

Protocol

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SUPPLEMENTARY APPENDIX

This supplement contains the following items:

APOLLO (ALN-TTR02-004) Protocol

- Original protocol (version 1)
- Final protocol (version 6)
- Summary of protocol changes


APOLLO (ALN-TTR02-004) Statistical analysis plan (SAP)

- Original SAP (version 1)
- Final SAP (version 2.1)
- Summary of SAP changes



**CLINICAL STUDY PROTOCOL
ALN-TTR02-004**

APOLLO: A Phase 3 Multicenter, Multinational, Randomized, Double-blind, Placebo-controlled Study to Evaluate the Efficacy and Safety of ALN-TTR02 in Transthyretin (TTR)-Mediated Polyneuropathy (Familial Amyloidotic Polyneuropathy-FAP)

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| Sponsor Contact: |  |

CONFIDENTIAL

The concepts and information contained in this document or generated during the study are considered proprietary and may not be disclosed in whole or in part without expressed written authorization of Anylam Pharmaceuticals, Inc.

The study will be completed according to guidelines of Good Clinical Practice. Compliance with this practice provides public assurance that the rights, safety, and well-being of study patients are protected consistent with the principles that have their origin in the Declaration of Helsinki.

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PROTOCOL SYNOPSIS

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| Protocol Title | APOLLO: A Phase 3 Multicenter, Multinational, Randomized, Double-blind, Placebo-controlled Study to Evaluate the Efficacy and Safety of ALN-TTR02 in Transthyretin (TTR)-Mediated Polyneuropathy (Familial Amyloidotic Polyneuropathy-FAP) |
| Indication | Treatment of transthyretin-mediated amyloidosis (ATTR) in patients with symptomatic polyneuropathy |
| Protocol Number | ALN-TTR02-004 |
| Phase of Development | 3 |
| Design | <p>This is a multicenter, multinational, randomized, double-blind study comparing ALN-TTR02 to placebo in ATTR patients with symptomatic Familial Amyloidotic Polyneuropathy (FAP).</p> <p>Consented eligible patients will be randomized to receive either 0.3 mg/kg ALN-TTR02 or placebo in a 2:1 ratio (ALN-TTR02 to placebo) in a blinded manner. Treatment arms will be balanced at entry for Neuropathy Impairment Score (NIS; 10-49 vs 50-100), early onset V30M (<50 years of age at onset) vs all other mutations (including late onset V30M), and previous tetramer stabilizer use (tafamidis or diflunisal) vs no previous tetramer stabilizer use. Patients will receive ALN-TTR02 or placebo once every 21 days for 78 weeks.</p> <p>Patients will have efficacy assessments at screening/baseline, 9 months, and 18 months.</p> <p>At the 9-month time point, if the clinical adjudication committee determines that a patient is exhibiting rapid disease progression (defined as ≥ 24 point increase in mNIS+7 from baseline [based on an average of 2 measurements] <u>and</u> FAP stage progression relative to baseline), the patient's treating physician will provide the patient with the option of discontinuing study drug and receiving local standard of care treatment for FAP. Patients who discontinue study drug will remain on study, following a modified schedule of visits, through completion of the 18-month efficacy assessments (blinding will be maintained throughout).</p> <p>Patients who complete the 18-month efficacy assessments can elect to participate in an extension study in which patients would receive open-label administration of 0.3 mg/kg ALN-TTR02 once every 21 days.</p> |

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| | A Data Monitoring Committee (DMC) will be implemented for the study and will operate under a prespecified charter. |
| Study Sites | This study will be conducted at multiple sites worldwide. |
| Investigational Drug | ALN-TTR02 (comprising an siRNA targeting mutant and wild-type TTR mRNA, in a lipid nanoparticle formulation for intravenous [IV] administration) |
| Dosage, Route of Administration and Duration of Treatment of Investigational Drug | <p>Patients randomized to the active treatment group will receive 0.3 mg/kg ALN-TTR02 once every 21 (± 3) days administered as an IV infusion over 70 minutes (approximately 1 mL/minute for the first 15 minutes followed by approximately 3 mL/minutes for the remainder of the infusion) by a controlled infusion device.</p> <p>All patients will receive the following premedication regimen at least 60 minutes prior to start of infusion of study drug:</p> <ul style="list-style-type: none"> • intravenous dexamethasone (10 mg) or equivalent, • oral paracetamol/acetaminophen (500 mg) or equivalent, • intravenous H2 blocker (e.g., ranitidine 50 mg, famotidine 20 mg, or equivalent), and • intravenous H1 blocker (diphenhydramine 50 mg or equivalent). |
| Control Drug | Placebo (normal saline 0.9% for IV administration) |
| Dosage, Route of Administration and Duration of Treatment of Control Drug | <p>Patients randomized to placebo will receive IV normal saline (0.9%) using the same dosing schedule and infusion rate as the active treatment group.</p> <p>Placebo patients will also receive the same premedication regimen as the active treatment group.</p> |
| Time on Study | The duration of patient participation in this study is approximately 21 months (inclusive of a 28-day screening window and up to a 56-day post last dose study visit). |
| Primary Objective | The primary objective of the study is to determine the efficacy of ALN-TTR02 by evaluating the difference between the ALN-TTR02 and placebo groups in the change from baseline of modified NIS+7 (mNIS+7) score at 18 months. |

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| <p>Secondary Objectives</p> | <p>The secondary objectives of the study are to determine the effect of ALN-TTR02 on various clinical parameters by assessing the difference between ALN-TTR02 and placebo in the change from baseline in the following measurements at 18 months:</p> <ul style="list-style-type: none"> • Norfolk Quality of Life-Diabetic Neuropathy (Norfolk QOL-DN) and EuroQOL (EQ-5D) questionnaires • Modified Body Mass Index (mBMI) and autonomic symptoms questionnaire (Composite Autonomic Symptom Score [COMPASS-31]) • NIS-weakness (NIS-W) and timed 10-meter walk test |
| <p>Exploratory Objectives:</p> | <p>The exploratory objectives of the study are:</p> <ul style="list-style-type: none"> • To determine the difference between the ALN-TTR02 and placebo groups in the change from baseline in the following measurements at 18 months: <ul style="list-style-type: none"> ○ NIS+7 score; ○ Grip strength; ○ Level of disability (Rasch-built Overall Disability Scale [R-ODS]); ○ Large vs small nerve fiber function including nerve conduction studies (NCS) 5 attributes ($\Sigma 5$), quantitative sensory testing by body surface area including touch pressure and heat pain (QST), vibration detection threshold (VDT), heart rate response to deep breathing (HRdb), postural blood pressure; ○ Pathologic evaluation of sensory and autonomic innervation through voluntary skin punch biopsies and analysis of intraepidermal nerve fiber density (IENFD) and sweat gland nerve fiber density (SGNFD); ○ Assessment of ambulation through FAP stage and Polyneuropathy Disability (PND) score; ○ Cardiac assessment through echocardiogram, troponin I, and N-terminal prohormone of B-type natriuretic peptide (NT-proBNP) levels; ○ Pharmacodynamic (PD) biomarkers (TTR, retinol binding protein [RBP], vitamin A); • To compare the proportion of patients in the ALN-TTR02 and placebo groups who meet the pre-defined criterion for rapid disease progression (defined as ≥ 24 point increase in mNIS+7 from baseline [based on an average of 2 measurements] <u>and</u> FAP stage progression relative to baseline) at 9 months. |

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| <p>Sample Size:</p> | <p>Approximately 200 patients will be enrolled in this study. An mNIS+7 progression rate (primary endpoint) in the placebo group of 24 ± 16 points in 18 months was estimated using natural history data from FAP patients. A sample of 154 patients provides 90% power for a 2-sided test with an 8.95 point (37.5%) mean difference between treatment groups in the primary endpoint at 2-sided $\alpha = 0.05$. Assuming a 25% random premature discontinuation rate (due to liver transplantation or other factors), the sample size for this study is approximately 200. Additional patients may be enrolled based on a recommendation to increase the sample size in the interim analysis.</p> |
| <p>Inclusion and Exclusion Criteria:</p> | <p>To be enrolled in the study, each patient must meet the following criteria within the Screening and Screening/Baseline Visits:</p> <ol style="list-style-type: none"> 1. Male or female of 18 to 80 years of age (inclusive); 2. Have a diagnosis of FAP with documented TTR mutation; 3. Have a NIS of 10 to 100 (inclusive); 4. The NCS ($\Sigma 5$) must, at a minimum, show a clear abnormality (≤ 5th percentile) of the sural nerve action potential (SNAP); 5. Have a Karnofsky performance status of $\geq 60\%$; 6. Have an absolute neutrophil count (ANC) ≥ 1500 cells/mm³, a platelet count $\geq 100,000$ cells/mm³, and hemoglobin ≥ 10 g/dL (or ≥ 100 g/L); 7. Have aspartate transaminase (AST) and alanine transaminase (ALT) levels $\leq 2.5 \times$ the upper limit of normal (ULN), total bilirubin within normal limits, albumin > 3 g/dL (or > 30 g/L), international normalized ratio (INR) ≤ 1.2; 8. Have a serum creatinine $\leq 1.5 \times$ ULN; 9. Have negative serology for hepatitis B virus (HBV) and hepatitis C virus (HCV); 10. Women of child-bearing potential must have a negative pregnancy test, cannot be breastfeeding, and must be using 2 highly effective methods of contraception prior to screening, throughout study participation, and for 1 month after last dose of study drug. Highly effective methods of birth control are defined as: hormonal (e.g., oral, implantable, injectable, or transdermal contraceptives in conjunction with spermicide, condom, or diaphragm), mechanical (e.g., spermicide in conjunction with a barrier such as a condom or diaphragm), intrauterine device in |

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| | <p>conjunction with spermicide or condom, or surgical sterilization of partner in conjunction with spermicide, condom, or diaphragm;</p> <ol style="list-style-type: none"> 11. Males with partners of child-bearing potential, must agree to use 1 barrier method (e.g., condom) and 1 additional method (e.g., spermicide) of contraception throughout study participation and for 1 month after the last dose of study drug; males must also abstain from sperm donation after the first dose of study drug through study participation and for 1 month after last dose of study drug; 12. Must be willing and able to comply with protocol-required visit schedule and visit requirements and provide written informed consent. <p>A patient will be excluded if they meet any of the following criteria at the time of the Screening and Screening/Baseline Visits:</p> <ol style="list-style-type: none"> 1. Has vitamin A levels below the lower limit of normal (LLN); 2. Had a prior liver transplant or is planned to undergo liver transplant during the study period; 3. Has other known causes of sensorimotor or autonomic neuropathy (e.g., autoimmune disease, monoclonal gammopathy, etc.); 4. Has known primary amyloidosis or leptomeningeal amyloidosis; 5. Has known type I diabetes; 6. Has had type II diabetes mellitus for ≥ 5 years; 7. Has vitamin B12 levels below LLN; 8. Has untreated hypo- or hyperthyroidism; 9. Has had a major surgery within the past 3 months or has a major surgery planned during any point of the study period; 10. Has known human immunodeficiency virus (HIV) infection; 11. Has an active infection requiring systemic antiviral or antimicrobial therapy that will not be completed prior to the first dose of study drug administration; 12. Had a malignancy within 2 years, except for basal or squamous cell carcinoma of the skin or carcinoma in situ of |
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| | <p>the cervix that has been successfully treated;</p> <ol style="list-style-type: none"> 13. Has a New York Heart Association heart failure classification >2; 14. Had acute coronary syndrome within the past 3 months; 15. Has uncontrolled clinically significant cardiac arrhythmia or unstable angina; 16. Has a known history of alcohol abuse or daily heavy alcohol consumption (females: more than 14 units of alcohol per week; males: more than 21 units of alcohol per week [unit: 1 glass of wine [125 mL] = 1 measure of spirits = ½ pint of beer]); 17. Received an investigational agent or device within 30 days of anticipated study drug administration or 5 half-lives of the investigational drug, whichever is longer; 18. Participated in a clinical trial with an antisense oligonucleotide for more than 3 months; if in a clinical trial with antisense oligonucleotide for ≤3 months, must have completed a 3-month wash-out prior to start of study drug administration in this study; 19. Is currently taking diflunisal, tafamidis, doxycycline, or tauroursodeoxycholic acid; if previously on any of these agents, must have completed a 14-day wash-out prior to start of study drug administration in this study; 20. Had a prior severe reaction to a liposomal product or a known hypersensitivity to oligonucleotides or any component of ALN-TTR02; 21. Is unable to take the required premedications; 22. Anticipated survival is less than 2 years, in the opinion of the Investigator; 23. Is considered unfit for the study by the Investigator. |
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| <p>Efficacy Assessments</p> | <p>Efficacy parameters will include the following (all evaluations will be conducted at baseline and at 9 and 18 months):</p> <ul style="list-style-type: none"> • Neurologic impairment will be assessed using the mNIS+7 composite score. The mNIS+7 includes the modified NIS (weakness and reflexes), NCS Σ5, QST, as well as autonomic assessment through postural blood pressure. • Patient-reported QOL will be evaluated using the Norfolk QOL-DN and the EQ-5D. Disability will be reported by patients using the R-ODS. • Autonomic symptoms will be assessed using the COMPASS-31. • Motor function assessments to be evaluated include NIS-W, timed 10-meter walk test, and grip strength test. • PND score and FAP stage. • Nutritional status will be assessed using mBMI. • Pathologic evaluation of sensory and autonomic innervation will be evaluated by IENFD analysis and quantitation of dermal sweat gland nerve fibers (SGNFD) via tandem 3 mm skin punch biopsies taken from the leg. • Neurologic impairment will also be assessed by NIS+7 (including full NIS, NCS, vibratory detection threshold [VDT], and heart rate variation with deep breathing [HRdb]). • Cardiac structure and function will be assessed through echocardiograms as well as measurement of serum levels of NT-proBNP and troponin I. |
| <p>Pharmacodynamic Assessments</p> | <p>Pharmacodynamic markers assessed serially will include serum TTR, vitamin A, and RBP. Additional blood samples will be collected for exploratory biomarkers related to FAP.</p> |

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| <p>Pharmacokinetic Assessments</p> | <p>The plasma pharmacokinetic (PK) evaluation will include, whenever possible, plasma-concentration time profiles for siRNA and the novel lipid components in ALN-TTR02: DLin-MC3-DMA and polyethylene glycol (PEG)₂₀₀₀-C-DMG. The siRNA, DLin-MC3-DMA, and PEG₂₀₀₀-C-DMG concentration will be determined for all patients at time points specified in Table 1-1, Table 1-2, and Table 1-3.</p> <p>Urine will be collected with void volume recorded for all patients at time points specified in Table 1-1, Table 1-2, and Table 1-3 to determine renal clearance (CL_R) of siRNA and 4-dimethylaminodibutyric acid (the metabolite of DLin-MC3-DMA) after dosing with study drug.</p> |
| <p>Safety Assessments</p> | <p>Safety will be assessed throughout the study by collecting adverse events (AEs; including serious adverse events [SAEs]); clinical laboratory tests, including hematology, clinical chemistry (including liver function tests), thyroid function parameters, and urinalysis; measurement of anti-drug antibodies; electrocardiograms; vital signs; physical examination findings; and ophthalmology examinations.</p> |
| <p>Other Assessments</p> | <p>Disease burden and healthcare utilization will be assessed using a patient-reported pharmacoeconomics questionnaire. The investigator will periodically assess mental status as it relates to suicidal ideation and behavior by using the Columbia–Suicide Severity Rating Scale (C-SSRS) questionnaire.</p> |
| <p>Follow-Up</p> | <p>Patients who complete the 18-month efficacy assessments can elect to participate in an extension study in which patients would receive open-label administration of 0.3 mg/kg ALN-TTR02 once every 21 days. Eligible patients who elect to participate will return for a follow-up visit 21 days after last dose of study drug.</p> <p>If the patient decides not to participate in the extension study, the patient will complete 2 follow-up visits at 21 and 56 days after the last dose of study drug. Each follow-up visit includes safety assessments and the 21-day follow-up also includes PD measurements.</p> |
| <p>Statistical Methods</p> | <p>A full statistical analysis plan (SAP) will be finalized prior to database lock.</p> <p>The primary analysis will compare patients administered ALN-TTR02 versus those administered placebo in the modified intent-to-treat (mITT) population. Specifically, subjects who are randomized and receive at least 1 dose of study drug will be included in this analysis. The primary analysis will compare change in mNIS+7 from baseline at 18 months between</p> |

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| | <p>ALN-TTR02 and placebo groups, adjusted for the stratification factors. Analysis of covariance (ANCOVA) will be used to analyze the primary endpoint. Primary endpoint data that are missing will be inferred using multiple imputations. Sensitivity analyses, including mixed model repeated measures (MMRM) analyses, will assess the robustness of the primary analysis for the mITT and per protocol (PP) populations.</p> <p>Type I error control for secondary endpoints will be achieved by a grouped hierarchical ordering procedure. Briefly, endpoints will be evaluated in families (functionally linked efficacy assessments), with multiplicity controlled within family via the Benjamini-Hochberg procedure.</p> <p>An independent interim analysis committee (IAC) will conduct a blinded interim analysis when approximately 50% of patients have completed their 9-month mNIS+7 assessments. This interim analysis will estimate the overall variance in the mNIS+7 score. Based on the results of the interim analysis, the committee can recommend either increasing the study sample size or making no adjustment to the sample size.</p> <p>PK analyses will be conducted using non-compartmental and/or compartmental evaluation. The PK parameters of siRNA, DLin-MC3-DMA, and PEG₂₀₀₀-C-DMG in plasma will be evaluated.</p> <p>Population PK analyses will be performed whenever possible on available siRNA, DLin-MC3-DMA, and PEG₂₀₀₀-C-DMG from sparse samples collected at various time points during the duration of the study.</p> <p>Inferential statistics of PD and PK/PD parameters, correlation between siRNA, DLin-MC3-DMA or PEG₂₀₀₀C-DMG exposure versus TTR, RBP, or vitamin A, and between TTR versus RBP and vitamin A will be provided.</p> |
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Table 1-1: Schedule of Assessments; Screening to 9-Month Efficacy Assessment

| Procedure | Visit Type | Screening | Screening/ Baseline ^(a) | Baseline ^(a) | Predosing | Dosing | | | | | | | | | | | 9-Month Efficacy Assessment ^(v) | | |
|--|------------|------------------|---------------------------------------|-------------------------|---------------|--------|-----|-----|-----|-----|------|------|------|------|------|------|--|------|---------------|
| | Study Day | Day -28 to 0 | | | D0 Predose | D0 | D21 | D42 | D63 | D84 | D105 | D126 | D147 | D168 | D189 | D210 | D231 | D252 | D259- D266 |
| | Study Week | NA | | | 0 | 0 | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 | 37-38 |
| | Windows | NA | | | 0 | 0 | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | NA |
| Informed Consent | X | | | | | | | | | | | | | | | | | | |
| Inclusion/Exclusion Criteria | X | X | X | | | | | | | | | | | | | | | | |
| Medical History | X | X ^(b) | X ^(b) | | | | | | | | | | | | | | | | |
| Demographics | X | | | | | | | | | | | | | | | | | | |
| Review Documentation of TTR Genotype | X | | | | | | | | | | | | | | | | | | |
| HIV Status Review | X | | | | | | | | | | | | | | | | | | |
| Karnofsky Performance Status | X | | | | | | | | | | | | | | | | | | |
| New York Heart Classification | X | | | | | | | | | | | | | | | | | | |
| Serology Testing ^(c) | X | | | | | | | | | | | | | | | | | | |
| Paraprotein by IFE | X | | | | | | | | | | | | | | | | | | |
| Vitamin B12 | X | | | | | | | | | | | | | | | | | | |
| Efficacy Assessments | | | | | | | | | | | | | | | | | | | |
| NIS ^(d) / NCS ^(d) | X | | | | | | | | | | | | | | | | | | |
| mNIS+7 ^(e) | | X | X | | | | | | | | | | | | | | | X | X |
| NIS + 7 ^(f) | | X | X | | | | | | | | | | | | | | | X | X |
| PND Score and FAP Score | | | X | | | | | | | | | | | | | | | | X |
| Skin Punch Biopsy (IENFD and SGNFD) ^(g) | | | X | | | | | | | | | | | | | | | | X |
| mBMI ^(h) | | | X | | | | | | | | | | | | | | | | X |
| 10-meter Walk Test ⁽ⁱ⁾ | | X | X | | | | | | | | | | | | | | | X | X |
| Grip Strength Test ⁽ⁱ⁾ | | X | X | | | | | | | | | | | | | | | X | X |
| Norfolk QOL-DN; E5-QD; R-ODS; COMPASS 31 Questionnaires | | | X | | | | | | | | | | | | | | | | X |
| Echocardiogram | | | X | | | | | | | | | | | | | | | | |
| NT-proBNP and Troponin I | | | X | | | | | | | | | | | | | | | | X |

Table 1-1: Schedule of Assessments; Screening to 9-Month Efficacy Assessment (continued)

| Procedure | Visit Type | Screening | Screening/ Baseline ^(a) | Baseline ^(a) | Predosing | Dosing | | | | | | | | | | | | 9-Month Efficacy Assessment ^(c) | |
|--|------------------|--------------|---------------------------------------|-------------------------|---------------|--------|-----|-----|-----|-----|------|------|------|------|------|------|------|---|---------------|
| | Study Day | Day -28 to 0 | | | D0 Predose | D0 | D21 | D42 | D63 | D84 | D105 | D126 | D147 | D168 | D189 | D210 | D231 | D252 | D259- D266 |
| | Study Week | NA | | | 0 | 0 | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 | 37-38 |
| | Windows | NA | | | 0 | 0 | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | NA |
| PD Assessments ^(k) | | | | | | | | | | | | | | | | | | | |
| TTR Protein, Vitamin A, and RBP | X ^(l) | X | X | X | | X | | | | | X | | | | | | | X | X |
| Obtain Blood Sample for Long-term Storage | | X | X | X | | X | | | | | X | | | | | | | X | X |
| Safety Assessments ^(s) | | | | | | | | | | | | | | | | | | | |
| Physical Examination | X | | | | | | | | | | | | | | | | | | X |
| Weight ^(m) | X | | X | X | | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Height | X | | | | | | | | | | | | | | | | | | |
| Vital Signs ⁽ⁿ⁾ | X | | | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| 12-Lead ECG | | | X | | | | | | | | | | | | | | | | X |
| Serum Chemistry | X | | | X | | | | | X | | | | | | X | | | | |
| Hematology, Urinalysis | X | | | | | | | | | | | | | | | | | | X |
| Thyroid Function Tests | X | | | | | | | | | | | | | | | | | | X |
| Coagulation Studies | X | | | | | | | | | | | | | | | | | | |
| Anti-drug Antibody Testing ^(o) | | | | X | | X | | | | | X | | | | | | | | X |
| Pregnancy Test ^(p) | X | | | | | | | | | | | | | | | | | | |
| Ophthalmology Exam ^(q) | | | X | | | | | | | | | | | | | | | | X |
| Concomitant Medications/Treatments | X | X | X | | | | | | | | X | | | | | | | | |
| Adverse Events | | | | | | | | | | | X | | | | | | | | |
| PK Assessments | | | | | | | | | | | | | | | | | | | |
| Plasma PK Sampling ^(r) | | | | X | X | X | | | | | X | | | | | | | | X |
| Urine PK Sampling ^(s) | | | | X | | X | | | | | X | | | | | | | | X |
| Other Assessments | | | | | | | | | | | | | | | | | | | |
| Pharmacoeconomic Questionnaire | | | X | | | | | | | | | | | | | | | | X |
| CSSR Questionnaire | | | X | | | | | | | | | | | | | | | | X |
| Drug Administration | | | | | | | | | | | | | | | | | | | |
| Randomization ^(t) | | | | X | | | | | | | | | | | | | | | |
| Pre-medication Administration ^(u) | | | | X | | X | X | X | X | X | X | X | X | X | X | X | X | X | |
| Study Drug Administration ^(v) | | | | | X | X | X | X | X | X | X | X | X | X | X | X | X | X | |

Table 1-1 Footnotes:

- a. The Screening/Baseline and Baseline visits will be performed on separate days. The Screening/Baseline visit must be performed within 14 days prior to the first dose of study drug (Day 0). The Baseline visit must be conducted at least 24 hours and not more than 7 days after the Screening/Baseline visit.
- b. An interval medical history will be collected at the Screening/Baseline and Baseline Visit. Only changes since the Screening Visit will be collected.
- c. Serologies will include hepatitis B surface antibody (HbsAb), hepatitis B surface antigen (HbsAg), and anti-hepatitis C virus antibody (anti-HCV Ab).
- d. The documented results of previously performed NIS and nerve conduction studies (NCS) may be used to qualify a patient for this study if these tests were performed within 60 days prior to the date of informed consent.
- e. The mNIS+7 consists of the modified NIS tool (weakness and reflexes), NCS 5 attributes ($\Sigma 5$), quantitative sensory testing (QST) by body surface area including touch pressure (TP) and heat pain (HP), and postural blood pressure. At the 9-month efficacy assessment, 2 assessments of the mNIS+7 will be performed on separate days (1 assessment on each day). The second (repeat) assessment must be conducted at least 24 hours after the first assessment, but not more than 7 days apart. Components that are shared between the mNIS+7 and NIS+7 (including NIS and NCS) will be performed once at each assessment (e.g., the weakness component should not be performed more than once on any given day).
- f. The NIS+7 consists of the NIS tool (weakness, sensation, and reflexes), NCS $\Sigma 5$, VDT, and heart rate response to deep breathing. At the 9-month efficacy assessment, 2 assessments of the NIS+7 will be performed on separate days (1 assessment on each day). The second (repeat) assessment must be conducted at least 24 hours after the first assessment, but not more than 7 days apart. Components that are shared between the mNIS+7 and NIS+7 (including NIS and NCS) will be performed once at each assessment (e.g., the weakness component should not be performed more than once on any given day).
- g. If the patient has provided separate informed consent for skin biopsies, 2 sets of tandem 3-mm skin punch biopsies are to be obtained (4 biopsies total). One set of biopsies will be taken from the distal lower leg, when a patient's clinical status allows, and one set from the distal thigh at each time point.
- h. The mBMI calculation will take place programmatically in the clinical database; the site will not perform the calculation.
- i. The patient will be asked to walk 10 meters. The walk must be completed without assistance from another person; ambulatory aids such as canes and walkers are permitted. The time required for the patient to walk 10 meters will be recorded. At the 9-month efficacy assessment, 2 assessments of the 10-meter walk test will be performed on separate days (1 assessment on each day). The second (repeat) assessment must be conducted at least 24 hours after the first assessment, but not more than 7 days apart.
- j. Grip strength will be measured in triplicate using a dynamometer held in the dominant hand. Every effort will be made to use the same device for a patient throughout the duration of the study. At the 9-month efficacy assessment, 2 assessments of the grip strength will be performed on separate days (1 assessment on each day). The second (repeat) assessment must be conducted at least 24 hours after the first assessment, but not more than 7 days apart.
- k. On dosing days, blood samples for PD assessments will be obtained prior to dosing and vitamin A supplementation.
- l. At screening, only Vitamin A is to be tested.
- m. Weight from previous visit should be used for calculating dose. Weight must be collected pre-dose.
- n. Vital signs to include: blood pressure, pulse rate, oral body temperature, and respiratory rate. Parameters are to be measured in the supine position using an automated instrument after the patient has rested comfortably for 10 minutes. Vital signs must be collected pre-dose. On Day 0, vital signs will also be collected post-dose.
- o. Blood samples for anti-drug antibody testing will be collected prior to study drug dosing.
- p. A pregnancy test (urine- or serum-based) will be performed on all females of child-bearing potential.
- q. The baseline ophthalmology examination may be performed any time after the patient is deemed eligible for participation in the study, but before the first administration of study drug.
- r. Plasma PK samples will be collected as follows: Day 0: pre-dose (within 1 hour of planned study drug dosing) and at the end of infusion (+5 minutes). Day 21 and Day 252: pre-dose (within 1 hour of planned study drug dosing) and 30 minutes after the end of the infusion (+15 minutes). Day 126: pre-dose (within 1 hour of planned study drug dosing) and at the end of infusion (+5 minutes).
- s. Urine PK samples will be collected pre-dose (within 1 hour of planned study drug dosing).

(continued)

Table 1-1 Footnotes (continued):

- t. Randomization procedures are described in [Section 4.4.1](#).
- u. At least 60 minutes prior to the start of infusion of study drug, a premedication regimen will be administered. The regimen is described in [Section 5.3.1](#).
- v. The patient's infusion site should be assessed for signs of any localized reaction during the infusion and for 30 minutes after the end of the infusion. The patient will remain at the study site for 1 hour following completion of dosing for observation and completion of assessments.
- w. Patients who discontinue study drug due to rapid disease progression based on the 9-month efficacy assessments will continue on to the Modified Visit Schedule (See [Table 1-3](#)).
- x. On dosing day, all safety assessments are performed pre-dose.

Table 1-2: Schedule of Assessments; Week 39 to Week 86 (Follow-up) / Early Withdrawal

| Procedure | Visit Type | Dosing | | | | | | | | | | | | | | 18-Month Efficacy Assessment | End of Study ^(a) | Follow-up ^(a) | Early Withdrawal ^(b) | |
|---|------------|--------|------|------|------|------|------|------|------|------|------|------|------|------|------|------------------------------|-----------------------------|--------------------------|---------------------------------|---|
| | Study Day | D273 | D294 | D315 | D336 | D357 | D378 | D399 | D420 | D441 | D462 | D483 | D504 | D525 | D546 | D553-D560 | D567 | D602 | NA | |
| | Study Week | 39 | 42 | 45 | 48 | 51 | 54 | 57 | 60 | 63 | 66 | 69 | 72 | 75 | 78 | 79-80 | 81 | 86 | NA | |
| | Windows | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | NA | ±7D | ±10D | 2D to 7D | |
| Efficacy Assessments | | | | | | | | | | | | | | | | | | | | |
| mNIS+7 ^(c) | | | | | | | | | | | | | | | | X | X | | X | X |
| NIS + 7 ^(d) | | | | | | | | | | | | | | | | X | X | | X | X |
| PND Score and FAP Score | | | | | | | | | | | | | | | | X | | | | X |
| Skin Punch Biopsy (IENFD and SGNFD) ^(e) | | | | | | | | | | | | | | | | X | | | | X |
| mBMI ^(f) | | | | | | | | | | | | | | | | X | | | | X |
| 10-meter Walk Test ^(g) | | | | | | | | | | | | | | | | X | X | | X | X |
| Grip Strength Test ^(h) | | | | | | | | | | | | | | | | X | X | | X | X |
| Norfolk QOL-DN; E5-QD; R-ODS; COMPASS 31 Questionnaires | | | | | | | | | | | | | | | | X | | | | X |
| Echocardiogram | | | | | | | | | | | | | | | | X | | | | X |
| NF-proBNP and Troponin I | | | | | | | | | | | | | | | | X | | | | X |
| PD Assessments⁽ⁱ⁾ | | | | | | | | | | | | | | | | | | | | |
| TTR Protein, Vitamin A, and RBP | | X | | | | | | X | | | | | | | X | X | X | | X | X |
| Obtain Blood Sample for Long-term Storage | | X | | | | | | X | | | | | | | X | X | X | | X | X |

Table 1-2: Schedule of Assessments; Week 39 to Week 86 (Follow-up) / Early Withdrawal (continued)

| Procedure | Visit Type | Dosing | | | | | | | | | | | | | | 18-Month Efficacy Assessment | End of Study ^(a) | Follow-up ^(a) | Early Withdrawal ^(b) | | |
|--|------------|--------|------|------|------|------|------|------|------|------|------|------|------|------|------|------------------------------|-----------------------------|--------------------------|---------------------------------|---|---|
| | Study Day | D273 | D294 | D315 | D336 | D357 | D378 | D399 | D420 | D441 | D462 | D483 | D504 | D525 | D546 | D553-D560 | D567 | D602 | NA | | |
| | Study Week | 39 | 42 | 45 | 48 | 51 | 54 | 57 | 60 | 63 | 66 | 69 | 72 | 75 | 78 | 79-80 | 81 | 86 | NA | | |
| | Windows | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | NA | ±7D | ±10D | 2D to 7D | | |
| Safety Assessments^(r) | | | | | | | | | | | | | | | | | | | | | |
| Physical Examination | | | | | | | | | | | | | | | X | | X | X | X | | |
| Weight ⁽ⁱ⁾ | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | | X | X |
| Vital Signs ^(k) | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| 12-Lead ECG | | | | | | | | | | | | | | | | X | | | | | X |
| Serum Chemistry | | | | | | X | | | | | X | | | | | X | | X | X | | X |
| Hematology, Urinalysis | | | | | | | | | | | | | | | | X | | | | | X |
| Thyroid Function Tests | | | | | | | | | | | | | | | | X | | | | | X |
| Anti-drug Antibody Testing ^(l) | | | | | | | | X | | | | | | | | X | | | | | X |
| Pregnancy Test ^(m) | | | | | | | | | | | | | | | | | X | X | | | X |
| Ophthalmology Examination | | | | | | | | | | | | | | | | X | | | | | |
| Concomitant Medications/Treatments | | | | | | | | | | | X | | | | | | | | | | |
| Adverse Events | | | | | | | | | | | X | | | | | | | | | | |
| PK Assessments | | | | | | | | | | | | | | | | | | | | | |
| Plasma PK Sampling ⁽ⁿ⁾ | | | | | | | | X | | | | | | | | X | | | | | X |
| Urine PK Sampling ^(o) | | | | | | | | X | | | | | | | | X | | | | | X |
| Other Assessments | | | | | | | | | | | | | | | | | | | | | |
| Pharmacoeconomic Questionnaire | | | | | | | | | | | | | | | | X | | | | | X |
| CSSR Questionnaire | | | | | | | | | | | | | | | | X | | | | | X |
| Drug Administration | | | | | | | | | | | | | | | | | | | | | |
| Pre-medication Administration ^(p) | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | | | | | | |
| Study Drug Administration ^(q) | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | | | | | | |

Table 1-2 Footnotes:

- a. If a patient enrolls in the extension study, the patient will only have to complete the 21-day follow-up assessments (EOS; Day 567) and not the 56-day follow-up assessments (Day 602). Patients who do not enroll in the extension study will need to complete both follow-up visits (Days 567 and 602).
- b. The Early Withdrawal Visit will take place over 2 days to allow for the repeat assessment of the mNIS+7, NIS+7, Timed 10-meter Walk, and Grip Strength Test.
- c. The mNIS+7 consists of the modified NIS tool (weakness and reflexes), NCS, 5 attributes ($\Sigma 5$), quantitative sensory testing (QST) by body surface area including touch pressure (TP) and heat pain (HP), and postural blood pressure. Two assessments will be performed on separate days (1 assessment on each day). The second (repeat) assessment must be conducted at least 24 hours after the first assessment, but not more than 7 days apart. Components that are shared between the mNIS+7 and NIS+7 (including NIS and nerve conduction studies [NCS]) will be performed once at each assessment (e.g., the weakness component should not be performed more than once on any given day).
- d. The NIS+7 consists of the NIS tool (weakness, sensation, and reflexes), NCS $\Sigma 5$, VDT, and heart rate response to deep breathing. Two assessments will be performed on separate days (1 assessment on each day). The second (repeat) assessment must be conducted at least 24 hours after the first assessment, but not more than 7 days apart. Components that are shared between the mNIS+7 and NIS+7 (including NIS and nerve conduction studies [NCS]) will be performed once at each assessment (e.g., the weakness component should not be performed more than once on any given day).
- e. If the patient has provided separate informed consent for skin biopsies, 2 sets of tandem 3-mm skin punch biopsies are to be obtained (4 biopsies total). One set of biopsies will be taken from the distal lower leg, when a patient's clinical status allows, and 1 set from the distal thigh at each time point.
- f. The mBMI calculation will take place programmatically in the clinical database; the site will not perform the calculation.
- g. The patient will be asked to walk 10 meters. The walk must be completed without assistance from another person; ambulatory aids such as canes and walkers are permitted. The time required for the patient to walk 10 meters will be recorded. Two assessments will be performed on separate days (1 assessment on each day). The second (repeat) assessment must be conducted at least 24 hours after the first assessment, but not more than 7 days apart.
- h. Grip strength will be measured using a dynamometer held in the dominant hand. Every effort will be made to use the same device for a patient throughout the duration of the study. Two assessments will be performed on separate days (1 assessment on each day). The second (repeat) assessment must be conducted at least 24 hours after the first assessment, but not more than 7 days apart.
- i. On dosing days, blood samples for PD assessments will be obtained prior to dosing and vitamin A supplementation.
- j. Weight from previous visit should be used for calculating dose. Weight must be collected pre-dose.
- k. Vital signs to include: blood pressure, pulse rate, oral body temperature, and respiratory rate. Parameters are to be measured in the supine position using an automated instrument after the patient has rested comfortably for 10 minutes. Vital signs must be collected pre-dose.
- l. On study drug dosing days, blood samples for anti-drug antibody testing will be collected pre-dose.
- m. A pregnancy test (urine- or serum-based) will be performed on all females of child-bearing potential.
- n. Plasma PK samples will be collected as follows: Day 399: pre-dose (within 1 hour of planned study drug dosing) and at the end of infusion (+5 minutes). Day 546: pre-dose (within 1 hour of planned study drug dosing) and 30 minutes after the end of the infusion (+15 minutes). Early Withdrawal: any time within the visit window.
- o. Urine PK samples will be collected as follows: Day 399 and Day 546: pre-dose (within 1 hour of planned study drug dosing). Early Withdrawal: any time within the visit window.
- p. At least 60 minutes prior to the start of infusion of study drug, a premedication regimen will be administered. The regimen is described in [Section 5.3.1](#).
- q. The patient's infusion site should be assessed for signs of any localized reaction during the infusion and for 30 minutes after the end of the infusion. The patient will remain at the study site for 1 hour following completion of dosing for observation and completion of assessments.
- r. On dosing day, all safety assessments are performed pre-dose.

