (one 61-mg tafamidis capsule) orally once daily for at least 6 months (with a ±3-month window). All patients meeting the below study enrollment criteria will be included in the full analysis dataset. It is estimated that at least 30 patients will be included in the analysis.

4.2. Safety Analysis Set

Not applicable.

4.3. Other Analysis Set

An analysis of available data may be conducted for scientific presentation at an international congress.

4.4. Subgroups

Subgroup analysis may be considered by patient genotype if sample size allows.

5. ENDPOINTS AND INDEPENDENT VARIABLES

5.1. Effectiveness Endpoint(s)

The primary study endpoint is neurologic disease progression, which includes neuropathy impairment score–composite score (NIS-CS), neuropathy impairment score–lower limbs (NIS-LL), polyneuropathy disability (PND) score, Medical Research Council (MRC) Scale for Muscle Strength score, and the Charcot-Marie-Tooth neuropathy score v2 (CMTNS2).

The secondary study endpoint is mBMI.

Table 1. Study Endpoints for Primary and Secondary Objectives

Variable	Role	Data source(s)	Operational Definition
Neurologic progression			
Neuropathy impairment score– composite score (NIS-CS)	Outcome	Research database	Data contains an assessment score for each muscle group or sensation area for motor strength/weakness, muscle stretch reflexes, and sensation. A final NIS-CS score will be calculated as a sum of these scores from the 3 sections ranging from 0 (normal) to 244 (total impairment), if data permits.
Neuropathy impairment score–lower limbs (NIS-LL)	Outcome	Research database	Same as above, NIS-LL will be calculated using individual assessment scores for motor strength/weakness, muscle stretch reflexes and sensation, from lower body only, ranging 0 (normal) to 88 (total impairment), if data permits.
Polyneuropathy disability (PND) score	Outcome	Research database	A scoring system of the patient's walking capacity. It consists of four stages from stage 0 (no impairment) to stage IV (confined to a wheelchair or bedridden).
Medical Research Council (MRC) Scale for Muscle Strength	Outcome	Research database	The MRC scale of muscle strength uses a score of 0 to 5 to grade the power of a particular muscle group in relation to the movement of a single joint.
Charcot-Marie-Tooth neuropathy score v2 (CMTNS2)	Outcome	Research database	Composite of nine assessments: symptoms (three items), signs (four items), and neurophysiology (two items). It is designed to measure length-dependent motor and sensory impairment in genetic neuropathies. Each assessment is scored on a 0 – 4 point scale, reflecting severity of impairment. Patients are classified as mild (CMTNS ≤ 10), moderate (CMTNS 11 – 20), or severe (CMTNS > 20).
Overall health			
Height	Outcome	Research database	Height in centimeter or inches
Weight	Outcome	Research database	Weight in kilograms or pounds

Variable	Role	Data source(s)	Operational Definition
Body mass index	Outcome	Research database	Recorded or calculated in kg/m^2
Modified BMI (mBMI)	Outcome	Research database	Calculated as the product of BMI in kg/m^2 and serum albumin in g/L to compensate for peripheral edema.

5.2. Safety Endpoints

Not applicable.

5.3. Other Endpoints

Not applicable.

5.4. Covariates

Patients' demographic and clinical characteristics will be collected in the pre-treatment baseline period.

Table 2. Study Covariates

Variable	Role	Data source(s)	Operational Definition
Patient demographics			
Age	Baseline characteristic	Research database	Patient age at baseline,* age groups will be determined by distribution of age.
Gender	Baseline characteristic	Research database	Male/Female
Race	Baseline characteristic	Research database	American Indian or Alaska Native/Asian/Black or African American/Native Hawaiian or Other Pacific Islander/White
Ethnicity	Baseline characteristic	Research database	Hispanic or Latino/Not Hispanic or Latino
Clinical characteristics			
Age at ATTR-CM diagnosis	Baseline characteristic	Research database	Age at diagnosis in years
TTR genotype	Baseline characteristic	Research database	Mutation in TTR gene associated with ATTR amyloidosis (eg, Val122Ile, Val30Met, Leu58His, Leu111Met)
Family history	Baseline characteristic	Research database	No family history/Parent/Siblings/Grandparent/ Other/Unknown
Comorbidities			
Charlson Comorbidity Index (CCI)**	Baseline characteristic	Research database	Based on comorbidities present at baseline*; grouped as 0, 1, 2, 3+

*Baseline is defined as within 3 months prior to or 3 months following initiation of tafamidis. In the absence of baseline data within a ± 3-month window, data outside this window may be used to align with real world practice.
**Individual comorbid conditions may be assessed based on feasibility assessment instead of CCI.

6. HANDLING OF MISSING VALUES

For NIS-CS and NIS-LL neurologic progression endpoints, two assessments (one for left side, one for right side of body) should be reported for each muscle group or sensation area for motor strength/weakness, muscle stretch reflexes, and sensation subscales for each patient at the relevant timepoint. If the score on the left side of the body for a specific group/area is missing, and the score on the right is not missing, then the score on the right side could be used as score for the left side. The imputation could be applied vice versa. No further imputation will be performed when calculating final NIS-CS and NIS-LL total scores.