Table 1-3: Modified Schedule of Assessments for Subjects who Discontinue Study Drug for Rapid Disease Progression; Week 39 to End of Study

| Procedure | Visit Type | Dosing | | | | | 18-Month Efficacy Assessment | | End of Study | Early Withdrawal ^(a) | |
|--|------------|---------------------|------|------|------|------|------------------------------|---|--------------|---------------------------------|---|
| | Study Day | D273 ^(b) | D294 | D378 | D462 | D546 | D553-D560 | | D567 | NA | |
| | Study Week | 39 | 42 | 54 | 66 | 78 | 79-80 | | 81 | NA | |
| | Windows | ±3D | ±3D | ±3D | ±3D | ±3D | NA | | ±7D | 2D to 7D | |
| Consultation on treatment options^(c) | | X | | | | | | | | | |
| Efficacy Assessments | | | | | | | | | | | |
| mNIS+7 ^(d) | | | | | | | X | X | | X | X |
| NIS + 7 ^(e) | | | | | | | X | X | | X | X |
| PND Score and FAP Score | | | | | | | X | | | X | |
| Skin Punch Biopsy (IENFD and SGNFD) ^(f) | | | | | | | X | | | X | |
| mBMI ^(g) | | | | | | | X | | | X | |
| 10-meter Walk Test ^(h) | | | | | | | X | X | | X | X |
| Grip Strength Test ⁽ⁱ⁾ | | | | | | | X | X | | X | X |
| Norfolk QOL-DN; E5-QD; R-ODS; COMPASS 31 Questionnaires | | | | | | | X | | | X | |
| Echocardiogram | | | | | | | X | | | X | |
| NT-proBNP and Troponin I | | | | | | | X | | | X | |
| PD Assessments | | | | | | | | | | | |
| TTR Protein, Vitamin A, and RBP | | | X | | | X | | | X | X | |
| Obtain Blood Sample for Long-term Storage | | | X | | | X | | | X | X | |

Table 1-3: Modified Schedule of Assessments for Subjects who Discontinue Study Drug for Rapid Disease Progression; Week 39 to End of Study (continued)

| Procedure | Visit Type | Dosing | | | | | 18-Month Efficacy Assessment | End of Study | Early Withdrawal ^(a) |
|---|------------|---------------------|------------------|------------------|------|------|------------------------------|--------------|---------------------------------|
| | Study Day | D273 ^(b) | D294 | D378 | D462 | D546 | D553-D560 | D567 | NA |
| | Study Week | 39 | 42 | 54 | 66 | 78 | 79-80 | 81 | NA |
| | Windows | ±3D | ±3D | ±3D | ±3D | ±3D | NA | ±7D | 2D to 7D |
| Safety Assessments | | | | | | | | | |
| Physical Examination | | X | | | | X | | X | X |
| Weight | | | | | | X | X | X | X |
| Vital Signs ⁽ⁱ⁾ | X | X | | | | X | X | X | X |
| 12-Lead ECG | | | | | | | X | | X |
| Serum Chemistry | | X | | | | X | | | X |
| Hematology, Urinalysis | | | | | | X | | | |
| Thyroid Function Tests | | | | | | X | | | X |
| Anti-drug Antibody Testing | | | | | | X | | | X |
| Pregnancy Test ^(k) | | X | | | | X | | | X |
| Concomitant Medications/Treatments ^(l) | | X | | | | | | | |
| Adverse Events ^(m) | | X | | | | | | | |
| Study-procedure-related Adverse Events ⁽ⁿ⁾ | | | | | | | X | | |
| PK Assessments | | | | | | | | | |
| Plasma PK Sampling | | X | | | | X | | | |
| Urine PK Sampling | | X | | | | X | | | |
| Other Assessments | | | | | | | | | |
| Pharmacoeconomic Questionnaire | | | | | | X | | | X |
| CSSR Questionnaire | | | | | | | X | | X |
| Collect Data on Subsequent FAP Treatment Regimens | | X | X ^(o) | X ^(o) | | X | | X | X |
| Phone Contact to Obtain Health Status Update ^(p) | | | X | X | | | | | |

Table 1-3 Footnotes:

- a. The Early Withdrawal Visit will be conducted for any subject who discontinues from the study after the Day 273 visit (e.g., from Day 274 onward). This visit will take place over 2 days (at least 24 hours, but not more than 7 days apart) to allow for the repeat assessment of the mNIS+7, NIS+7, timed 10-meter walk, and grip strength test.
- b. Patients who discontinue study drug and also decide to discontinue from the study at the time of this visit will not have any additional visits after the Day 273 assessments are completed.
- c. The patient will consult with the Investigator on a subsequent plan of care, which may include receiving therapy for FAP as per the local standard of care.
- d. The mNIS+7 consists of the modified NIS tool (weakness and reflexes), NCS, 5 attributes ($\Sigma 5$), quantitative sensory testing (QST) by body surface area including touch pressure (TP) and heat pain (HP), and postural blood pressure. Two assessments will be performed on separate days (1 assessment on each day). The second (repeat) assessment must be conducted at least 24 hours after the first assessment, but not more than 7 days apart. Components that are shared between the mNIS+7 and NIS+7 (including NIS and nerve conduction studies [NCS]) will be performed once at each assessment (e.g., the weakness component should not be performed more than once on any given day).
- e. The NIS+7 consists of the NIS tool (weakness, sensation, and reflexes), NCS $\Sigma 5$, VDT, and heart rate response to deep breathing. Two assessments will be performed on separate days (1 assessment on each day). The second (repeat) assessment must be conducted at least 24 hours after the first assessment, but not more than 7 days apart. Components that are shared between the mNIS+7 and NIS+7 (including NIS and nerve conduction studies [NCS]) will be performed once at each assessment (e.g., the weakness component should not be performed more than once on any given day).
- f. If the patient has provided separate informed consent for skin biopsies, 2 sets of tandem 3-mm skin punch biopsies are to be obtained (4 biopsies total). One set of biopsies will be taken from the distal lower leg, when a patient's clinical status allows, and 1 set from the distal thigh at each time point.
- g. The mBMI calculation will take place programmatically in the clinical database; the site will not perform the calculation.
- h. The patient will be asked to walk 10 meters. The walk must be completed without assistance from another person; ambulatory aids such as canes and walkers are permitted. The time required for the patient to walk 10 meters will be recorded. Two assessments will be performed on separate days (1 assessment on each day). The second (repeat) assessment must be conducted at least 24 hours after the first assessment, but not more than 7 days apart.
- i. Grip strength will be measured using a dynamometer held in the dominant hand. Every effort will be made to use the same device for a patient throughout the duration of the study. Two assessments will be performed on separate days (1 assessment on each day). The second (repeat) assessment must be conducted at least 24 hours after the first assessment, but not more than 7 days apart.
- j. Vital signs to include: blood pressure, pulse rate, oral body temperature, and respiratory rate. Parameters are to be measured in the supine position using an automated instrument after the patient has rested comfortably for 10 minutes.
- k. A pregnancy test (urine- or serum-based) will be performed on all females of child-bearing potential.
- l. Concomitant medications/treatments will be collected through Day 294 (Week 42) only. Data on subsequent FAP treatment regimens will be collected separately.
- m. Adverse events will be collected through Day 294 (Week 42) only. See [Section 8.5](#) for SAE reporting periods.
- n. Following the Day 294 (Week 42) visit, only adverse events that are considered related to the study procedures will be collected (e.g., skin biopsies, venipunctures).
- o. At these time points, data will be collected through phone contact.
- p. Study personnel will contact patients by phone to query for general health status and information on subsequent FAP treatment regimens.

ABBREVIATIONS

| Abbreviation | Definition |
|-----------------|---|
| Σ5 | 5 attributes |
| ADA | Anti-drug antibodies |
| AE | Adverse event |
| ALT | Alanine transaminase |
| ANC | Absolute neutrophil count |
| ANCOVA | Analysis of covariance |
| aPTT | Activated partial thromboplastin time |
| AST | Aspartate transaminase |
| ATTR | Transthyretin-mediated amyloidosis |
| BUN | Blood urea nitrogen |
| CAS | Central Assessment Site |
| CC | Complete case |
| CFR | Code of Federal Regulations |
| CL _R | Renal clearance |
| COMPASS-31 | Composite Autonomic Symptom Score |
| CRF | Case Report Form |
| CRO | Contract research organization |
| C-SSRS | Columbia–Suicide Severity Rating Scale |
| DEHP | di(2-ethylhexyl)phthalate |
| DLin-MC3-DMA | 1,2-Dilinoleyloxy-N,N-dimethylpropylamine |
| DSPC | 1,2-Distearoyl-sn-glycero-3-phosphocholine |
| ECG | Electrocardiogram |
| EDC | Electronic data capture |
| ELISA | Enzyme linked immunosorbent assay |
| EU | European Union |
| FAC | Familial amyloidotic cardiomyopathy |
| FAP | Familial amyloidotic polyneuropathy |
| GCP | Good Clinical Practice |
| GMP | Good Manufacturing Practice |
| HBV | Hepatitis B virus |
| HCV | Hepatitis C virus |
| H1/H2 blocker | Histamine H1/H2 receptor antagonist |
| HIPAA | Health Insurance Portability and Accountability Act of 1996 |
| HIV | Human immunodeficiency virus |
| HP | Heat pain |

| Abbreviation | Definition |
|----------------------------|---|
| HPLC | High performance liquid chromatography |
| HRdb | Heart rate response to deep breathing |
| IB | Investigator's Brochure |
| ICF | Informed consent form |
| ICH | International Conference on Harmonization |
| IEC | Independent Ethics Committee |
| IENFD | Intraepidermal nerve fiber density |
| IFE | immunofixation electrophoresis |
| INR | International normalized ratio |
| IRB | Institutional Review Board |
| IRR | Infusion-related reaction |
| IRS | Interactive response system |
| IV | Intravenous(ly) |
| LLN | Lower limit of normal |
| LOCF | Last observation carried forward |
| LNP | Lipid nanoparticles |
| mBMI | Modified body mass index |
| MedDRA [®] | Medical Dictionary for Regulatory Activities |
| mITT | Modified Intent to Treat |
| MMRM | Mixed model repeated measures |
| mNIS | Modified Neuropathy Impairment Score |
| mRNA | Messenger ribonucleic acid |
| NIS | Neuropathy Impairment Score |
| NCS | Nerve conduction studies |
| NOAEL | No observed adverse effect limit |
| Norfolk QOL-DN | Norfolk Quality of Life-Diabetic Neuropathy |
| NSAID | Nonsteroidal anti-inflammatory |
| NT-proBNP | N-terminal prohormone of B-type natriuretic peptide |
| OTC | Over-the-counter |
| PCS | Patient Care Site |
| PD | Pharmacodynamic |
| PEG ₂₀₀₀ -C-DMG | 3-N-[(ω-methoxy poly(ethylene glycol)2000) carbamoyl]-1,2-dimyristyloxy-propylamine |
| PI | Principal Investigator |
| PK | Pharmacokinetic(s) |
| PND | Polyneuropathy disability |

| Abbreviation | Definition |
|---------------------|---|
| PO | Per os (orally) |
| PP | Per protocol |
| PVC | Polyvinyl chloride |
| PT | Prothrombin time |
| QST | Quantitative sensory testing |
| QTc | QT interval corrected for heart rate |
| RBC | Red blood cell |
| RBP | Retinol binding protein |
| RNAi | RNA interference |
| R-ODS | Rausch-built Overall Disability Scale |
| SAE | Serious adverse event |
| SGNFD | Sweat gland nerve fiber density |
| siRNA | Small interfering ribonucleic acid |
| SNAP | Sural nerve action potential |
| SUSAR | Suspected unexpected serious adverse reaction |
| T3 | Triiodothyronine |
| T4 | Thyroxine |
| TP | Touch pressure |
| TSH | Thyroid stimulating hormone |
| TTR | Transthyretin |
| ULN | Upper limit of normal |
| US/USA | United States |
| USP/EP | United States Pharmacopeia/European Pharmacopoeia |
| V30M | Val30Met |
| VDT | Vibration detection threshold |
| WBC | White blood cell |
| WHO | World Health Organization |
| WT | Wild type |

3 INTRODUCTION

1.1 Background and Rationale

1.1.1 Disease Overview

Transthyretin-mediated amyloidosis (ATTR) is an inherited, autosomal dominant, systemic disease caused by a mutation in the transthyretin (TTR) gene.¹ Transthyretin is a tetrameric 127 amino acid protein that is secreted predominantly (>95%) by hepatocytes, with a smaller fraction produced by the choroid plexus and retina.² Physiologically, TTR is a major serum carrier for retinol binding protein (RBP) and a minor carrier of thyroxine (T4). Mutations in the TTR protein lead to destabilization of the tetrameric form and dissociation into dimers and monomers; misfolding of mutated monomers from the α -helical to the β -pleated sheet structure results in tissue deposition of amyloid fibrils.³ Amyloid deposits typically contain both mutant and wild-type (WT) TTR. The particular TTR mutation and site of amyloid deposition determines the clinical manifestations of the disease, which include sensory and motor neuropathy, autonomic neuropathy, and/or cardiomyopathy. ATTR is a progressive disease associated with severe morbidity, with a life expectancy limited to 5 to 15 years from symptom onset.⁴ There are over 100 reported TTR mutations which are associated with 2 clinical syndromes: familial amyloidotic polyneuropathy (FAP) and familial amyloidotic cardiomyopathy (FAC).^{5,6,7}

ALN-TTR02 is being developed for the treatment of ATTR patients with symptomatic FAP.

The estimated worldwide prevalence of FAP is 5,000 to 10,000, with the majority of cases in Portugal, Sweden, France, Japan, Brazil, and the United States.^{8,9} The most common causative mutation of FAP is TTR Val30Met (V30M), with the onset of symptoms typically occurring between 30 and 55 years of age.¹⁰ Amyloid deposition occurs largely in the peripheral nerves, starting as a nerve length-dependent sensory polyneuropathy in the feet causing numbness and pain and progressing to painful dysesthesias. Disabling motor neuropathy follows, characterized by leg weakness and eventual inability to walk. Autonomic neuropathy is another common feature of the disease, resulting in severe gastrointestinal pathology (including diarrhea or constipation and malabsorption, leading to severe malnutrition), orthostatic hypotension, and bladder dysfunction with recurring urinary tract infections.^{11,12,13,14} For several mutations, cardiac pathology also occurs due to amyloid infiltration of the sinus node, atrioventricular conduction system, and infiltration of the myocardium.^{15,16} Involvement of the conduction system can lead to sudden death due to dysrhythmias, and myocardial infiltration can lead to diastolic dysfunction and right-sided heart failure.¹⁷ The cardiomyopathy proceeds inexorably, leading to death typically within 10 years.¹⁸

There are multiple lines of evidence demonstrating that reduction of circulating TTR improves outcomes in patients with ATTR. Because the liver is the primary source of WT and mutant TTR, orthotopic liver transplantation has been used since 1990 in an attempt to treat FAP,¹⁹ and is the current standard of care in patients who are eligible for

transplant (patients with minimal neuropathy symptoms and no cardiac involvement). When liver transplantation is performed early in the course of the disease, it can stabilize and slow the course of neuropathic disease in patients with FAP due to V30M, but is less effective in patients with other TTR mutations.²⁰ However, it is less effective in patients with more advanced disease, especially those with heart involvement, due to the continued production and deposition of WT TTR in tissues with pre-existing amyloid.^{21,22,23}

It is estimated that approximately two-thirds of FAP patients are not transplant-eligible. Furthermore, liver transplant poses risks from the surgical procedure and from life-threatening complications due to graft rejection or infections. The 1-year mortality rate post-transplant is 10%.²⁴

Nonsurgical options that are used for the treatment of FAP (depending on geographic location) include tafamidis (Vyndaqel[®]) and diflunisal. Tafamidis is a small molecule TTR stabilizer that binds to the thyroxine binding sites of the TTR tetramer, thus preventing its dissociation to monomers and potentially preventing fibril formation. While tafamidis is approved in the European Union (EU) for the treatment of ATTR in adult patients with Stage 1 symptomatic polyneuropathy to delay peripheral neurologic impairment, it is not considered the standard of care throughout the EU and it has not been approved for use outside the EU.²⁵

Diflunisal is a generic, nonsteroidal anti-inflammatory drug (NSAID) that is also a tetramer stabilizer and binds to TTR in a similar manner as tafamidis. A multinational, placebo-controlled Phase 3 study in patients with all stages of FAP was recently completed; however, the results have not been released. Due to the restricted use of liver transplantation and tafamidis in patients with early stage of disease, and the non-standard use of diflunisal among practitioners, there remains an unmet medical need for a potent and effective therapy for FAP that will have an impact on patients across a broad range of neurologic impairment, regardless of their mutation (V30M or non-V30M).

1.1.2 RNA Interference

Ribonucleic acid interference (RNAi) is a naturally occurring cellular mechanism for regulating gene expression that is mediated by “small interfering ribonucleic acids” (siRNAs).²⁶ Typically, synthetic siRNAs are 19 to 23 base pair double-stranded oligonucleotides in a staggered duplex with a 2-nucleotide overhang at one or both of the 3' ends. Such siRNAs can be designed to target an endogenous or virally-expressed gene. When introduced into cells, the net effect of an RNAi-based pharmacological approach is the binding of the siRNA to its complementary messenger ribonucleic acid (mRNA) sequence, cleavage of this target mRNA, and suppression of the target protein.²⁷ The ability to selectively and potently degrade the mRNA encoding the TTR protein using an siRNA offers a potent and specific approach for the treatment of ATTR.

Unformulated siRNAs, and those without chemical modification, are rapidly degraded and eliminated upon systemic administration, and thus do not achieve significant tissue distribution.²⁸ As a result, various formulations are used to target siRNA distribution to tissues, and to facilitate their uptake into the relevant cell type. One approach that has

been used successfully *in vivo*, including in rodents, non-human primates, and humans, employs IV delivery of siRNAs in lipid nanoparticles (LNPs).^{29,30,31,32} These LNPs, with their small size (<100 nm) and low surface charge, can pass through the fenestrated vascular endothelium of the liver. Endocytosis of the intact LNPs, followed by fusion with the endosomal membrane and release of the siRNA into the cytoplasm, results in the siRNA engaging the endogenous RNAi machinery described above leading to targeted degradation of the mRNA, and a consequent reduction in target protein levels.^{33,34}

1.1.3 ALN-TTR02

ALN-TTR02 comprises a small interfering ribonucleic acid (siRNA) which is specific for TTR, and is formulated in a hepatotropic lipid nanoparticle (LNP) for intravenous (IV) administration.³⁵ This TTR siRNA has a target region within the 3'UTR region of TTR gene to ensure and confirm homology with WT TTR as well as all reported TTR mutations. Following LNP-mediated delivery to the liver, ALN-TTR02 targets TTR mRNA for degradation, resulting in the potent and sustained reduction of mutant and WT TTR protein via the RNAi mechanism.

Since circulating TTR is almost exclusively synthesized in the liver, the IV administration of ALN-TTR02 is postulated to reduce the level of precursors that lead to amyloid fibril deposition, resulting in clinical benefit to patients with FAP.

1.1.4 Therapeutic Hypothesis

ALN-TTR02 is a novel investigational agent intended for the treatment of FAP, a serious and life-threatening orphan disease. The therapeutic hypothesis that systemic amyloidoses can be managed by reduction in circulating levels of amyloidogenic protein has been validated in other acquired (e.g., immunoglobulin light chain systemic [AL], or amyloid A [AA]) and hereditary (e.g. Fibrinogen A α -chain, ApoA1) amyloidosis. The experience from these systemic amyloidotic disorders,^{36,37,38,39} as well as the liver transplant data in FAP, suggest that lowering of the circulating amyloidogenic protein by at least 50% is required to impact the clinical course of the disease, with reductions in protein beyond 50% providing further incremental improvements in outcomes. It is therefore postulated that the >80% suppression in *both* WT and mutant TTR observed upon administration of 0.3 mg/kg ALN-TTR02 once every 21 days will result in clinical benefit in FAP patients with mild to moderate polyneuropathy. This hypothesis is further supported by evidence from tafamidis suggesting that reduction in free TTR monomer can slow neuropathy progression in early-stage V30M patients with FAP.¹⁰

1.2 Summary of ALN-TTR02 Nonclinical Data

[REDACTED]



Further information can be found in the ALN-TTR02 IB.

1.3 Summary of Clinical Data with ALN-TTR02

A Phase 1 multicenter, randomized, placebo-controlled, single-blind, single-ascending dose clinical study of ALN-TTR02 in healthy volunteers was completed in the UK. ALN-TTR02 was administered as a single 60-minute IV infusion to healthy volunteers at doses of 0.01 to 0.05 mg/kg. Patients were premedicated with dexamethasone, H1 and H2 blockers, and paracetamol/acetaminophen prior to dosing to minimize the risk of infusion-related reactions (IRRs). Significant pharmacology in terms of TTR protein lowering (>80% reduction from pretreatment baseline) was observed at doses ≥ 0.15 mg/kg. Transthyretin levels showed evidence of recovery beginning at around Day 21 to 28, returning to baseline by Day 70.

An open-label, Phase 2, multiple-ascending dose study of ALN-TTR02 in ATTR patients with FAP (Study ALN-TTR02-002) to determine the safety and tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) of a 60-70 minute IV infusion of ALN-TTR02 administered once every 3 or 4 weeks for 2 doses has completed enrollment (N=29). Preliminary data show >80% TTR knockdown with 0.3 mg/kg after the first and second doses ($p < 0.001$), with better sustained suppression of >80% observed throughout the dosing interval with the once every 3 week dosing schedule compared with once every 4 weeks. In V30M patients, both mutant and wild-type TTR were suppressed to the same extent. Multiple doses of ALN-TTR02 have been generally safe and well-tolerated, including 0.3 mg/kg administered once every 3 or 4 weeks with either a 60-minute infusion and original premedication regimen (total of 28 mg dexamethasone or equivalent, administered the night before and morning of infusion) or a 70-minute step-

wise infusion (approximately 1 mL/minute for the first 15 minutes followed by approximately 3 mL/minutes for the remainder of the infusion) and a simplified premedication regimen (total of 10 mg dexamethasone or equivalent, administered at least 60 minutes prior to infusion).

Patients completing Study ALN-TTR02-002 are eligible to enroll into a Phase 2, open-label, extension study (ALN-TTR02-003) designed to evaluate the safety and tolerability of long-term ALN-TTR02 dosing administered to patients with FAP; additional information will be evaluated including PK, PD, and clinical activity. Patients will receive 0.3 mg/kg ALN-TTR02 once every 21 days for approximately 2 years.

Further details on these clinical studies can be found in the ALN-TTR02 IB.

1.4 Study Design Rationale

This is a Phase 3 randomized, double-blind, placebo-controlled, multicenter, multinational study of ALN-TTR02 in FAP patients. The patients proposed for this study are reflective of the FAP population encountered by clinicians in various different countries worldwide, including patients with a range of neuropathy severity and broad spectrum of TTR mutations. Disease progression will be assessed by neurological measures and functional tests; therefore, the range of baseline neuropathy severity (NIS of 10-100) is selected such that the lower end is advanced enough to show significant progression in the placebo group, while the upper end is not so advanced as to preclude detection of change as a result of a ceiling effect of neuropathy measures or to be confounded by other comorbidities.

The inclusion of placebo as a control allows for a rigorous analysis of the treatment effect of ALN-TTR02. However, given the 18-month duration of the study, those patients who have evidence of rapid disease progression at 9 months (defined as ≥ 24 point increase in mNIS+7 from baseline [based on an average of 2 measurements] and FAP stage progression relative to baseline) will be given the option of discontinuing study drug and receiving local standard of care treatment for their FAP. Such patients will be asked to follow a modified study visit schedule and return for their 18-month efficacy assessment (blinding will be maintained throughout). It is expected that fewer than 5% of patients involved in the ALN-TTR02-004 study will meet the definition of rapid disease progression at 9 months.

Given the orphan nature of the disease and the significant, progressive morbidities associated with FAP, randomization to ALN-TTR02 or placebo will be performed in a 2:1 ratio (ALN-TTR02:placebo) to increase the probability that patients will receive active drug. Treatment groups will be balanced at entry for NIS (10-49 vs 50-100), early onset V30M (<50 years of age at onset) vs all other mutations (including late onset V30M), and previous tetramer stabilizer use (tafamidis or diflunisal) vs no previous tetramer stabilizer use.

The primary endpoint for this study is the change from baseline at 18 months in a composite measure of neurologic impairment termed modified NIS+7 (mNIS+7), which includes a clinical exam-based assessment of neurologic impairment (NIS) combined with electrophysiologic measures of small and large nerve fiber function (NCS and QST),

and measurement of autonomic function (postural blood pressure). The utility of various neuropathy endpoints in demonstrating a treatment effect in randomized, controlled clinical trials in patients with FAP has been established through Phase 3 studies using the small molecule TTR tetramer stabilizers tafamidis and diflunisal. For these studies, the primary efficacy endpoint utilized variations of NIS (NIS-Lower Limbs in the case of tafamidis and NIS+7 for diflunisal). Composite endpoints, such as NIS+7, have been found to be more sensitive in detecting abnormalities in patients with generalized peripheral neuropathy, such as diabetic polyneuropathy and chronic idiopathic demyelinating polyneuropathy, and the reproducibility of composite scores has been shown to be greater than for individual tests. The use of mNIS+7 in this study is expected to increase the sensitivity and reproducibility of the measurement of neuropathy progression in a heterogeneous group of FAP patients presenting with a broad spectrum of disease severity and TTR mutations.

The 18-month endpoint was selected based on the expected rate of neuropathy progression in patients with FAP, derived from a global natural history dataset of 283 patients from Alnylam collaborators in the USA, Portugal, France, and Italy. From these data, it is estimated that a patient's mNIS+7 score will have increased by approximately 24 points in the placebo group after 18 months, thereby providing adequate disease progression for the detection of a treatment effect in the ALN-TTR02 group.

1.5 Dose Selection and Dosing Schedule Rationale

Given the fundamental role of TTR in the pathogenesis of the disease and the data from liver transplantation in FAP, it is postulated that the optimal dose and schedule for ALN-TTR02 is one that will result in the greatest level of sustained TTR suppression with an acceptable safety profile. Based on the data to date from the nonclinical and clinical studies with ALN-TTR02, >80% maintained suppression of circulating TTR is consistently achieved at the 0.3 mg/kg dose administered once every 3 weeks, and this dose was generally well tolerated. ALN-TTR02 will be administered every 21 days as a 70-minute IV infusion (approximately 1 mL/minute for the first 15 minutes followed by approximately 3 mL/minutes for the remainder of the infusion) with a premedication regimen consisting of IV dexamethasone and H1/H2 blockers along with oral paracetamol/acetaminophen given at least 60 minutes prior to each infusion.

The chronic toxicology studies in rodents (6-month duration) and monkeys (9-month duration), in which animals safely tolerated 14 doses of ALN-TTR02 at ≥ 0.3 mg/kg once every 2 or 3 weeks (rats or monkeys, respectively), also supports long-term dosing in FAP patients at 0.3 mg/kg once every 21 days.

1.6 Risk-Benefit Assessment

Please see the IB for expanded risk/benefit assessment.

1.6.1 Infusion-Related Reactions

Infusion-related reactions (IRRs) can occur with systemic administration of certain biological agents such as liposomes or monoclonal antibodies. In order to reduce the

potential for an IRR with ALN-TTR02, all patients in this study will be premedicated prior to dosing with ALN-TTR02 or placebo. The step-wise infusion regimen over approximately 70 minutes (starting with a slower infusion rate of approximately 1 mL/minute for the first 15 minutes before increasing to 3 mL/minute for the remainder of the infusion), may further reduce the risk of an IRR.

1.6.2 Vitamin A Lowering

The suppression of serum levels of TTR and RBP is expected to result in the lowering of circulating vitamin A levels. Preclinical and clinical data have shown that the lowering of circulating vitamin A associated with suppression of RBP does not result in severe vitamin A deficiency. Furthermore, there are individuals with RBP/TTR mutations who have life-long low levels of circulating vitamin A and are essentially in good health, suggesting that there are compensatory transport mechanisms for vitamin A that are yet undescribed.⁴⁰ This has also been confirmed in TTR knockout mice, which do not exhibit any manifestations of vitamin A deficiency, with vitamin A uptake by most tissues continuing in the absence of RBP.⁴¹ Provided there is adequate vitamin A in the diet, tissue stores of vitamin A should not be affected by the lowering of TTR and RBP. However, as the vitamin A content of the diet may vary between different countries and different individuals within a country, all patients on the study will be asked to take a supplement containing the recommended daily allowance of vitamin A.

4" STUDY OBJECTIVES

2.1 Primary Objective

The primary objective of the study is to determine the efficacy of ALN-TTR02 by evaluating the difference between the ALN-TTR02 and placebo groups in the change from baseline of mNIS+7 score at 18 months.

2.2 Secondary Objectives

The secondary objectives of the study are to determine the effect of ALN-TTR02 on various clinical parameters by assessing the difference between ALN-TTR02 and placebo in the change from baseline in the following measurements at 18 months:

- Norfolk Quality of Life-Diabetic Neuropathy (Norfolk QOL-DN) and EuroQOL (EQ-5D) questionnaires
- Modified Body Mass Index (mBMI) and autonomic symptoms questionnaire (Composite Autonomic Symptom Score [COMPASS-31])
- NIS-weakness (NIS-W) and timed 10-meter walk test

2.3 Exploratory Objectives

The exploratory objectives of the study are:

- To determine the difference between the ALN-TTR02 and placebo groups in the change from baseline in the following measurements at 18 months:
 - NIS+7 score;
 - Grip strength;
 - Level of disability (Rasch-built Overall Disability Scale [R-ODS]);
 - Large vs small nerve fiber function including nerve conduction studies (NCS) 5 attributes ($\Sigma 5$), quantitative sensory testing by body surface area including touch pressure and heat pain (QST), vibration detection threshold (VDT), heart rate response to deep breathing (HRdb), postural blood pressure;
 - Pathologic evaluation of sensory and autonomic innervation through voluntary skin punch biopsies and analysis of intraepidermal nerve fiber density (IENFD) and sweat gland nerve fiber density (SGNFD);
 - Assessment of ambulation through FAP stage and Polyneuropathy Disability (PND) score;
 - Cardiac assessment through echocardiogram, troponin I, and N terminal prohormone of B-type natriuretic peptide (NT-proBNP) levels;
 - Pharmacodynamic (PD) biomarkers (TTR, RBP, vitamin A);
- To compare the proportion of patients in the ALN-TTR02 and placebo groups who meet the pre-defined criterion for rapid disease progression (defined as ≥ 24 point

increase in mNIS+7 from baseline [based on an average of 2 measurements] and FAP stage progression relative to baseline) at 9 months.

5 STUDY PLAN

3.1 Overall Design

This is a multicenter, multinational, randomized, double-blind, placebo-controlled, Phase 3 study designed to demonstrate the clinical efficacy of ALN-TTR02 and to establish the safety of chronic dosing in adult patients with FAP. A schematic of the study design is presented in [Figure 1](#).

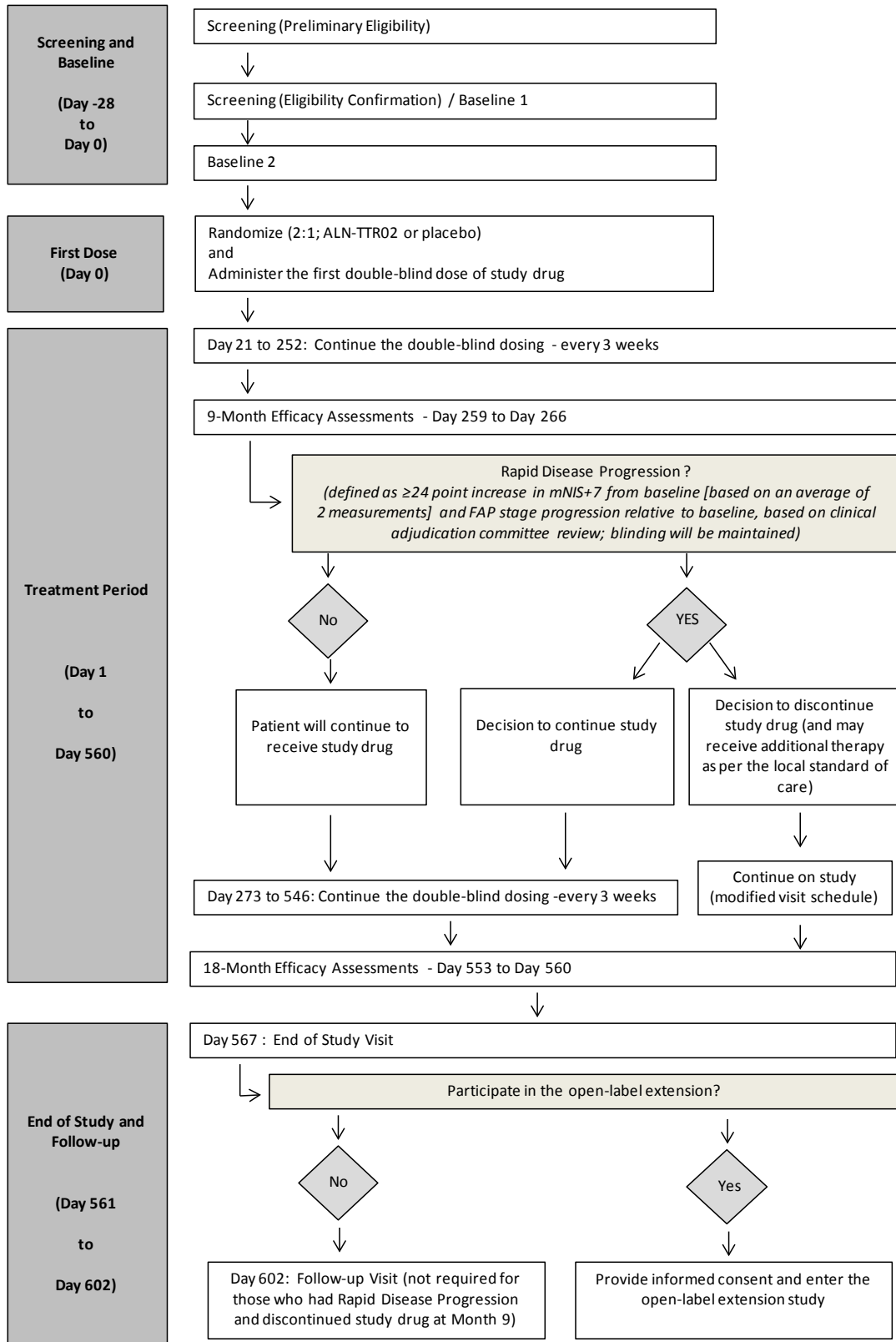
The duration of patient participation in this study is approximately 21 months. Patients will be screened within 28 days prior to administration of study drug. Consented eligible subjects will be randomized to receive either ALN-TTR02 or placebo (2:1 ratio, ALN-TTR02 to placebo) once every 21 days for 78 weeks. Treatment groups will be balanced at entry for NIS (10-49 vs 50-100), early onset V30M (<50 years of age at onset) vs all other mutations (including late onset V30M), and previous tetramer stabilizer use (tafamidis or diflunisal) vs no previous tetramer stabilizer use.

On each dosing day, patients will receive a premedication regimen at least 60 minutes prior to the start of infusion of study drug in order to reduce the risk of patients experiencing an IRR. Blinded study drug will be administered as a 70-minute IV infusion (approximately 1 mL/minute for the first 15 minutes followed by approximately 3 mL/minute for the remainder of the infusion). In addition to returning to the site for dosing of study drug once every 21 days, patients will also return for out-patient visits at 9 and 18 months for efficacy assessments (see [Section 6](#) for details).

At the 9-month time point, if the clinical adjudication committee determines that a patient is exhibiting rapid disease progression (defined as ≥ 24 point increase in mNIS+7 from baseline [based on an average of 2 measurements] and FAP stage progression relative to baseline, see [Section 4.6](#)), the patient's treating physician will provide the patient with the option of discontinuing study drug and receiving local standard of care treatment for FAP. Patients who discontinue study drug will remain on study through completion of the 18-month efficacy assessments, following a modified visit schedule as shown in [Table 1-3](#) and described in [Section 6.4](#). Blinding will be maintained throughout. Patients who complete the 18-month efficacy assessments can elect to participate in an extension study in which patients would receive open-label administration of 0.3 mg/kg ALN-TTR02 once every 21 days.

A Data Monitoring Committee (DMC) will be implemented for the study and will operate under a prespecified charter.

Figure 1: Study Schematic



3.2 Efficacy Assessments

Efficacy parameters will include the following (all evaluations will be conducted at baseline and at 9 and 18 months):

- Neurologic impairment will be assessed using the mNIS+7 composite score. The mNIS+7 includes the modified NIS (weakness and reflexes), NCS $\Sigma 5$, QST, as well as autonomic assessment through postural blood pressure.
- Patient-reported QOL will be evaluated using the Norfolk QOL-DN and the EQ-5D. Disability will be reported by patients using the R-ODS.
- Autonomic symptoms will be assessed using the COMPASS-31.
- Motor function assessments to be evaluated include NIS-W, timed 10-meter walk test, and grip strength test.
- PND score and FAP stage.
- Nutritional status will be assessed using mBMI.
- Pathologic evaluation of sensory and autonomic innervation will be evaluated by IENFD analysis and quantitation of dermal sweat gland nerve fibers (SGNFD) via tandem 3 mm skin punch biopsies taken from the leg.
- Neurologic impairment will also be assessed by NIS+7 (including full NIS, NCS, vibratory detection threshold [VDT], and heart rate variation with deep breathing [HRdb]).
- Cardiac structure and function will be assessed through echocardiograms as well as measurement of serum levels of NT-proBNP and troponin I.

3.3 Safety Assessments

Safety will be assessed throughout the study by collecting adverse events (AEs; including serious adverse events [SAEs]); clinical laboratory tests, including hematology, clinical chemistry (including liver function tests), thyroid function parameters, and urinalysis; measurement of anti-drug antibodies; electrocardiograms; vital signs; physical examination findings; and ophthalmology examinations.

3.4 Pharmacodynamic Assessments

Pharmacodynamic markers assessed serially will include serum TTR, vitamin A, and RBP. Additional blood samples will be collected for exploratory biomarkers related to FAP.

3.5 Pharmacokinetic Assessments

The plasma PK evaluation will include, whenever possible, plasma-concentration time profiles for siRNA and the novel lipid components in ALN-TTR02: DLin-MC3-DMA and polyethylene glycol (PEG)₂₀₀₀-C-DMG. The siRNA, DLin-MC3-DMA, and PEG₂₀₀₀-C-DMG concentration will be determined for all patients at time points specified in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#).

Urine will be collected with void volume recorded for all patients at time points specified in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#) to determine renal clearance (CL_R) of siRNA and 4-dimethylaminodibutyric acid (the metabolite of DLin-MC3-DMA) after dosing with study drug.

3.6 Other Assessments

Disease burden and healthcare utilization will be assessed using a patient-reported pharmacoeconomics questionnaire. The investigator will periodically assess mental status as it relates to suicidal ideation and behavior by using the Columbia–Suicide Severity Rating Scale (C-SSRS) questionnaire.

6" PATIENT POPULATION

4.1 Eligibility of Patients

Approximately 200 patients are expected to be enrolled at multiple centers worldwide. All centers will be selected on the basis of their experience in the treatment of patients with FAP.

4.2 Inclusion Criteria

To be enrolled in the study, each patient must meet the following criteria within the Screening and Screening/Baseline Visits:

1. Male or female of 18 to 80 years of age (inclusive);
2. Have a diagnosis of FAP with documented TTR mutation;
3. Have a NIS of 10 to 100 (inclusive);
4. The NCS ($\Sigma 5$) must, at a minimum, show a clear abnormality (≤ 5 th percentile) of the sural nerve action potential (SNAP);
5. Have a Karnofsky performance status of $\geq 60\%$;
6. Have an absolute neutrophil count (ANC) ≥ 1500 cells/mm³, a platelet count $\geq 100,000$ cells/mm³, and hemoglobin ≥ 10 g/dL (or ≥ 100 g/L);
7. Have aspartate transaminase (AST) and alanine transaminase (ALT) levels $\leq 2.5 \times$ the upper limit of normal (ULN), total bilirubin within normal limits, albumin > 3 g/dL (or > 30 g/L), international normalized ratio (INR) ≤ 1.2 ;
8. Have a serum creatinine $\leq 1.5 \times$ ULN;
9. Have negative serology for hepatitis B virus (HBV) and hepatitis C virus (HCV);
10. Women of child-bearing potential must have a negative pregnancy test, cannot be breastfeeding, and must be using 2 highly effective methods of contraception prior to screening, throughout study participation, and for 1 month after last dose of study drug. Highly effective methods of birth control are defined as: hormonal (e.g., oral, implantable, injectable, or transdermal contraceptives in conjunction with spermicide, condom, or diaphragm), mechanical (e.g., spermicide in conjunction with a barrier such as a condom or diaphragm), intrauterine device in conjunction with spermicide or condom, or surgical sterilization of partner in conjunction with spermicide, condom, or diaphragm;
11. Males with partners of child-bearing potential, must agree to use 1 barrier method (e.g., condom) and 1 additional method (e.g., spermicide) of contraception throughout study participation and for 1 month after the last dose of study drug; males must also abstain from sperm donation after the first dose of study drug through study participation and for 1 month after last dose of study drug;
12. Must be willing and able to comply with protocol-required visit schedule and visit requirements and provide written informed consent.

4.3 Exclusion Criteria

A patient will be excluded if they meet any of the following criteria at the time of Screening and Screening/Baseline Visits:

1. Has vitamin A levels below the lower limit of normal (LLN);
2. Had a prior liver transplant or is planning to undergo liver transplant during the study period;
3. Has other known causes of sensorimotor or autonomic neuropathy (e.g., autoimmune disease, monoclonal gammopathy, etc.);
4. Has known primary amyloidosis or leptomeningeal amyloidosis;
5. Has known type I diabetes;
6. Has had type II diabetes mellitus for ≥ 5 years;
7. Has vitamin B12 levels below LLN;
8. Has untreated hypo- or hyperthyroidism;
9. Has had a major surgery within the past 3 months or has a major surgery planned during any point of the study period;
10. Has known human immunodeficiency virus (HIV) infection;
11. Has an active infection requiring systemic antiviral or antimicrobial therapy that will not be completed prior to the first dose of study drug administration;
12. Had a malignancy within 2 years, except for basal or squamous cell carcinoma of the skin or carcinoma in situ of the cervix that has been successfully treated;
13. Has a New York Heart Association heart failure classification > 2 ;
14. Had acute coronary syndrome within the past 3 months;
15. Has uncontrolled clinically significant cardiac arrhythmia or unstable angina;
16. Has a known history of alcohol abuse or daily heavy alcohol consumption (females: more than 14 units of alcohol per week; males: more than 21 units of alcohol per week [unit: 1 glass of wine [125 mL] = 1 measure of spirits = $\frac{1}{2}$ pint of beer]);
17. Received an investigational agent or device within 30 days of anticipated study drug administration or 5 half-lives of the investigational drug, whichever is longer;
18. Participated in a clinical trial with an antisense oligonucleotide for more than 3 months; if in a clinical trial with antisense oligonucleotide for ≤ 3 months, must have completed a 3-month wash-out prior to start of study drug administration in this study;

19. Is currently taking diflunisal, tafamidis, doxycycline, or tauroursodeoxycholic acid; if previously on any of these agents, must have completed a 14-day wash-out prior to start of study drug administration in this study;
20. Had a prior severe reaction to a liposomal product or a known hypersensitivity to oligonucleotides or any component of ALN-TTR02;
21. Is unable to take the required premedications;
22. Anticipated survival is less than 2 years, in the opinion of the Investigator;
23. Is considered unfit for the study by the Investigator.

4.4 Assignment to Treatment Group/Patient Number

4.4.1 Randomization Procedures

Patients will be randomly assigned in a 2:1 ratio to receive either 0.3 mg/kg ALN-TTR02 or placebo (normal saline 0.9%).

Patients will be randomized via an interactive response system (IRS). Either designated unblinded site personnel or the pharmacist may request randomization for the patient, but only the pharmacist or unblinded personnel will be allowed to receive the treatment code. The treatment code will be delivered to the unblinded personnel and the pharmacist will use the necessary number of vials for that patient based on their weight.

Treatment arms will be balanced at entry for NIS (10-49 vs 50-100), early onset V30M (<50 years of age at onset) vs all other mutations (including late onset V30M), and previous tetramer stabilizer use (tafamidis or diflunisal) vs no previous tetramer stabilizer use.

4.4.2 Patient Numbering

At enrollment, each patient will be uniquely identified in the study by a combination of his/her center number and screening number. The center number will be assigned by the Sponsor. Upon signing the informed consent form, the patient will be assigned a screening number by the IRS. The Investigator or his/her delegate will contact the IRS (via phone or web) after confirming that the patient fulfills all the inclusion criteria and none of the exclusion criteria. The patient will be randomized via the IRS, assigned a subject number and a study treatment. A combination of the center number, screening number, enrollment number, and patient initials will create the unique patient identifier. In countries with regulations prohibiting the use of patient initials, a strategy consistent with local regulations will be used to generate replacement values for the patient initials.

4.4.3 Blinding Procedure

Only the pharmacist and designated site personnel who dispense or administer study drug will be unblinded to the study treatment. All other site personnel will be blinded to the treatment. Study personnel performing assessments related to the efficacy endpoints will be different from the Investigator and other personnel managing the patient, all of whom will also remain blinded to any clinical laboratory results that could potentially unblind them (e.g., TTR levels, vitamin A levels, thyroid function tests).

All patients will be blinded to treatment and will receive an IV infusion once every 21 days using identical volumes for placebo and ALN-TTR02. The tubing and the lines will be covered so that it will not be possible to detect a difference in active versus placebo drug.

Furthermore, unblinded source documentation containing all descriptions of pharmacy preparations and infusions or distribution of study drug or randomization data will be stored separate from all other study data/records and from other pharmacy staff not participating on the study.

Unblinding is only to occur in the case of patient emergencies or when necessary from a regulatory reporting perspective (e.g., Suspected Unexpected Serious Adverse Reactions [SUSAR] occurring in the EU), and at the conclusion of the study.

Patients who discontinue study drug at 9 months due to rapid disease progression will remain blinded throughout the study.

4.4.4 Breaking the Blind

In the event that the Investigator requests to know a patient's study treatment assignment, the Investigator will first contact the CRO Medical Monitor to discuss the need for unblinding. In case of an emergency, the treatment allocation for each patient will be available from the unblinded site personnel, pharmacist, or the IRS system.

If a patient becomes pregnant or seriously ill during the study, the blind should be broken only if knowledge of the treatment administered will affect treatment options available to the patient. Before breaking the blind, the PI or Sub-investigator should attempt to contact the CRO Medical Monitor. If the Medical Monitor is immediately unreachable, the PI or Sub-investigator should break the blind as necessary using the code breaking information provided and contact the Medical Monitor as soon as possible. A record should be kept of when the blind was broken, who broke it, and why.

4.5 Early Patient Withdrawal

Patients are free to withdraw from the study at any time and for any reason, without penalty to their continuing medical care. For those patients who withdraw early, every effort will be made to determine their reason for dropping out, and to complete the Early Withdrawal visit.

A patient will be considered to have completed the study if the patient completes protocol-specified procedures up through the 18-month efficacy assessment visit.

4.5.1 Reasons for Withdrawal

The Investigator may withdraw a patient from the study if the patient:

- Is in violation of the protocol.
- Experiences a serious or intolerable adverse event (AE).
- Becomes pregnant.
- Requires a prohibited medication (see [Section 5.8](#)).

- Requests to be withdrawn from the study.
- Is found to be considerably noncompliant with the protocol required ALN-TTR02 dosing visits.

A patient may also be withdrawn from the study if, in the Investigator's opinion, they are unable to continue. The Investigator will also withdraw the patient from the study upon the request of Alnylam, including if Alnylam terminates the study. Upon occurrence of a serious or intolerable AE, the Investigator will confer with Alnylam before discontinuing the patient. A patient may withdraw consent to participate in the study at any time.

Missing an occasional dose of study drug will not necessarily result in the patient being withdrawn from the study. However, if a patient misses 2 consecutive doses of study drug, the Investigator at the site and the Medical Monitor will determine whether the patient should be withdrawn from the study. If the patient is withdrawn from the study, the reason will be noted in the patient medical record and on the case report form (CRF).

4.5.2 Handling of Withdrawals

In the event a patient is withdrawn from the study, the CRO Medical Monitor must be informed immediately. If there is a medical reason for withdrawal, the patient will remain under the supervision of the Investigator for protocol-specified safety follow-up procedures.

If a patient is withdrawn or withdraws, every effort should be made to conduct the Early Withdrawal visit. Patients who fail to return for final evaluations will be contacted by the site in an attempt to have them comply with the protocol.

When a patient withdraws from the study, the primary reason for discontinuation must be recorded in the appropriate section of the CRF and all efforts will be made to complete and report the observations as thoroughly as possible.

4.5.3 Replacements

No replacements will be allowed for patients who withdraw early from the study.

4.6 Discontinuation Due to Rapid Disease Progression

At the 9-month time point, if the clinical adjudication committee determines that a patient is exhibiting rapid disease progression (defined as ≥ 24 point increase in mNIS+7 from baseline [based on an average of 2 measurements] and FAP stage progression relative to baseline), the patient's treating physician will provide the patient with the option of discontinuing study drug and receiving local standard of care treatment for FAP.

Patients who discontinue study drug will remain on study through completion of the 18-month efficacy assessments (blinding will be maintained throughout), following a modified visit schedule as shown in [Table 1-3](#). Patients who complete the 18-month efficacy assessments can elect to participate in an extension study in which patients would receive open-label administration of 0.3 mg/kg ALN-TTR02 once every 21 days. Patients who discontinue treatment and also decide to discontinue from the study will not have any additional visits after the Day 273 assessments are completed.

4.7 Pregnancy and Breastfeeding Restrictions / Contraception Requirements

Women of child-bearing potential must have a negative pregnancy test, cannot be breastfeeding, and must be using 2 highly effective methods of contraception prior to screening, throughout study participation, and for 1 month after last dose of study drug. Highly effective methods of birth control are defined as: hormonal (e.g., oral, implantable, injectable, or transdermal contraceptives in conjunction with spermicide, condom, or diaphragm), mechanical (e.g., spermicide in conjunction with a barrier such as a condom or diaphragm), intrauterine device in conjunction with spermicide or condom, or surgical sterilization of partner in conjunction with spermicide, condom, or diaphragm.

Males with partners of child-bearing potential, must agree to use 1 barrier method (e.g., condom) and 1 additional method (e.g., spermicide) of contraception throughout study participation and for 1 month after the last dose of study drug. Males must also abstain from sperm donation after the first dose of study drug through study participation and for 1 month after last dose of study drug.

Pregnancy reporting guidelines are provided in [Section 8.12](#).

7 STUDY MEDICATION

5.1 Presentation of Study Drug

ALN-TTR02 Solution for Injection is an RNAi therapeutic consisting of an siRNA targeting TTR mRNA formulated in a LNP. The ALN-TTR02 drug product is a sterile formulation of TTR siRNA with lipid excipients (DLin-MC3-DMA, DSPC, cholesterol, and PEG₂₀₀₀-C-DMG) in isotonic phosphate buffered saline. ALN-TTR02 Solution for Injection contains 2 mg/mL of TTR siRNA drug substance.

The ALN-TTR02 drug product is packaged in 10 mL glass vials with a fill volume of 5.5 mL. The container closure system consists of a United States Pharmacopeia/European Pharmacopoeia (USP/EP) Type I borosilicate glass vial, a Teflon-faced butyl rubber stopper, and an aluminium flip-off cap.

The control drug for this study will be a placebo (normal saline 0.9% for IV administration). Control drug will be provided by a central supplier.

5.2 Preparation of Study Drug

Each investigational site will be responsible for IV preparation and labeling, according to separate handling instructions, and allocating treatments to the patients.

The pharmacist or unblinded study personnel will prepare the study drug under aseptic conditions. The total amount to be infused into each patient at each dosing visit will be 200 mL. To maintain the blind, all IV infusion bags and lines will have amber-colored covers added prior to leaving the pharmacy.

Additional study drug preparation details are provided in the ALN-TTR02 Pharmacy Manual.

5.2.1 Preparation of ALN-TTR02

The amount (in mg) of ALN-TTR02 to be administered will be determined based on the patient's body weight (kg). For each dose administered, the weight obtained during the previous dosing day will be used to calculate the dose of study drug. In the event that the weight obtained on the previous dosing day is not available, the weight obtained predose on the dosing day can be used for dose calculation. Patients who weigh 105 kg or more will receive ALN-TTR02 dosing based on an assumption of a body weight of 104 kg.

Sterile normal saline (0.9% NaCl) will be added to a sterile infusion bag, and the calculated volume of ALN-TTR02 will be withdrawn from the vials and injected into the infusion bag.

5.2.2 Preparation of Placebo

Normal saline (0.9% NaCl), provided by a central supplier, will be infused into patients randomized into the control group. Depending on the availability, saline may need to be withdrawn from a larger bag or additional saline may need to be added to a sterile infusion bag to ensure the 200 mL volume.

5.3 Drug Administration

5.3.1 Premedication

Prior to study drug, all patients will receive the following premedications:

- Intravenous dexamethasone (10 mg) or equivalent, administered at least 60 minutes prior to the start of the infusion of study drug;
- Oral paracetamol/acetaminophen (500 mg) or equivalent at least 60 minutes prior to the start of the infusion of study drug;
- Intravenous H2 blocker (e.g., ranitidine 50 mg, famotidine 20 mg, or equivalent other H2 blocker dose) at least 60 minutes prior to the start of the infusion of study drug; and
- Intravenous H1 blocker: diphenhydramine 50 mg (or equivalent other IV H1 blocker available at the study site) at least 60 minutes prior to the start of the infusion of study drug. Hydroxyzine or fexofenadine 25 mg per os (PO, orally) or cetirizine 10 mg PO may be substituted for any patient who does not tolerate IV diphenhydramine or other IV H1 blocker.

As noted in [Section 5.9](#), following a discussion with the Investigator and Medical Monitor, the premedication regimen may be modified for patients who continue to have mild to moderate reactions to an infusion, or who experience a severe reaction. Further details (including a table of equivalent premedications) can be found in the ALN-TTR02 Pharmacy Manual.

5.3.2 Study Drug Administration

Patients who are randomized into the active treatment group will receive 0.3 mg/kg ALN-TTR02 once every 21 days (\pm 3 days). Patients who are randomized into the control group will receive placebo (normal saline 0.9%) once every 21 days (\pm 3 days).

The body weight that was obtained during the previous dosing day must be used to calculate the dose of study drug. In the event that the body weight obtained on the previous dosing day is not available, the body weight obtained pre-dose on the dosing day can be used for the dose calculation. Patients who weigh 105 kg or more will receive ALN-TTR02 dosing based on an assumption of a body weight of 104 kg.

Study drug (either ALN-TTR02 or placebo) will be administered under the supervision of the unblinded site personnel, as a 70-minute IV infusion (approximately 1 mL/minute for the first 15 minutes followed by approximately 3 mL/minute for the remainder of the infusion). The study drug will be administered via a controlled infusion device with an extension set containing a 1.2 micron filter supplied by Alnylam or designee. Infusion products must not contain polyvinyl chloride, di(2-ethylhexyl)phthalate (PVC, DEHP). As described in [Section 4.4.3](#), the tubing and the lines will be covered so that it will not be possible to detect a difference in active versus placebo drug.

The infusion time may be extended up to 3 hours in the event of a mild or moderate IRR (study drug administration will not be resumed for any patient following a severe IRR until the case is discussed with the Medical Monitor). The patient's infusion site should

be assessed for signs of any localized reaction during the infusion and for 30 minutes after the end of the infusion. The patient will remain at the study site for 1 hour following completion of dosing for observation and completion of assessments.

If a patient does not receive a dose of study drug within the dosing window (± 3 days), the dose will be considered missed and not made up.

Additional details can be found in the ALN-TTR02 Pharmacy Manual.

In addition, patients will receive an oral daily supplemental dose of the recommended daily allowance of vitamin A.

5.4 Storage of Study Drug

All study drug must be stored in a secure, temperature controlled location and may be dispensed only by a staff member specifically authorized by the Investigator, or by a pharmacist, as appropriate. All study drug will be stored upright and refrigerated at approximately $5 \pm 3^{\circ}\text{C}$. Any deviation from the recommended storage conditions must be reported to the CRO and/or Alnylam and use of the study drug halted until authorization for its continued use has been given by Alnylam or designee.

No special procedures for the safe handling of ALN-TTR02 are required. An unblinded Alnylam Monitor or designee will be permitted, upon request, to audit the supplies, storage, dispensing procedures, and records.

No study product(s) may be administered to any person not enrolled in the study.

Additional preparation details are provided in the ALN-TTR02 Pharmacy Manual.

5.5 Labeling and Packaging of Study Drug

All packaging and labeling as well as the preparation of ALN-TTR02 and placebo will be in compliance with Good Manufacturing Practice (GMP) specifications, as described in the Manufacture of Investigational Medicinal Products Volume 4 Annex 13, and any other or local applicable regulations.

Study drug labels will include all appropriate local labeling requirements on the vial and external label. Sample labels will be submitted to health authorities, per local country submission requirements.

5.6 Measurement of Patient Compliance

Treatment compliance with study drug administration is dependent on the proper preparation and administration of IV infusions by unblinded study site personnel as well as attendance by the patient to the clinic. Treatment compliance with study drug administration will be verified by unblinded study staff observation. A dose will be considered completed if 80% or more of the total volume of the IV solution has been administered to the patient. Patients will be permitted to miss an occasional dose of study drug. However, if a patient misses 2 consecutive doses, the PI, in consultation with the Medical Monitor, will discuss whether the patient will be able to continue on the study.

Patients failing to complete the 18-month efficacy assessment visit will not be eligible to receive ALN-TTR02 on the open-label extension study.

5.7 Study Drug Accountability

The Investigator will maintain accurate records of receipt and the condition of all study drugs including dates of receipt. In addition, accurate records will be kept of the weight used to calculate each dispensed ALN-TTR02 dose, and when and how much study drug is dispensed and used by each patient in the study. Any reasons for departure from the protocol dispensing regimen must also be recorded.

Drug accountability records and inventory will be available for verification by unblinded Alnylam Monitor or designee that will not have a role in any other aspect of managing the study. Remaining study drug (all used, partially used, and unused vials) will be returned to Alnylam or its specified designee/depot or destroyed at the site according to applicable regulations.

Study drug must not be used for any purpose other than the present study. Study drug which has been dispensed for a patient and returned unused must not be redispensed.

Further instructions about study drug accountability are detailed in the ALN-TTR02 Pharmacy Manual.

5.8 Concomitant Medication / Treatment

Use of the following medications/treatments is prohibited during study participation (with the exclusion of patients who have rapid disease progression and discontinue study drug after the 9-month efficacy assessments):

- Any investigational agent other than ALN-TTR02;
- Tafamidis (use prior to screening permitted);
- Diflunisal (use prior to screening permitted);
- Doxycycline/TUDCA (use prior to screening permitted);
- Corticosteroids other than those administered as premedications prior to the dose of ALN-TTR02, those used to treat an infusion reaction, or topical or inhaled corticosteroids. However, for patients with chronic inflammatory disorders (e.g., asthma, rheumatoid arthritis, etc.), systemically administered steroids may be permitted provided that: 1) the dose is <20 mg/day prednisone or equivalent if administered chronically, or 2) for doses \geq 20 mg/day, administration is limited to no more than 5 consecutive days.

Medications and treatments other than those specified above, including palliative and supportive care approved by the investigator for disease-related symptoms, are permitted during the study.

Investigator should review over-the-counter (OTC) and or herbal preparations to ensure that these are not potentially disease modifying.

Use of all concomitant medications and treatments will be recorded on the patient's CRF through the time points shown in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#). This will include all prescription drugs, herbal preparations, OTC medications, vitamins, and minerals. Any changes in medications during the study will also be recorded on the CRF.

Any concomitant medication or treatment that is required for the patient's welfare may be given by the Investigator. However, it is the responsibility of the Investigator to ensure that details regarding the medication or treatment are recorded on the CRF, and coded using an internationally recognized and accepted coding dictionary.

5.9 Suggested Guidelines for Management of Infusion-related Reactions

Criteria for categorizing IRRs are provided in [Appendix 3](#).

In the event of an IRR, the infusion of study drug will be stopped and the patient closely monitored until resolution of the reaction. Drugs that may be used to facilitate resolution and permit resumption of study drug administration include, but are not limited to: paracetamol/acetaminophen (or equivalent), additional H1/H2 blockers (e.g., ranitidine), NSAIDs, adrenaline, supplemental oxygen, IV fluids, and/or corticosteroids.

Following resolution of a mild or moderate IRR that required interruption of the study drug infusion, resumption of administration may occur at the Investigator's discretion at a slower infusion rate (but not to exceed 3 hours) for that dose and for all subsequent doses of study drug. If the infusion is delayed, the administration of the infusion should be completed no more than 6 hours from the initial start of the infusion. Study drug administration will not be resumed for any patient following a severe IRR until the case is discussed with the Medical Monitor.

The patient's infusion site should be assessed for signs of any localized reaction during the infusion and for 30 minutes after the end of the infusion. The patient will remain at the study site for 1 hour following completion of dosing for observation and completion of assessments. Patients will be instructed to call the Investigator if they experience symptoms such as fever, chills, myalgia, or nausea/vomiting after discharge from the site.

For those patients who continue to have mild to moderate reactions to an infusion or who experience a severe reaction, the premedication regimen may be modified, following a discussion with the Investigator and Medical Monitor, to the following:

- Dexamethasone 8 mg or equivalent self-administered PO the evening before the dose.
- Intravenous dexamethasone (20 mg) or equivalent, administered at least 60 minutes prior to the start of the infusion of study drug;
- Oral paracetamol/acetaminophen (500 mg) or equivalent at least 60 minutes prior to the start of the infusion of study drug;
- Intravenous H2 blocker (e.g., ranitidine 50 mg, famotidine 20 mg, or equivalent other H2 blocker dose) at least 60 minutes prior to the start of the infusion of study drug; and
- Intravenous H1 blocker: diphenhydramine 50 mg (or equivalent other IV H1 blocker available at the study site) at least 60 minutes prior to the start of the infusion of study drug. Hydroxyzine or fexofenadine 25 mg PO or cetirizine

10 mg PO may be substituted for any patient who does not tolerate IV diphenhydramine or other IV H1 blocker.

8 STUDY VISITS

The duration of a patient's participation in this study is approximately 21 months (inclusive of a 28-day screening period and up to a 56-day post last dose study visit).

Screening evaluations are to be performed within 28 days before receiving the first dose of study drug, as indicated in [Table 1-1](#). Patients determined to be eligible based on Screening assessments will receive blinded study drug (IV infusion of either ALN-TTR02 or placebo) once every 21 days. Patients will remain at the study site for 1 hour following completion of dosing for observation and completion of assessments.

In order to decrease the variability in the efficacy assessment testing, there will be a limited number of sites within any one territory that conduct efficacy assessments at baseline, 9 months, and 18 months (referred to as "Central Assessment Sites [CAS]"); these sites can also screen, dose and manage patients. There will also be sites that screen, dose, and manage the patients ("Patient Care Sites [PCS]"), while sending the patients to the nearest CAS for their baseline, 9- and 18-month efficacy assessments.

Prior to starting the study and screening patients in the protocol, the CAS and PCS will create a delegation of responsibilities document that clearly details which site is responsible for which tests and safety parameters to ensure adherence to the protocol. This document will become part of the study site specific documentation.

All patients who discontinue from the study early will return to the study site for their Early Withdrawal assessments.

6.1 Screening, Screening/Baseline, and Baseline Visits (Days -28 to Day 0)

Screening evaluations will be conducted over 3 visits (Screening, Screening/Baseline, and Baseline). All screening visits must occur within 28 days of the first dose of study drug (Day 0). [Table 1-1](#) provides an overview of the schedule of events required at each screening visit.

Prior to screening activities, the patient will sign and date an informed consent form (ICF) and receive a copy of the signed ICF. No study procedures should be performed prior to informed consent being obtained. The Investigator or another person authorized by the Investigator will also sign and date the informed consent prior to giving a copy to the patient. The ICF will be filed in the patient's medical record.

6.1.1 Screening

The following activities must be performed during the Screening visit as part of the initial review of patient eligibility:

- Assess study eligibility using the inclusion and exclusion criteria.
- Obtain medical history information, including inquiry into HIV status.
- Obtain demographic information.
- Determine Karnofsky Performance Status (see [Appendix 1](#)).

- Determine New York Heart Association classification of heart failure (see [Appendix 2](#)).
- Collect and review documentation for TTR genotype.
- Perform a physical examination.
- Measure weight.
- Measure height.
- Measure vital signs.
- Collect blood samples for clinical laboratory tests, including:
 - Paraprotein (assessed by immunofixation electrophoresis [IFE]);
 - Vitamin A;
 - Vitamin B₁₂;
 - Serology;
 - Hematology;
 - Serum chemistries;
 - Coagulation studies;
 - Thyroid function tests.
- Collect urine sample for urinalysis.
- Perform a pregnancy test (for females of child-bearing potential only).
- Obtain information on concomitant medications.
- Perform NIS and NCS. Note: The documented results of previously performed NIS and NCS may be used to qualify a patient for this study if these tests were performed within 60 days prior to the date of informed consent.

6.1.2 Screening/Baseline Visit

Patients who meet all of the Screening criteria (including NIS and NCS assessments) will complete the screening process during 2 subsequent visits (which will be conducted at CAS for patients screened at PCS): a Screening/Baseline visit and a Baseline visit.

The Screening/Baseline visit must be performed within 14 days prior to the first dose of study drug (Day 0).

The following assessments must be performed during the Screening/Baseline visit:

- Perform the following efficacy assessments:
 - mNIS+7*;
 - NIS+7*;

*Note: Components that are shared between the mNIS+7 and NIS+7 (including NIS and NCS) will be performed only once.

- Timed 10-meter walk test;
- Grip strength test.
- Collect blood sample for serum TTR protein, RBP, and vitamin A. Additionally, aliquots of serum samples will be taken for long-term frozen storage, to permit testing of additional proteins related to FAP.
- Obtain information on concomitant medications.
- Obtain an interval medical history.
- Re-assess study eligibility using the inclusion and exclusion criteria.

6.1.3 Baseline Visit

The Baseline visit will occur no less than 24 hours but no greater than 7 days from the Screening/Baseline visit. The following activities should be performed during the Baseline Visit:

- Perform an ophthalmology exam. Note: The ophthalmology examination may be performed any time after the patient is deemed eligible for participation in the study, but before the first administration of study drug.
- Perform a 12-lead ECG.
- Measure weight.
- Collect blood sample for serum TTR protein, RBP, and vitamin A. Additionally, aliquots of serum samples will be taken for long-term frozen storage, to permit testing of additional proteins related to FAP.
- Obtain information on concomitant medications.
- Perform the following efficacy assessments:
 - mNIS+7*;
 - NIS+7*;
 - *Note: Components that are shared between the mNIS+7 and NIS+7 (including NIS and NCS) will be performed only once.
 - PND stage (see [Appendix 5](#));
 - FAP score (see [Appendix 6](#));
 - If the patient has provided separate informed consent for the skin biopsies, obtain 2 sets of tandem 3-mm skin punch biopsies (4 biopsies total; 1 set from the distal lower leg, when a patient's clinical status allows, and 1 set from the distal thigh);
 - Timed 10-meter walk test;
 - Grip strength test;

- mBMI (Note: The site will only measure the patient's weight. Using this weight, mBMI will be calculated programmatically in the clinical database; the site will not perform the calculation.);
 - Echocardiogram;
 - Collect blood samples for NT-proBNP and troponin I;
 - Norfolk QOL-DN;
 - EQ-5D;
 - R-ODS;
 - COMPASS-31.
- Complete the pharmacoeconomics and C-SSRS questionnaires.
 - Obtain an interval medical history.
 - Re-assess study eligibility using the inclusion and exclusion criteria.

6.2 Treatment Visits

Patients who are determined to be eligible for the study will be enrolled on Day 0.

On all study visit days, patients should take their vitamin A supplement after completing the blood draws.

6.2.1 Day 0

6.2.1.1 Pre-dose on Day 0

On Day 0, patients will undergo the following procedures prior to study drug administration:

- Measure weight.
- Measure vital signs.
- Collect blood samples for clinical laboratory tests, including:
 - Serum chemistries;
 - Anti-drug antibody testing.
- Collect blood sample for serum TTR protein, RBP, and vitamin A. Additionally, aliquots of serum samples will be taken for long-term frozen storage, to permit testing of additional proteins related to FAP.
- Collect blood sample for plasma PK assessment within 1 hour of planned study drug dosing start.
- Collect urine sample for PK assessment within 1 hour of planned study drug dosing start.
- Obtain information on concomitant medications.
- Randomize the patient (See [Section 4.4.1](#))

- Administer premedications at least 60 minutes prior to the start of administration of study drug (see [Section 5.3.1](#)).
- Document any AEs.

6.2.1.2 Administration of Study Drug on Day 0

As described in [Section 5.3.2](#), after completion of all pre-dose evaluations and procedures, study drug will be administered by a controlled infusion device as a 70-minute IV infusion (approximately 1 mL/minute for the first 15 minutes followed by approximately 3 mL/minute for the remainder of the infusion) or at a more prolonged infusion rate (up to 3 hours) if required due to prior IRR. The patient's infusion site should be assessed for signs of any localized reaction during the infusion and for 30 minutes after the end of the infusion.

The infusion time may be extended up to 3 hours in the event of a mild or moderate IRR. Study drug administration will not be resumed for any patient following a severe infusion reaction until the case is discussed with the Medical Monitor.

Suggested guidelines for management of IRRs are provided in [Section 5.9](#).

6.2.1.3 Post-dose on Day 0

The patient's infusion site should be assessed for signs of any localized reaction during the infusion and for 30 minutes after the end of the infusion. The patient will remain at the study site for 1 hour following completion of dosing for observation and completion of assessments:

- Measure vital signs.
- Collect a PK sample at the end of infusion (+5 minutes).
- Obtain information on concomitant medications.
- Document any AEs.

6.2.2 Routine Study Visits

The procedures described below are to be performed for all routine study visits (from Day 21 through Day 546), with the exception of the 9-month and 18-month efficacy assessments (occurring on Days 259-266 and Days 553-560, respectively).

Patients will undergo the following procedures before study drug dosing:

- At all dosing visits, administer premedications at least 60 minutes prior to the start of administration of study drug (see [Section 5.3.1](#)).
- At all dosing visits, measure weight.
- At all dosing visits, measure vital signs.
- On Days 252 and 546, perform a physical examination.
- On Days 21, 126, 252, 273, 399, and 546, collect blood sample for serum TTR protein, RBP, and vitamin A. Additionally, aliquots of serum samples will be taken for long-term frozen storage, to permit testing of additional proteins related to FAP.

- On Days 84, 189, 357, 462, and 546, collect blood samples for serum chemistry tests.
- On Days 21, 126, 252, 399, and 546, collect a blood sample for anti-drug antibody assessment.
- On Days 252 and 546, collect blood samples for clinical laboratory tests, including:
 - Hematology
 - Thyroid function tests.
- On Days 252 and 546, collect urine sample for urinalysis.
- On Days 21, 126, 252, 399, and 546, collect a plasma PK sample pre-dose (within 1 hour of planned study drug dosing).
- On Days 21, 126, 252, 399, and 546, collect a urine PK sample pre-dose (within 1 hour of planned dosing start).
- On Days 252 and 546, complete the pharmacoeconomics questionnaire.
- Perform an ophthalmology examination (2 exams with 1 being completed from Day 252 through Day 266, and 1 completed from Day 546 through Day 560).
- At all dosing visits, obtain information on concomitant medications.
- At all dosing visits, document any AEs.

Following the completion of all required pre-dose activities and assessments, administer the study drug at all dosing visits, as described in [Section 6.2.1.2](#).

Following dosing, collect plasma PK samples as follows:

- On Days 126 and 399, collect a plasma PK sample at the end of the infusion (+5 minutes).
- On Days 21, 252, and 546, collect a plasma PK sample 30 minutes after the end of the infusion (+15 minutes).

6.2.3 Efficacy Assessment Visits (9 Months and 18 Months)

Efficacy assessment will be performed on all patients at approximately 9 and 18 months (Days 259-266 and 553-560, respectively). Patients will not receive any study drug on these days.

As described in [Section 4.6](#), in consultation with the Investigator, patients who meet the criteria for Rapid Disease Progression based on the 9-month efficacy assessments may continue study drug dosing or discontinue study drug dosing. Patients who discontinue study drug dosing may receive additional therapy for FAP, as per the local standard of care. These patients will remain on the study and follow the modified schedule of assessments shown in [Table 1-3](#) and described in [Section 6.4](#). Blinding will be maintained throughout.

The 9- and 18-month efficacy assessment visits will take place over 2 days.

- Perform the following efficacy assessments:

- mNIS+7*;
- NIS+7*;
- Timed 10-meter walk test*;
- Grip strength test*;

*Note: For the mNIS+7, NIS+7, timed 10-meter walk test, and grip strength test, an independent assessment will be performed on each day. Each site will make every effort to have these assessments performed by the same Investigator. Assessments are to be performed no less than 24 hours apart from each other but no greater than 7 days apart. For the mNIS+7 and NIS+7, components that are shared (including NIS and NCS) will be performed only once on each assessment day.

- PND stage (see [Appendix 5](#));
 - FAP score (see [Appendix 6](#));
 - If the patient has provided separate informed consent for the skin biopsies, obtain 2 sets of tandem 3-mm skin punch biopsies (4 biopsies total); 1 set from the distal lower leg when a patient's clinical status allows, and 1 set from the distal thigh;
 - mBMI (Note: The site will only measure the patient's weight. Using this weight, mBMI will be calculated programmatically in the clinical database; the site will not perform the calculation.);
 - Echocardiogram (only at 18 months);
 - Collect blood samples for NT-proBNP and troponin I;
 - Norfolk QOL-DN;
 - EQ-5D;
 - R-ODS;
 - COMPASS-31.
- Measure weight on both efficacy assessment days.
 - Measure vital signs on both efficacy assessment days.
 - Complete the C-SSR questionnaire.
 - Perform a 12-lead ECG.
 - Collect blood samples for:
 - TTR protein, RBP, and vitamin A;
 - Additional aliquots of serum for long-term frozen storage, to permit testing of additional proteins related to FAP.
 - Obtain information on concomitant medications.

- Document any AEs.

6.2.4 Follow-Up Visits

Patients will return to the study site for 2 follow-up visits, 21 days and 56 days after receiving their last dose of study drug. If a patient enrolls in the extension study, they will only have to complete the 21-day follow-up assessments (End of Study [EOS]; Day 567) and not the 56-day follow-up assessments (Day 602). Patients who do not enroll in the extension study will need to complete both follow-up visits (EOS [Day 567] and Day 602).

6.2.4.1 Twenty-One-Day Follow-up Visit (End of Study)

The following procedures will be performed at the 21-Day Follow-up Visit (EOS; Day 567).

- Perform a physical examination.
- Measure weight.
- Measure vital signs.
- Collect blood sample for serum chemistry testing.
- Collect blood sample for serum TTR protein, RBP, and vitamin A. Additionally, aliquots of serum samples will be taken for long-term frozen storage, to permit testing of additional proteins related to FAP.
- Perform a pregnancy test (for females of child-bearing potential only).
- Obtain information on concomitant medications.
- Document any AEs.

6.2.4.2 Fifty-Six-Day Follow-up Visit

This visit will only be conducted for patients who do not enroll in the extension study.

The following procedures will be performed at the 56-Day Follow-up Visit (Day 602):

- Perform a physical examination.
- Measure vital signs.
- Collect blood sample for serum chemistry testing.
- Perform a pregnancy test (only for females of child-bearing potential).
- Obtain concomitant medications information.
- Document any AEs.

6.3 Early Withdrawal Visit

For patients who withdraw early from the study, every effort will be made to have them return to the study site to have the following procedures performed. These visits will take place over 2 days.

- Perform the following efficacy assessments:

- mNIS+7*;
- NIS+7*;
- Timed 10-meter walk test*;
- Grip strength test*.

*Note: For the mNIS+7, NIS+7, timed 10-meter walk test, and grip strength test, an independent assessment will be performed on each day. Each site will make every effort to have these assessments performed by the same Investigator. Assessments are to be performed no less than 24 hours apart from each other but no greater than 7 days apart. For the mNIS+7 and NIS+7, components that are shared (including NIS and NCS) will be performed only once on each assessment day.

- PND stage (see [Appendix 5](#));
 - FAP score (see [Appendix 6](#));
 - If the patient has provided separate informed consent for the skin biopsies, obtain 2 sets of tandem 3-mm skin punch biopsies (4 biopsies total); 1 set from the distal lower leg when a patient's clinical status allows, and 1 set from the distal thigh;
 - mBMI (Note: The site will only measure the patient's weight. Using this weight, mBMI will be calculated programmatically in the clinical database; the site will not perform the calculation.);
 - Echocardiogram;
 - Collect blood samples for NT-proBNP and troponin I;
 - Norfolk QOL-DN;
 - EQ-5D;
 - R-ODS;
 - COMPASS-31.
- Complete the pharmacoeconomics and C-SSRS questionnaires.
 - Measure weight on both efficacy assessment days.
 - Measure vital signs on both efficacy assessment days.
 - Perform a physical examination.
 - Collect a 12-lead ECG.
 - Collect blood samples for clinical laboratory tests, including:
 - Hematology;
 - Serum chemistries;
 - Thyroid function tests;

- Anti-drug antibody testing.
- Collect blood sample for serum TTR protein, RBP, and vitamin A. Additionally, aliquots of serum samples will be taken for long-term frozen storage, to permit testing of additional proteins related to FAP.
- Collect urine sample for urinalysis.
- Perform a pregnancy test (only for females of child-bearing potential).
- Collect a blood sample for plasma PK assessment.
- Collect a urine sample for PK assessment.
- Obtain information on concomitant medications.
- Document any AEs.

6.4 Modified Visit Schedule for Subjects who Discontinue Study Drug for Rapid Disease Progression at Month 9

As described in [Section 4.6](#), in consultation with the Investigator, patients who meet the criteria for Rapid Disease Progression based on the 9-month efficacy assessments may continue study drug dosing or discontinue study drug dosing. Patients who discontinue study drug dosing may receive additional therapy for FAP, as per the local standard of care. These patients will remain on the study and follow a modified visit schedule as shown in [Table 1-3](#) and described in detail below. Blinding will be maintained throughout.

6.4.1 Modified Day 273

The following procedures will be performed:

- Consult with the patient on a subsequent plan of care, which may include receiving therapy for FAP as per the local standard of care;
- Measure vital signs;
- Collect information on concomitant medications;
- Document any AEs.

Patients who discontinue treatment and also decide to discontinue from the study at the time of this visit will not have any additional visits after the Day 273 assessments are completed.

6.4.2 Modified Day 294

The following procedures will be performed:

- Perform a physical examination.
- Measure vital signs.
- Collect blood sample for serum chemistry testing.

- Collect blood sample for serum TTR protein, RBP, and vitamin A. Additionally, aliquots of serum samples will be taken for long-term frozen storage, to permit testing of additional proteins related to FAP.
- Collect a blood sample for plasma PK assessment.
- Collect a urine sample for PK assessment.
- Perform a pregnancy test (only for females of child-bearing potential).
- Obtain concomitant medications information. (This will be the final collection of concomitant medications.)
- Document any AEs. (Following this visit, only adverse events that are considered related to study procedures will be collected.)
- Collect data on subsequent FAP treatment regimens.

6.4.3 Modified Day 378 and Day 462

Study personnel will contact the patient by phone to query for general health status and data on subsequent FAP treatment regimens.

6.4.4 Modified Day 546

The following procedures will be performed:

- Perform a physical examination.
- Measure weight.
- Measure vital signs.
- Collect blood samples for clinical laboratory tests, including:
 - Hematology;
 - Serum chemistries;
 - Thyroid function tests;
 - Anti-drug antibody testing.
- Collect urine sample for urinalysis.
- Perform a pregnancy test (only for females of child-bearing potential).
- Collect blood sample for serum TTR protein, RBP, and vitamin A. Additionally, aliquots of serum samples will be taken for long-term frozen storage, to permit testing of additional proteins related to FAP.
- Collect a blood sample for plasma PK assessment.
- Collect a urine sample for PK assessment.
- Complete the pharmacoeconomics questionnaire.
- Collect data on subsequent FAP treatment regimens.

6.4.5 Modified 18-Month Efficacy Assessment Visit

This visit will take place over 2 days. The following procedures will be performed:

- Perform the following efficacy assessments:

- mNIS+7*;
- NIS+7*;
- Timed 10-meter walk test*;
- Grip strength test*;

*Note: For the mNIS+7, NIS+7, timed 10-meter walk test, and grip strength test, an independent assessment will be performed on each day. Each site will make every effort to have these assessments performed by the same Investigator.

Assessments are to be performed no less than 24 hours apart from each other but no greater than 7 days apart. For the mNIS+7 and NIS+7, components that are shared (including NIS and NCS) will be performed only once on each assessment day.

- PND stage (see [Appendix 5](#));
 - FAP score (see [Appendix 6](#));
 - If the patient has provided separate informed consent for the skin biopsies, obtain 2 sets of tandem 3-mm skin punch biopsies (4 biopsies total); 1 set from the distal lower leg when a patient's clinical status allows, and 1 set from the distal thigh;
 - mBMI (Note: The site will only measure the patient's weight. Using this weight, mBMI will be calculated programmatically in the clinical database; the site will not perform the calculation.);
 - Echocardiogram;
 - Collect blood samples for NT-proBNP and troponin I;
 - Norfolk QOL-DN;
 - EQ-5D;
 - R-ODS;
 - COMPASS-31.
- Measure weight on both efficacy assessment days.
 - Measure vital signs on both efficacy assessment days.
 - Collect a 12-lead ECG.
 - Complete the C-SSRS questionnaire.
 - Document any study-procedure-related AEs.

6.4.6 Modified Twenty-One-Day Follow-up Visit (End of Study)

The following procedures will be performed:

- Perform a physical examination.
- Measure weight.
- Measure vital signs.
- Collect blood sample for serum TTR protein, RBP, and vitamin A. Additionally, aliquots of serum samples will be taken for long-term frozen storage, to permit testing of additional proteins related to FAP.
- Collect data on subsequent FAP treatment regimens.

6.4.7 Modified Early Withdrawal Visit

If the patient on the modified visit schedule discontinues from the study any time after the Day 273 assessments are completed (i.e., Day 274 onward), every effort will be made to have the patient return to the study site for the Modified Early Withdrawal Visit. This visit will take place over 2 days (at least 24 hours, but not more than 7 days apart). The following procedures will be performed:

- Perform the following efficacy assessments:
 - mNIS+7*;
 - NIS+7*;
 - Timed 10-meter walk test*;
 - Grip strength test*;

*Note: For the mNIS+7, NIS+7, timed 10-meter walk test, and grip strength test, an independent assessment will be performed on each day. Each site will make every effort to have these assessments performed by the same Investigator. Assessments are to be performed no less than 24 hours apart from each other but no greater than 7 days apart. For the mNIS+7 and NIS+7, components that are shared (including NIS and NCS) will be performed only once on each assessment day.

- PND stage (see [Appendix 5](#));
- FAP score (see [Appendix 6](#));
- If the patient has provided separate informed consent for the skin biopsies, obtain 2 sets of tandem 3-mm skin punch biopsies (4 biopsies total); 1 set from the distal lower leg when a patient's clinical status allows, and 1 set from the distal thigh;
- mBMI (Note: The site will only measure the patient's weight. Using this weight, mBMI will be calculated programmatically in the clinical database; the site will not perform the calculation.);
- Echocardiogram;

- Collect blood samples for NT-proBNP and troponin I;
 - Norfolk QOL-DN;
 - EQ-5D;
 - R-ODS;
 - COMPASS-31.
- Complete the pharmacoeconomics and C-SSRS questionnaires.
 - Perform a physical examination.
 - Measure weight on both efficacy assessment days.
 - Measure vital signs on both efficacy assessment days.
 - Collect a 12-lead ECG.
 - Collect blood sample for serum TTR protein, RBP, and vitamin A. Additionally, aliquots of serum samples will be taken for long-term frozen storage, to permit testing of additional proteins related to FAP.
 - Collect blood samples for clinical laboratory tests, including:
 - Serum chemistries;
 - Thyroid function tests;
 - Anti-drug antibody testing.
 - Perform a pregnancy test (only for females of child-bearing potential).
 - Document any study-procedure-related AEs.
 - Collect data on subsequent FAP treatment regimens.

6.5 **Unscheduled Visits**

Unscheduled visits for study assessments may occur if deemed necessary by the study personnel.

9" STUDY ASSESSMENTS

7.1 Demographic Data and Medical History

At the Screening Visit, patient demographic data and a complete medical history will be obtained. At the Screening/Baseline Visit and Baseline Visit, an interval medical history will be obtained, capturing only changes since the Screening Visit.

At the Screening Visit, HIV status will be obtained. The Investigator will assess the patient according to the Karnofsky Scale (see [Appendix 1](#)) and the New York Heart Association Classification of Heart Failure (see [Appendix 2](#)).

7.2 Efficacy Assessments

Efficacy assessments will occur at Screening/Baseline, Baseline, 9 months, 18 months, and Early Withdrawal (if applicable). A central neurologic testing core facility will review the data derived from the various neuropathy measures at each participating site to ensure compliance with testing procedures and quality of the data. A central echocardiography core lab will analyze the echocardiogram data. A core lab will also be responsible for processing and analyzing skin punch biopsy samples.

Further details on performing these assessments will be provided in the Study Reference Manual.

7.2.1 Screening Neurological Impairment Score and Nerve Conduction Studies

Unless done within 60 days prior to the date of informed consent, the NIS and NCS will be performed during the Screening Visit. The NIS consists of a clinical assessment that tests weakness, reflexes, sensation and cranial nerves. Nerve conduction studies $\sum 5$ will assess SNAP.