For mBMI, the pre-treatment value can be used to impute the missing post-treatment value for the same patient.

No missing data imputation will be performed for other neurologic progression or overall health endpoints.

7. STATISTICAL METHODOLOGY AND STATISTICAL ANALYSES

7.1. Statistical Methods

7.1.1. Analyses for Categorical/Binary Data

Categorical or binary variables including sex, race, ethnicity, age group at tafamidis initiation and at ATTR diagnosis, TTR genotype, family history, individual comorbid conditions, and CCI group will be summarized by using frequencies and percentages.

7.1.2. Analyses for Continuous Data

Continuous variables including age at tafamidis initiation, age at ATTR-CM diagnosis, height, CCI score, and neuropathy scores will be reported using the summary statistics of mean, standard deviation (SD), median, first and third quartiles, and range. Change from baseline in neuropathy scores will be described using descriptive statistics. Continuous age and age at ATTR-CM diagnosis will be further categorized into age groups based on clinical knowledge and literature review. Variable distributions will be examined using summary measures, tables, and graphics, such as histograms and scatter plots, where appropriate. Variables will initially be used as they were obtained; however, transformation may be necessary or prove more informative. For example, continuous variables may be categorized, using common category boundaries found in the literature to stratify the data.

7.1.3. Analyses for Change from Baseline and Repeated Measured Endpoints

Continuous variables representing neurologic disease progression, such as NIS-CS, NIS-LL, PND, MRC, CMTNS2 and mBMI may be collected at multiple time points. Measures may be reported in the pre-treatment baseline period (at least 6 months (and up to 12 months) before treatment initiation), tafamidis initiation (within 3 months before or after treatment initiation), and post-treatment (at least 6 months after treatment initiation with a ±3-month window) as population mean, SD, median, first and third quartiles, and range in a table or chart format. If a participant had multiple values in one or more of the pre-defined periods, the mean value will be used in calculating the population-level summary statistics. More details of the number of assessments and assessment schedule will be finalized once the data structure is known.

PND and CMTNS2 scores will be summarized using a frequency table for both the pre-treatment and tafamidis treatment periods. Additionally, a shift table will be used to capture the change in PND and CMTNS2 scores between the two periods. The shift table will capture the changes at each visit relative to start of the period, ie, baseline for the pre-treatment period and tafamidis initiation visit for the tafamidis treatment period (within 3 months before or after treatment initiation).

7.2. Statistical Analyses

For the primary and secondary objectives, neurologic function, ie, NIS-CS, NIS-LL, MRC, CMTNS2, and mBMI will be examined in the full analysis set. If there is a sufficient number of patients with at least three neurologic assessments a linear mixed-effect model will be conducted to compare the rate of disease progression (within patient) between pre-treatment and tafamidis treatment periods. Patients must have at least three assessments to be included in the linear mixed-effect model (one in the pre-treatment period, one in the tafamidis treatment period, and one additional that can be in either pre-treatment or in the tafamidis treatment period). The linear mixed-effect model includes pre-treatment baseline score, treatment period, and their interactions with time as fixed effects, and the time-slope and intercept for each patient are included as random effects. Time is defined as number of months from pre-treatment baseline. The estimates of the monthly rate of change, along with the 95% confidence interval and the p value associated with the within group comparison comparing the pre-treatment and tafamidis treatment periods will be summarized.

However, if there is not a sufficient number of patients with at least three neurologic assessments, and many patients only have two assessments, then an approach is to take the first measurement and the measurement that fell within the window of the measurement in which majority of the patients had for the post-treatment assessment (whether they only have two or three or more) and test for significant statistical differences between the two assessments using an unpaired, or independent samples, t-test.

Alternatively, in the event there is an insufficient number of patients with three or more neurologic assessments, separate subgroup statistical analyses may be performed. This approach may use a between-subjects analysis of variance (ANOVA) for patients with at least three neurologic assessments and conduct unpaired, or independent samples, t-test for

patients with only two neurologic assessments. Descriptive summaries will be provided by treatment (pre-treatment or post-treatment), or by subgroups (if data permits) for each neurologic function endpoint.

It is anticipated that there will be patients who do not satisfy inclusion criteria for pre and post treatment comparisons for various reasons such as not being on treatment for at least 6 months (with ±3-month window) because they passed away, not having a pre-treatment or post-treatment neurologic assessment, not having a NIS score or other missing data points and/or reasons. While pre and post treatment comparisons cannot be conducted for these patients, descriptive statistics on the composite score and/or its sub-scale will be reported to characterize patient and treatment characteristics.

Additional post-hoc analyses of final data from this study may be performed against final data from comparable tafamidis-treated and untreated ATTRv mixed phenotype patients from the Transthyretin Amyloidosis Outcomes Survey (THAOS; Pfizer Study B3461001). If performed, analysis methods will be provided as part of an amendment to this SAP.

All data analysis will be executed using statistical software SAS version 9.4.

7.2.1. Safety Analyses

Not applicable.

8. REFERENCES

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