7.2.2 Modified Neurological Impairment Score + 7

The mNIS+7 assessment tool is a composite measure of neurologic impairment which includes the following measures:

- Clinical exam-based neurologic impairment score (NIS-weakness and reflexes)
- Electrophysiologic measures of small and large nerve fiber function (+7) including:
 - Nerve conduction studies (NCS $\sum 5$)
 - Quantitative sensory testing (QST) by body surface area including touch pressure (TP) and heat pain (HP)
- Autonomic function (postural blood pressure)

A summary of the scoring of the components of the mNIS+7 is provided in [Appendix 4](#).

At each time point, 2 independent assessments will be performed. Each site will make every effort to have these assessments performed by the same blinded study personnel, who will be different from the Investigator and other personnel managing the patient. Assessments are to be performed no less than 24 hours apart from each other but no greater than 7 days apart. In order to limit potential intra-operator bias, results of any of

the prior assessments will not be available to the examiner when performing the second assessment.

Every effort will be made to use the same devices for NCS and QST for a patient throughout the duration of the study.

7.2.3 Neurological Impairment Score + 7

The NIS+7 consists of the following measures:

- Full NIS (including weakness, sensation, and reflexes)
- Electrophysiologic measures of small and large nerve fiber function (+7) including:
 - NCS Σ 5
 - Vibration detection threshold (VDT)
- Heart rate response to deep breathing (HRdb)

A summary of the scoring of the components of the NIS+7 is provided in [Appendix 4](#).

At each time point, 2 independent assessments will be performed in the same manner as described above for mNIS+7. Every effort will be made to use the same devices for NCS and VDT for a patient throughout the duration of the study.

Components that are shared between the mNIS+7 and NIS+7 (including NIS and NCS) will be performed once at each assessment.

7.2.4 Familial Amyloidotic Polyneuropathy Stage and Polyneuropathy Disability Score

Changes in ambulation will be evaluated through the PND score and FAP stage (see [Appendix 5](#) and [Appendix 6](#), respectively).^{42,43}

7.2.5 Intraepidermal Nerve Fiber Density and Sweat Gland Nerve Fiber Density

Quantification of nerve fibers in skin will be assessed via IENFD and SGNFD. Patients who have provided additional voluntary consent for skin biopsy will undergo tandem 3-mm skin punch biopsies for IENFD and SGNFD assessment. At each time point, 1 set of biopsies will be taken from the distal lower leg, when a patient's clinical status allows, and 1 set from the distal thigh (4 biopsies total).

A repeat baseline biopsy may be performed if the initial sample is not of sufficient quality.

Details on sample collection, processing, and storage will be provided in the Study Laboratory Manual.

7.2.6 Modified Body Mass Index

Sites will measure body weight at the time points specified in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#). Using that data, the mBMI will be calculated ($\text{BMI} \times \text{albumin}$). This calculation will take place programmatically in the clinical database; the sites will not perform the calculation.

7.2.7 Timed Ten-meter Walk Test

To perform the timed 10-meter walk, the patient will be asked to walk 10 meters. The walk must be completed without assistance from another person; ambulatory aids such as canes and walkers are permitted. The time required for the patient to walk 10 meters will be recorded. At 9 Months, 18 Months, and Early Withdrawal, the assessments will be conducted on 2 days, at least 24 hours, but not more than 7 days apart.

7.2.8 Grip Strength Test

Hand grip strength will be measured by dynamometer. Sites will be trained on dynamometer and testing for the study. When performing the test, patients will stand, holding the dynamometer in their dominant hand, with their arm parallel to the body without squeezing the arm against the body. Tests will be performed in triplicate on the same day for each time point.

Every effort will be made to use the same dynamometer for a patient throughout the duration of the study.

At 9 Months, 18 Months, and Early Withdrawal, the assessments will be conducted on 2 days, at least 24 hours, but not more than 7 days apart.

7.2.9 Composite Autonomic Symptom Score

To evaluate changes in autonomic symptoms, patients will complete the COMPASS-31 questionnaire. The questionnaire consists of 31 clinically selected questions evaluating 6 autonomic domains (orthostatic intolerance, secretomotor, gastrointestinal, bladder, and pupillomotor).⁴⁴

7.2.10 Norfolk Quality of Life - Diabetic Neuropathy Questionnaire

Quality of life will be assessed through the Norfolk QOL-DN questionnaire, a standardized 47-item patient-reported outcomes measure that is sensitive to the different features of diabetic neuropathy (DN)-small fiber, large fiber, and autonomic nerve function.⁴⁵

7.2.11 EuroQoL Quality of Life Questionnaire

Quality of life will be assessed through the use of the EQ-5D, a standardized 5-question instrument for use as a measure of health outcomes.⁴⁶

7.2.12 Rasch-built Overall Disability Scale

An assessment of the disability each patient experiences will be assessed through the R-ODS.⁴⁷ The R-ODS is comprised of a 24-item linearly weighted scale that specifically captures activity and social participation limitations in patients.

7.2.13 Echocardiogram and Biomarkers of Cardiac Function

Cardiac structure and function will be assessed through echocardiograms as well as measurement of serum levels of the cardiac biomarkers NT-proBNP and troponin I.

Echocardiograms will be performed at the time points specified in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#) and interpreted at a central echocardiography core lab. Image acquisition, storage, and transfer guidelines will be provided in a Study Manual.

Blood samples will be drawn to measure levels of NT-proBNP and troponin I at the time points specified in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#). Details on sample collection, processing, and storage will be provided in a Study Laboratory Manual.

7.3 Pharmacodynamic Assessments

Pharmacodynamic sample collection, processing, and storage guidelines will be provided in a Study Laboratory Manual.

7.3.1 Transthyretin

Blood for serum TTR levels will be collected at Screening and prior to the administration of study drug at the time points specified in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#).

Serum TTR will be assessed using both ELISA (enzyme linked immunosorbent assay) and turbidimetric assays.

7.3.2 Retinol Binding Protein

Blood for serum RBP levels will be collected at Screening and prior to the administration of study drug at the time points specified in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#).

Serum RBP will be quantified using nephelometry.

7.3.3 Vitamin A

Blood for serum vitamin A levels will be collected at Screening and prior to the administration of study drug and supplemental vitamin A at the time points specified in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#).

7.3.4 Exploratory Biomarkers

To explore the expression of hepatocyte derived proteins to further characterize the biological effects of siRNA and/ or to explore possible metabolite profiling of ALN-TTR02, serum and plasma samples will be collected at the time points specified in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#).

Details on biomarker sample collection, processing, and storage will be provided in a Laboratory Manual.

Biological samples for biomarker research and possible metabolic profiling can be retained on behalf of Alnylam for a maximum of 15 years following the last patient's last visit in the study.

7.4 Pharmacokinetic Evaluations

Details on PK sample collection, processing, and storage will be provided in a Laboratory Manual. Plasma and urine samples will be evaluated using a validated ATTO-Probe-HPLC (high performance liquid chromatography) assay to determine siRNA concentration and by LC/MS/MS for DLin-MC3-DMA and PEG₂₀₀₀-C-DMG concentrations.

7.4.1 Plasma Pharmacokinetics

Plasma samples will be collected at the time points shown in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#) for assessment of siRNA, DLin-MC3-DMA and PEG₂₀₀₀-C-DMG. PK parameters, including population PK will be analyzed, whenever possible as outlined in [Section 9.2.6](#).

7.4.2 Urine Pharmacokinetics

Urine samples and urine volume void will be obtained at specified time points as specified in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#). Renal clearance (CL_R) will be determined whenever possible for siRNA and 4-dimethylaminobutyric acid, metabolite of DLin-MC3-DMA, excreted in the urine. PK parameters will be analyzed, whenever possible, as outlined in [Section 9.2.6](#).

7.5 Safety Assessments

All safety assessment measures will be recorded in the patient's medical record and CRF.

7.5.1 Physical Examination

A complete physical examination (including general appearance; head, ears, eyes, nose, and throat [HEENT]; cardiovascular; dermatologic; abdominal; genito-urinary; lymph nodes; hepatic; musculoskeletal; respiratory; and neurological) is to be at the study visits listed in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#).

7.5.2 Body Weight and Height

Body weight will be measured at the study visits listed in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#). The rules for using body weight to calculate dose are described in [Section 5.3.2](#).

Height will only be measured at Screening.

7.5.3 Vital Signs

Vital signs will be measured at the time points specified in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#). Vital signs include systolic/diastolic blood pressure, pulse rate, respiratory rate, and oral body temperature, and will be measured in the supine position using an automated instrument after the patient has rested comfortably for 10 minutes. Each patient's blood pressure should be taken using the same arm. Oral temperature will be recorded in Celsius or Fahrenheit. Heart rate will be counted for a full minute and recorded in beats per minute. Respirations will be counted for a full minute and recorded in breaths per minute.

For the safety of the patient, additional vital signs may be added at the discretion of the Investigator.

7.5.4 Electrocardiogram

Computerized 12-lead ECG recordings will be obtained in triplicate at each time point listed in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#) and read locally. Each lead shall be recorded for at least 3 beats at a speed of 25 mm/s.

The following electrophysiologic parameters will be assessed: rhythm, ventricular rate, PR interval, QRS duration, and QT interval. Either Bazett's and/or Fridericia's formula will be used to calculate the heart rate corrected QT interval (QTc).

For any clinically significant abnormal results, the Investigator must contact the CRO Medical Monitor to discuss continued participation of the patient in the study (e.g., ischemic ECG changes, wave/interval changes, or arrhythmia).

7.5.5 Clinical Laboratory Tests

Blood samples for clinical laboratory testing will be collected prior to study drug dosing at the time points listed in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#). Samples will be sent to a central laboratory for analysis. Details on sample collection, processing, and shipping will be provided in a Laboratory Manual.

In the event of an unexplained clinically relevant abnormal laboratory test occurring after study drug administration, the test should be repeated and followed up at the discretion of the Investigator until it has returned to the normal range or stabilized, and/or a diagnosis is made to adequately explain the abnormality.

The following clinical laboratory parameters are to be determined:

| Hematology | |
|---|--------------------------------|
| • Hematocrit | • Neutrophils, absolute and % |
| • Hemoglobin | • Lymphocytes, absolute and % |
| • Red blood cell (RBC) count | • Monocytes, absolute and % |
| • White blood cell (WBC) count | • Eosinophils, absolute and % |
| • Mean corpuscular volume | • Basophils, absolute and % |
| • Mean corpuscular hemoglobin | • Platelet count |
| • Mean corpuscular hemoglobin concentration | |
| Serum Chemistries | |
| • Aspartate transaminase (AST) | • Alkaline phosphatase |
| • Alanine transaminase (ALT) | • Bilirubin (total and direct) |
| • Sodium | • Glucose |
| • Potassium | • Phosphate |
| • Blood urea nitrogen (BUN) | • Albumin |
| • Creatinine | • Calcium |

| | |
|---|--|
| Coagulation Studies | |
| <ul style="list-style-type: none"> Prothrombin time (PT) Activated partial thromboplastin time (aPTT) | <ul style="list-style-type: none"> International Normalized Ratio (INR) |
| Thyroid Function Tests | |
| <ul style="list-style-type: none"> Thyroid stimulating hormone (TSH) Thyroxine (Free T4) | <ul style="list-style-type: none"> Triiodothyronine (Free T3) |
| Anti-drug Antibodies | |
| <ul style="list-style-type: none"> Anti-PEG antibodies | |
| Urinalysis | |
| <ul style="list-style-type: none"> Visual inspection for color and appearance pH Specific gravity Ketones Protein Glucose | <ul style="list-style-type: none"> Leukocytes Bilirubin Nitrite Urobilinogen Microscopic inspection of sediment |
| Serology | |
| <ul style="list-style-type: none"> Hepatitis B surface antibody (HbsAb) Hepatitis B surface antigen (HbsAg) | <ul style="list-style-type: none"> Anti-hepatitis C virus antibody (anti-HCVAb) |
| Cardiac Biomarkers | |
| <ul style="list-style-type: none"> N-terminal prohormone of B-type natriuretic peptide (NT-proBNP) Troponin I | |
| Other | |
| <ul style="list-style-type: none"> β-human chorionic gonadotropin (women of child-bearing potential only; may be a urine- or serum-based test) Vitamin B12 Paraprotein by IFE | |

7.5.5.1 Pregnancy Test

The pregnancy test may be urine- or serum-based, at the discretion of the Investigator. The test will only be performed for women of child-bearing potential. The timing of the tests is specified in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#); additional testing may be done any time pregnancy is suspected. The results must be known prior to administration of

study drug. Patients who are pregnant are not eligible for study participation. Patients who become pregnant while on study will be followed until the pregnancy outcome is known (see [Section 8.12](#)).

7.5.6 Ophthalmology Examination

The timing of the ophthalmology examinations is specified in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#). These examinations will include assessment of visual acuity, slit-lamp evaluation, intraocular pressure, dilated indirect ophthalmoscopy, color fundus photography, and visual field. Visual acuity should be evaluated at the beginning of each specified visit in the study (i.e., prior to slit-lamp examination). Manifest refraction will be performed at each specified visit prior to visual acuity testing and will be used to obtain a correction for visual acuity evaluations. Visual acuity testing should be done with best (most recent) correction.

Further details regarding the ophthalmology examinations will be provided in the Study Reference Manual.

7.5.7 Adverse Events and Study-Procedure-Related Adverse Events

Adverse events will be assessed and recorded at the time points specified in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#).

As shown in [Table 1-3](#), only study-procedure-related AEs (e.g., skin-biopsy-related AE, venipuncture-related AE) will be collected after Day 294 for patients who meet the criteria for rapid disease progression at Month 9 and receive their last dose of study drug on Day 252 but remain on the study.

[Section 8](#) provides assessment and reporting guidelines.

7.5.8 Concomitant Medications and Treatments

Use of all concomitant medications and treatments will be recorded on the patient's CRF through the time points shown in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#). This will include all prescription drugs, herbal preparations, OTC medications, vitamins, and minerals. Any changes in medications during the study will also be recorded on the CRF. [Section 5.8](#) provides guidelines on concomitant medications and treatments.

7.6 Other Assessments

7.6.1 Pharmacoeconomics Questionnaire

The burden of disease and healthcare utilization will be assessed using a patient-reported, 16-question pharmacoeconomics questionnaire.

7.6.2 Suicidality Questionnaire

The Columbia–Suicide Severity Rating Scale (C-SSRS) will be used to assess patient's mental status as it related to suicidal ideation and behavior.⁴⁸ This questionnaire will be administered to the patient by trained study personnel.

7.6.3 Phone Contact for Health Status Update and FAP Treatment

Patients following the Modified Schedule of Assessments will be called on the telephone by study personnel at the time points specified in [Table 1-3](#). Patients will be asked about their general health status and any treatments they may have received for FAP.

: **REPORTING ADVERSE EVENTS****8.1 Adverse Event Definition**

An AE is any untoward medical occurrence in a patient or clinical investigational patient administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment.

An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Disease progression (including worsening of neuropathy impairment) will not be considered an AE.

All IRRs will be recorded as AEs.

For patients who meet the criteria for rapid disease progression at Month 9 and receive their last dose of study drug on Day 252 but remain on the study, AEs will be followed through Day 294. Following Day 294, only study-procedure-related AEs will be collected (e.g., skin-biopsy-related AE, venipuncture-related AE).

8.2 Serious Adverse Event Definition

An SAE is any untoward medical occurrence that at any dose:

- Results in death;
- Is life-threatening (an event which places the patient at immediate risk of death from the event as it occurred. It does not include an event that had it occurred in a more severe form might have caused death);
- Requires inpatient hospitalization or prolongation of existing hospitalization;
- Results in persistent or significant disability/incapacity;
- Is a congenital anomaly or birth defect;
- An important medical event that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above (e.g., such events include allergic bronchospasm, blood dyscrasias or convulsions, or the development of drug dependency or abuse).

8.3 Eliciting Adverse Event Information

The patient should be asked about medically relevant changes in his/her health since the last visit. The patient should also be asked if he/she has been hospitalized, had any accidents, used any new medications, or changed concomitant medication routines (both prescription and OTC).

In addition to patient observations, AEs will be documented from any clinically relevant laboratory findings, physical examination findings, ECG changes, or other findings.

8.4 Adverse Event Reporting

The Investigator is responsible for reporting all AEs that are observed or reported after the first dose of study drug regardless of their relationship to study drug through until the end of the reporting periods defined in [Section 8.5](#). For patients who meet the criteria for Rapid Disease Progression at Month 9 and receive their last dose of study drug on Day 252 but remain on the study, AEs will be followed through Day 294. Following Day 294, only study-procedure-related AEs will be collected (e.g., skin-biopsy-related AE, venipuncture-related AE).

Any medical condition that is present when a patient is screened and does not deteriorate should not be reported as an AE. However, if it does deteriorate at any time during the study, it should be reported as an AE.

All AEs must be fully recorded in the site's source records and in the patients' CRF, whether or not they are considered to be drug-related. Each AE should be described in detail: onset time and date, description of event, severity, relationship to investigational product, action taken, and outcome (including time and date of resolution, if applicable).

Adverse events should be followed through until the end of the reporting periods defined in [Section 8.5](#) or until recovery to the normal state has been achieved, whichever occurs first. In the event of a patient not returning to the clinical unit, the outcome of this event will be recorded as lost at follow-up.

For patients who withdraw from the study early, ongoing AEs will be followed until resolution or 28 days from last dose, whichever occurs first.

8.5 Adverse Event Reporting Period

AEs and SAEs will be reported according to the following timeframes:

- For patients who complete the study, AEs will be assessed through the End of Study visit on Day 567 or the Follow-up Visit on Day 602 (depending whether or not the patient plans to roll over to the open-label extension study). All AEs that occur after the start of study drug administration on Day 0 must be reported in detail on the appropriate CRF page and followed to satisfactory resolution, or through the end of study visit on Day 567 or Day 602 (depending whether or not the patient plans to roll over to the open label extension study) after the last dose of study drug administration. SAEs will be followed through the end of study visit on Day 567 or Day 602 (depending whether or not the patient plans to roll over to the open label extension study), or until satisfactory resolution, or until the SAE is considered by the Investigator to be chronic or the patient is stable, whichever occurs first.
- For patients who meet the criteria for Rapid Disease Progression at Month 9 and receive their last dose of study drug on Day 252 but remain on the study, AEs will be followed through Day 294. Following Day 294, only study-procedure-related AEs will be collected (e.g., skin-biopsy-related AE, venipuncture-related AE). SAEs will be followed through Day 294, or until satisfactory resolution, or until the SAE is considered by the Investigator to be chronic or the patient is stable, whichever occurs first.

- For patients who withdraw from the study early, ongoing AEs will be followed until resolution or 28 days from last dose, whichever occurs first. SAEs will be followed through 28 days from the last dose of study drug, or until satisfactory resolution, or until the SAE is considered by the Investigator to be chronic or the patient is stable, whichever occurs first.

8.6 Assessment of Causality

Causal relationship assessment to drug treatments is required for purposes of reporting AEs. To promote consistency, the following guidelines should be taken into consideration along with good clinical and scientific judgment when determining the relationship of drug treatments to an AE:

Definitely Related: A clinical event, including laboratory test abnormality, occurring in a plausible time relationship to the medication administration, and which cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug should be clinically plausible.

Possibly Related: A clinical event, including laboratory test abnormality, with a reasonable time sequence to the medication administration, but which could also be explained by concurrent disease or other drugs or chemicals. Information on the drug withdrawal may be lacking or unclear.

Unlikely Related: A clinical event, including laboratory test abnormality, with little or no temporal relationship to medication administration, and which other drugs, chemicals, or underlying disease provide plausible explanations.

Not Related: A clinical event, including laboratory test abnormality, that has no temporal relationship to the medication or has more likely alternative etiology.

8.7 Assessment of Severity

Adverse events are to be graded according to the categories detailed below.

Mild: Mild events are those which are easily tolerated with no disruption of normal daily activity.

Moderate: Moderate events are those which cause sufficient discomfort to interfere with daily activity.

Severe: Severe events are those which incapacitate and prevent usual activity.

Changes in severity should be documented in the medical record to allow assessment of the duration of the event at each level of severity. Adverse events characterized as intermittent require documentation of the start and stop of each incidence. When changes in the severity of an AE occur more frequently than once a day, the maximum severity for the experience that day should be noted. If the severity category changes over a number of days, then those changes should be recorded separately (with distinct onset dates).

8.8 Action Taken for Adverse Event

Action taken in regards to study drug will be defined as:

- None;
- Infusion interrupted and restarted at a later time;
- Infusion stopped and was not restarted at a later time;
- Infusion cycle delayed.

8.9 Outcome of Adverse Event

Outcome will be defined as:

- Resolved (with or without sequelae);
- Ongoing;
- Lost to follow-up.

8.10 Coding of Adverse Events

The Medical Dictionary for Regulatory Activities (MedDRA[®]) will be used to code AEs.

8.11 Serious Adverse Event Reporting

An assessment of the seriousness of each AE will be made by the Investigator. Any AE and laboratory abnormality that meets the above seriousness criteria ([Section 8.2](#)) must be reported immediately to the CRO, always within 24 hours from the time that site personnel first learn of the event. All SAEs must be reported regardless of the relationship to study drug.

The initial report should include at least the following information:

- Patient's study number,
- Description and date of onset of the event,
- Criterion for serious, and
- Preliminary assignment of causality to study drug.

SAE reporting will be via electronic data capture (EDC).

To report the SAE, complete the SAE form electronically in the electronic data capture (EDC) system for the study. When the form is completed, [REDACTED] Safety personnel will be notified electronically and will retrieve the form. If the event meets serious criteria and it is not possible to access the EDC system, send an email to [REDACTED]

████████████████████ or call the ██████████ SAE hotline (phone number will be provided in the Study Manual), and fax the completed back-up paper SAE form to ██████████ (fax number will be provided in the Study Manual) within 24 hours of awareness. When the EDC system becomes available, the SAE information must be entered into the EDC system within 24 hours of the system becoming available. ██████████ Safety personnel will be available for SAE reporting on a 24-hour basis. Incoming reports will be reviewed during normal business hours.

Within 24 hours of receipt of follow-up information, the investigator must update the SAE form electronically in the EDC system for the study and submit any supporting documentation (e.g., patient discharge summary or autopsy reports) to ██████████ Safety personnel via fax or e-mail. If it is not possible to access the EDC system, refer to the procedures outlined above for initial reporting of SAEs.

Appropriate remedial measures should be taken by the Investigator using his/her best medical judgment to treat the SAE. These measures and the patient's response to these measures should be recorded. All SAEs, regardless of relationship to study drug, will be followed by the Investigator until satisfactory resolution or the Investigator deems the SAE to be chronic or stable. Clinical, laboratory, and diagnostic measures should be employed by the Investigator as needed to adequately determine the etiology of the event.

The Investigator will be responsible for reporting all SAEs to the local independent ethics committee (IEC) or Institutional Review Board (IRB) when required by national regulations.

Alnylam or its representative will be responsible for the reporting of all relevant events to the concerned regulatory authorities according to all applicable regulations.

In Europe, in accordance with the Directive 2001/20/EC, the Competent Authorities and the Ethics Committees in the concerned Member States will be notified of fatal and life-threatening Suspected Unexpected Serious Adverse Reactions (SUSARs) as soon as possible but no later than 7 calendar days after Alnylam or its representative has first knowledge of the minimum criteria for expedited reporting. Non-fatal and non-life-threatening SUSARs should be reported no later than 15 calendar days after Alnylam or its representative has first knowledge of them.

The Investigator may be informed by Alnylam or its representative of SAEs from other Investigators or clinical studies which may have relevance to this clinical study. These SAEs should also be reported promptly to the IEC/IRB that approved the study. All SAE reports should be transmitted to the IEC/IRB with a cover letter or transmittal form, and a copy of that transmittal should be maintained in the Investigator's files and forwarded to Alnylam as part of the TMF on study completion.

8.12 Pregnancy Reporting

A female patient with a positive pregnancy test at Screening is ineligible for this study. If a female patient is found to be pregnant during the course of the study or during the first month after receiving the last dose of study drug, the Investigator should report the pregnancy to the CRO within 24 hours of being notified of the pregnancy. Details of the

pregnancy will be recorded on the pregnancy reporting form. The patient should receive any necessary counseling regarding the risks of continuing the pregnancy and the possible effects on the fetus.

The pregnancy should be followed by the Investigator until completion. At the completion of the pregnancy, the Investigator will document the outcome of the pregnancy. If the outcome of the pregnancy meets the criteria for an SAE (i.e., postpartum complication, spontaneous abortion, stillbirth, neonatal death, or congenital anomaly), then the Investigator should follow the procedures for reporting an SAE outlined above.

Pregnancy occurring in the partner of a patient participating in the study should also be reported to the Investigator, who will then report this to the CRO and follow to outcome as described above.

; **STATISTICAL METHODS****9.1 Sample Size**

Approximately 200 patients will be enrolled in this study. An mNIS+7 progression rate (primary endpoint) in the placebo group of 24 ± 16 points in 18 months was estimated using natural history data from FAP patients. A sample of 154 patients provides 90% power for a 2-sided test with an 8.95 point (37.5%) mean difference between treatment groups in the primary endpoint at 2-sided $\alpha = 0.05$. Assuming a 25% random premature discontinuation rate (due to liver transplantation or other factors), the sample size for this study is approximately 200. Additional patients may be enrolled based on a recommendation to increase the sample size in the interim analysis.

9.2 Statistical Methodology

A full statistical analysis plan will be finalized prior to database lock.

9.2.1 Populations to be Analyzed

The following patient populations (i.e., analysis sets) may be evaluated and used for presentation of the data:

- Modified ITT (mITT) population: All patients who were randomized and received at least 1 dose of study drug.
- Per protocol (PP) population: All patients who completed the 18-month efficacy assessment visit and did not have any major protocol violations.
- Safety population: All patients who received at least 1 dose of study drug (analyzed as treated, not as randomized).

The primary population for efficacy analyses will be the mITT population; key efficacy results will also be analyzed secondarily for the PP population. For efficacy analyses, subjects will be grouped according to the treatment to which they were randomized. The primary population for safety analysis will be the safety population. Subjects will be grouped according to treatment received for summaries of safety.

9.2.2 Baseline Evaluations

Demographic and baseline disease characteristic data will be summarized. Data to be tabulated will include sex, age, and race, as well as disease-specific information.

9.2.3 Efficacy Analyses**9.2.3.1 Primary Efficacy Endpoint**

The primary analysis will compare change in mNIS+7 from baseline between treatment groups of the mITT population. An analysis of covariance (ANCOVA) model, with baseline mNIS+7 value and age as continuous covariates and genotype (V30M vs Non-V30M), prior tetramer stabilizer (tafamidis or diflunisal) use (Yes vs No), and treatment group (ALN-TTR02 vs Placebo) as factors will be employed to analyze the primary mNIS+7 endpoint. The mNIS+7 change from baseline will be assessed at 78 weeks. Primary endpoint data that are missing will be inferred using multiple imputation (MI)

(see [Section 9.2.10](#)). Analyses will be conducted using PROC MI and PROC MIANALYZE in SAS 9.2 (or later). The efficacy of ALN-TTR02 will have been established if the estimate of the parameter associated with treatment variable demonstrates that ALN-TTR02 improves mNIS+7 relative to placebo with a p-value (2-sided) less than or equal to 0.05, based on the replicated imputations from the MI methodology.

Sensitivity analyses will assess the robustness of the primary analysis in the mITT and PP populations for different methods of handling missing data, including mixed model repeated measures (MMRM), complete cases (CC), and last observation carried forward (LOCF).

9.2.3.2 Secondary Efficacy Endpoints

Secondary efficacy endpoints will be analyzed using methods similar to those employed for the primary analysis, e.g. ANCOVA with multiple imputations, as appropriate.

Type I error control for secondary endpoints will be achieved by a grouped hierarchical ordering procedure. Specifically, the 6 secondary endpoints will be analyzed in 33 families of 2 endpoints each. The families will be tested in the following prespecified hierarchy:

- Norfolk Quality of Life-Diabetic Neuropathy (Norfolk QOL-DN) and EuroQOL (EQ-5D) questionnaires
- Modified Body Mass Index (mBMI) and autonomic symptoms questionnaire (Composite Autonomic Symptom Score [COMPASS-31])
- NIS-weakness (NIS-W) and timed 10-meter walk test

Within each family, multiplicity will be controlled by the Benjamini-Hochberg procedure. If one or more comparisons are significant within a family, the next family in the hierarchy may be tested.

9.2.3.3 Exploratory Efficacy Endpoints

Continuous exploratory efficacy variables, including those closely related to the mNIS+7 primary endpoint, may be compared using methods similar to those employed for the primary analysis, e.g. ANCOVA with multiple imputations, as appropriate. Binary secondary endpoints will be assessed by the Cochran-Mantel-Haenszel test, stratified by the randomization stratification factors. Exploratory efficacy endpoints will be analyzed using methods similar to those employed for the primary analysis, e.g. ANCOVA with multiple imputations, as appropriate.

9.2.4 Safety Analyses

A summary of study drug exposure, including the durations of the infusions and doses, and the proportions of patients with modifications in the durations of infusions will be produced.

Adverse events will be summarized by MedDRA system organ class and preferred term. Separate tabulations will be produced for all treatment emergent AEs, treatment-related

AEs (those considered by the Investigator as at least possibly drug related), SAEs, and discontinuations due to AEs. By-patient listings will be provided for deaths, SAEs, and events leading to discontinuation of treatment.

Descriptive statistics will be provided for clinical laboratory data and vital signs data, presented as both actual values and changes from baseline relative to each on-study evaluation and to the last evaluation on study.

Descriptive statistics will be provided for ECG interval data and presented as both actual values and changes from baseline relative to each on-study evaluation and to the last evaluation on study. Details of any abnormalities will be included in patient listings.

9.2.5 Pharmacodynamics

Summary tables and graphical displays of observed values and changes from baseline in serum TTR will be used to assess the durability of suppression over the course of the study. Similar analyses will be performed for the secondary PD biomarkers (RBP and vitamin A).

9.2.6 Pharmacokinetics

Pharmacokinetic analyses will be conducted using non-compartmental and/or compartmental evaluation. Whenever possible, the PK parameters of siRNA, DLin-MC3-DMA, and PEG₂₀₀₀-DMG (lipid) in sparse plasma samples collected from all subjects. PK parameters will be calculated using a validated version of WinNonlin® Enterprise (Version 5.2 or higher) with NCA Model 200.

Population PK analyses, will be performed whenever possible, on available siRNA, DLin-MC3-DMA, and PEG₂₀₀₀-C-DMG from sparse plasma samples obtained from all subjects during the duration of the study using Phoenix NLME (Version 1.1 or later). Summary tables and figures and inferential statistics will be generated with Phoenix NLME (Version 1.1 or later) or similar software.

Pharmacokinetic/PD analysis will be conducted whenever possible, of sparse plasma samples collected during the study period. The analysis will include, but not limited to, the determination of the relationship between exposure to siRNA, DLin-MC3-DMA, and PEG₂₀₀₀-C-DMG and the extent of suppression of TTR, RBP, and vitamin A and their correlation will be evaluated. Correlation between TTR versus RBP and vitamin A will also be performed. The strength of the relationship will be assessed using statistical estimators. As part of the PK/PD analysis, additional PD baseline analysis on serum TTR, RBP, and vitamin A may be conducted to include the PD data obtained post-PD recovery period. The PD and PK/PD analysis will be performed with WinNonLin or Phoenix NLME (Version 1.1 or later) or similar software. The PD and PK/PD parameters summary tables and figures and inferential statistics will be generated and will not be limited to descriptive statistics.

9.2.7 Summary of Efficacy Assessments

Summary statistics of observed values and changes from baseline will be provided for the mNIS +7 composite score. Summaries will also be provided for the components of the composite score (e.g., the NIS weakness and reflex scores, the $\sum 5$ NCS, and QST

values). The NIS+7 score, including its components (e.g. full NIS, HRdb, VDT) will also be summarized.

Patient reported quality of life and disability will be assessed by summary statistics for the Norfolk QOL-DN, EQ-5D, and R-ODS. Summary statistics will be provided for observed values and changes from baseline. Patient reported autonomic neuropathy symptoms will be assessed by descriptive statistics for the COMPASS-31.

Descriptive statistics will also be provided for observed values and changes from baseline in motor function (10-meter walk test and test of grip strength), nutritional status (mBMI), sensory and autonomic innervation (IENFD and SGNFD), and ambulation (FAP stage and PND score). Descriptive statistics will also be provided for proportions of patients in each group that meet the rapid progression definition at 9 months and those that meet the responder criterion at 18 months.

9.2.8 Other Assessments

The observed values and changes from Baseline in burden of disease and healthcare utilization will be evaluated and summarized using descriptive statistics. Data on suicidality will be summarized by treatment group using descriptive statistics.

9.2.9 Interim Analysis

Because the sample size estimates are based on limited data for variance and disease progression, it is intended that an interim analysis will be conducted by an independent committee when approximately 50% of patients have completed their 9-month mNIS+7 assessment. This interim analysis will be blinded and will only estimate the overall variance observed in the primary endpoint. Since the interim analysis examines only the pooled variance of all patients in a blinded fashion, it does not require an adjustment to the overall alpha level for the test of the primary endpoint. Based on the results of the interim analysis, the committee can recommend either increasing the study size or making no adjustment to the sample size. Details regarding the Interim Analysis Committee are provided in [Section 10.3.3](#).

9.2.10 Missing Data

Subjects that prematurely discontinue from the study will have their 78-week mNIS+7 change from baseline value imputed using a stepwise regression approach for identification of explanatory variables (e.g., demographics, stratifying variables, and baseline/9-month mNIS+7 data when available); treatment assignment will not be included in the imputation. A minimum of 100 imputed datasets will then be analyzed as complete cases via the ANCOVA model specified for the primary analysis, and then combined to produce inferential results. Further details on imputations and sensitivity analyses will be included in the SAP that will be finalized before database lock.

32 STUDY MANAGEMENT

The Investigator is accountable for the conduct of the study. If any responsibilities are delegated, the Investigator should maintain a list of appropriately qualified staff to whom he/she has delegated specified significant trial related duties.

10.1 Data Handling and Quality Assurance

10.1.1 Case Report Forms

The Investigator and designees agree to maintain accurate CRFs and source documentation as part of these case histories. Source documents are the originals of any documents used by the Investigator or hospital/institution that allow verification of the existence of the patient and substantiate the integrity of the data collected during the trial.

Anylam will supply CRFs for each patient. Case report forms must be completed only by persons designated by the Investigator. Corrections must be made so as not to obliterate original data and must be identified and dated by the person who made the correction. All data entered into the CRF must also be available in the source documents. The Investigator will allow designated Anylam representatives and regulatory bodies to have direct access to the source documents to verify the data reported in the CRFs.

Each completed CRF must be reviewed and signed by the Investigator or designee in a timely manner. The completed CRF will be the records maintained by Anylam. A copy of the CRF will remain in the Investigator's files.

10.1.2 Monitoring

The clinical monitor, as a representative of Anylam, has an obligation to follow the study closely. In doing so, the monitor will visit the Investigator and site periodically as well as maintain frequent telephone and written contact. The monitor will maintain current personal knowledge of the study through observation, review of study records and source documentation and discussion of the conduct of the study with the Investigator and staff.

All aspects of the study will be carefully monitored by Anylam or its designee for compliance with applicable government regulations in respect to Good Clinical Practice (GCP) and current standard operating procedures.

10.1.3 Inspections

The Investigator will permit trial-related monitoring, audits and review by the IEC or IRB and/or Regulatory Authorities, providing direct access to source data/documents. The study may be subject to audit by Anylam or its representatives or by regulatory authorities. If such an audit occurs, the Investigator must agree to allow access to the required patient records. In the event of an audit, the Investigator agrees to allow Anylam, representatives from Anylam, or regulatory agencies access to all study records.

10.2 Regulatory Guidelines

This study will be performed in accordance with the clinical trial agreement, the protocol, all applicable government laws, regulations, and guidances where the study is being

conducted including policies with foundations in the World Health Organization (WHO) Declaration of Helsinki, the International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use, the Health Insurance Portability and Accountability Act of 1996 (“HIPAA”), and all other applicable medical privacy laws and regulations.

10.2.1 Institutional Review Board/Independent Ethics Committee

National regulations and ICH require that approval be obtained from an IRB or an IEC prior to participation of patients in research studies. Prior to the study onset, the protocol, any protocol amendments, ICFs, advertisements to be used for patient recruitment, and any other written information regarding this study to be provided to a patient or patient’s legal guardian must be approved by the IRB or IEC.

All IRB and IEC approvals must be dated and contain IRB/IEC Chairman or designee authorization and must identify the IRB/IEC (e.g., name and address), the clinical protocol by title and/or protocol number, and the date of approval or favorable opinion was granted for the clinical research.

No drug will be released to the site to dose a patient until written IRB/IEC authorization has been received by Alnylam or designee.

The Investigator is responsible for obtaining continuing review of the clinical research at least annually or more often if specified by the IRB or IEC. The Investigator must supply Alnylam with written documentation of the approval of the continued clinical research.

The Investigator will make all attempts to ensure that the IRB or IEC is constituted and operates in accordance with Federal and ICH GCP and any local regulations.

10.2.2 Regulatory Authorities

Regulatory authorities will receive the protocol, amendments, reports on SAEs, and the Integrated Clinical Trial Report according to national and any local regulations.

10.2.3 Modification of the Protocol

Major changes in this research activity, except those to remove an apparent immediate hazard to the patient, must be reviewed and approved by Alnylam and the IRB or IEC that approved the study. Amendments to the protocol must be submitted in writing to the Investigator’s IRB or IEC and the Regulatory Authority for approval prior to patients being enrolled under the amended protocol.

10.2.4 Informed Consent Form

Written informed consent in compliance with 21 Code of Federal Regulations (CFR) § 50 and ICH will be obtained from each patient prior to undergoing any protocol-specific tests or procedures that are not part of routine care.

Alnylam or the CRO designee will provide an ICF template to the Investigator for use in developing a site-specific ICF. Prior to submission of the site-specific ICF to the IRB or IEC, the site-specific ICF must be reviewed and approved by Alnylam or designee. Any changes requested by the IRB or IEC must also be agreed upon. The final IRB/IEC

approved ICF must be provided to Alnylam. Revisions to the ICF required during the study must be agreed upon, and a copy of the revised ICF provided to Alnylam.

At the time of recruitment, each prospective patient (or legal guardian) will be given a full explanation of the study and be allowed to read the ICF. Once the Investigator is assured that the patient/legal guardian understands the commitments of participating in the study, the patient/legal guardian will be asked to sign and date the ICF. A copy of the fully signed and dated ICF will be given to the patient. The original will be maintained in the patient's medical record at the site. All active patients will sign an updated ICF if revisions are made to the ICF during the course of the study.

10.2.5 Study Reporting Requirements

The Investigator will submit reports of SAEs as outlined in this protocol. In addition, the Investigator agrees to submit progress reports to his/her IRB or IEC per their local reporting requirements, or at least annually and at the conclusion of the study. The reports will be made available to Alnylam or designee.

Deviations from the protocol necessary to protect patient safety should be reported to the CRO within 24 hours of knowledge of the event.

Any communications from regulatory agencies in regard to inspections, other studies that impact this protocol or the qualifications of study personnel should be promptly reported to the CRO.

10.2.6 Financial Disclosure Reporting Obligations

Each Investigator (including principal and any sub-investigators) directly involved in the treatment or evaluation of study patients is required to provide financial disclosure information according to all applicable legal requirements. In addition, Investigators must commit to promptly updating this information if any relevant changes occur during the study and for a period of one year after the completion of the study.

10.3 Study Committees

10.3.1 Data Monitoring Committee

A Data Monitoring Committee (DMC) will be involved in the conduct of this study. The DMC has the responsibility for monitoring the progress of the clinical study and the safety of the study participants. The DMC will perform periodic reviews of data and study conduct during the course of the clinical trial, as defined in the DMC Charter for this clinical trial. The membership of the DMC and reporting structure are defined in the DMC Charter.

10.3.2 Clinical Adjudication Committee

An independent clinical adjudication committee will perform a blinded adjudication of the results of those patients who have clinical evidence of rapid disease progression (defined as ≥ 24 point increase in mNIS+7 from baseline [based on an average of 2 measurements] and FAP stage progression relative to baseline) at 9 months. Each review will follow procedures detailed in the committee's charter.

10.3.3 Interim Analysis Committee

An Interim Analysis Committee (IAC), which will include at least 1 statistician (blinded) comprised of 2 statisticians (1 blinded and 1 unblinded) independent of the conduct of the study, will be responsible for the implementation of the interim analysis and for the calculations and recommendations surrounding whether an adjustment to the sample size is warranted, and if so, the appropriate adjustment, based on the study's primary endpoint data from the interim analysis. The Sponsor, the CROs, and all other parties conducting the study will remain blinded to all interim analyses until study completion. The IAC will follow the procedure outlined in the committee's charter.

10.4 Ancillary Research

Research ancillary to this main protocol may not be performed by individual study sites without prior discussion and approval by Alnylam.

10.5 Study Record Retention

Essential documents should be retained for the period of time required by applicable local law. The essential documents include the signed and dated final protocol, signed and dated amendments(s), if applicable, signed and dated Curriculum Vitae (CVs) of the Investigators, copies of the completed CRFs, signed ICFs, IEC/IRB approval and all related correspondence, financial agreements, regulatory approval, drug accountability, study correspondence, and patient identification codes. Records will not be destroyed without informing Alnylam in writing and giving Alnylam the opportunity to store the records for a longer period of time at Alnylam's expense.

The International Conference on Harmonization requires that patient identification codes be retained for at least 15 years after the completion or discontinuation of the study.

10.6 Discontinuation of the Study by Alnylam

Alnylam reserves the right to discontinue the study for clinical or administrative reasons at any time. If the site does not recruit at a reasonable rate, the study may be discontinued at that site. Should the study be terminated and/or the site closed for whatever reason, all documentation and study drug pertaining to the study must be returned to Alnylam or its representative, and the Investigators, IEC/IRB and Regulatory Authorities will be promptly informed of the termination and the reason for the decision. The Investigator should promptly inform the patients and assure appropriate therapy and follow-up.

10.7 Study Documentation

Prior to beginning the study, the Investigator will be asked to comply with ICH E6 and 21 CFR by providing at least the following essential documents:

- An original signed Investigator agreement page of the protocol and any amendments;
- An IEC/IRB and Alnylam approved ICF;
- IEC/IRB approval of the protocol, and any amendments;
- Completed and signed FDA form 1572;

- Curriculum vitae for the Investigator signed and dated by the Investigator indicating that it is current;
- Financial disclosure information (if applicable);
- Other documents which the Investigator should provide before study start include:
 - Curriculum vitae for all Sub-investigators; these should be signed and dated by the Sub-investigators indicating that they are current;
 - Financial disclosure information for all Sub-investigators (if applicable);
 - Advertisements for patient recruitment and any other written information to be given to patients, family members or legal guardians and IEC/IRB approval of any advertisements and any other written information;
 - IEC/IRB composition: If the Investigator or any of the Sub-investigators is a member of the IEC/IRB, assurance that he/she refrained from voting should be provided;
 - Laboratory accreditation and reference ranges for any laboratory values for local laboratories.

10.8 Confidentiality

The Investigator must ensure that the patients' anonymity will be maintained. On the CRFs or other documents submitted to Alnylam or designees, patients should not be identified by their names, but by the assigned patient number and initials. If patient names are included on copies of documents submitted to Alnylam or designees, the names (except for initials) will be obliterated and the assigned patient number added to the document. Documents not for submission to Alnylam (e.g. signed ICFs) should be maintained by the Investigator in strict confidence.

Following the principles of the Good Clinical Practice, if local regulations specify a patient's number and initials will be used to identify the patient on their study records. Laboratory samples may be labeled with an independent numbering code, and the label will not contain any other personal identification information. The numbering code associated with these labels will be held by the study CRO and Alnylam, thereby allowing no unwarranted access to the information. When reporting results for interim safety assessment, the interim analysis, and at the end of the study, the code will be shared per standard operating procedures with the responsible member of the Biostatistical and Data Management Departments of the CRO. The numbering code will also be held for samples in storage until marketing approval of ALN-TTR02 in the countries where this study was conducted, or until clinical development of ALN-TTR02 is halted. Throughout sample collection, storage (limited, staff only access area containing locked sample storage, and limited access sample tracking) and processing, the samples will only be handled by appropriate personnel per the laboratory's standard operating procedures.

The Investigator must treat all of the information related to the study and the compiled data as confidential, whose use is for the purpose of conducting the study. Alnylam must approve any transfer of information not directly involved in the study.

10.9 Publications/Reports

Following completion of the study, the data may be considered for publication in a scientific journal or for reporting at a scientific meeting. A copy of the manuscript must be provided and confirmed received at Alnylam at least 30 days prior to its submission.

No submission of a manuscript may be made until the results from all of the study sites have been received and analyzed by Alnylam, or the study has been terminated at all centers. A separate, individual publication of the results of the study will be delayed until initial publication of the results of the multicenter study, or a decision not to publish is made. If an initial draft is not produced within 18 months of completion of the study at all centers, or the timeframe for publication is not satisfactory, the Investigator may disclose the results after providing a copy and Alnylam confirms receipt of the manuscript 30 days prior to submission.

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34 APPENDICES

Appendix 1: Karnofsky Scale

| | | |
|---|-----|---|
| Able to carry on normal activity and to work; no special care needed. | 100 | Normal no complaints; no evidence of disease. |
| | 90 | Able to carry on normal activity; minor signs or symptoms of disease. |
| | 80 | Normal activity with effort; some signs or symptoms of disease. |
| Unable to work; able to live at home and care for most personal needs; varying amount of assistance needed. | 70 | Cares for self; unable to carry on normal activity or to do active work. |
| | 60 | Requires occasional assistance, but is able to care for most of his personal needs. |
| | 50 | Requires considerable assistance and frequent medical care. |
| Unable to care for self; requires equivalent of institutional or hospital care; disease may be progressing rapidly. | 40 | Disabled; requires special care and assistance. |
| | 30 | Severely disabled; hospital admission is indicated although death not imminent. |
| | 20 | Very sick; hospital admission necessary; active supportive treatment necessary. |
| | 10 | Moribund; fatal processes progressing rapidly. |
| | 0 | Dead |

Appendix 2: New York Heart Association Classification of Heart Failure

| Class | Symptomatology |
|--------------|---|
| I | No symptoms. Ordinary physical activity such as walking and climbing stairs does not cause fatigue or dyspnea. |
| II | Symptoms with ordinary physical activity. Walking or climbing stairs rapidly, walking uphill, walking or stair climbing after meals, in cold weather, in wind or when under emotional stress causes undue fatigue or dyspnea. |
| III | Symptoms with less than ordinary physical activity. Walking one to two blocks on the level and climbing more than one flight of stairs in normal conditions causes undue fatigue or dyspnea. |
| IV | Symptoms at rest. Inability to carry on any physical activity without fatigue or dyspnea. |

Appendix 3: Categorization of Infusion-Related Reactions

Signs and symptoms of an infusion-related reaction (IRR) usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Signs/symptoms may include: allergic reaction/hypersensitivity (including drug fever), arthralgia (joint pain), bronchospasm, cough, dizziness, dyspnea (shortness of breath), fatigue (asthenia, lethargy, malaise), headache, hypertension, hypotension, myalgia (muscle pain), nausea, pruritus/itching, rash/desquamation, rigors/chills, sweating (diaphoresis), tachycardia, urticaria (hives, welts, wheals), vomiting.

Categorization of IRRs is as follows:

| Categorization | Description |
|-----------------------|---|
| Mild | Mild reaction: infusion may be continued; if intervention is indicated it is minimal and additional treatment (other than paracetamol for delayed reactions) is not required. |
| Moderate | Moderate reaction: requires treatment including more intensive therapy (e.g., IV fluids, nonsteroidal anti-inflammatory [NSAIDs]) in addition to infusion interruption but responds promptly to medication. Treatment is indicated for ≤ 24 hours. |
| Severe | More than moderate reaction: not rapidly responsive to medication or to interruption of infusion; and/ or prolonged (treatment is indicated for > 24 hours); recurrence of severe symptoms following initial improvement. |

Appendix 4: Neuropathy Scores and Their Components

| Assessment Tool | Total Points | Components (points) |
|-----------------|--------------|--|
| NIS+7 | 270 | <ul style="list-style-type: none"> • Neurologic exam of lower limbs, upper limbs and cranial nerves (NIS^a) <ul style="list-style-type: none"> • Weakness (192) • Sensation (32) • Reflexes (20) • Nerve conduction studies $\sum 5$ (18.6)^a <ul style="list-style-type: none"> • Sural SNAP, tibial motor n. distal latency, peroneal SNAP/motor n. conduction velocity/motor n. distal latency • Vibration detection threshold (3.7) • Heart rate response to deep breathing (3.7) |
| Modified NIS+7 | 304 | <ul style="list-style-type: none"> • Neurologic exam of lower limbs, upper limbs and cranial nerves (mNIS^a) <ul style="list-style-type: none"> • Weakness (192) • Reflexes (20) • Nerve conduction studies $\sum 5$ (10)^a <ul style="list-style-type: none"> • Ulnar CMAP and SNAP, sural SNAP, tibial CMAP, peroneal CMAP • Quantitative sensory testing: QST-BSA_{TP+HP5} (80) • Postural blood pressure (2) |

a Components that are shared between the mNIS+7 and NIS+7 (including NIS and NCS) will be performed once at each assessment.

Appendix 5: Polyneuropathy Disability Score

| Stage | Description |
|--------------|---|
| 0 | No symptoms |
| I | Sensory disturbances but preserved walking capability |
| II | Impaired walking capacity but ability to walk without a stick or crutches |
| IIIA | Walking with the help of one stick or crutch. |
| IIIB | Walking with the help of two sticks or crutches. |
| IV | Confined to a wheelchair or bedridden. |

Appendix 6: Familial Amyloidotic Polyneuropathy Stage

| Stage | Description |
|--------------|---|
| 0 | No symptoms |
| I | Unimpaired ambulation; mostly mild sensory, motor, and autonomic neuropathy in the lower limbs |
| II | Assistance with ambulation required, mostly moderate impairment progression to the lower limbs, upper limbs, and trunk. |
| III | Wheelchair-bound or bedridden; severe sensory, motor, and autonomic involvement of all limbs. |



**CLINICAL STUDY PROTOCOL
ALN-TTR02-004**

APOLLO: A Phase 3 Multicenter, Multinational, Randomized, Double-blind, Placebo-controlled Study to Evaluate the Efficacy and Safety of Patisiran (ALN-TTR02) in Transthyretin (TTR)-Mediated Polyneuropathy (Familial Amyloidotic Polyneuropathy-FAP)

| | |
|------------------|--|
| Protocol Version | Version 6.0 (Incorporating Amendment 5) |
| Protocol Date | 08 September 2015 |
| IND Number | 117395 |
| EudraCT Number | 2013-002987-17 |
| Sponsor: | Alnylam Pharmaceuticals, Inc. 300 Third Street Cambridge, MA 02142 USA |
| Sponsor Contact: | [REDACTED] |

CONFIDENTIAL

The concepts and information contained in this document or generated during the study are considered proprietary and may not be disclosed in whole or in part without expressed written authorization of Alnylam Pharmaceuticals, Inc.

The study will be completed according to guidelines of Good Clinical Practice. Compliance with this practice provides public assurance that the rights, safety, and well-being of study patients are protected consistent with the principles that have their origin in the Declaration of Helsinki.

AUTHORIZED SIGNATORIES

INVESTIGATOR'S STATEMENT: I agree to conduct this study as outlined in the protocol and in accordance with the guidelines and all applicable government regulations. I have read all parts of the protocol.

Principal Investigator

Signature _____ Date _____

Name (print) _____

Sponsor

Signature  Date 08 Sept 2015

Name (print) 
 

Version History:

| Version Number | Date | Comment |
|----------------|-------------------|----------------------------------|
| 1.0 (Original) | 15 August 2013 | Initial Release |
| 2.0 | 18 October 2013 | Incorporating Global Amendment 1 |
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PROTOCOL SYNOPSIS

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| Protocol Title | APOLLO: A Phase 3 Multicenter, Multinational, Randomized, Double-blind, Placebo-controlled Study to Evaluate the Efficacy and Safety of Patisiran (ALN-TTR02) in Transthyretin (TTR)-Mediated Polyneuropathy (Familial Amyloidotic Polyneuropathy-FAP) |
| Indication | Treatment of transthyretin-mediated amyloidosis (ATTR) in patients with symptomatic polyneuropathy |
| Protocol Number | ALN-TTR02-004 |
| Phase of Development | 3 |
| Design | <p>This is a multicenter, multinational, randomized, double-blind study comparing patisiran (ALN-TTR02) to placebo in ATTR patients with symptomatic Familial Amyloidotic Polyneuropathy (FAP).</p> <p>Consented eligible patients will be randomized to receive either 0.3 mg/kg patisiran or placebo in a 2:1 ratio (patisiran to placebo) in a blinded manner. Treatment arms will be balanced at entry for Neuropathy Impairment Score (NIS; 5-49 vs 50-130), early onset V30M (<50 years of age at onset) vs. all other mutations (including late onset V30M), and previous tetramer stabilizer use (tafamidis or diflunisal) vs no previous tetramer stabilizer use. Patients will receive patisiran or placebo once every 21 days for 78 weeks.</p> <p>Patients will have baseline efficacy assessments and efficacy assessments at 9 and 18 months. The study personnel performing these assessments will be blinded to the results of any previous assessments (e.g., Screening/Baseline, Baseline, or 9-month assessments).</p> <p>At the 9-month time point, if the clinical adjudication committee determines that a patient is exhibiting rapid disease progression (defined as ≥ 24 point increase in modified NIS+7 (mNIS+7) from baseline [based on an average of 2 measurements] and FAP stage progression relative to baseline), the patient's treating physician will provide the patient with the option of discontinuing study drug and receiving local standard of care treatment for FAP. Patients who discontinue study drug will remain on study, following a modified schedule of visits, through completion of the 18-month efficacy assessments (blinding will be maintained throughout).</p> |

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| | <p>Patients who complete the 18-month efficacy assessments can elect to participate in an extension study in which patients would receive open-label administration of 0.3 mg/kg patisiran once every 21 days.</p> <p>A Data Monitoring Committee (DMC) will be implemented for the study and will operate under a prespecified charter.</p> |
| Study Sites | This study will be conducted at multiple sites worldwide. |
| Investigational Drug | Patisiran (comprising a small interfering ribonucleic acid [siRNA] targeting mutant and wild-type TTR mRNA, in a lipid nanoparticle formulation for intravenous [IV] administration) |
| Dosage, Route of Administration and Duration of Treatment of Investigational Drug | <p>Patients randomized to the active treatment group will receive 0.3 mg/kg patisiran once every 21 (\pm3) days administered as an IV infusion over 70 minutes (approximately 1 mL/minute for the first 15 minutes followed by approximately 3 mL/minute for the remainder of the infusion) by a controlled infusion device.</p> <p>Prior to each dose of study drug, patients will receive the following premedications at least 60 minutes prior to the infusion:</p> <ul style="list-style-type: none"> • Intravenous dexamethasone (10 mg) or equivalent; • Oral paracetamol/acetaminophen (500 mg) or equivalent; • Intravenous H2 blocker (e.g., ranitidine 50 mg, famotidine 20 mg, or equivalent other H2 blocker dose); and • Intravenous H1 blocker: diphenhydramine 50 mg (or equivalent other IV H1 blocker available at the study site). Hydroxyzine or fexofenadine 25 mg per oral suspension (PO, orally) or cetirizine 10 mg PO may be substituted for any patient who does not tolerate intravenous diphenhydramine or other intravenous H1 blocker. |
| Control Drug | Placebo (normal saline 0.9% for IV administration) |
| Dosage, Route of Administration and Duration of Treatment of Control Drug | <p>Patients randomized to placebo will receive IV normal saline (0.9%) using the same dosing schedule and infusion rate as the active treatment group.</p> <p>Placebo patients will also receive the same premedication regimen as the active treatment group.</p> |
| Time on Study | The duration of patient participation in this study is approximately 21 months (inclusive of a 42-day screening window and up to a 56-day post last dose study visit). |
| Primary Objective | The primary objective of the study is to determine the efficacy of patisiran by evaluating the difference between the patisiran and placebo groups in the change from baseline of mNIS+7 score at 18 months. |

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| <p>Secondary Objectives</p> | <p>The secondary objectives of the study are to determine the effect of patisiran on various clinical parameters by assessing the difference between patisiran and placebo in the change from baseline in the following measurements at 18 months:</p> <ul style="list-style-type: none"> • Norfolk Quality of Life-Diabetic Neuropathy (Norfolk QOL-DN) questionnaire; • NIS-weakness (NIS-W) score; • Modified body mass index (mBMI); • Timed 10-meter walk test; • Autonomic symptoms questionnaire (Composite Autonomic Symptom Score [COMPASS-31]). |
| <p>Exploratory Objectives:</p> | <p>The exploratory objectives of the study are:</p> <ul style="list-style-type: none"> • To determine the difference between the patisiran and placebo groups in the change from baseline in the following measurements at 18 months: <ul style="list-style-type: none"> • NIS+7 score; • Grip strength; • EuroQOL (EQ-5D) questionnaire; • Level of disability (Rasch-built Overall Disability Scale [R-ODS]); • Large vs small nerve fiber function including nerve conduction studies (NCS) 5 attributes ($\Sigma 5$), quantitative sensory testing by body surface area including touch pressure and heat pain (QST), vibration detection threshold (VDT), heart rate response to deep breathing (HRdb), postural blood pressure; • Pathologic evaluation of sensory and autonomic innervation through voluntary skin punch biopsies and analysis of intraepidermal nerve fiber density (IENFD) and sweat gland nerve fiber density (SGNFD); • Assessment of ambulation through FAP stage and Polyneuropathy Disability (PND) score; • Cardiac assessment through echocardiogram, troponin I, and N-terminal prohormone of B-type natriuretic peptide (NT-proBNP) levels; • Pharmacodynamic (PD) biomarkers (TTR, retinol binding protein [RBP], vitamin A); • To compare the proportion of patients in the patisiran and placebo groups who meet the pre-defined criterion for rapid disease progression (defined as ≥ 24 point increase in mNIS+7 from baseline [based on an average of |

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| | <p>2 measurements] <u>and</u> FAP stage progression relative to baseline) at 9 months.</p> |
| <p>Sample Size:</p> | <p>Approximately 200 patients will be enrolled in this study. Of those 200 patients, no more than 40 patients will have a NIS range of 101 to 130. An mNIS+7 progression rate (primary endpoint) in the placebo group of 24 ± 16 points in 18 months was estimated using natural history data from FAP patients. A sample of 154 patients provides 90% power for a 2-sided test with an 8.95 point (37.5%) mean difference between treatment groups in the primary endpoint at 2-sided alpha = 0.05. Assuming a 25% random premature discontinuation rate (due to liver transplantation or other factors), the sample size for this study is approximately 200. Additional patients may be enrolled based on a recommendation to increase the sample size in the interim analysis.</p> |
| <p>Inclusion and Exclusion Criteria:</p> | <p>To be enrolled in the study, each patient must meet the following criteria at the Screening visit, except where specified:</p> <ol style="list-style-type: none"> 1. Male or female of 18 to 85 years of age (inclusive); 2. Have a diagnosis of FAP with documented TTR mutation; 3. Have an NIS of 5 to 130 (inclusive) and a PND score of $\leq 3b$ (Note: This criterion must be met at the Screening/Baseline visit); 4. Have an NCS sum of the sural sensory nerve action potential (SNAP), tibial compound muscle action potential (CMAP), ulnar SNAP, ulnar CMAP, and peroneal CMAP of ≥ 2 points; (Note: This criterion must be met at the Screening/Baseline visit); 5. Have a Karnofsky performance status of $\geq 60\%$; 6. Have an absolute neutrophil count (ANC) ≥ 1500 cells/mm³, and a platelet count $\geq 50,000$ cells/mm³; 7. Have aspartate transaminase (AST) and alanine transaminase (ALT) levels $\leq 2.5 \times$ the upper limit of normal (ULN), total bilirubin within normal limits, international normalized ratio (INR) ≤ 2.0 (patients on anticoagulant therapy with an INR of ≤ 3.5 will be allowed). Patients with total bilirubin $\leq 2 \times$ ULN are eligible if the elevation is secondary to documented Gilbert's syndrome (elevation of unconjugated bilirubin with normal conjugated bilirubin) and the patient has ALT and AST levels within normal ranges; 8. Have a serum creatinine $\leq 2 \times$ ULN; |

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| | <p>9. No active infection with hepatitis B or hepatitis C by serology;</p> <p>10. Women of child-bearing potential must have a negative pregnancy test, cannot be breastfeeding, and must be using 2 highly effective methods of contraception prior to screening, throughout study participation, and for 75 days after the last dose of study drug. Highly effective methods of birth control are defined in Section 4.7;</p> <p>11. Males with partners of child-bearing potential must agree to use 1 barrier method (e.g., condom) and 1 additional method (e.g., spermicide) of contraception throughout study participation and for 75 days after the last dose of study drug; males must also abstain from sperm donation after the first dose of study drug through study participation and for 75 days after the last dose of study drug;</p> <p>12. Must be willing and able to comply with protocol-required visit schedule and visit requirements and provide written informed consent.</p> <p>A patient will be excluded if they meet any of the following criteria at the time of the Screening visit:</p> <ol style="list-style-type: none"> 1. Had a prior liver transplant or is planning to undergo liver transplant during the study period; 2. Has other known causes of sensorimotor or autonomic neuropathy (e.g., autoimmune disease, monoclonal gammopathy); 3. Has known primary amyloidosis or leptomeningeal amyloidosis; 4. Has known type I diabetes; 5. Has had type II diabetes mellitus for ≥ 5 years; 6. Has vitamin B12 levels below the lower limit of normal (LLN); 7. Has untreated hypo- or hyperthyroidism; 8. Has had a major surgery within the past 3 months or has a major surgery planned during any point of the study period; 9. Has known human immunodeficiency virus (HIV) infection; 10. Has an active infection requiring systemic antiviral or antimicrobial therapy that will not be completed prior to the first dose of study drug administration; |
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| | <ol style="list-style-type: none"> 11. Had a malignancy within 2 years, except for basal or squamous cell carcinoma of the skin or carcinoma in situ of the cervix that has been successfully treated; 12. Has a New York Heart Association heart failure classification >2; 13. Had acute coronary syndrome within the past 3 months; 14. Has uncontrolled cardiac arrhythmia or unstable angina; 15. Has a known history of alcohol abuse within the past 2 years or daily heavy alcohol consumption (females: more than 14 units of alcohol per week; males: more than 21 units of alcohol per week [unit: 1 glass of wine [125 mL] = 1 measure of spirits = ½ pint of beer]); 16. Received an investigational agent or device within 30 days of anticipated study drug administration or 5 half-lives of the investigational drug, whichever is longer; 17. Participated in a clinical trial with antisense oligonucleotide, must have completed a 3-month wash-out prior to start of the study drug administration in this study; 18. Is currently taking tafamidis, doxycycline, or tauroursodeoxycholic acid; if previously on any of these agents, must have completed a 14-day wash-out prior to start of study drug administration in this study; 19. Is currently taking diflunisal; if previously on this agent, must have at least a 3-day wash-out prior to start of study drug administration in this study; 20. Had a prior severe reaction to a liposomal product or a known hypersensitivity to oligonucleotides or any component of patisiran; 21. Is unable to take the required premedications; 22. Anticipated survival is less than 2 years, in the opinion of the Investigator; 23. Is considered unfit for the study by the Investigator; 24. Is under legal protection (defined as “any person who becomes incapable of protecting his/her interests due to a medically diagnosed impairment of his/her mental faculties that may limit or prevent the expression of his/her will”). |
| <p>Efficacy Assessments</p> | <p>Efficacy parameters will include the following (baseline evaluations will be conducted as well as evaluations at 9 and 18 months):</p> |

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| | <ul style="list-style-type: none"> • Neurologic impairment will be assessed using the mNIS+7 composite score. The mNIS+7 includes the modified NIS (weakness and reflexes), NCS Σ5, QST, as well as autonomic assessment through postural blood pressure; • Patient-reported QOL will be evaluated using the Norfolk QOL-DN and the EQ-5D. Disability will be reported by patients using the R-ODS; • Autonomic symptoms will be assessed using the COMPASS-31; • Motor function assessments to be evaluated include NIS-W, timed 10-meter walk test, and grip strength test; • PND score and FAP stage; • Nutritional status will be assessed using mBMI; • Pathologic evaluation of sensory and autonomic innervation will be evaluated by IENFD analysis and quantitation of dermal SGNFD via tandem 3 mm skin punch biopsies taken from the leg; • Neurologic impairment will also be assessed by NIS+7 (including full NIS, NCS, VDT, and HRdb); • Cardiac structure and function will be assessed through echocardiograms as well as measurement of serum levels of NT-proBNP and troponin I. |
| <p>Pharmacodynamic Assessments</p> | <p>Pharmacodynamic markers assessed serially will include serum TTR, vitamin A, and RBP. Additional blood samples will be collected for exploratory biomarkers related to FAP.</p> |
| <p>Pharmacokinetic Assessments</p> | <p>The plasma pharmacokinetic (PK) evaluation will include, whenever possible, plasma-concentration time profiles for siRNA and the novel lipid components in patisiran: DLin-MC3-DMA and polyethylene glycol (PEG)₂₀₀₀-C-DMG. The siRNA, DLin-MC3-DMA, and PEG₂₀₀₀-C-DMG concentrations will be determined for all patients at time points specified in Table 1-1, Table 1-2, and Table 1-3.</p> <p>Urine will be collected with void volume recorded for all patients at time points specified in Table 1-1, Table 1-2, and Table 1-3 and to determine renal clearance (CL_R) of siRNA and 4-dimethylaminodibutyric acid (the metabolite of DLin-MC3-DMA) after dosing with study drug.</p> |

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| <p>Safety Assessments</p> | <p>Safety will be assessed throughout the study by collecting adverse events (AEs; including serious adverse events [SAEs]); clinical laboratory tests, including hematology, clinical chemistry (including liver function tests), thyroid function parameters, and urinalysis; measurement of anti-drug antibodies; electrocardiograms; vital signs; physical examination findings; and ophthalmology examinations.</p> |
| <p>Other Assessments</p> | <p>Disease burden and healthcare utilization will be assessed using a patient-reported pharmacoeconomics questionnaire. The investigator will periodically assess mental status as it relates to suicidal ideation and behavior by using the Columbia–Suicide Severity Rating Scale (C-SSRS) questionnaire.</p> |
| <p>Follow-Up</p> | <p>Patients who complete the 18-month efficacy assessments can elect to participate in an extension study in which patients would receive open-label administration of 0.3 mg/kg patisiran once every 21 days. Eligible patients who elect to participate will return for a follow-up visit 21 days after last dose of study drug.</p> <p>If the patient decides not to participate in the extension study, the patient will complete 2 follow-up visits at 21 and 56 days after the last dose of study drug. Each follow-up visit includes safety assessments and the 21-day follow-up also includes PD measurements.</p> |
| <p>Statistical Methods</p> | <p>A full statistical analysis plan (SAP) will be finalized prior to database lock.</p> <p>The primary analysis will compare patients administered patisiran versus those administered placebo in the modified intent-to-treat (mITT) population. Specifically, patients who are randomized and receive at least 1 dose of study drug will be included in this analysis. The primary analysis will compare change in mNIS+7 from baseline at 18 months between patisiran and placebo groups, adjusted for the stratification factors.</p> <p>Analysis of covariance (ANCOVA) will be used to analyze the primary endpoint. Primary endpoint data that are missing will be inferred using multiple imputations. Sensitivity analyses, including mixed model repeated measures (MMRM) analyses, will assess the robustness of the primary analysis for the mITT and per protocol (PP) populations.</p> <p>Type I error control for secondary endpoints will be achieved by a hierarchical ordering procedure. Briefly, endpoints will be evaluated in order to control for multiplicity.</p> <p>An independent interim analysis committee (IAC) will conduct a blinded interim analysis when approximately 50% of patients have completed their 9-month mNIS+7 assessments. This</p> |

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| | <p>interim analysis will estimate the overall variance in the mNIS+7 score. Based on the results of the interim analysis, the committee can recommend either increasing the study sample size or making no adjustment to the sample size.</p> <p>PK analyses will be conducted using non-compartmental and/or compartmental evaluation. The PK parameters of siRNA, DLin-MC3-DMA, and PEG₂₀₀₀-C-DMG in plasma will be evaluated.</p> <p>Population PK analyses will be performed whenever possible on available siRNA, DLin-MC3-DMA, and PEG₂₀₀₀-C-DMG from sparse samples collected at various time points during the duration of the study.</p> <p>Inferential statistics of PD and PK/PD parameters, correlation between siRNA, DLin-MC3-DMA or PEG₂₀₀₀-C-DMG exposure versus TTR, RBP, or vitamin A, and between TTR versus RBP and vitamin A will be provided.</p> |
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Table 1-1: Schedule of Assessments; Screening to 9-Month Efficacy Assessment

| Procedure | Visit Type | Screening ^(a) | Screening/ Baseline ^(a) | Baseline ^(a) | Predosing | Dosing | | | | | | | | | | | | 9-Month Efficacy Assessment ^(g) | | | |
|--|------------|--------------------------|---------------------------------------|-------------------------|---------------|--------|-----|-----|-----|-----|------|------|------|------|------|------|------|--|-------|------|---------------|
| | Study Day | Day -42 to 0 | | | D0 Predose | D0 | D21 | D42 | D63 | D84 | D105 | D126 | D147 | D168 | D189 | D210 | D231 | | | D252 | D253- D272 |
| | Study Week | NA | | | 0 | 0 | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 | 36-39 | | |
| | Windows | NA | | | 0 | 0 | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | NA | | |
| Informed Consent | X | | | | | | | | | | | | | | | | | | | | |
| Inclusion/Exclusion Criteria | X | X ^(a) | | | | | | | | | | | | | | | | | | | |
| Medical History | X | X ^(b) | X ^(b) | | | | | | | | | | | | | | | | | | |
| Demographics | X | | | | | | | | | | | | | | | | | | | | |
| Review Documentation of TTR Genotype | X | | | | | | | | | | | | | | | | | | | | |
| HIV Status Review | X | | | | | | | | | | | | | | | | | | | | |
| Karnofsky Performance Status | X | | | | | | | | | | | | | | | | | | | | |
| New York Heart Classification | X | | | | | | | | | | | | | | | | | | | | |
| Serology Testing ^(c) | X | | | | | | | | | | | | | | | | | | | | |
| Paraprotein by IFE | X | | | | | | | | | | | | | | | | | | | | |
| Vitamin B12 | X | | | | | | | | | | | | | | | | | | | | |
| Efficacy Assessments | | | | | | | | | | | | | | | | | | | | | |
| NIS ^(d) / NCS ^(d) | X | | | | | | | | | | | | | | | | | | | | |
| mNIS+7 ^(e) | | X | X | | | | | | | | | | | | | | | | X | X | |
| NIS + 7 ^(f) | | X | X | | | | | | | | | | | | | | | | X | X | |
| PND Score and FAP Stage | | X ^(g) | X | | | | | | | | | | | | | | | | | X | |
| Skin Punch Biopsy (IENFD and SGNFD) ^(h) | | | X | | | | | | | | | | | | | | | | | X | |
| mBMI ⁽ⁱ⁾ | | | X | | | | | | | | | | | | | | | | | X | |
| 10-meter Walk Test ^(j) | | | X ^(z) | | | | | | | | | | | | | | | | | X | X |
| Grip Strength Test ^(k) | | | X ^(z) | | | | | | | | | | | | | | | | | X | X |
| Norfolk QOL-DN; COMPASS 31 Questionnaires | | | X ^(z) | | | | | | | | | | | | | | | | | | X |
| EQ-5D; R-ODS Questionnaires | | | X ^(z) | | | | | | | | | | | | | | | | | | X |
| Echocardiogram | | | X ^(z) | | | | | | | | | | | | | | | | | | X |
| NT-proBNP and Troponin I | | | X | | | | | | | | | | | | | | | | | | X |
| Pharmacodynamic Assessments^(l) | | | | | | | | | | | | | | | | | | | | | |
| TTR Protein, Vitamin A, and RBP | | | X | X | | X | | | | | X | | | | | | | | X | X | |
| Obtain Blood Sample for Long-term Storage | | X | X | X | | X | | | | | X | | | | | | | X | X | | |

Table 1-1: Schedule of Assessments; Screening to 9-Month Efficacy Assessment (continued)

| Procedure | Visit Type | Screening ^(a) | Screening/ Baseline ^(a) | Baseline ^(a) | Predosing | Dosing | | | | | | | | | | | | 9-Month Efficacy Assessment ^(g) | | |
|---|-----------------------|--------------------------|---------------------------------------|-------------------------|------------------|--------|-----|-----|-----|-----|------|------|------|------|------|------|------|--|---------------|---|
| | Study Day | Day -42 to 0 | | | D0 Pre-dose | D0 | D21 | D42 | D63 | D84 | D105 | D126 | D147 | D168 | D189 | D210 | D231 | D252 | D253- D272 | |
| | Study Week Windows | NA | | | 0 | 0 | 3 | 6 | 9 | 12 | 15 | 18 | 21 | 24 | 27 | 30 | 33 | 36 | 36-39 | |
| Safety Assessments^(m) | | | | | | | | | | | | | | | | | | | | |
| Physical Examination | X | | | | | | | | | | | | | | | | | X | | |
| Weight ⁽ⁿ⁾ | X | X | X | X | X | | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Height | X | | | | | | | | | | | | | | | | | | | |
| Vital Signs ^(o) | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| 12-Lead ECG ^(p) | | X ^(z) | | | | | | | | | X | | | | | | | | X | |
| Serum Chemistry | X | | | | X | | | | X | | | | | | X | | | | | |
| Hematology, Urinalysis | X | | | | | | | | | | | | | | | | | X | | |
| Thyroid Function Tests | X | | | | | | | | | | | | | | | | | X | | |
| Coagulation Studies | X | | | | | | | | | | | | | | | | | | | |
| Anti-drug Antibody Testing ^(q) | | | | | X | | X | | | | X | | | | | | | X | | |
| Pregnancy Test ^(r) | X | | | | | | | | | | | | | | | | | | | |
| Ophthalmology Exam ^(s) | | X | | | | | | | | | | | | | | | | X | | |
| Concomitant Medications | X | X | X | | | | | | | | X | | | | | | | | | |
| Adverse Events | | | | | | | | | | | X | | | | | | | | | |
| Pharmacokinetic Assessments | | | | | | | | | | | | | | | | | | | | |
| Plasma PK Sampling ^(t) | | | | | X | X | X | | | | | X | | | | | | X | | |
| Urine PK Sampling ^(u) | | | | | X | | X | | | | | X | | | | | | X | | |
| Other Assessments | | | | | | | | | | | | | | | | | | | | |
| Pharmacoeconomic Questionnaire | | | | | X ^(z) | | | | | | | | | | | | | | X | |
| C-SSRS Questionnaire | | X ^(z) | | | | | | | | | | | | | | | | | X | |
| Drug Administration | | | | | | | | | | | | | | | | | | | | |
| Randomization ^(v) | | | | | X | | | | | | | | | | | | | | | |
| Premedication Administration ^(w) | | | | | X | | X | X | X | X | X | X | X | X | X | X | X | X | X | |
| Study Drug Administration ^(x) | | | | | | X | X | X | X | X | X | X | X | X | X | X | X | X | X | |

Table 1-1 Footnotes:

- Abbreviations: COMPASS 31 = Composite Autonomic Symptom Score; EQ-5D = EuroQOL-5 Dimensions; ECG = electrocardiogram; FAP = familial amyloidotic polyneuropathy; HIV = human immunodeficiency virus; IENFD = Intraepidermal nerve fiber density; IFE = immunofixation electrophoresis; mBMI = modified body mass index; mNIS = Modified Neuropathy Impairment Score; NCS = nerve conduction studies; NIS = Neuropathy Impairment Score; NT-proBNP = N-terminal prohormone of B-type natriuretic peptide; PND = polyneuropathy disability; QOL-DN = Quality of Life-Diabetic Neuropathy; RBP = retinol binding protein; R-ODS = Rausch-built Overall Disability Scale; SGNFD = Sweat gland nerve fiber density; TTR = transthyretin.
- a. The Screening/Baseline and Baseline visits will be performed on separate days. The Screening/Baseline visit must be performed within 21 days prior to the first dose of study drug (Day 0). The Baseline visit must be conducted at least 24 hours (approximately), but not more than 7 days, after the Screening/Baseline visit. In conjunction with the decision of the Medical Monitor(s), patients may be allowed to rescreen after a minimum of 5 days have elapsed from their last screening assessment. Note: Inclusion Criteria 3 (i.e., NIS of 5 to 130 [inclusive] and PND score $\leq 3b$) and 4 (i.e., NCS sum of the sural sensory nerve action potential [SNAP], tibial compound muscle action potential [CMAP], ulnar SNAP, ulnar CMAP, and peroneal CMAP of ≥ 2 points) must be met at the Screening/Baseline visit. All other entry criteria (inclusion and exclusion) will be assessed at the Screening visit only.
 - b. An interval medical history will be collected at the Screening/Baseline and Baseline visit. Only changes since the Screening visit will be collected.
 - c. Serologies will include hepatitis B surface antibody (HbsAb), hepatitis B surface antigen (HbsAg), and anti-hepatitis C virus antibody (anti-HCV Ab).
 - d. The NIS and NCS will be assessed for the likelihood of a patient meeting the NIS and NCS eligibility criteria at the Screening/Baseline visit. The documented results of previously performed NIS and NCS may be used to qualify a patient for this study if these tests were performed within 60 days prior to the date of informed consent.
 - e. The mNIS+7 consists of the modified NIS tool (weakness and reflexes), NCS 5 attributes ($\Sigma 5$), quantitative sensory testing (QST) by body surface area including touch pressure (TP) and heat pain (HP), and postural blood pressure. At the 9-month efficacy assessment, 2 assessments of the mNIS+7 will be performed on separate days (1 assessment on each day). The second (repeat) assessment must be conducted at least 24 hours (approximately) after the first assessment, but not more than 7 days apart. Components that are shared between the mNIS+7 and NIS+7 (including NIS and NCS) will be performed once at each assessment (e.g., the weakness component should not be performed more than once on any given day).
 - f. The NIS+7 consists of the NIS tool (weakness, sensation, and reflexes), NCS $\Sigma 5$, vibration detection threshold (VDT), and heart rate response to deep breathing (HRdB). At the 9-month efficacy assessment, 2 assessments of the NIS+7 will be performed on separate days (1 assessment on each day). The second (repeat) assessment must be conducted at least 24 hours (approximately) after the first assessment, but not more than 7 days apart. Components that are shared between the mNIS+7 and NIS+7 (including NIS and NCS) will be performed once at each assessment (e.g., the weakness component should not be performed more than once on any given day).
 - g. At the Screening/Baseline visit, only PND score is required.
 - h. If the patient has provided separate informed consent for skin biopsies, 2 sets of tandem 3-mm skin punch biopsies are to be obtained (4 biopsies total). One set of biopsies will be taken from the distal lower leg, when a patient's clinical status allows, and one set from the distal thigh at each time point. Skin biopsies will be performed at a central assessment site (CAS).
 - i. The mBMI calculation will take place programmatically in the clinical database; the site will not perform the calculation.
 - j. The patient will be asked to walk 10 meters. The walk must be completed without assistance from another person; ambulatory aids such as canes and walkers are permitted. The time required for the patient to walk 10 meters will be recorded. At the 9-month efficacy assessment, 2 assessments of the 10-meter walk test will be performed on separate days (1 assessment on each day). The second (repeat) assessment must be conducted at least 24 hours (approximately) after the first assessment, but not more than 7 days apart.
 - k. Grip strength will be measured in triplicate using a dynamometer held in the dominant hand. Every effort will be made to use the same device for a patient throughout the duration of the study. At the 9-month efficacy assessment, 2 assessments of the grip strength will be performed on separate days (1 assessment on each day). The second (repeat) assessment must be conducted at least 24 hours (approximately) after the first assessment, but not more than 7 days apart.
 - l. On dosing days, blood samples for PD assessments will be obtained prior to dosing and vitamin A supplementation.

Table 1-1 Footnotes (continued):

- m. On dosing days, all safety assessments are performed pre-dose.
- n. Weight from previous visit should be used for calculating dose. Weight must be collected pre-dose.
- o. Vital signs to include: blood pressure, pulse rate, temperature, and respiratory rate. Parameters are to be measured in the supine position using an automated instrument after the patient has rested comfortably for 10 minutes. Vital signs must be collected pre-dose. On Day 0, vital signs will also be collected post-dose.
- p. All ECGs are to be obtained in triplicate.
- q. Blood samples for anti-drug antibody testing will be collected prior to study drug dosing.
- r. A pregnancy test (urine- or serum-based) will be performed on all females of child-bearing potential.
- s. The baseline ophthalmology examination may be performed any time after the patient is deemed eligible for participation in the study through Day 21. The 9-month ophthalmology examination will be performed between Days 231(\pm 3) and 272 at a CAS.
- t. Plasma PK samples will be collected as follows: Day 0: pre-dose (within 1 hour of planned study drug dosing) and at the end of infusion (+5 minutes). Day 21 and Day 252: pre-dose (within 1 hour of planned study drug dosing) and 30 minutes after the end of the infusion (+15 minutes). Day 126: pre-dose (within 1 hour of planned study drug dosing) and at the end of infusion (+5 minutes).
- u. Urine PK samples will be collected pre-dose (within 1 hour of planned study drug dosing).
- v. Randomization procedures are described in [Section 4.4.1](#).
- w. Prior to dosing, all patients will receive premedications administered at least 60 minutes prior to the start of infusion of study drug. The regimen is described in [Section 5.3.1](#).
- x. The patient's infusion site should be assessed for signs of any localized reaction during the infusion and for 30 minutes after the end of the infusion. The patient will remain at the study site for 1 hour following completion of dosing for observation and completion of assessments.
- y. Patients who discontinue study drug due to rapid disease progression based on the 9-month efficacy assessments will continue on to the Modified Visit Schedule (See [Table 1-3](#)).
- z. Assessment must be completed at a single time point during one of the specified visits, at the discretion of the investigator.

Table 1-2: Schedule of Assessments; Week 39 to Week 86 (Follow-up) / Early Withdrawal

| Procedure | Visit Type | Dosing | | | | | | | | | | | | | | 18-Month Efficacy Assessment | End of Study ^(a) | Follow-up ^(a) | Early Withdrawal ^(b) | Follow-up for Patients who Discontinue Treatment but Return at 9 and/or 18 mo ^(c) | | | | | |
|---|------------|--------|------|------|------|------|------|------|------|------|------|------|------|------|------|------------------------------|-----------------------------|--------------------------|---------------------------------|--|-----------|------------------|---|------------------|---|
| | Study Day | D273 | D294 | D315 | D336 | D357 | D378 | D399 | D420 | D441 | D462 | D483 | D504 | D525 | D546 | D553-D560 | D567 | D602 | NA | D253-D272 | D553-D560 | | | | |
| | Study Week | 39 | 42 | 45 | 48 | 51 | 54 | 57 | 60 | 63 | 66 | 69 | 72 | 75 | 78 | 79-80 | 81 | 86 | NA | 36-39 | 79-80 | | | | |
| | Windows | +3 D | +3 D | +3 D | +3 D | +3 D | +3 D | +3 D | +3D | +3D | +3D | +3D | +3D | +3D | +3D | NA | ±7D | ±10D | 2D to 7D | ±10D | ±10D | | | | |
| Efficacy Assessments | | | | | | | | | | | | | | | | | | | | | | | | | |
| mNIS+7 ^(d) | | | | | | | | | | | | | | | | X | X | | | X | X | X | X | X | X |
| NIS+7 ^(e) | | | | | | | | | | | | | | | | X | X | | | X | X | X | X | X | X |
| PND Score and FAP Stage | | | | | | | | | | | | | | | | X | | | | X | | X | | X | |
| Skin Punch Biopsy (IENFD and SGNFD) ^(f) | | | | | | | | | | | | | | | | X | | | | X | | | | | |
| mBMI ^(g) | | | | | | | | | | | | | | | | X | | | | X | | X | | X | |
| 10-meterWalkTest ^(h) | | | | | | | | | | | | | | | | X | X | | | X | X | | | | |
| Grip Strength Test ⁽ⁱ⁾ | | | | | | | | | | | | | | | | X | X | | | X | X | | | | |
| Norfolk QOL-DN; EQ-5D; R-ODS Disability; COMPASS31 Questionnaires | | | | | | | | | | | | | | | | X | | | | X | | X ^(j) | | X ^(j) | |
| Echocardiogram | | | | | | | | | | | | | | | | X | | | | X | | | | | |
| NT-pro BNP and Troponin I | | | | | | | | | | | | | | | | X | | | | X | | | | | |
| Pharmacodynamic Assessments^(k) | | | | | | | | | | | | | | | | | | | | | | | | | |
| TTR Protein, Vitamin A, and RBP | X | | | | | | | X | | | | | | | X | X | X | | | X | | X | | X | |
| Obtain Blood Sample for Long-term Storage | X | | | | | | | X | | | | | | | X | X | X | | | X | | | | | |

| Procedure | Visit Type | Dosing | | | | | | | | | | | | | | 18-Month Efficacy Assessment | End of Study ^(a) | Follow-up ^(a) | Early Withdrawal ^(b) | Follow-up for Patients who Discontinue Treatment but Return at 9 and/or 18 mo ^(c) | | | |
|--|------------|--------|------|------|------|------|------|------|------|------|------|------|------|------|------|------------------------------|-----------------------------|--------------------------|---------------------------------|--|-----------|---|---|
| | Study Day | D273 | D294 | D315 | D336 | D357 | D378 | D399 | D420 | D441 | D462 | D483 | D504 | D525 | D546 | D553-D560 | D567 | D602 | NA | D253-D272 | D553-D560 | | |
| | Study Week | 39 | 42 | 45 | 48 | 51 | 54 | 57 | 60 | 63 | 66 | 69 | 72 | 75 | 78 | 79-80 | 81 | 86 | NA | 36-39 | 79-80 | | |
| | Windows | +3 D | +3 D | +3 D | +3 D | +3 D | +3 D | +3 D | +3D | +3D | +3D | +3D | +3D | +3D | +3D | NA | ±7D | ±10D | 2D to 7D | ±10D | ±10D | | |
| Safety Assessment^(l) | | | | | | | | | | | | | | | | | | | | | | | |
| Physical Examination | | | | | | | | | | | | | | | X | | X | X | X | X | X | | |
| Weight ^(m) | | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | | X | X | X | X |
| Vital Signs ⁽ⁿ⁾ | | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | | |
| 12-Lead ECG ^(o) | | | | | | | | X | | | | | | | | X | | | | X | | | |
| Serum Chemistry | | | | | | X | | | | X | | | | | X | | | X | X | | | | |
| Coagulation ^(w) | | | | | | | | | | | | | | | X | | | | | | | | |
| Hematology, Urinalysis | | | | | | | | | | | | | | | X | | | | | X | | | |
| Thyroid Function Tests | | | | | | | | | | | | | | | X | | | | | X | | | |
| Anti-drug Antibody Testing ^(p) | | | | | | | | X | | | | | | | X | | | | | X | | | |
| Pregnancy Test ^(q) | | | | | | | | | | | | | | | | | X | X | X | | | | |
| Ophthalmology ^(r) | | | | | | | | | | | | | | | | X | | | | | | | |
| Concomitant Medications | | X | | | | | | | | | | | | | | | | | | | | | |
| Adverse Events | | X | | | | | | | | | | | | | | | | | | | | | |
| Pharmacokinetic Assessments | | | | | | | | | | | | | | | | | | | | | | | |
| Plasma Pharmacokinetic Sampling ^(s) | | | | | | | | X | | | | | | | X | | | | | X | X | X | |
| Urine Pharmacokinetic Sampling ^(t) | | | | | | | | X | | | | | | | X | | | | | X | | | |
| Other Assessments | | | | | | | | | | | | | | | | | | | | | | | |
| Pharmacoeconomic Questionnaire | | | | | | | | | | | | | | | | X | | | | X | | | |

| Procedure | Visit Type | Dosing | | | | | | | | | | | | | | 18-Month Efficacy Assessment | End of Study ^(a) | Follow-up ^(a) | Early Withdrawal ^(b) | Follow-up for Patients who Discontinue Treatment but Return at 9 and/or 18 mo ^(c) | |
|---|------------|--------|------|------|------|------|------|------|------|------|------|------|------|------|------|------------------------------|-----------------------------|--------------------------|---------------------------------|--|-----------|
| | Study Day | D273 | D294 | D315 | D336 | D357 | D378 | D399 | D420 | D441 | D462 | D483 | D504 | D525 | D546 | D553-D560 | D567 | D602 | NA | D253-D272 | D553-D560 |
| | Study Week | 39 | 42 | 45 | 48 | 51 | 54 | 57 | 60 | 63 | 66 | 69 | 72 | 75 | 78 | 79-80 | 81 | 86 | NA | 36-39 | 79-80 |
| | Windows | +3 D | ±3 D | ±3 D | ±3 D | ±3 D | ±3 D | ±3 D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | NA | ±7D | ±10D | 2D to 7D | ±10D | ±10D |
| C-SSRS Questionnaire | | | | | | | | | | | | | | | | X | | | X | | |
| Drug Administration | | | | | | | | | | | | | | | | | | | | | |
| Premedication Administration ^(u) | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X | | | | | | |
| Study Drug Administration ^(v) | X | X | X | X | X | X | X | X | X | X | X | X | X | X | | | | | | | |
| Karnosky performance status | | | | | | | | | | | | | | | | | | | | X | X |
| NYHA class | | | | | | | | | | | | | | | | | | | | X | X |

Table 1-2 Footnotes:

- Abbreviations: COMPASS 31 = Composite Autonomic Symptom Score; C-SSRS = Columbia–Suicide Severity Rating Scale; EQ-5D = EuroQOL; ECG = electrocardiogram; FAP = familial amyloidotic polyneuropathy; IENFD = Intraepidermal nerve fiber density; mBMI = modified body mass index; mNIS = Modified Neuropathy Impairment Score; NIS = Neuropathy Impairment Score; NT-proBNP = N-terminal prohormone of B-type natriuretic peptide; PND = polyneuropathy disability; QOL-DN = Quality of Life-Diabetic Neuropathy; RBP = retinol binding protein; R-ODS = Rausch-built Overall Disability Scale; SGNFD = Sweat gland nerve fiber density; TTR = transthyretin.
- a. If a patient enrolls in the extension study, the patient will only have to complete the 21-day follow-up assessments (EOS; Day 567) and not the 56-day follow-up assessments (Day 602). Patients who do not enroll in the extension study will need to complete both follow-up visits (Days 567 and 602).
 - b. The Early Withdrawal visit will take place over 2 days to allow for the repeat assessment of the mNIS+7, NIS+7, timed 10-meter walk, and grip strength test.
 - c. Patients who discontinue treatment may return for follow-up visits at 9 and/or 18 months.
 - d. The mNIS+7 consists of the modified NIS tool (weakness and reflexes), NCS $\Sigma 5$, QST by body surface area including TP and HP, and postural blood pressure. Two assessments will be performed on separate days (1 assessment on each day). The second (repeat) assessment must be conducted at least 24 hours (approximately) after the first assessment, but not more than 7 days apart. Components that are shared between the mNIS+7 and NIS+7 (including NIS and NCS) will be performed once at each assessment (e.g., the weakness component should not be performed more than once on any given day).
 - e. The NIS+7 consists of the NIS tool (weakness, sensation, and reflexes), NCS $\Sigma 5$, VDT, and heart rate response to deep breathing. Two assessments will be performed on separate days (1 assessment on each day). The second (repeat) assessment must be conducted at least 24 hours (approximately) after the first assessment, but not more than 7 days apart. Components that are shared between the mNIS+7 and NIS+7 (including NIS and NCS) will be performed once at each assessment (e.g., the weakness component should not be performed more than once on any given day).
 - f. If the patient has provided separate informed consent for skin biopsies, 2 sets of tandem 3-mm skin punch biopsies are to be obtained (4 biopsies total). One set of biopsies will be taken from the distal lower leg, when a patient's clinical status allows, and 1 set from the distal thigh at each time point.
 - g. The mBMI calculation will take place programmatically in the clinical database; the site will not perform the calculation.
 - h. The patient will be asked to walk 10 meters. The walk must be completed without assistance from another person; ambulatory aids such as canes and walkers are permitted. The time required for the patient to walk 10 meters will be recorded. Two assessments will be performed on separate days (1 assessment on each day). The second (repeat) assessment must be conducted at least 24 hours (approximately) after the first assessment, but not more than 7 days apart.
 - i. Grip strength will be measured using a dynamometer held in the dominant hand. Every effort will be made to use the same device for a patient throughout the duration of the study. Two assessments will be performed on separate days (1 assessment on each day). The second (repeat) assessment must be conducted at least 24 hours (approximately) after the first assessment, but not more than 7 days apart.
 - j. Only the Norfolk and EQ-5D are to be performed for patients who discontinue treatment and return for the 9 and 18 month visits.
 - k. On dosing days, blood samples for PD assessments will be obtained prior to dosing and vitamin A supplementation.
 - l. On dosing day, all safety assessments are performed pre-dose.
 - m. Weight from previous visit should be used for calculating dose. Weight must be collected pre-dose.
 - n. Vital signs to include: blood pressure, pulse rate, temperature, and respiratory rate. Parameters are to be measured in the supine position using an automated instrument after the patient has rested comfortably for 10 minutes. Vital signs must be collected pre-dose.
 - o. All ECGs are to be obtained in triplicate.
 - p. On study drug dosing days, blood samples for anti-drug antibody testing will be collected pre-dose.
 - q. A pregnancy test (urine- or serum-based) will be performed on all females of child-bearing potential.
 - r. The 18-month ophthalmology examination will be performed between Days 546(± 3) and 560 at a CAS.

Table 1-2 Footnotes (continued):

- s. Plasma PK samples will be collected as follows: Day 399: pre-dose (within 1 hour of planned study drug dosing) and at the end of infusion (+5 minutes). Day 546: pre-dose (within 1 hour of planned study drug dosing) and 30 minutes after the end of the infusion (+15 minutes). Early Withdrawal: any time within the visit window.
- t. Urine PK samples will be collected as follows: Day 399 and Day 546: pre-dose (within 1 hour of planned study drug dosing). Early Withdrawal: any time within the visit window.
- u. Prior to dosing, all patients will receive premedications administered at least 60 minutes prior to the start of infusion of study drug. The regimen is described in [Section 5.3.1](#).
- v. The patient's infusion site should be assessed for signs of any localized reaction during the infusion and for 30 minutes after the end of the infusion. The patient will remain at the study site for 1 hour following completion of dosing for observation and completion of assessments.
- w. INR assessment only, which is to be used for qualification for Study TTR02-006.

Table 1-3: Modified Schedule of Assessments for Patients who Discontinue Study Drug for Rapid Disease Progression; Week 39 to End of Study

| Procedure | Visit Type | Dosing | | | | | 18-Month Efficacy Assessment | End of Study | Early Withdrawal ^(a) | | |
|--|------------|---------------------|------|------|------|------|------------------------------|--------------|---------------------------------|---|---|
| | Study Day | D273 ^(b) | D294 | D378 | D462 | D546 | D553-D560 | D567 | NA | | |
| | Study Week | 39 | 42 | 54 | 66 | 78 | 79-80 | 81 | NA | | |
| | Windows | +3D | ±3D | ±3D | ±3D | ±3D | NA | ±7D | 2D to 7D | | |
| Consultation on treatment options^(c) | | X | | | | | | | | | |
| Efficacy Assessments | | | | | | | | | | | |
| mNIS+7 ^(d) | | | | | | X | X | | X | X | |
| NIS + 7 ^(e) | | | | | | X | X | | X | X | |
| PND Score and FAP Stage | | | | | | | X | | | X | |
| Skin Punch Biopsy (IENFD and SGNFD) ^(f) | | | | | | | X | | | X | |
| mBMI ^(g) | | | | | | | X | | | X | |
| 10-meter Walk Test ^(h) | | | | | | | X | X | | X | X |
| Grip Strength Test ⁽ⁱ⁾ | | | | | | | X | X | | X | X |
| Norfolk QOL-DN; EQ-5D; R-ODS Disability; COMPASS 31 Questionnaires | | | | | | | X | | | X | |
| Echocardiogram | | | | | | | X | | | X | |
| NT-proBNP and Troponin I | | | | | | | X | | | X | |
| PD Assessments | | | | | | | | | | | |
| TTR Protein, Vitamin A, and RBP | | | X | | | X | | | X | X | |
| Obtain Blood Sample for Long-term Storage | | | X | | | X | | | X | X | |

Table 1-3: Modified Schedule of Assessments for Patients who Discontinue Study Drug for Rapid Disease Progression; Week 39 to End of Study (continued)

| Procedure | Visit Type | Dosing | | | | | 18-Month Efficacy Assessment | End of Study | Early Withdrawal ^(a) |
|---|------------|---------------------|------------------|------------------|------|------|------------------------------|--------------|---------------------------------|
| | Study Day | D273 ^(b) | D294 | D378 | D462 | D546 | D553-D560 | D567 | NA |
| | Study Week | 39 | 42 | 54 | 66 | 78 | 79-80 | 81 | NA |
| | Windows | +3D | ±3D | ±3D | ±3D | ±3D | NA | ±7D | 2D to 7D |
| Safety Assessments | | | | | | | | | |
| Physical Examination | | X | | | | X | | X | X |
| Weight | | | | | | X | X | X | X |
| Vital Signs ^(j) | X | X | | | | X | X | X | X |
| 12-Lead ECG ^(k) | | | | | | | X | | X |
| Serum Chemistry | | X | | | | X | | | X |
| Coagulation ^(r) | | | | | | X | | | |
| Hematology, Urinalysis | | | | | | X | | | |
| Thyroid Function Tests | | | | | | X | | | X |
| Anti-drug Antibody Testing | | | | | | X | | | X |
| Pregnancy Test ^(l) | | X | | | | X | | | X |
| Concomitant Medications ^(m) | | X | | | | | | | |
| Adverse Events ⁽ⁿ⁾ | | X | | | | | | | |
| Study-procedure-related Adverse Events ^(o) | | | | | | | X | | |
| PK Assessments | | | | | | | | | |
| Plasma PK Sampling | | X | | | | X | | | |
| Urine PK Sampling | | X | | | | X | | | |
| Other Assessments | | | | | | | | | |
| Pharmacoeconomic Questionnaire | | | | | | X | | | X |
| C-SSRS Questionnaire | | | | | | | X | | X |
| Collect Data on Subsequent FAP Treatment Regimens | | X | X ^(p) | X ^(p) | | X | | X | X |
| Phone Contact to Obtain Health Status Update ^(q) | | | X | X | | | | | |

Table 1-3 Footnotes:

- Abbreviations: COMPASS 31 = Composite Autonomic Symptom Score; C-SSRS = Columbia–Suicide Severity Rating Scale; EQ-5D = EuroQOL; ECG = electrocardiogram; FAP = familial amyloidotic polyneuropathy; IENFD = Intraepidermal nerve fiber density; mNIS = Modified Neuropathy Impairment Score; NIS = Neuropathy Impairment Score; NT-proBNP = N-terminal prohormone of B-type natriuretic peptide; PND = polyneuropathy disability; QOL-DN = Quality of Life-Diabetic Neuropathy; RBP = retinol binding protein; R-ODS = Rausch-built Overall Disability Scale; SGNFD = Sweat gland nerve fiber density; TTR = transthyretin.
- a. The Early Withdrawal visit will be conducted for any patient who discontinues from the study after the Day 273 visit (e.g., from Day 274 onward). This visit will take place over 2 days (at least 24 hours [approximately], but not more than 7 days apart) to allow for the repeat assessment of the mNIS+7, NIS+7, timed 10-meter walk, and grip strength test.
 - b. Patients who discontinue study drug and also decide to discontinue from the study at the time of this visit will not have any additional visits after the Day 273 assessments are completed.
 - c. The patient will consult with the Investigator on a subsequent plan of care, which may include receiving therapy for FAP as per the local standard of care.
 - d. The mNIS+7 consists of the modified NIS tool (weakness and reflexes), NCS $\Sigma 5$, QST by body surface area including TP and HP, and postural blood pressure. Two assessments will be performed on separate days (1 assessment on each day). The second (repeat) assessment must be conducted at least 24 hours (approximately) after the first assessment, but not more than 7 days apart. Components that are shared between the mNIS+7 and NIS+7 (including NIS and NCS) will be performed once at each assessment (e.g., the weakness component should not be performed more than once on any given day).
 - e. The NIS+7 consists of the NIS tool (weakness, sensation, and reflexes), NCS $\Sigma 5$, VDT, and heart rate response to deep breathing. Two assessments will be performed on separate days (1 assessment on each day). The second (repeat) assessment must be conducted at least 24 hours (approximately) after the first assessment, but not more than 7 days apart. Components that are shared between the mNIS+7 and NIS+7 (including NIS and NCS) will be performed once at each assessment (e.g., the weakness component should not be performed more than once on any given day).
 - f. If the patient has provided separate informed consent for skin biopsies, 2 sets of tandem 3-mm skin punch biopsies are to be obtained (4 biopsies total). One set of biopsies will be taken from the distal lower leg, when a patient's clinical status allows, and 1 set from the distal thigh at each time point.
 - g. The mBMI calculation will take place programmatically in the clinical database; the site will not perform the calculation.
 - h. The patient will be asked to walk 10 meters. The walk must be completed without assistance from another person; ambulatory aids such as canes and walkers are permitted. The time required for the patient to walk 10 meters will be recorded. Two assessments will be performed on separate days (1 assessment on each day). The second (repeat) assessment must be conducted at least 24 hours (approximately) after the first assessment, but not more than 7 days apart.
 - i. Grip strength will be measured using a dynamometer held in the dominant hand. Every effort will be made to use the same device for a patient throughout the duration of the study. Two assessments will be performed on separate days (1 assessment on each day). The second (repeat) assessment must be conducted at least 24 hours (approximately) after the first assessment, but not more than 7 days apart.
 - j. Vital signs to include: blood pressure, pulse rate, temperature, and respiratory rate. Parameters are to be measured in the supine position using an automated instrument after the patient has rested comfortably for 10 minutes.
 - k. All ECGs are to be obtained in triplicate.
 - l. A pregnancy test (urine- or serum-based) will be performed on all females of child-bearing potential.
 - m. Concomitant medications/treatments will be collected through Day 294 (Week 42) only. Data on subsequent FAP treatment regimens will be collected separately.
 - n. Adverse events will be collected through Day 294 (Week 42) only. See [Section 8.5](#) for SAE reporting periods.
 - o. Following the Day 294 (Week 42) visit, only adverse events that are considered related to the study procedures will be collected (e.g., skin biopsies, venipunctures).
 - p. At these time points, data will be collected through phone contact.
 - q. Study personnel will contact patients by phone to query for general health status and information on subsequent FAP treatment regimens.
 - r. INR assessment only, which is to be used for qualification visit for Study TTR02-006.

ABBREVIATIONS

| Abbreviation | Definition |
|---------------------|--|
| Σ5 | 5 attributes |
| ADA | Anti-drug antibodies |
| AE | Adverse event |
| ALT | Alanine transaminase |
| ANC | Absolute neutrophil count |
| ANCOVA | Analysis of covariance |
| aPTT | Activated partial thromboplastin time |
| AST | Aspartate transaminase |
| ATTR | Transthyretin-mediated amyloidosis |
| BUN | Blood urea nitrogen |
| CAS | Central Assessment Site |
| CC | Complete case |
| CFR | Code of Federal Regulations |
| CL _R | Renal clearance |
| CMAP | Compound muscle action potential |
| COMPASS-31 | Composite Autonomic Symptom Score |
| CRF | Case Report Form |
| CRO | Contract research organization |
| C-SSRS | Columbia–Suicide Severity Rating Scale |
| DEHP | di(2-ethylhexyl)phthalate |
| DLin-MC3-DMA | 1,2-Dilinoleyloxy-N,N-dimethylpropylamine |
| DSPC | 1,2-Distearoyl-sn-glycero-3-phosphocholine |
| EQ-5D | EuroQOL |
| ECG | Electrocardiogram |
| EDC | Electronic data capture |
| ELISA | Enzyme linked immunosorbent assay |
| ERG | Electroretinography |
| EU | European Union |
| FAC | Familial amyloidotic cardiomyopathy |
| FAP | Familial amyloidotic polyneuropathy |
| GCP | Good Clinical Practice |
| GMP | Good Manufacturing Practice |
| HBV | Hepatitis B virus |
| HCV | Hepatitis C virus |
| H1/H2 blocker | Histamine H1/H2 receptor antagonist |

| Abbreviation | Definition |
|----------------------------|---|
| HIPAA | Health Insurance Portability and Accountability Act of 1996 |
| HIV | Human immunodeficiency virus |
| HP | Heat pain |
| HPLC | High performance liquid chromatography |
| HRdb | Heart rate response to deep breathing |
| IB | Investigator's Brochure |
| ICF | Informed consent form |
| ICH | International Conference on Harmonization |
| IEC | Independent Ethics Committee |
| IENFD | Intraepidermal nerve fiber density |
| IFE | Immunofixation electrophoresis |
| INR | International normalized ratio |
| IRB | Institutional Review Board |
| IRR | Infusion-related reaction |
| IRS | Interactive response system |
| IV | Intravenous(ly) |
| LLN | Lower limit of normal |
| LOCF | Last observation carried forward |
| LNP | Lipid nanoparticles |
| mBMI | Modified body mass index |
| MedDRA® | Medical Dictionary for Regulatory Activities |
| mITT | Modified Intent to Treat |
| MMRM | Mixed model repeated measures |
| mNIS | Modified Neuropathy Impairment Score |
| mRNA | Messenger ribonucleic acid |
| NIS | Neuropathy Impairment Score |
| NCS | Nerve conduction studies |
| NOAEL | No observed adverse effect limit |
| Norfolk QOL-DN | Norfolk Quality of Life-Diabetic Neuropathy |
| NSAID | Nonsteroidal anti-inflammatory |
| NT-proBNP | N-terminal prohormone of B-type natriuretic peptide |
| OTC | Over-the-counter |
| PCS | Patient Care Site |
| PD | Pharmacodynamic |
| PEG ₂₀₀₀ -C-DMG | 3-N-[(ω-methoxy poly(ethylene glycol)2000) carbamoyl]-1,2-dimyristyloxy-propylamine |

| Abbreviation | Definition |
|---------------------|---|
| PI | Principal Investigator |
| PK | Pharmacokinetic(s) |
| PND | Polyneuropathy disability |
| PO | Per os (orally) |
| PP | Per protocol |
| PVC | Polyvinyl chloride |
| PT | Prothrombin time |
| QST | Quantitative sensory testing |
| QTc | QT interval corrected for heart rate |
| RBC | Red blood cell |
| RBP | Retinol binding protein |
| RNAi | RNA interference |
| R-ODS | Rausch-built Overall Disability Scale |
| SAE | Serious adverse event |
| SGNFD | Sweat gland nerve fiber density |
| siRNA | Small interfering ribonucleic acid |
| SNAP | Sensory nerve action potential |
| SUSAR | Suspected unexpected serious adverse reaction |
| T3 | Triiodothyronine |
| T4 | Thyroxine |
| TP | Touch pressure |
| TSH | Thyroid stimulating hormone |
| TTR | Transthyretin |
| TUDCA | Tauroursodeoxycholic acid |
| ULN | Upper limit of normal |
| US/USA | United States |
| USP/EP | United States Pharmacopeia/European Pharmacopoeia |
| V30M | Val30Met |
| VDT | Vibration detection threshold |
| WBC | White blood cell |
| WHO | World Health Organization |
| WT | Wild type |

1 INTRODUCTION

1.1 Background and Rationale

1.1.1 Disease Overview

Transthyretin-mediated amyloidosis (ATTR) is an inherited, autosomal dominant, systemic disease caused by a mutation in the transthyretin (TTR) gene.¹ Transthyretin is a tetrameric 127 amino acid protein that is secreted predominantly (>95%) by hepatocytes, with a smaller fraction produced by the choroid plexus and retina.² Physiologically, TTR is a major serum carrier for retinol binding protein (RBP) and a minor carrier of thyroxine (T4). Mutations in the TTR protein lead to destabilization of the tetrameric form and dissociation into dimers and monomers; misfolding of mutated monomers from the α -helical to the β -pleated sheet structure results in tissue deposition of amyloid fibrils.³ Amyloid deposits typically contain both mutant and wild-type (WT) TTR. The particular TTR mutation and site of amyloid deposition determines the clinical manifestations of the disease, which include sensory and motor neuropathy, autonomic neuropathy, and/or cardiomyopathy. ATTR is a progressive disease associated with severe morbidity, with a life expectancy limited to 5 to 15 years from symptom onset.⁴ There are over 100 reported TTR mutations which are associated with 2 clinical syndromes: familial amyloidotic polyneuropathy (FAP) and familial amyloidotic cardiomyopathy (FAC).^{5,6,7}

“Patisiran” (the International Nonproprietary Name [INN] name for the drug product previously referred to as ALN-TTR02) is being developed for the treatment of ATTR patients with symptomatic FAP.

The estimated worldwide prevalence of FAP is 5,000 to 10,000, with the majority of cases in Portugal, Sweden, France, Japan, Brazil, and the United States.^{8,9} The most common causative mutation of FAP is TTR Val30Met (V30M), with the onset of symptoms typically occurring between 30 and 55 years of age.¹⁰ Amyloid deposition occurs largely in the peripheral nerves, starting as a nerve length-dependent sensory polyneuropathy in the feet causing numbness and pain and progressing to painful dysesthesias. Disabling motor neuropathy follows, characterized by leg weakness and eventual inability to walk. Autonomic neuropathy is another common feature of the disease, resulting in severe gastrointestinal pathology (including diarrhea or constipation and malabsorption, leading to severe malnutrition), orthostatic hypotension, and bladder dysfunction with recurring urinary tract infections.^{11,12,13,14} For several mutations, cardiac pathology also occurs due to amyloid infiltration of the sinus node, atrioventricular conduction system, and infiltration of the myocardium.^{15,16} Involvement of the conduction system can lead to sudden death due to dysrhythmias, and myocardial infiltration can lead to diastolic dysfunction and right-sided heart failure.¹⁷ The cardiomyopathy proceeds inexorably, leading to death typically within 10 years.¹⁸

There are multiple lines of evidence demonstrating that reduction of circulating TTR improves outcomes in patients with ATTR. Because the liver is the primary source of WT and mutant TTR, orthotopic liver transplantation has been used since 1990 in an attempt to treat FAP,¹⁹ and is the current standard of care in patients who are eligible for transplant (patients with minimal neuropathy symptoms and no cardiac involvement).

When liver transplantation is performed early in the course of the disease, it can stabilize and slow the course of neuropathic disease in patients with FAP due to V30M, but is less effective in patients with other TTR mutations.²⁰ However, it is less effective in patients with more advanced disease, especially those with heart involvement, due to the continued production and deposition of WT TTR in tissues with pre-existing amyloid.^{21,22,23}

It is estimated that approximately two-thirds of FAP patients are not transplant-eligible. Furthermore, liver transplant poses risks from the surgical procedure and from life-threatening complications due to graft rejection or infections. The 1-year mortality rate post-transplant is 10%.²⁴

Nonsurgical options that are used for the treatment of FAP (depending on geographic location) include tafamidis (Vyndaqel[®]) and diflunisal. Tafamidis is a small molecule TTR stabilizer that binds to the thyroxine binding sites of the TTR tetramer, thus preventing its dissociation to monomers and potentially preventing fibril formation. While tafamidis is approved in the European Union (EU) for the treatment of ATTR in adult patients with Stage 1 symptomatic polyneuropathy to delay peripheral neurologic impairment, it is not considered the standard of care throughout the EU and it has not been approved for use outside the EU.²⁵

Diflunisal is a generic, nonsteroidal anti-inflammatory drug (NSAID) that is also a tetramer stabilizer and binds to TTR in a similar manner as tafamidis. A multinational, placebo-controlled Phase 3 study in patients with all stages of FAP was recently completed; however, the results have not been released. Due to the restricted use of liver transplantation and tafamidis in patients with early stage of disease, and the non-standard use of diflunisal among practitioners, there remains an unmet medical need for a potent and effective therapy for FAP that will have an impact on patients across a broad range of neurologic impairment, regardless of their mutation (V30M or non-V30M).

1.1.2 RNA Interference

Ribonucleic acid interference (RNAi) is a naturally occurring cellular mechanism for regulating gene expression that is mediated by “small interfering ribonucleic acids” (siRNAs).²⁶ Typically, synthetic siRNAs are 19 to 23 base pair double-stranded oligonucleotides in a staggered duplex with a 2-nucleotide overhang at one or both of the 3' ends. Such siRNAs can be designed to target an endogenous or virally-expressed gene. When introduced into cells, the net effect of an RNAi-based pharmacological approach is the binding of the siRNA to its complementary messenger ribonucleic acid (mRNA) sequence, cleavage of this target mRNA, and suppression of the target protein.²⁷ The ability to selectively and potently degrade the mRNA encoding the TTR protein using an siRNA offers a potent and specific approach for the treatment of ATTR.

Unformulated siRNAs, and those without chemical modification, are rapidly degraded and eliminated upon systemic administration, and thus do not achieve significant tissue distribution.²⁸ As a result, various formulations are used to target siRNA distribution to tissues, and to facilitate their uptake into the relevant cell type. One approach that has been used successfully *in vivo*, including in rodents, non-human primates, and humans, employs IV delivery of siRNAs in lipid nanoparticles (LNPs).^{29,30,31,32} These LNPs, with

their small size (<100 nm) and low surface charge, can pass through the fenestrated vascular endothelium of the liver. Endocytosis of the intact LNPs, followed by fusion with the endosomal membrane and release of the siRNA into the cytoplasm, results in the siRNA engaging the endogenous RNAi machinery described above leading to targeted degradation of the mRNA, and a consequent reduction in target protein levels.^{33,34}

1.1.3 PATISIRAN

Patisiran comprises a small interfering ribonucleic acid (siRNA) which is specific for TTR, and is formulated in a hepatotropic lipid nanoparticle (LNP) for intravenous (IV) administration.³⁵ This TTR siRNA has a target region within the 3'UTR region of TTR gene to ensure and confirm homology with WT TTR as well as all reported TTR mutations. Following LNP-mediated delivery to the liver, patisiran targets TTR mRNA for degradation, resulting in the potent and sustained reduction of mutant and WT TTR protein via the RNAi mechanism.

Since circulating TTR is almost exclusively synthesized in the liver, the IV administration of patisiran is postulated to reduce the level of precursors that lead to amyloid fibril deposition, resulting in clinical benefit to patients with FAP.

1.1.4 Therapeutic Hypothesis

Patisiran is a novel investigational agent intended for the treatment of FAP, a serious and life-threatening orphan disease. The therapeutic hypothesis that systemic amyloidoses can be managed by reduction in circulating levels of amyloidogenic protein has been validated in other acquired (e.g., immunoglobulin light chain systemic [AL], or amyloid A [AA]) and hereditary (e.g. Fibrinogen A α -chain, ApoA1) amyloidosis. The experience from these systemic amyloidotic disorders,^{36,37,38,39} as well as the liver transplant data in FAP, suggest that lowering of the circulating amyloidogenic protein by at least 50% is required to impact the clinical course of the disease, with reductions in protein beyond 50% providing further incremental improvements in outcomes. It is therefore postulated that the >80% suppression in *both* WT and mutant TTR observed upon administration of 0.3 mg/kg patisiran once every 21 days will result in clinical benefit in FAP patients with mild to moderate polyneuropathy. This hypothesis is further supported by evidence from tafamidis suggesting that reduction in free TTR monomer can slow neuropathy progression in early-stage V30M patients with FAP.¹⁰

1.2 Summary of Patisiran Nonclinical Data

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

Further information can be found in the patisiran (ALN-TTR02) IB.

1.3 Summary of Clinical Data with Patisiran

A Phase 1 multicenter, randomized, placebo-controlled, single-blind, single-ascending dose clinical study of patisiran in healthy volunteers was completed in the UK. Patisiran was administered as a single 60-minute IV infusion to healthy volunteers at doses of 0.01 to 0.05 mg/kg. Patients were premedicated with dexamethasone, H1 and H2 blockers, and paracetamol/acetaminophen prior to dosing to minimize the risk of infusion-related reactions (IRRs). Significant pharmacology in terms of TTR protein lowering (>80% reduction from pretreatment baseline) was observed at doses ≥ 0.15 mg/kg. Transthyretin levels showed evidence of recovery beginning at around Day 21 to 28, returning to baseline by Day 70.

An open-label, Phase 2, multiple-ascending dose study of patisiran in ATTR patients with FAP (Study ALN-TTR02-002) to determine the safety and tolerability, pharmacokinetics (PK), and pharmacodynamics (PD) of a 60-70 minute IV infusion of patisiran administered once every 3 or 4 weeks for 2 doses has completed enrollment (N=29). Preliminary data show >80% TTR knockdown with 0.3 mg/kg after the first and second doses ($p < 0.001$), with better sustained suppression of >80% observed throughout the dosing interval with the once every 3 week dosing schedule compared with once every 4 weeks. In V30M patients, both mutant and wild-type TTR were suppressed to the same extent. Multiple doses of patisiran have been generally safe and well-tolerated, including 0.3 mg/kg administered once every 3 or 4 weeks with either a 60-minute infusion and original premedication regimen (total of 28 mg dexamethasone or equivalent, administered the night before and morning of infusion) or a 70-minute step-wise infusion (approximately 1 mL/minute for the first 15 minutes followed by approximately 3 mL/minutes for the remainder of the infusion) and a simplified premedication regimen (total of 10 mg dexamethasone or equivalent, administered at least 60 minutes prior to infusion).

Patients completing Study ALN-TTR02-002 are eligible to enroll into a Phase 2, open-label, extension study (ALN-TTR02-003) designed to evaluate the safety and tolerability of long-term patisiran dosing administered to patients with FAP; additional information will be evaluated including PK, PD, and clinical activity. Patients will receive 0.3 mg/kg patisiran once every 21 days for approximately 2 years. Data from this study of March 13, 2015 demonstrate a sustained mean serum TTR knockdown of approximately 80%, with mean nadir up to 88% between doses, for approximately 16 months in patients receiving patisiran 0.3 mg/kg once every 3 weeks (n=20). Neuropathy impairment scores were stable through 12 months with mean change in mNIS+7 and NIS of -2.5 and +0.4 points, respectively.

Further details on these clinical studies can be found in the patisiran (ALN-TTR02) IB.

1.4 Study Design Rationale

This is a Phase 3 randomized, double-blind, placebo-controlled, multicenter, multinational study of patisiran in FAP patients. The patients proposed for this study are reflective of the FAP population encountered by clinicians in various different countries worldwide, including patients with a range of neuropathy severity and broad spectrum of TTR mutations. Disease progression will be assessed by neurological measures and functional tests; therefore, the range of baseline neuropathy severity (NIS of 5-130) is selected such that the lower end is advanced enough to show significant progression in the placebo group, while the upper end is not so advanced as to preclude detection of change as a result of a ceiling effect of neuropathy measures or to be confounded by other comorbidities.

The inclusion of placebo as a control allows for a rigorous analysis of the treatment effect of patisiran. However, given the 18-month duration of the study, those patients who have evidence of rapid disease progression at 9 months (defined as ≥ 24 point increase in mNIS+7 from baseline [based on an average of 2 measurements] and FAP stage progression relative to baseline) will be given the option of discontinuing study drug and receiving local standard of care treatment for their FAP. Such patients will be asked to follow a modified study visit schedule and return for their 18-month efficacy assessment (blinding will be maintained throughout). It is expected that fewer than 5% of patients involved in the ALN-TTR02-004 study will meet the definition of rapid disease progression at 9 months.

Given the orphan nature of the disease and the significant, progressive morbidities associated with FAP, randomization to patisiran or placebo will be performed in a 2:1 ratio (patisiran:placebo) to increase the probability that patients will receive active drug. Treatment groups will be balanced at entry for NIS (5-49 vs 50-130), early onset V30M (<50 years of age at onset) vs all other mutations (including late onset V30M), and previous tetramer stabilizer use (tafamidis or diflunisal) vs no previous tetramer stabilizer use.

The primary endpoint for this study is the change from baseline at 18 months in a composite measure of neurologic impairment termed modified NIS+7 (mNIS+7), which includes a clinical exam-based assessment of neurologic impairment (NIS) combined with electrophysiologic measures of small and large nerve fiber function (NCS and QST),

and measurement of autonomic function (postural blood pressure). The utility of various neuropathy endpoints in demonstrating a treatment effect in randomized, controlled clinical trials in patients with FAP has been established through Phase 3 studies using the small molecule TTR tetramer stabilizers tafamidis and diflunisal. For these studies, the primary efficacy endpoint utilized variations of NIS (NIS-Lower Limbs in the case of tafamidis and NIS+7 for diflunisal). Composite endpoints, such as NIS+7, have been found to be more sensitive in detecting abnormalities in patients with generalized peripheral neuropathy, such as diabetic polyneuropathy and chronic idiopathic demyelinating polyneuropathy, and the reproducibility of composite scores has been shown to be greater than for individual tests. The use of mNIS+7 in this study is expected to increase the sensitivity and reproducibility of the measurement of neuropathy progression in a heterogeneous group of FAP patients presenting with a broad spectrum of disease severity and TTR mutations.

The 18-month endpoint was selected based on the expected rate of neuropathy progression in patients with FAP, derived from a global natural history dataset of 283 patients from Alnylam collaborators in the USA, Portugal, France, and Italy. From these data, it is estimated that a patient's mNIS+7 score will have increased by approximately 24 points in the placebo group after 18 months, thereby providing adequate disease progression for the detection of a treatment effect in the patisiran group.

1.5 Dose Selection and Dosing Schedule Rationale

Given the fundamental role of TTR in the pathogenesis of the disease and the data from liver transplantation in FAP, it is postulated that the optimal dose and schedule for patisiran is one that will result in the greatest level of sustained TTR suppression with an acceptable safety profile. Based on the data to date from the nonclinical and clinical studies with patisiran, >80% maintained suppression of circulating TTR is consistently achieved at the 0.3 mg/kg dose administered once every 3 weeks, and this dose was generally well tolerated. Patisiran will be administered every 21 days as a 70-minute IV infusion (approximately 1 mL/minute for the first 15 minutes followed by approximately 3 mL/minute for the remainder of the infusion) with a premedication regimen consisting of IV dexamethasone and H1/H2 blockers along with oral paracetamol/acetaminophen given at least 60 minutes prior to each infusion.

The chronic toxicology studies in rodents (6-month duration) and monkeys (9-month duration), in which animals safely tolerated 14 doses of patisiran at ≥ 0.3 mg/kg once every 2 or 3 weeks (rats or monkeys, respectively), also supports long-term dosing in FAP patients at 0.3 mg/kg once every 21 days.

1.6 Risk-Benefit Assessment

Patisiran is a novel investigational agent intended for the treatment of FAP, a serious and life-threatening orphan disease with limited treatment options. The therapeutic hypothesis that systemic amyloidoses can be managed by reducing circulating levels of amyloidogenic protein has been established in other acquired and hereditary amyloidoses. The experience from these systemic amyloidotic disorders, as well as the liver transplant data in FAP, suggest that lowering of the circulating amyloidogenic protein could impact the clinical course of the disease. Based on nonclinical and clinical data that showed suppression in

levels of WT and mutant TTR upon administration of 0.3 mg/kg patisiran once every 21 days, it is possible that long term treatment with patisiran may have a clinical benefit in FAP patients with mild to moderate polyneuropathy and that further study is warranted.

In completed and ongoing clinical studies (ALN-TTR02-001, ALN-TTR02-002 and ALN-TTR02-003), single and multiple doses of patisiran have been generally well tolerated. Potential risks of patisiran include the following:

- **Infusion-Related Reactions**

Infusion-related reactions (IRRs) can occur with systemic administration of certain biological agents such as liposomes or monoclonal antibodies. In order to reduce the potential for an IRR with patisiran, all patients in this study will be premedicated prior to dosing with patisiran or placebo. The step-wise infusion regimen over approximately 70 minutes (starting with a slower infusion rate of approximately 1 mL/minute for the first 15 minutes before increasing to 3 mL/minute for the remainder of the infusion), may further reduce the risk of an IRR.

- **Liver function test abnormalities**

In preclinical studies, patisiran-related increased serum liver markers and histopathology in the liver were observed at dosages > 0.1 mg/kg in rats and > 1.0 mg/kg in monkeys. In general, all of the findings were not observed or were observed at lower severity at the end of the 60-90 day dose free period, indicating that the toxicities were reversible. In clinical studies to date, there have been no clinically significant changes in liver function tests in either the single-dose or multiple-dose studies. Patients in the study are monitored for liver function via serial laboratory assessments.

- **Vitamin A Lowering**

The suppression of serum levels of TTR and RBP is expected to result in the lowering of circulating vitamin A levels. Preclinical and clinical data have shown that the lowering of circulating vitamin A associated with suppression of RBP does not result in severe vitamin A deficiency. Furthermore, there are individuals with RBP/TTR mutations who have life-long low levels of circulating vitamin A and are essentially in good health, suggesting that there are compensatory transport mechanisms for vitamin A that are yet undescribed.⁴⁰ This has also been confirmed in TTR knockout mice, which do not exhibit any manifestations of vitamin A deficiency, with vitamin A uptake by most tissues continuing in the absence of RBP.⁴¹ Provided there is adequate vitamin A in the diet, tissue stores of vitamin A should not be affected by the lowering of TTR and RBP. However, as the vitamin A content of the diet may vary between different countries and different individuals within a country, all patients on the study will be asked to take a daily supplement containing the recommended daily allowance of vitamin A. Patients should have started vitamin A supplementation by the time they start his/her first dose of patisiran or placebo.

- **Osteoporosis**

Patients with FAP may be at risk for osteoporosis.⁴² In addition, as part of the premedication regimen administered prior to patisiran dosing every three weeks, FAP

patients participating in this study are given glucocorticoids, a drug known to have the potential to reduce bone mineralization. If there are no medical contraindications, and per investigator judgment and local standard of care, study participants should, if appropriate, receive therapy for the prevention and early treatment of osteoporosis such as: calcium and vitamin D supplementation, bisphosphonates, calcitonin, parathyroid hormone, estrogen agonist/antagonist or combination therapies.

Further guidance to the investigator can be found in the IB.

2 STUDY OBJECTIVES

2.1 Primary Objective

The primary objective of the study is to determine the efficacy of patisiran (ALN-TTR02) by evaluating the difference between the patisiran and placebo groups in the change from baseline of mNIS+7 score at 18 months.

2.2 Secondary Objectives

The secondary objectives of the study are to determine the effect of patisiran on various clinical parameters by assessing the difference between patisiran and placebo in the change from baseline in the following measurements at 18 months:

- Norfolk Quality of Life-Diabetic Neuropathy (Norfolk QOL-DN) questionnaire;
- NIS-weakness (NIS-W) score;
- Modified body mass index (mBMI);
- Timed 10-meter walk test;
- Autonomic symptoms questionnaire (Composite Autonomic Symptom Score [COMPASS-31]).

2.3 Exploratory Objectives

The exploratory objectives of the study are:

- To determine the difference between the patisiran and placebo groups in the change from baseline in the following measurements at 18 months:
 - NIS+7 score;
 - Grip strength;
 - EuroQOL (EQ-5D) questionnaire;
 - Level of disability (Rasch-built Overall Disability Scale [R-ODS]);
 - Large vs small nerve fiber function including nerve conduction studies (NCS) 5 attributes ($\Sigma 5$), quantitative sensory testing (QST) by body surface area including touch pressure (TP) and heat pain (HP), vibration detection threshold (VDT), heart rate response to deep breathing (HRdb), postural blood pressure;
 - Pathologic evaluation of sensory and autonomic innervation through voluntary skin punch biopsies and analysis of intraepidermal nerve fiber density (IENFD) and sweat gland nerve fiber density (SGNFD);
 - Assessment of ambulation through FAP stage and Polyneuropathy Disability (PND) score;
 - Cardiac assessment through echocardiogram, troponin I, and N terminal prohormone of B-type natriuretic peptide (NT-proBNP) levels;
 - Pharmacodynamic (PD) biomarkers (TTR, RBP, vitamin A);

- To compare the proportion of patients in the patisiran and placebo groups who meet the pre-defined criterion for rapid disease progression (defined as ≥ 24 point increase in mNIS+7 from baseline [based on an average of 2 measurements] and FAP stage progression relative to baseline) at 9 months.

3 STUDY PLAN

3.1 Overall Design

This is a multicenter, multinational, randomized, double-blind, placebo-controlled, Phase 3 study designed to demonstrate the clinical efficacy of patisiran (ALN-TTR02) and to establish the safety of chronic dosing in adult patients with FAP. A schematic of the study design is presented in [Figure 1](#).

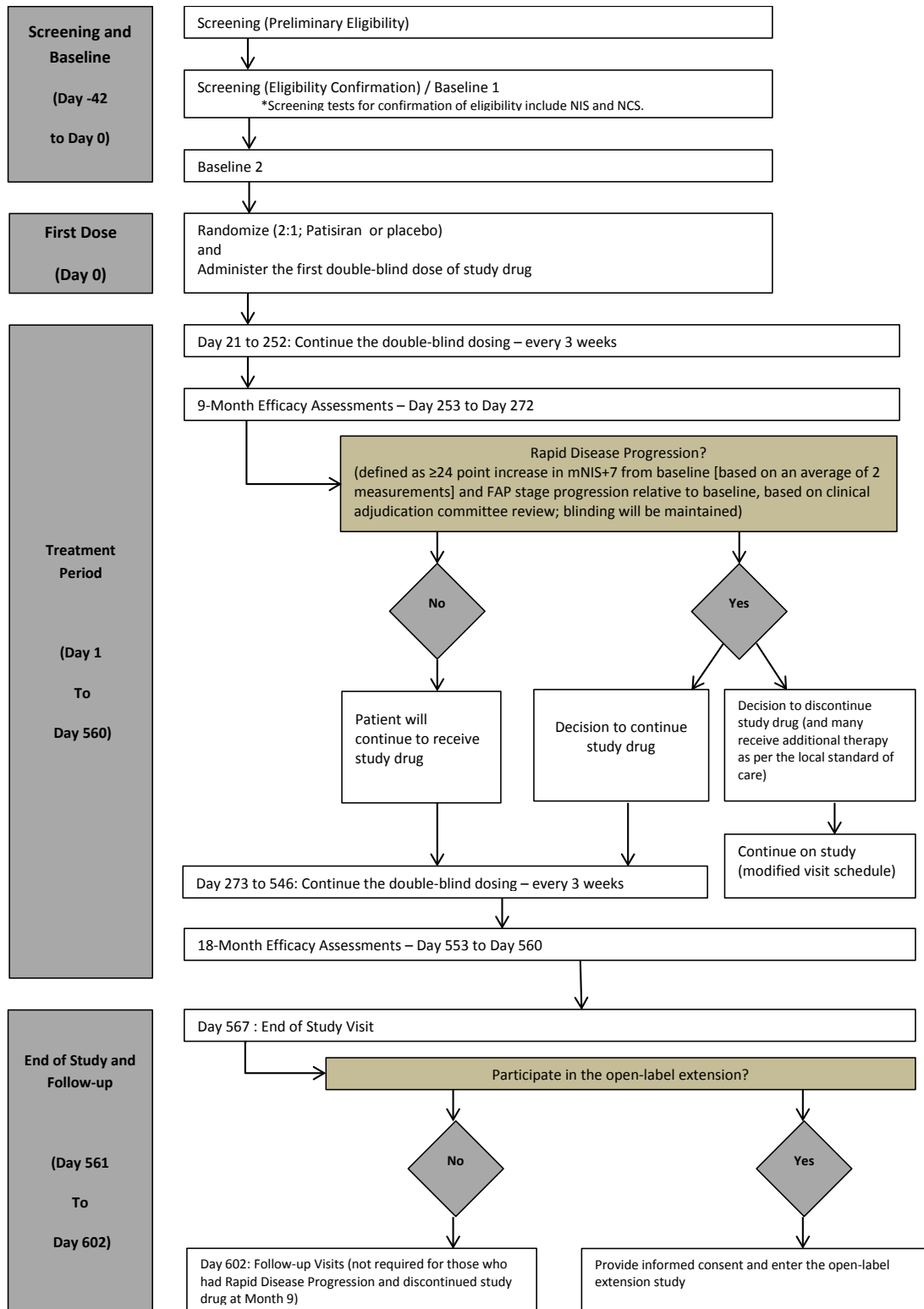
The duration of patient participation in this study is approximately 21 months. Patients will be screened within 42 days prior to administration of study drug. Consented eligible patients will be randomized to receive either patisiran or placebo (2:1 ratio, patisiran to placebo) once every 21 days for 78 weeks. Treatment groups will be balanced at entry for NIS (5-49 vs 50-130), early onset V30M (<50 years of age at onset) vs all other mutations (including late onset V30M), and previous tetramer stabilizer use (tafamidis or diflunisal) vs no previous tetramer stabilizer use.

Prior to dosing, patients will receive premedications in order to reduce the risk of experiencing an IRR. The premedications will be administered at least 60 minutes prior to the start of infusion of study drug. Blinded study drug will be administered as a 70-minute IV infusion (approximately 1 mL/minute for the first 15-minutes followed by approximately 3 mL/minute for the remainder of the infusion). In addition to returning to the site for dosing of study drug once every 21 days, patients will also return for out-patient visits at 9 and 18 months for efficacy assessments (see [Section 6](#) for details). The study personnel performing these assessments will be blinded to the results of any previous assessments (e.g., Screening/Baseline, Baseline, or 9-month assessments).

At the 9-month time point, if the clinical adjudication committee determines that a patient is exhibiting rapid disease progression (defined as ≥ 24 point increase in mNIS+7 from baseline [based on an average of 2 measurements] and FAP stage progression relative to baseline, see [Section 4.6](#)), the patient's treating physician will provide the patient with the option of discontinuing study drug and receiving local standard of care treatment for FAP. Patients who discontinue study drug will remain on study through completion of the 18-month efficacy assessments, following a modified visit schedule as shown in [Table 1-3](#) and described in [Section 6.4](#). Blinding will be maintained throughout. Patients who complete the 18-month efficacy assessments can elect to participate in an extension study in which patients will receive open-label administration of 0.3 mg/kg patisiran once every 21 days.

A Data Monitoring Committee (DMC) will be implemented for the study and will operate under a prespecified charter.

Figure 1: Study Schematic



3.2 Efficacy Assessments

Efficacy parameters will include the following (baseline evaluations will be conducted as well as evaluations at 9 and 18 months):

- Neurologic impairment will be assessed using the mNIS+7 composite score. The mNIS+7 includes the modified NIS (weakness and reflexes), NCS Σ 5, QST, as well as autonomic assessment through postural blood pressure;
- Patient-reported QOL will be evaluated using the Norfolk QOL-DN and the EQ-5D. Disability will be reported by patients using the R-ODS;
- Autonomic symptoms will be assessed using the COMPASS-31;
- Motor function assessments to be evaluated include NIS-W, timed 10-meter walk test, and grip strength test;
- PND score and FAP stage;
- Nutritional status will be assessed using mBMI;
- Pathologic evaluation of sensory and autonomic innervation will be evaluated by IENFD analysis and quantitation of SGNFD via tandem 3 mm skin punch biopsies taken from the leg;
- Neurologic impairment will also be assessed by NIS+7 (including full NIS, NCS, VDT, and HRdb);
- Cardiac structure and function will be assessed through echocardiograms as well as measurement of serum levels of NT-proBNP and troponin I.

3.3 Safety Assessments

Safety will be assessed throughout the study by collecting adverse events (AEs; including serious adverse events [SAEs]); clinical laboratory tests, including hematology, clinical chemistry (including liver function tests), thyroid function parameters, and urinalysis; measurement of anti-drug antibodies; electrocardiograms; vital signs; physical examination findings; and ophthalmology examinations.

3.4 Pharmacodynamic Assessments

Pharmacodynamic markers assessed serially will include serum TTR, vitamin A, and RBP. Additional blood samples will be collected for exploratory biomarkers related to FAP.

3.5 Pharmacokinetic Assessments

The plasma PK evaluation will include, whenever possible, plasma-concentration time profiles for siRNA and the novel lipid components in patisiran: DLin-MC3-DMA and polyethylene glycol (PEG)₂₀₀₀-C-DMG. The siRNA, DLin-MC3-DMA, and PEG₂₀₀₀-C-DMG concentration will be determined for all patients at time points specified in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#).

Urine will be collected with void volume recorded for all patients at time points specified in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#) and to determine renal clearance (CL_R) of siRNA

and 4-dimethylaminodibutyric acid (the metabolite of DLin-MC3-DMA) after dosing with study drug.

3.6 Other Assessments

Disease burden and healthcare utilization will be assessed using a patient-reported pharmacoeconomics questionnaire. The investigator will periodically assess mental status as it relates to suicidal ideation and behavior by using the Columbia–Suicide Severity Rating Scale (C-SSRS) questionnaire.

4 PATIENT POPULATION

4.1 Eligibility of Patients

Approximately 200 patients are expected to be enrolled at multiple centers worldwide. All centers will be selected on the basis of their experience in the treatment of patients with FAP.

4.2 Inclusion Criteria

To be enrolled in the study, each patient must meet the following criteria at the Screening visit, except where specified:

1. Male or female of 18 to 85 years of age (inclusive);
2. Have a diagnosis of FAP with documented TTR mutation;
3. Have an NIS of 5 to 130 (inclusive) and a PND score of $\leq 3b$ (Note: This criterion must be met at the Screening/Baseline visit);
4. Have an NCS sum of the sural sensory nerve action potential (SNAP), tibial compound muscle action potential (CMAP), ulnar SNAP, ulnar CMAP, and peroneal CMAP of ≥ 2 points (Note: This criterion must be met at the Screening/Baseline visit);
5. Have a Karnofsky performance status of $\geq 60\%$;
6. Have an absolute neutrophil count (ANC) ≥ 1500 cells/mm³, and a platelet count $\geq 50,000$ cells/mm³;
7. Have aspartate transaminase (AST) and alanine transaminase (ALT) levels $\leq 2.5 \times$ the upper limit of normal (ULN), total bilirubin within normal limits, international normalized ratio (INR) ≤ 2.0 (patients on anticoagulant therapy with an INR of ≤ 3.5 will be allowed). Patients with total bilirubin $\leq 2 \times$ ULN are eligible if the elevation is secondary to documented Gilbert's syndrome (elevation of unconjugated bilirubin with normal conjugated bilirubin) and the patient has ALT and AST levels within normal ranges;
8. Have a serum creatinine $\leq 2 \times$ ULN;
9. No active infection with hepatitis B or hepatitis C by serology;
10. Women of child-bearing potential must have a negative pregnancy test, cannot be breastfeeding, and must be using 2 highly effective methods of contraception prior to screening, throughout study participation, and for 75 days after the last dose of study drug. Highly effective methods of birth control are defined in [Section 4.7](#);
11. Males with partners of child-bearing potential, must agree to use 1 barrier method (e.g., condom) and 1 additional method (e.g., spermicide) of contraception throughout study participation and for 75 days after the last dose of study drug; males must also abstain from sperm donation after the first dose of study drug through study participation and for 75 days after the last dose of study drug;

12. Must be willing and able to comply with protocol-required visit schedule and visit requirements and provide written informed consent.

4.3 Exclusion Criteria

A patient will be excluded if they meet any of the following criteria at the time of Screening visit:

1. Had a prior liver transplant or is planning to undergo liver transplant during the study period;
2. Has other known causes of sensorimotor or autonomic neuropathy (e.g., autoimmune disease, monoclonal gammopathy, etc.);
3. Has known primary amyloidosis or leptomeningeal amyloidosis;
4. Has known type I diabetes;
5. Has had type II diabetes mellitus for ≥ 5 years;
6. Has vitamin B12 levels below the lower limit of normal (LLN);
7. Has untreated hypo- or hyperthyroidism;
8. Has had a major surgery within the past 3 months or has a major surgery planned during any point of the study period;
9. Has known human immunodeficiency virus (HIV) infection;
10. Has an active infection requiring systemic antiviral or antimicrobial therapy that will not be completed prior to the first dose of study drug administration;
11. Had a malignancy within 2 years, except for basal or squamous cell carcinoma of the skin or carcinoma in situ of the cervix that has been successfully treated;
12. Has a New York Heart Association heart failure classification > 2 ;
13. Had acute coronary syndrome within the past 3 months;
14. Has uncontrolled cardiac arrhythmia or unstable angina;
15. Has a known history of alcohol abuse within the past 2 years or daily heavy alcohol consumption (females: more than 14 units of alcohol per week; males: more than 21 units of alcohol per week [unit: 1 glass of wine [125 mL] = 1 measure of spirits = $\frac{1}{2}$ pint of beer]);
16. Received an investigational agent or device within 30 days of anticipated study drug administration or 5 half-lives of the investigational drug, whichever is longer;
17. Participated in a clinical trial with antisense oligonucleotide, must have completed a 3-month wash-out prior to start of the study drug administration in this study;
18. Is currently taking tafamidis, doxycycline, or tauroursodeoxycholic acid (TUDCA); if previously on any of these agents, must have completed a 14-day wash-out prior to start of study drug administration in this study;

19. Is currently taking diflunisal; if previously on this agent, must have at least a 3-day wash-out prior to start of study drug administration in this study;
20. Had a prior severe reaction to a liposomal product or a known hypersensitivity to oligonucleotides or any component of patisiran (ALN-TTR02);
21. Is unable to take the required premedications;
22. Anticipated survival is less than 2 years, in the opinion of the Investigator;
23. Is considered unfit for the study by the Investigator.
24. Is under legal protection (defined as “any person who becomes incapable of protecting his/her interests due to a medically diagnosed impairment of his/her mental faculties that may limit or prevent the expression of his/her will”).

4.4 Assignment to Treatment Group/Patient Number

4.4.1 Randomization Procedures

Patients will be randomly assigned in a 2:1 ratio to receive either 0.3 mg/kg patisiran or placebo (normal saline 0.9%).

Patients will be randomized via an interactive response system (IRS). Either designated unblinded site personnel or the pharmacist may request randomization for the patient, but only the pharmacist or unblinded personnel will be allowed to receive the treatment code. The treatment code will be delivered to the unblinded personnel and the pharmacist will use the necessary number of vials for that patient based on their weight.

Treatment arms will be balanced at entry for NIS (5-49 vs 50-130), early onset V30M (<50 years of age at onset) vs all other mutations (including late onset V30M), and previous tetramer stabilizer use (tafamidis or diflunisal) vs no previous tetramer stabilizer use.

4.4.2 Patient Numbering

At enrollment, each patient will be uniquely identified in the study by a combination of his/her center number and screening number. The center number will be assigned by the Sponsor. Upon signing the informed consent form, the patient will be assigned a screening number by the IRS. The Investigator or his/her delegate will contact the IRS (via phone or web) after confirming that the patient fulfills all the inclusion criteria and none of the exclusion criteria. The patient will be randomized via the IRS, assigned a patient number and a study treatment. A combination of the center number, screening number, enrollment number, and patient initials will create the unique patient identifier. In countries with regulations prohibiting the use of patient initials, a strategy consistent with local regulations will be used to generate replacement values for the patient initials.

4.4.3 Blinding Procedure

Only the pharmacist and designated site personnel who dispense or administer study drug will be unblinded to the study treatment. All other site personnel will be blinded to the treatment. Study personnel performing assessments related to the efficacy endpoints will be different from the Investigator and other personnel managing the patient, and all of these study personnel will be blinded to any clinical laboratory results that could

potentially unblind them (e.g., TTR levels, vitamin A levels, thyroid function tests). In addition, the study personnel performing assessments related to the efficacy endpoints will also be blinded to the results of any previous assessments (e.g., Screening/Baseline, Baseline, or 9-month assessments).

All patients will be blinded to treatment and will receive an IV infusion once every 21 days using identical volumes for placebo and patisiran. The tubing and the lines will be covered so that it will not be possible to detect a difference in active versus placebo drug.

Furthermore, unblinded source documentation containing all descriptions of pharmacy preparations and infusions or distribution of study drug or randomization data will be stored separate from all other study data/records and from other pharmacy staff not participating on the study.

Unblinding is only to occur in the case of patient emergencies or when necessary from a regulatory reporting perspective (e.g., Suspected Unexpected Serious Adverse Reactions [SUSAR] occurring in the EU), and at the conclusion of the study.

Patients who discontinue study drug at 9 months due to rapid disease progression will remain blinded throughout the study.

4.4.4 Breaking the Blind

In the event that the Investigator requests to know a patient's study treatment assignment, the Investigator will first contact the CRO Medical Monitor to discuss the need for unblinding. In case of an emergency, the treatment allocation for each patient will be available from the unblinded site personnel, pharmacist, or the IRS system.

If a patient becomes pregnant or seriously ill during the study, the blind should be broken only if knowledge of the treatment administered will affect treatment options available to the patient. Before breaking the blind, the PI or Sub-investigator should attempt to contact the CRO Medical Monitor. If the Medical Monitor is immediately unreachable, the PI or Sub-investigator should break the blind as necessary using the code breaking information provided and contact the Medical Monitor as soon as possible. A record should be kept of when the blind was broken, who broke it, and why.

4.5 Early Patient Discontinuation or Withdrawal

Patients are free to discontinue treatment or withdraw from the study at any time and for any reason, without penalty to their continuing medical care.

There are 3 ways for a patient to discontinue treatment and/or withdraw from the study:

- 1) The patient or investigator decides to discontinue study treatment, but the patient agrees to remain in the study and undergo follow-up assessments (as described in [Section 6.3](#)).
- 2) The patient experiences protocol-defined rapid disease progression at Month 9 and elects to discontinue study treatment but remain in the study and return for protocol-specified visits, including follow-up assessment at Month 18 (as described in [Section 4.6](#)).
- 3) The patient decides to no longer participate in the study and withdraws consent.

A patient will be considered to have completed the study if the patient does not withdraw consent from the study and completes protocol-specified procedures up through the 18-month efficacy assessment visit.

4.5.1 Reasons for Treatment Discontinuation or Withdrawal

The Investigator may discontinue treatment or withdraw a patient from the study if the patient:

- Is in violation of the protocol;
- Experiences a serious or intolerable AE;
- Becomes pregnant;
- Requires a prohibited medication (see [Section 5.8](#));
- Requests to discontinue treatment or be withdrawn from the study;
- Is found to be considerably noncompliant with the protocol-required patisiran dosing visits.

A patient may discontinue treatment or be withdrawn from the study if, in the Investigator's opinion, they are unable to continue. The Investigator will also withdraw the patient from the study upon the request of Alnylam, including if Alnylam terminates the study. Upon occurrence of a serious or intolerable AE, the Investigator will confer with Alnylam before discontinuing treatment.

In general, patients who discontinue treatment will be encouraged to remain on the study to complete study assessments, particularly the Month 9 and Month 18 assessments, so that their experience is captured in the final analyses. However, a patient may withdraw consent to participate in the study at any time.

Missing an occasional dose of study drug will not necessarily result in the patient being withdrawn from the study. However, if a patient misses 2 consecutive doses of study drug, the Investigator at the site and the Medical Monitor will determine whether treatment should be discontinued.

4.5.2 Handling of Withdrawals or Patients who Discontinue from Treatment

In the event a patient discontinues treatment, withdraws, or is withdrawn from the study, the CRO Medical Monitor must be informed immediately. If there is a medical reason for withdrawal, the patient will remain under the supervision of the Investigator for protocol-specified safety follow-up procedures.

If a patient discontinues treatment, withdraws, or is withdrawn from the study, the primary reason for discontinuation must be recorded in the appropriate section of the CRF and all efforts will be made to complete and report the observations as thoroughly as possible.

If a patient withdraws or is withdrawn from the study, the site staff is to encourage the patient to complete the Early Withdrawal visit. Patients who withdraw or are withdrawn from the study, or patients who remain on the study and permanently discontinue study treatment for reasons other than rapid disease progression at Month 9 (see [Section 4.6](#)),

will not be eligible to participate in the planned extension study in which patients will receive open-label administration of 0.3 mg/kg patisiran once every 21 days.

4.5.3 Replacements

No replacements will be allowed for patients who withdraw early from the study.

4.6 Discontinuation Due to Rapid Disease Progression

At the 9-month time point, if the clinical adjudication committee determines that a patient is exhibiting rapid disease progression (defined as ≥ 24 point increase in mNIS+7 from baseline [based on an average of 2 measurements] and FAP stage progression relative to baseline), the patient's treating physician will provide the patient with the option of discontinuing study drug and receiving local standard of care treatment for FAP.

Patients who discontinue due to rapid disease progression will remain on study through completion of the 18-month efficacy assessments (blinding will be maintained throughout), following a modified visit schedule as shown in [Table 1-3](#). Patients who complete the 18-month efficacy assessments can elect to participate in an extension study in which patients will receive open-label administration of 0.3 mg/kg patisiran once every 21 days. Patients who discontinue study drug due to rapid disease progression and also decide to withdraw from the study will not have any additional visits after the Day 273 assessments are completed and will not be eligible to participate in the planned extension study.

4.7 Pregnancy and Breastfeeding Restrictions / Contraception Requirements

Women of child-bearing potential must have a negative pregnancy test, cannot be breastfeeding, and must be using 2 highly effective methods of contraception prior to screening, throughout study participation, and for 75 days after the last dose of study drug. Highly effective methods of birth control result in a low failure rate (i.e., less than 1% per year)⁴³. Acceptable forms of effective contraception are defined as follows:

- Hormonal: established use of oral, implantable, injectable, or transdermal hormonal methods of conception;
- Placement of an intrauterine device (IUD);
- Placement of an intrauterine system (IUS);
- Mechanical/barrier method of contraception: condom or occlusive cap (diaphragm or cervical/vault cap) in conjunction with spermicide (foam, gel, film, cream or suppository);

Note: Failure rates indicate that, when used alone, the diaphragm and condom are not highly effective forms of contraception. Therefore the use of additional spermicides does confer additional theoretical contraceptive protection. However, spermicides alone are inefficient at preventing pregnancy when the whole ejaculate is spilled. Therefore, spermicides are not a barrier method of contraception and should not be used alone.

- Surgical sterilization of male partner (with the appropriate post-vasectomy documentation of the absence of sperm in the ejaculate; for female patients on the study, the vasectomized male partner should be the sole partner for that patient);

- Sexual true abstinence: when this is in line with the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.

Patients should be instructed to use two different forms of effective contraception from the list above. Examples of two forms of highly effective contraception are as follows:

- Oral, implantable, injectable, or transdermal contraceptives in conjunction with condom or diaphragm and spermicide;
- IUD in conjunction with condom or diaphragm and spermicide;
- Surgical sterilization of partner in conjunction with condom or diaphragm and spermicide.

Males (including men who have had vasectomies) with partners of child-bearing potential, must agree to use 1 barrier method (e.g., condom) and 1 additional method (e.g., spermicidal foam, gel, film, cream, or suppository) of contraception throughout study participation and for 75 days (i.e., a whole spermatogenic cycle) after the last dose of study drug. Males must also abstain from sperm donation after the first dose of study drug through study participation and for 75 days after last dose of study drug.

Pregnancy reporting guidelines are provided in [Section 8.12](#).

5 STUDY MEDICATION

5.1 Presentation of Study Drug

Patisiran (ALN-TTR02) Solution for Injection is an RNAi therapeutic consisting of an siRNA targeting TTR mRNA formulated in a LNP. The patisiran drug product is a sterile formulation of TTR siRNA with lipid excipients (DLin-MC3-DMA, DSPC, cholesterol, and PEG₂₀₀₀-C-DMG) in isotonic phosphate buffered saline. Patisiran Solution for Injection contains 2 mg/mL of TTR siRNA drug substance.

The patisiran drug product is packaged in 10 mL glass vials with a fill volume of 5.5 mL. The container closure system consists of a United States Pharmacopeia/European Pharmacopoeia (USP/EP) Type I borosilicate glass vial, a Teflon-faced butyl rubber stopper, and an aluminium flip-off cap.

The control drug for this study will be a placebo (normal saline 0.9% for IV administration). Control drug will be provided by a central supplier.

5.2 Preparation of Study Drug

Each investigational site will be responsible for IV preparation and labeling, according to separate handling instructions, and allocating treatments to the patients.

The pharmacist or unblinded study personnel will prepare the study drug under aseptic conditions. The total amount to be infused into each patient at each dosing visit will be 200 mL. To maintain the blind, all IV infusion bags and lines will have amber-colored covers added prior to leaving the pharmacy.

Additional study drug preparation details are provided in the patisiran (ALN-TTR02) Pharmacy Manual.

5.2.1 Preparation of Patisiran

The amount (in mg) of patisiran to be administered will be determined based on the patient's body weight (kg). For each dose administered, the weight obtained during the previous visit will be used to calculate the dose of study drug. In the event that the weight obtained on the previous dosing day is not available, the weight obtained predose on the dosing day can be used for dose calculation. Patients who weigh 105 kg or more will receive patisiran dosing based on an assumption of a body weight of 104 kg.

Sterile normal saline (0.9% NaCl) will be added to a sterile infusion bag, and the calculated volume of patisiran will be withdrawn from the vials and injected into the infusion bag.

5.2.2 Preparation of Placebo

Normal saline (0.9% NaCl), provided by a central supplier, will be infused into patients randomized into the control group. Depending on the availability, saline may need to be withdrawn from a larger bag or additional saline may need to be added to a sterile infusion bag to ensure the 200 mL volume.

5.3 Drug Administration

5.3.1 Premedication

Prior to each dose of study drug, in order to reduce the risk of experiencing an IRR, patients will receive the following premedications at least 60 minutes prior to the infusion:

- Intravenous dexamethasone (10 mg) or equivalent;
- Oral paracetamol/acetaminophen (500 mg) or equivalent;
- Intravenous H2 blocker (e.g., ranitidine 50 mg, famotidine 20 mg, or equivalent other H2 blocker dose);
- Intravenous H1 blocker: diphenhydramine 50 mg (or equivalent other IV H1 blocker available at the study site). Hydroxyzine or fexofenadine 25 mg per os (PO, orally) or cetirizine 10 mg PO may be substituted for any patient who does not tolerate IV diphenhydramine or other IV H1 blocker.

Patients will be started on the above premedication regimen. However, modifications may be made to the premedication regimen after consultation with the medical monitor either due to a patient's inability to tolerate one or more of the premedications or to the occurrence of IRRs unresponsive to slowing of the infusion rate.

- If a patient is having difficulty tolerating the steroid premedication regimen (e.g., patient develops uncontrolled hyperglycemia, altered mental status, or other complication), then lowering of the steroid premedication may be allowed for that patient after consultation with the medical monitor.
- If after consultation with the medical monitor, it is agreed that an individual patient's steroid premedication will be lowered, then the following steps **must be followed**:
 - 1) If the current steroid dosage is 10 mg intravenous dexamethasone or equivalent, then the patient may be lowered in increments no greater than 2.5 mg intravenous dexamethasone or equivalent.
 - 2) After each incremental lowering below 10 mg intravenous dexamethasone or equivalent, the patient must receive three consecutive intravenous doses of patisiran without IRR and continued signs or symptoms of steroid intolerance before further reductions in steroid premedications.
 - 3) The premedication steroid dosage should not be reduced below 5 mg intravenous dexamethasone or equivalent.

Alternatively, if a patient experiences an IRR when 10 mg or less of intravenous dexamethasone or equivalent is used as the steroid premedication, then proceed to [Section 5.9](#).

Further details (including a table of equivalent premedications) can be found in the patisiran (ALN-TTR02) Pharmacy Manual.

5.3.2 Study Drug Administration

Patients who are randomized into the active treatment group will receive 0.3 mg/kg patisiran once every 21 days (\pm 3 days). Patients who are randomized into the control group will receive placebo (normal saline 0.9%) once every 21 days (\pm 3 days).

The body weight that was obtained during the previous visit must be used to calculate the dose of study drug. In the event that the body weight obtained on the previous dosing day is not available, the body weight obtained pre-dose on the dosing day can be used for the dose calculation. Patients who weigh 105 kg or more will receive patisiran dosing based on an assumption of a body weight of 104 kg.

Study drug (either patisiran or placebo) will be administered under the supervision of the unblinded site personnel, as a 70-minute IV infusion (approximately 1 mL/minute for the first 15 minutes followed by approximately 3 mL/minute for the remainder of the infusion). The study drug will be administered via a controlled infusion device with an extension set containing a 1.2 micron filter supplied by Alnylam or designee. Infusion products must not contain polyvinyl chloride, di(2-ethylhexyl)phthalate (PVC, DEHP). As described in [Section 4.4.3](#), the tubing and the lines will be covered so that it will not be possible to detect a difference in active versus placebo drug.

- If the patient experiences an IRR the infusion time may be extended up to 3 hours in the event of a mild or moderate IRR.
 - 1) If the patient experiences a mild or moderate IRR that resolves by slowing the infusion rate then no further premedication changes are recommended.
 - 2) If the patient experiences a severe IRR, then study drug administration will not be resumed until the case is discussed with the medical monitor (see [Section 5.9](#)).
- If the infusion time for a patient was extended due to an IRR, that modified infusion duration should be continued throughout the duration of the study for that particular patient. Returning the patient's infusion duration to 70 minutes will require a discussion with the medical monitor.
- For the first 3 infusions of study drug on this study, the patient's infusion site should be assessed for signs of any localized reaction during the infusion and for 30 minutes after the end of the infusion, and the patient will remain at the clinical site for 1 hour following completion of dosing.

If a patient does not receive a dose of study drug within the dosing window (\pm 3 days), the dose will be considered missed and not administered.

Additional details can be found in the patisiran (ALN-TTR02) Pharmacy Manual.

In addition, patients will receive an oral daily supplemental dose of the recommended daily allowance of vitamin A.

5.4 Storage of Study Drug

All study drug must be stored in a secure, temperature controlled location and may be dispensed only by a staff member specifically authorized by the Investigator or by a

pharmacist, as appropriate. All study drug will be stored upright and refrigerated at approximately $5 \pm 3^{\circ}\text{C}$. Any deviation from the recommended storage conditions must be reported to the CRO and/or Alnylam and use of the study drug halted until authorization for its continued use has been given by Alnylam or designee.

No special procedures for the safe handling of patisiran are required. An unblinded Alnylam Monitor or designee will be permitted, upon request, to audit the supplies, storage, dispensing procedures, and records.

No study product(s) may be administered to any person not enrolled in the study.

Additional preparation details are provided in the patisiran (ALN-TTR02) Pharmacy Manual.

5.5 Labeling and Packaging of Study Drug

All packaging and labeling as well as the preparation of patisiran and placebo will be in compliance with Good Manufacturing Practice (GMP) specifications, as described in the Manufacture of Investigational Medicinal Products Volume 4 Annex 13, and any other or local applicable regulations.

Study drug labels will include all appropriate local labeling requirements on the vial and external label. Sample labels will be submitted to health authorities, per local country submission requirements.

5.6 Measurement of Patient Compliance

Treatment compliance with study drug administration is dependent on the proper preparation and administration of IV infusions by unblinded study site personnel as well as attendance by the patient to the clinic. Treatment compliance with study drug administration will be verified by unblinded study staff observation. A dose will be considered completed if 80% or more of the total volume of the IV solution has been administered to the patient. Patients will be permitted to miss an occasional dose of study drug. However, if a patient misses 2 consecutive doses, the PI, in consultation with the Medical Monitor, will discuss whether the patient will be able to continue on the study.

Patients failing to complete the 18-month efficacy assessment visit will not be eligible to receive patisiran on the open-label extension study.

5.7 Study Drug Accountability

The Investigator will maintain accurate records of receipt and the condition of all study drugs including dates of receipt. In addition, accurate records will be kept of the weight used to calculate each dispensed patisiran dose, and when and how much study drug is dispensed and used by each patient in the study. Any reasons for departure from the protocol dispensing regimen must also be recorded.

Drug accountability records and inventory will be available for verification by unblinded Alnylam Monitor or designee that will not have a role in any other aspect of managing the study. Remaining study drug (all used, partially used, and unused vials) will be returned to Alnylam or its specified designee/depot or destroyed at the site according to applicable regulations.

Study drug must not be used for any purpose other than the present study. Study drug which has been dispensed for a patient and returned unused must not be redispensed.

Further instructions about study drug accountability are detailed in the patisiran (ALN-TTR02) Pharmacy Manual.

5.8 Concomitant Medication / Treatment

Use of the following medications/treatments is prohibited during study participation (with the exclusion of patients who have rapid disease progression and discontinue study drug after the 9-month efficacy assessments):

- Any investigational agent other than patisiran;
- Tafamidis (use prior to screening permitted);
- Diflunisal (use prior to screening permitted);
- Doxycycline/TUDCA (use prior to screening permitted);
- Corticosteroids other than those administered as premedications prior to the dose of patisiran, those used to treat an infusion reaction, or topical or inhaled corticosteroids. However, for patients with chronic inflammatory disorders (e.g., asthma, rheumatoid arthritis, etc.), systemically administered steroids may be permitted provided that: 1) the dose is <20 mg/day prednisone or equivalent if administered chronically, or 2) for doses \geq 20 mg/day, administration is limited to no more than 5 consecutive days.

Medications and treatments other than those specified above, including palliative and supportive care approved by the investigator for disease-related symptoms, are permitted during the study.

Investigator should review over-the-counter (OTC) and or herbal preparations to ensure that these are not potentially disease modifying.

Use of all concomitant medications and treatments will be recorded on the patient's CRF through the time points shown in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#). This will include all prescription drugs, herbal preparations, OTC medications, vitamins, and minerals. Any changes in medications during the study will also be recorded on the CRF.

Any concomitant medication or treatment that is required for the patient's welfare may be given by the Investigator. However, it is the responsibility of the Investigator to ensure that details regarding the medication or treatment are recorded on the CRF, and coded using an internationally recognized and accepted coding dictionary.

5.9 Suggested Guidelines for Management of Infusion-related Reactions

Criteria for categorizing IRRs are provided in [Appendix 3](#).

- In the event of an IRR, the infusion of study drug may be stopped and the patient closely monitored until resolution of the reaction. Drugs that may be used to facilitate resolution and permit resumption of study drug administration include, but are not limited to: paracetamol/acetaminophen (or equivalent), additional histamine H1/H2 receptor antagonists (eg, ranitidine), NSAIDs, adrenaline, supplemental oxygen, IV fluids, and/or corticosteroids.

- Following resolution of a mild or moderate IRR that required interruption of the study drug infusion, resumption of administration may occur at the Investigator's discretion at a slower infusion rate (but not to exceed 3 hours) for that dose and for all subsequent doses of study drug. If the infusion is delayed, the administration of the infusion should be completed no more than 6 hours from the initial start of the infusion.
- Study drug administration will not be resumed for any patient following a severe IRR until the case is discussed with the Medical Monitor.
- If after consultation with the medical monitor it is agreed that an individual patient's steroid premedication will be increased then the following steps are **recommended**:
 - 1) If the IRR occurred while the patient received 10 mg intravenous dexamethasone or equivalent at least 60 minutes before the infusion and did not resolve with slowing of the infusion rate, then the patient should be increased by multiples of 5 mg intravenous dexamethasone or equivalent at least 60 minutes before the infusion and/or 5 mg oral dexamethasone or equivalent the night before the intravenous infusion.
 - 2) Increased dose of premedication steroids should NOT exceed the combination of 20 mg intravenous dexamethasone or equivalent on the day of infusion and 8 mg oral dexamethasone or equivalent taken the night before the infusion.
 - 3) If the IRR occurred while the patient received less than 10 mg intravenous dexamethasone or equivalent, then the patient should return to the prior dose of intravenous dexamethasone or equivalent that did not result in an IRR.

The patient's infusion site should be assessed for signs of any localized reaction during the infusion and for 30 minutes after the end of the infusion. The patient will remain at the study site for 1 hour following completion of dosing for observation and completion of assessments. Patients will be instructed to call the Investigator if they experience symptoms such as fever, chills, myalgia, or nausea/vomiting after discharge from the site.

6 STUDY VISITS

The duration of a patient's participation in this study is approximately 21 months (inclusive of a 42-day screening period and up to a 56-day post last dose study visit).

Screening evaluations are to be performed within 42 days before receiving the first dose of study drug, as indicated in [Table 1-1](#). Patients determined to be eligible based on Screening assessments will receive blinded study drug IV infusion of either patisiran (ALN-TTR02) or placebo once every 21 days. Patients will remain at the study site for 1 hour following completion of dosing for observation and completion of assessments.

In order to decrease the variability in the efficacy assessment testing, there will be a limited number of sites within any one territory that conduct efficacy assessments at Screening/Baseline, Baseline, 9-month, and 18-month visits (referred to as "Central Assessment Sites [CAS]"); these sites can also screen, dose, and manage patients. There will also be sites that screen, dose, and manage the patients ("Patient Care Sites [PCS]"), while sending the patients to the nearest CAS for their Screening/Baseline, Baseline, 9- and 18-month efficacy assessments.

Prior to starting the study and screening patients in the protocol, the CAS and PCS will create a delegation of responsibilities document that clearly details which site is responsible for which tests and safety parameters to ensure adherence to the protocol. This document will become part of the study site specific documentation.

All patients who discontinue from the study early will return to the study site for their Early Withdrawal assessments.

6.1 Screening, Screening/Baseline, and Baseline Visits (Days –42 to Day 0)

Screening evaluations will be conducted over 3 or more visits (Screening, Screening/Baseline, and Baseline). All screening visits must occur within 42 days of the first dose of study drug (Day 0). [Table 1-1](#) provides an overview of the schedule of events required at each screening visit.

Prior to screening activities, the patient will sign and date an informed consent form (ICF) and receive a copy of the signed ICF. No study procedures should be performed prior to informed consent being obtained. The Investigator or another person authorized by the Investigator will also sign and date the informed consent prior to giving a copy to the patient. The ICF will be filed in the patient's medical record.

In conjunction with the decision of the Medical Monitor(s), patients may be allowed to rescreen after a minimum of 5 days have elapsed from their last screening assessment.

6.1.1 Screening

The following activities must be performed during the Screening visit as part of the initial review of patient eligibility:

- Assess study eligibility using the inclusion and exclusion criteria;
- Obtain medical history information, including inquiry into HIV status;
- Obtain demographic information;

- Determine Karnofsky Performance Status (see [Appendix 1](#));
- Determine New York Heart Association classification of heart failure (see [Appendix 2](#));
- Collect and review documentation for TTR genotype;
- Perform a physical examination;
- Measure weight;
- Measure height;
- Measure vital signs;
- Collect blood samples for clinical laboratory tests, including:
 - Paraprotein (assessed by immunofixation electrophoresis [IFE]);
 - Vitamin B₁₂;
 - Serology;
 - Hematology;
 - Serum chemistries;
 - Coagulation studies;
 - Thyroid function tests.
- Collect urine sample for urinalysis;
- Perform a pregnancy test (for females of child-bearing potential only);
- Obtain information on concomitant medications;
- Perform NIS and NCS. Note: The documented results of previously performed NIS and NCS may be used to qualify a patient for this study if these tests were performed within 60 days prior to the date of informed consent. These studies may be completed as per local protocol.

6.1.2 Screening/Baseline Visit

Patients who meet all of the Screening criteria (including NIS and NCS assessments) will complete the screening process during 2 subsequent visits (which will be conducted at CAS for patients screened at PCS): a Screening/Baseline visit and a Baseline visit.

The Screening/Baseline visit must be performed within 21 days prior to the first dose of study drug (Day 0).

The following assessments must be performed during the Screening/Baseline visit:

- Perform the following efficacy assessments:
 - mNIS+7*;
 - NIS+7*;

*Note: Components that are shared between the mNIS+7 and NIS+7 (including NIS and NCS) will be performed only once.

- PND score;
- Collect blood sample for long-term frozen storage of serum to permit testing of additional proteins related to FAP;
- Measure vital signs;
- Measure weight;
- Obtain information on concomitant medications;
- Obtain an interval medical history;
- Confirm that NIS, PND, and NCS results meet study eligibility requirements. Inclusion Criteria 3 (i.e., NIS of 5 to 130 [inclusive] and PND score of $\leq 3b$) and 4 (i.e., NCS sum of the sural SNAP, tibial CMAP, ulnar SNAP, ulnar CMAP, and peroneal CMAP of ≥ 2 points).

Note: The following assessments may be performed at a single time point during either the Screening/Baseline visit or as noted below, at the discretion of the investigator.

At the Screening/Baseline or Baseline visit:

- Perform a 12-lead ECG;
- Complete the Norfolk QOL-DN, and COMPASS-31 questionnaires;
- Complete the C-SSRS questionnaire;

At any time after the patient is deemed eligible for participation in the study, but before the first administration of study drug:

- Perform a timed 10-meter walk test.

At any time after the patient is deemed eligible for participation in the study through the Day 0 visit:

- Perform a grip strength test;
- Perform an echocardiogram;
- Complete the EQ-5D and R-ODS questionnaires;

At any time after the patient is deemed eligible for participation in the study through the Day 21 visit:

- Perform an ophthalmology exam;
- Complete the pharmacoeconomics questionnaire.

6.1.3 Baseline Visit

The Baseline visit will occur at least 24 hours (approximately), but no greater than 7 days, from the Screening/Baseline visit. The following activities should be performed during the Baseline visit:

- Perform a 12-lead ECG (if not completed at the Screening/Baseline visit);
- Measure vital signs;

- Measure weight;
- Collect blood sample for serum TTR protein, RBP, and vitamin A. Additionally, aliquots of serum samples will be taken for long-term frozen storage, to permit testing of additional proteins related to FAP;
- Obtain information on concomitant medications;
- Perform the following efficacy assessments:
 - mNIS+7*;
 - NIS+7*;
*Note: Components that are shared between the mNIS+7 and NIS+7 (including NIS and NCS) will be performed only once.
 - PND score (see [Appendix 5](#));
 - FAP stage (see [Appendix 6](#));
 - If the patient has provided separate informed consent for the skin biopsies, obtain 2 sets of tandem 3-mm skin punch biopsies (4 biopsies total; 1 set from the distal lower leg, when a patient's clinical status allows, and 1 set from the distal thigh). Skin biopsies will be performed at a CAS;
 - mBMI (Note: The site will only measure the patient's weight. Using this weight, mBMI will be calculated programmatically in the clinical database; the site will not perform the calculation.);
 - Collect blood samples for NT-proBNP and troponin I;
 - Norfolk QOL-DN (if not completed at the Screening/Baseline visit);
 - COMPASS-31 (if not completed at the Screening/Baseline visit).
- Complete the C-SSRS questionnaire (if not completed at the Screening/Baseline visit);
- Obtain an interval medical history.

The following activities may also be completed at the Baseline visit if not completed at the Screening/Baseline visit, as described in [Section 6.1.2](#):

- Timed 10-meter walk test;
- Grip strength test;
- Echocardiogram;
- EQ-5D and R-ODS questionnaires;
- Ophthalmology examination at a CAS;
- Pharmacoeconomics questionnaire.

6.2 Treatment Visits

Patients who are determined to be eligible for the study will be enrolled on Day 0.

On all study visit days, patients should take their vitamin A supplement after completing the blood draws.

Prior to dosing, all patients will receive premedications in order to reduce the risk of experiencing an IRR as described in [Section 5.3.1](#).

6.2.1 Day 0

6.2.1.1 Pre-dose on Day 0

On Day 0, patients will undergo the following procedures prior to study drug administration:

- Measure weight;
- Measure vital signs;
- Timed 10-meter walk test (if not completed at the Screening/Baseline or Baseline visits);
- Collect blood samples for clinical laboratory tests, including:
 - Serum chemistries;
 - Anti-drug antibody testing.
- Collect blood sample for serum TTR protein, RBP, and vitamin A. Additionally, aliquots of serum samples will be taken for long-term frozen storage, to permit testing of additional proteins related to FAP;
- Collect blood sample for plasma PK assessment within 1 hour of planned study drug dosing start;
- Collect urine sample for PK assessment within 1 hour of planned study drug dosing start;
- Obtain information on concomitant medications;
- Randomize the patient (See [Section 4.4.1](#));
- Administer premedications at least 60-minutes prior to the start of administration of study drug (see [Section 5.3.1](#));
- Document any AEs.

The following activities are to be completed on Day 0 pre- or post-dosing if not completed at the Screening/Baseline or Baseline visits, as described in [Section 6.1.2](#):

- Grip strength test;
- Echocardiogram;
- EQ-5D and R-ODS questionnaires.

6.2.1.2 Administration of Study Drug on Day 0

As described in [Section 5.3.2](#), after completion of all pre-dose evaluations and procedures, study drug will be administered by a controlled infusion device as a 70-minute IV infusion (approximately 1 mL/minute for the first 15 minutes followed by approximately 3 mL/minute for the remainder of the infusion) or at a more prolonged infusion rate (up to 3 hours) if required due to prior IRR. The patient's infusion site should be assessed for signs of any localized reaction during the infusion and for 30 minutes after the end of the infusion.

The infusion time may be extended up to 3 hours in the event of a mild or moderate IRR. Study drug administration will not be resumed for any patient following a severe infusion reaction until the case is discussed with the Medical Monitor.

Suggested guidelines for management of IRRs are provided in [Section 5.9](#).

6.2.1.3 Post-dose on Day 0

The patient's infusion site should be assessed for signs of any localized reaction during the infusion and for 30 minutes after the end of the infusion. The patient will remain at the study site for 1 hour following completion of dosing for observation and completion of assessments:

- Measure vital signs;
- Collect a PK sample at the end of infusion (+5 minutes);
- Obtain information on concomitant medications;
- Document any AEs.

The following activities may also be completed on Day 0 if not completed at the Screening/Baseline or Baseline visits, as described in [Section 6.1.2](#):

- Ophthalmology examination at a CAS;
- Pharmacoeconomics questionnaire.

6.2.2 Routine Study Visits

The procedures described below are to be performed for all routine study visits (from Day 21 through Day 546), with the exception of the 9-month and 18-month efficacy assessments (occurring on Days 253-272 and Days 553-560, respectively). All visits have a window of " ± 3 days" except for the Day 273 visit which has a window of "+3 days" to allow adequate time for the assessment of the patient's 9-month mNIS+7 and FAP stage testing, which will determine if the patient met the criteria for Rapid Disease Progression.

Patients will undergo the following procedures before study drug dosing:

- At all dosing visits, administer premedications at least 60 minutes prior to the start of administration of study drug (see [Section 5.3.1](#));
- At all dosing visits, measure weight;
- At all dosing visits, measure vital signs;

- On Days 252 and 546, perform a physical examination;
- On Days 126 and 399, perform a 12-lead ECG;
- On Days 21, 126, 252, 273, 399, and 546, collect blood sample for serum TTR protein, RBP, and vitamin A. Additionally, aliquots of serum samples will be taken for long-term frozen storage, to permit testing of additional proteins related to FAP;
- On Days 84, 189, 357, 462, and 546, collect blood samples for serum chemistry tests;
- On Day 546, collect blood sample for INR;
- On Days 21, 126, 252, 399, and 546, collect a blood sample for anti-drug antibody assessment;
- On Days 252 and 546, collect blood samples for clinical laboratory tests, including:
 - Hematology;
 - Thyroid function tests.
- On Days 252 and 546, collect urine sample for urinalysis;
- On Days 21, 126, 252, 399, and 546, collect a plasma PK sample pre-dose (within 1 hour of planned study drug dosing);
- On Days 21, 126, 252, 399, and 546, collect a urine PK sample pre-dose (within 1 hour of planned dosing start);
- Perform ophthalmology examinations on Day 21 (if baseline examination was not completed prior to the Day 21 visit), once between Days 231(\pm 3) and 272 at a CAS, and once between Days 546(\pm 3) and 560 at a CAS;
- Complete the pharmacoeconomics questionnaire on Day 21, if not completed prior to the Day 21 visit;
- At all dosing visits, obtain information on concomitant medications;
- At all dosing visits, document any AEs.

Following the completion of all required pre-dose activities and assessments, administer the study drug at all dosing visits, as described in [Section 6.2.1.2](#).

Following dosing, collect plasma PK samples as follows:

- On Days 126 and 399, collect a plasma PK sample at the end of the infusion (+5 minutes);
- On Days 21, 252, and 546, collect a plasma PK sample 30 minutes after the end of the infusion (+15 minutes).

6.2.3 Efficacy Assessment Visits (9 Months and 18 Months)

Efficacy assessment will be performed on all patients at approximately 9 and 18 months (Days 253-272 and 553-560, respectively). Patients will not receive any study drug on these days.

As described in [Section 4.6](#), in consultation with the Investigator on Day 273 (+3 days), patients who meet the criteria for Rapid Disease Progression based on the 9-month efficacy assessments may continue study drug dosing or discontinue study drug dosing. Patients who discontinue study drug dosing may receive additional therapy for FAP, as per the local standard of care. These patients will remain on the study and follow the modified schedule of assessments shown in [Table 1-3](#) and described in [Section 6.4](#). Blinding will be maintained throughout.

The 9- and 18-month efficacy assessment visits will take place over 2 days.

- Perform the following efficacy assessments:

- mNIS+7*;
- NIS+7*;
- Timed 10-meter walk test*;
- Grip strength test*;

*Note: For the mNIS+7, NIS+7, timed 10-meter walk test, and grip strength test, an independent assessment will be performed on each day. Each site will make every effort to have these assessments performed by the same Investigator. Assessments are to be performed at least 24 hours (approximately) apart from each other but no greater than 7 days apart. For the mNIS+7 and NIS+7, components that are shared (including NIS and NCS) will be performed only once on each assessment day.

- PND score (see [Appendix 5](#));
 - FAP stage (see [Appendix 6](#));
 - If the patient has provided separate informed consent for the skin biopsies, obtain 2 sets of tandem 3-mm skin punch biopsies (4 biopsies total); 1 set from the distal lower leg when a patient's clinical status allows, and 1 set from the distal thigh. Skin biopsies will be performed at a CAS;
 - mBMI (Note: The site will only measure the patient's weight. Using this weight, mBMI will be calculated programmatically in the clinical database; the site will not perform the calculation.);
 - Echocardiogram;
 - Collect blood samples for NT-proBNP and troponin I;
 - Norfolk QOL-DN;
 - EQ-5D;
 - R-ODS;
 - COMPASS-31.
- Measure weight on both efficacy assessment days;
 - Measure vital signs on both efficacy assessment days;

- Complete the C-SSRS questionnaire;
- Complete the pharmacoeconomics questionnaire;
- Perform a 12-lead ECG;
- Collect blood samples for:
 - TTR protein, RBP, and vitamin A;
 - Additional aliquots of serum for long-term frozen storage, to permit testing of additional proteins related to FAP.
- Perform an ophthalmology examination (2 exams with 1 being completed between Days 231(\pm 3) and 272 at a CAS, and 1 completed between Days 546(\pm 3) and 560 at a CAS);
- Obtain information on concomitant medications;
- Document any AEs.

6.2.4 Follow-Up Visits

Patients will return to the study site for 2 follow-up visits, 21 days and 56 days after receiving their last dose of study drug. If a patient enrolls in the extension study, they will only have to complete the 21-day follow-up assessments (End of Study [EOS]; Day 567) and not the 56-day follow-up assessments (Day 602). Patients who do not enroll in the extension study will need to complete both follow-up visits (EOS [Day 567] and Day 602).

6.2.4.1 Twenty-One-Day Follow-up Visit (End of Study)

The following procedures will be performed at the 21-Day Follow-up visit (EOS; Day 567).

- Perform a physical examination;
- Measure weight;
- Measure vital signs;
- Collect blood sample for serum TTR protein, RBP, and vitamin A. Additionally, aliquots of serum samples will be taken for long-term frozen storage, to permit testing of additional proteins related to FAP;
- Perform a pregnancy test (for females of child-bearing potential only);
- Obtain information on concomitant medications;
- Document any AEs.

6.2.4.2 Fifty-Six-Day Follow-up Visit

This visit will only be conducted for patients who do not enroll in the extension study.

The following procedures will be performed at the 56-Day Follow-up visit (Day 602):

- Perform a physical examination;

- Measure vital signs;
- Collect blood sample for serum chemistry testing;
- Perform a pregnancy test (only for females of child-bearing potential);
- Obtain concomitant medications information;
- Document any AEs.

6.3 Early Withdrawal Visit and Follow-up Visits for Patients who Permanently Discontinue Study Treatment

6.3.1 Early Withdrawal Visit

For patients who withdraw early from the study, every effort will be made to have them return to the study site to have the following procedures performed. These visits will take place over 2 days.

- Perform the following efficacy assessments:
 - mNIS+7*;
 - NIS+7*;
 - Timed 10-meter walk test*;
 - Grip strength test*.

*Note: For the mNIS+7, NIS+7, timed 10-meter walk test, and grip strength test, an independent assessment will be performed on each day. Each site will make every effort to have these assessments performed by the same Investigator. Assessments are to be performed at least 24 hours (approximately) apart from each other but no greater than 7 days apart. For the mNIS+7 and NIS+7, components that are shared (including NIS and NCS) will be performed only once on each assessment day.

- PND score (see [Appendix 5](#));
- FAP stage (see [Appendix 6](#));
- If the patient has provided separate informed consent for the skin biopsies, obtain 2 sets of tandem 3-mm skin punch biopsies (4 biopsies total); 1 set from the distal lower leg when a patient's clinical status allows, and 1 set from the distal thigh;
- mBMI (Note: The site will only measure the patient's weight. Using this weight, mBMI will be calculated programmatically in the clinical database; the site will not perform the calculation.);
- Echocardiogram;
- Collect blood samples for NT-proBNP and troponin I;
- Norfolk QOL-DN;
- EQ-5D;

- R-ODS;
- COMPASS-31.
- Complete the pharmacoeconomics and C-SSRS questionnaires.
- Measure weight on both efficacy assessment days.
- Measure vital signs on both efficacy assessment days.
- Perform a physical examination.
- Collect a 12-lead ECG.
- Collect blood samples for clinical laboratory tests, including:
 - Hematology;
 - Serum chemistries;
 - Thyroid function tests;
 - Anti-drug antibody testing.
- Collect blood sample for serum TTR protein, RBP, and vitamin A. Additionally, aliquots of serum samples will be taken for long-term frozen storage, to permit testing of additional proteins related to FAP.
- Collect urine sample for urinalysis.
- Perform a pregnancy test (only for females of child-bearing potential).
- Collect a blood sample for plasma PK assessment;
- Collect a urine sample for PK assessment;
- Obtain information on concomitant medications;
- Document any AEs.

6.3.2 Follow-up Visits for Patients who Permanently Discontinue Study Treatment

Patients who permanently discontinue study treatment at any time after their first dose of study drug (due to decision made by treating physician or by the patient) may consent to return for follow-up visits at Months 9 and/or 18. If the patient discontinues study treatment >24 weeks prior to the Month 9 evaluation visit, the patient will complete follow-up visits at 9 and 18 months; if the patient discontinues study treatment after the Month 9 evaluation visit and >24 weeks before the Month 18 evaluation visit, the patient will complete a follow-up visit at 18 months.

These visits will take place over 2 days (at least 24 hours [approximately], but not more than 7 days apart). The following procedures will be performed:

- Perform the following efficacy assessments:
 - mNIS+7*;
 - NIS+7*;

*Note: For the mNIS+7 and NIS+7, an independent assessment will be performed on each day. Each site will make every effort to have these assessments performed by the same Investigator. Assessments are to be performed at least 24 hours (approximately) apart from each other but no greater than 7 days apart. For the mNIS+7 and NIS+7, components that are shared (including NIS and NCS) will be performed only once on each assessment day.

- PND score (see [Appendix 5](#));
 - FAP stage (see [Appendix 6](#));
 - mBMI (Note: The site will only measure the patient's weight. Using this weight, mBMI will be calculated programmatically in the clinical database; the site will not perform the calculation.);
 - Norfolk QOL-DN;
 - EQ-5D
- Perform a physical examination;
 - Measure weight on both efficacy assessment days;
 - Measure vital signs on both efficacy assessment days;
 - Collect blood sample for serum TTR protein, RBP, and vitamin A.
 - Determine Karnofsky Performance Status (see [Appendix 1](#));
 - Determine New York Heart Association classification of heart failure (see [Appendix 2](#));
 - Collect a blood sample for plasma PK assessment;
 - Collect data on subsequent FAP treatment regimens.

6.4 Modified Visit Schedule for Patients who Discontinue Study Drug for Rapid Disease Progression at Month 9

As described in [Section 4.6](#), in consultation with the Investigator, patients who meet the criteria for Rapid Disease Progression based on the 9-month efficacy assessments may continue study drug dosing or discontinue study drug dosing. Patients who discontinue study drug dosing may receive additional therapy for FAP, as per the local standard of care. These patients will remain on the study and follow a modified visit schedule as shown in [Table 1-3](#) and described in detail below. Blinding will be maintained throughout.

6.4.1 Modified Day 273

The following procedures will be performed on Day 273 (+3 days):

- Consult with the patient on a subsequent plan of care, which may include receiving therapy for FAP as per the local standard of care;
- Measure vital signs;

- Collect information on concomitant medications;
- Document any AEs.

Patients who discontinue treatment and also decide to discontinue from the study at the time of this visit will not have any additional visits after the Day 273 assessments are completed.

6.4.2 Modified Day 294

The following procedures will be performed:

- Perform a physical examination;
- Measure vital signs;
- Collect blood sample for serum chemistry testing;
- Collect blood sample for serum TTR protein, RBP, and vitamin A. Additionally, aliquots of serum samples will be taken for long-term frozen storage, to permit testing of additional proteins related to FAP;
- Collect a blood sample for plasma PK assessment;
- Collect a urine sample for PK assessment;
- Perform a pregnancy test (only for females of child-bearing potential);
- Obtain concomitant medications information (this will be the final collection of concomitant medications);
- Document any AEs (following this visit, only AEs that are considered related to study procedures will be collected);
- Collect data on subsequent FAP treatment regimens.

6.4.3 Modified Day 378 and Day 462

Study personnel will contact the patient by phone to query for general health status and data on subsequent FAP treatment regimens.

6.4.4 Modified Day 546

The following procedures will be performed:

- Perform a physical examination;
- Measure weight;
- Measure vital signs;
- Collect blood samples for clinical laboratory tests, including:
 - Hematology;
 - Serum chemistries and INR;
 - Thyroid function tests;
 - Anti-drug antibody testing.

- Collect urine sample for urinalysis;
- Perform a pregnancy test (only for females of child-bearing potential);
- Collect blood sample for serum TTR protein, RBP, and vitamin A. Additionally, aliquots of serum samples will be taken for long-term frozen storage, to permit testing of additional proteins related to FAP;
- Collect a blood sample for plasma PK assessment;
- Collect a urine sample for PK assessment;
- Complete the pharmacoeconomics questionnaire;
- Document any study-procedure-related AEs;
- Collect data on subsequent FAP treatment regimens.

6.4.5 Modified 18-Month Efficacy Assessment Visit

This visit will take place over 2 days. The following procedures will be performed:

- Perform the following efficacy assessments:
 - mNIS+7*;
 - NIS+7*;
 - Timed 10-meter walk test*;
 - Grip strength test*;

*Note: For the mNIS+7, NIS+7, timed 10-meter walk test, and grip strength test, an independent assessment will be performed on each day. Each site will make every effort to have these assessments performed by the same Investigator. Assessments are to be performed at least 24 hours (approximately) apart from each other but no greater than 7 days apart. For the mNIS+7 and NIS+7, components that are shared (including NIS and NCS) will be performed only once on each assessment day.

- PND score (see [Appendix 5](#));
- FAP stage (see [Appendix 6](#));
- If the patient has provided separate informed consent for the skin biopsies, obtain 2 sets of tandem 3-mm skin punch biopsies (4 biopsies total); 1 set from the distal lower leg when a patient's clinical status allows, and 1 set from the distal thigh;
- mBMI (Note: The site will only measure the patient's weight. Using this weight, mBMI will be calculated programmatically in the clinical database; the site will not perform the calculation.);
- Echocardiogram;
- Collect blood samples for NT-proBNP and troponin I;
- Norfolk QOL-DN;

- EQ-5D;
 - R-ODS;
 - COMPASS-31.
- Measure weight on both efficacy assessment days;
 - Measure vital signs on both efficacy assessment days;
 - Collect a 12-lead ECG;
 - Complete the C-SSRS questionnaire;
 - Document any study-procedure-related AEs.

6.4.6 Modified Twenty-One-Day Follow-up Visit (End of Study)

The following procedures will be performed:

- Perform a physical examination;
- Measure weight;
- Measure vital signs;
- Collect blood sample for serum TTR protein, RBP, and vitamin A. Additionally, aliquots of serum samples will be taken for long-term frozen storage, to permit testing of additional proteins related to FAP;
- Document any study-procedure-related AEs;
- Collect data on subsequent FAP treatment regimens.

6.4.7 Modified Early Withdrawal Visit

If the patient on the modified visit schedule discontinues from the study any time after the Day 273 assessments are completed (i.e., Day 274 onward), every effort will be made to have the patient return to the study site for the Modified Early Withdrawal visit. This visit will take place over 2 days (at least 24 hours [approximately], but not more than 7 days apart). The following procedures will be performed:

- Perform the following efficacy assessments:
 - mNIS+7*;
 - NIS+7*;
 - Timed 10-meter walk test*;
 - Grip strength test*;

*Note: For the mNIS+7, NIS+7, timed 10-meter walk test, and grip strength test, an independent assessment will be performed on each day. Each site will make every effort to have these assessments performed by the same Investigator. Assessments are to be performed at least 24 hours (approximately) apart from each other but no greater than 7 days apart. For the mNIS+7 and NIS+7, components that are shared (including NIS and NCS) will be performed only once on each assessment day.

- PND score (see [Appendix 5](#));
 - FAP stage (see [Appendix 6](#));
 - If the patient has provided separate informed consent for the skin biopsies, obtain 2 sets of tandem 3-mm skin punch biopsies (4 biopsies total); 1 set from the distal lower leg when a patient's clinical status allows, and 1 set from the distal thigh;
 - mBMI (Note: The site will only measure the patient's weight. Using this weight, mBMI will be calculated programmatically in the clinical database; the site will not perform the calculation.);
 - Echocardiogram;
 - Collect blood samples for NT-proBNP and troponin I;
 - Norfolk QOL-DN;
 - EQ-5D;
 - R-ODS;
 - COMPASS-31.
- Complete the pharmacoeconomics and C-SSRS questionnaires;
 - Perform a physical examination;
 - Measure weight on both efficacy assessment days;
 - Measure vital signs on both efficacy assessment days;
 - Collect a 12-lead ECG;
 - Collect blood sample for serum TTR protein, RBP, and vitamin A. Additionally, aliquots of serum samples will be taken for long-term frozen storage, to permit testing of additional proteins related to FAP;
 - Collect blood samples for clinical laboratory tests, including:
 - Serum chemistries;
 - Thyroid function tests;
 - Anti-drug antibody testing.
 - Perform a pregnancy test (only for females of child-bearing potential);
 - Document any study-procedure-related AEs;
 - Collect data on subsequent FAP treatment regimens.

6.5 Unscheduled Visits

Unscheduled visits for study assessments may occur if deemed necessary by the study personnel.

7 STUDY ASSESSMENTS

7.1 Demographic Data and Medical History

At the Screening visit, patient demographic data and a complete medical history will be obtained, including review of documentation of TTR genotype. At the Screening/Baseline visit and Baseline visit, an interval medical history will be obtained, capturing only changes since the Screening visit.

At the Screening visit, HIV status will be obtained. The Investigator will assess the patient according to the Karnofsky Scale (see [Appendix 1](#)) and the New York Heart Association Classification of Heart Failure (see [Appendix 2](#)).

7.2 Efficacy Assessments

Efficacy assessments will occur at Screening/Baseline, Baseline, 9 months, 18 months, and Early Withdrawal (if applicable). A central neurologic testing core facility will review the data derived from the various neuropathy measures at each participating site to ensure compliance with testing procedures and quality of the data. A central echocardiography core lab will analyze the echocardiogram data. A core lab will also be responsible for processing and analyzing skin punch biopsy samples.

The study personnel performing assessments related to the efficacy endpoints will be blinded to the results of any previous assessments (e.g., Screening/Baseline, Baseline, or 9-month assessments).

Further details on performing these assessments will be provided in the Study Reference Manual.

7.2.1 Modified Neurological Impairment Score +7

The mNIS+7 assessment tool is a composite measure of neurologic impairment which includes the following measures:

- Clinical exam-based neurologic impairment score (NIS-weakness and reflexes);
- Electrophysiologic measures of small and large nerve fiber function (+7) including:
 - NCS Σ5;
 - QST by body surface area including TP and HP.
- Autonomic function (postural blood pressure).

A summary of the scoring of the components of the mNIS+7 is provided in [Appendix 4](#).

At each time point, 2 independent assessments will be performed. Each site will make every effort to have these assessments performed by the same blinded study personnel, who will be different from the Investigator and other personnel managing the patient. Assessments are to be performed at least 24 hours (approximately) apart from each other but no greater than 7 days apart. In order to limit potential intra-operator bias, results of any of the prior assessments will not be available to the examiner when performing the second assessment.

Every effort will be made to use the same devices for NCS and QST for a patient throughout the duration of the study.

7.2.2 Neurological Impairment Score +7

The NIS+7 consists of the following measures:

- Full NIS (including weakness, sensation, and reflexes);
- Electrophysiologic measures of small and large nerve fiber function (+7) including:
 - NCS $\Sigma 5$;
 - VDT.
- HRdb.

A summary of the scoring of the components of the NIS+7 is provided in [Appendix 4](#).

At each time point, 2 independent assessments will be performed in the same manner as described above for mNIS+7. Every effort will be made to use the same devices for NCS and VDT for a patient throughout the duration of the study.

Components that are shared between the mNIS+7 and NIS+7 (including NIS and NCS) will be performed once at each assessment.

7.2.3 Familial Amyloidotic Polyneuropathy Stage and Polyneuropathy Disability Score

Changes in ambulation will be evaluated through the PND score and FAP stage (see [Appendix 5](#) and [Appendix 6](#), respectively).^{44,45}

7.2.4 Intraepidermal Nerve Fiber Density and Sweat Gland Nerve Fiber Density

Quantification of nerve fibers in skin will be assessed via IENFD and SGNFD. Patients who have provided additional voluntary consent for skin biopsy will undergo tandem 3mm skin punch biopsies for IENFD and SGNFD assessment. At each time point, 1 set of biopsies will be taken from the distal lower leg, when a patient's clinical status allows, and 1 set from the distal thigh (4 biopsies total).

A repeat baseline biopsy may be performed if the initial sample is not of sufficient quality.

Details on sample collection, processing, and storage will be provided in the Study Laboratory Manual.

7.2.5 Modified Body Mass Index

Sites will measure body weight at the time points specified in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#). Using that data, the mBMI will be calculated ($BMI \times \text{albumin}$). This calculation will take place programmatically in the clinical database; the sites will not perform the calculation.

7.2.6 Timed Ten-meter Walk Test

To perform the timed 10-meter walk, the patient will be asked to walk 10 meters. The walk must be completed without assistance from another person; ambulatory aids such as

canes and walkers are permitted. The time required for the patient to walk 10 meters will be recorded. At the 9-month, 18-month, and Early Withdrawal visits, the assessments will be conducted on 2 days, at least 24 hours (approximately), but not more than 7 days apart.

7.2.7 Grip Strength Test

Hand grip strength will be measured by dynamometer. Sites will be trained on dynamometer and testing for the study. When performing the test, patients will stand, holding the dynamometer in their dominant hand, with their arm parallel to the body without squeezing the arm against the body. Tests will be performed in triplicate on the same day for each time point.

Every effort will be made to use the same dynamometer for a patient throughout the duration of the study.

At the 9-month, 18-month, and Early Withdrawal visits, the assessments will be conducted on 2 days, at least 24 hours (approximately), but not more than 7 days apart.

7.2.8 Composite Autonomic Symptom Score

To evaluate changes in autonomic symptoms, patients will complete the COMPASS-31 questionnaire. The questionnaire consists of 31 clinically selected questions evaluating 6 autonomic domains (orthostatic intolerance, secretomotor, gastrointestinal, bladder, and pupillomotor).⁴⁶

7.2.9 Norfolk Quality of Life - Diabetic Neuropathy Questionnaire

Quality of life will be assessed through the Norfolk QOL-DN questionnaire, a standardized 47-item patient-reported outcomes measure that is sensitive to the different features of diabetic neuropathy (DN)-small fiber, large fiber, and autonomic nerve function.⁴⁷

7.2.10 EuroQoL Quality of Life Questionnaire

Quality of life will be assessed through the use of the EQ-5D, a standardized 5-question instrument for use as a measure of health outcomes.⁴⁸

7.2.11 Rasch-built Overall Disability Scale

An assessment of the disability each patient experiences will be assessed through the R-ODS.⁴⁹ The R-ODS is comprised of a 24-item linearly weighted scale that specifically captures activity and social participation limitations in patients.

7.2.12 Echocardiogram and Biomarkers of Cardiac Function

Cardiac structure and function will be assessed through echocardiograms as well as measurement of serum levels of the cardiac biomarkers NT-proBNP and troponin I.

Echocardiograms will be performed at the time points specified in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#) and interpreted at a central echocardiography core lab. Image acquisition, storage, and transfer guidelines will be provided in a Study Manual.

Blood samples will be drawn to measure levels of NT-proBNP and troponin I at the time points specified in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#). Details on sample collection, processing, and storage will be provided in a Study Laboratory Manual.

7.3 Pharmacodynamic Assessments

Pharmacodynamic sample collection, processing, and storage guidelines will be provided in a Study Laboratory Manual.

7.3.1 Transthyretin

Blood for serum TTR levels will be collected at the Baseline visit and prior to the administration of study drug at the time points specified in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#).

Serum TTR will be assessed using both ELISA (enzyme linked immunosorbent assay) and turbidimetric assays.

7.3.2 Retinol Binding Protein

Blood for serum RBP levels will be collected at the Baseline visit and prior to the administration of study drug at the time points specified in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#).

Serum RBP will be quantified using nephelometry.

7.3.3 Vitamin A

Blood for serum vitamin A levels will be collected at the Baseline visit and prior to the administration of study drug at the time points specified in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#). Supplemental vitamin A will be taken at the time points specified in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#).

7.3.4 Exploratory Biomarkers

To explore the expression of hepatocyte derived proteins to further characterize the biological effects of siRNA and/ or to explore possible metabolite profiling of patisiran (ALN-TTR02), serum and plasma samples will be collected at the time points specified in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#).

Details on biomarker sample collection, processing, and storage will be provided in a Laboratory Manual.

Biological samples for biomarker research and possible metabolic profiling can be retained on behalf of Alnylam for a maximum of 15 years following the last patient's last visit in the study.

7.4 Pharmacokinetic Evaluations

Details on PK sample collection, processing, and storage will be provided in a Laboratory Manual. Plasma and urine samples will be evaluated using a validated ATTO-Probe-HPLC (high performance liquid chromatography) assay to determine siRNA concentration and by LC/MS/MS for DLin-MC3-DMA and PEG₂₀₀₀-C-DMG concentrations.

7.4.1 Plasma Pharmacokinetics

Plasma samples will be collected at the time points shown in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#) for assessment of siRNA, DLin-MC3-DMA and PEG₂₀₀₀-C-DMG. PK parameters, including population PK will be analyzed, whenever possible as outlined in [Section 9.2.6](#).

7.4.2 Urine Pharmacokinetics

Urine samples and urine volume void will be obtained at specified time points as specified in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#). Renal clearance (CL_R) will be determined whenever possible for siRNA and 4-dimethylaminobutyric acid, metabolite of DLin-MC3-DMA, excreted in the urine. PK parameters will be analyzed, whenever possible, as outlined in [Section 9.2.6](#).

7.5 Safety Assessments

All safety assessment measures will be recorded in the patient's medical record and CRF.

7.5.1 Physical Examination

A complete physical examination (including general appearance; head, ears, eyes, nose, and throat [HEENT]; cardiovascular; dermatologic; abdominal; genito-urinary; lymph nodes; hepatic; musculoskeletal; respiratory; and neurological) is to be at the study visits listed in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#).

7.5.2 Body Weight and Height

Body weight will be measured at the study visits listed in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#). The rules for using body weight to calculate dose are described in [Section 5.3.2](#).

Height will only be measured at Screening.

7.5.3 Vital Signs

Vital signs will be measured at the time points specified in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#). Vital signs include systolic/diastolic blood pressure, pulse rate, respiratory rate, and temperature, and will be measured in the supine position using an automated instrument after the patient has rested comfortably for 10 minutes. Each patient's blood pressure should be taken using the same arm. Temperature will be recorded in Celsius or Fahrenheit. Heart rate will be counted for a full minute and recorded in beats per minute. Respirations will be counted for a full minute and recorded in breaths per minute.

For the safety of the patient, additional vital signs may be added at the discretion of the Investigator.

7.5.4 Electrocardiogram

Computerized 12-lead ECG recordings will be obtained in triplicate at each time point listed in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#) and read locally by a cardiologist or qualified physician. Each lead shall be recorded for at least 3 beats at a speed of 25 mm/s.

The following electrophysiologic parameters will be assessed: rhythm, ventricular rate, PR interval, QRS duration, and QT interval. If the ECG machine does not calculate the

heart rate corrected QT interval (QTc), either Bazett's and/or Fridericia's formula will be used to calculate the QTc.

For any clinically significant abnormal results, the Investigator must contact the CRO Medical Monitor to discuss continued participation of the patient in the study (e.g., ischemic ECG changes, wave/interval changes, or arrhythmia).

7.5.5 Clinical Laboratory Tests

Blood samples for clinical laboratory testing will be collected prior to study drug dosing at the time points listed in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#). Samples will be sent to a central laboratory for analysis. Details on sample collection, processing, and shipping will be provided in a Laboratory Manual.

In the event of an unexplained clinically relevant abnormal laboratory test occurring after study drug administration, the test should be repeated and followed up at the discretion of the Investigator until it has returned to the normal range or stabilized, and/or a diagnosis is made to adequately explain the abnormality.

The following clinical laboratory parameters are to be determined:

| Hematology | |
|---|--------------------------------|
| • Hematocrit | • Neutrophils, absolute and % |
| • Hemoglobin | • Lymphocytes, absolute and % |
| • Red blood cell (RBC) count | • Monocytes, absolute and % |
| • White blood cell (WBC) count | • Eosinophils, absolute and % |
| • Mean corpuscular volume | • Basophils, absolute and % |
| • Mean corpuscular hemoglobin | • Platelet count |
| • Mean corpuscular hemoglobin concentration | |
| Serum Chemistries | |
| • Aspartate transaminase (AST) | • Alkaline phosphatase |
| • Alanine transaminase (ALT) | • Bilirubin (total and direct) |
| • Sodium | • Glucose |
| • Potassium | • Phosphate |
| • Blood urea nitrogen (BUN) | • Albumin |
| • Creatinine | • Calcium |

| | |
|---|--|
| Coagulation Studies | |
| <ul style="list-style-type: none"> Prothrombin time (PT) Activated partial thromboplastin time (aPTT) | <ul style="list-style-type: none"> International Normalized Ratio (INR) |
| Thyroid Function Tests | |
| <ul style="list-style-type: none"> Thyroid stimulating hormone (TSH) Thyroxine (Free T4) | <ul style="list-style-type: none"> Triiodothyronine (Free T3) |
| Anti-drug Antibodies | |
| <ul style="list-style-type: none"> Anti-PEG antibodies | |
| Urinalysis | |
| <ul style="list-style-type: none"> Visual inspection for color and appearance pH Specific gravity Ketones Protein Glucose | <ul style="list-style-type: none"> Leukocytes Bilirubin Nitrite Urobilinogen Microscopic inspection of sediment |
| Serology | |
| <ul style="list-style-type: none"> Hepatitis B surface antibody (HbsAb) Hepatitis B surface antigen (HbsAg) | <ul style="list-style-type: none"> Anti-hepatitis C virus antibody (anti-HCVAb) |
| Cardiac Biomarkers | |
| <ul style="list-style-type: none"> N-terminal prohormone of B-type natriuretic peptide (NT-proBNP) Troponin I | |
| Other | |
| <ul style="list-style-type: none"> β-human chorionic gonadotropin (women of child-bearing potential only; may be a urine- or serum-based test) | |
| <ul style="list-style-type: none"> Vitamin B12 | |
| <ul style="list-style-type: none"> Paraprotein by IFE | |

7.5.5.1 Pregnancy Test

The pregnancy test may be urine- or serum-based, at the discretion of the Investigator. The test will only be performed for women of child-bearing potential. The timing of the tests is specified in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#); additional testing may be done any time pregnancy is suspected. The results must be known prior to administration of

study drug. Patients who are pregnant are not eligible for study participation. Patients who become pregnant while on study will be followed until the pregnancy outcome is known (see [Section 8.12](#)).

7.5.6 Ophthalmology Examination

The timing of the ophthalmology examinations is specified in [Section 6.2.2](#) and in [Table 1-1](#) and [Table 1-2](#). These examinations will be performed at a CAS and will include assessment of visual acuity, slit-lamp evaluation, intraocular pressure, dilated indirect ophthalmoscopy, color fundus photography, visual field, and electroretinography (ERG). Visual acuity should be evaluated at the beginning of each specified visit in the study (i.e., prior to slit-lamp examination). Manifest refraction will be performed at each specified visit prior to visual acuity testing and will be used to obtain a correction for visual acuity evaluations. Visual acuity testing should be done with best (most recent) correction. ERG evaluations will be centrally read.

Further details regarding the ophthalmology examinations will be provided in the Study Reference Manual.

7.5.7 Adverse Events and Study-Procedure-Related Adverse Events

Adverse events will be assessed and recorded at the time points specified in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#).

As shown in [Table 1-3](#), only study-procedure-related AEs (e.g., skin-biopsy-related AE, venipuncture-related AE) will be collected after Day 294 for patients who meet the criteria for Rapid Disease Progression at Month 9 and receive their last dose of study drug on Day 252 but remain on the study.

[Section 8](#) provides assessment and reporting guidelines.

7.5.8 Concomitant Medications and Treatments

Use of all concomitant medications and treatments will be recorded on the patient's CRF through the time points shown in [Table 1-1](#), [Table 1-2](#), and [Table 1-3](#). This will include all prescription drugs, herbal preparations, OTC medications, vitamins, and minerals. Any changes in medications during the study will also be recorded on the CRF.

[Section 5.8](#) provides guidelines on concomitant medications and treatments.

7.6 Other Assessments

7.6.1 Pharmacoeconomics Questionnaire

The burden of disease and healthcare utilization will be assessed using a patient-reported, 21-question pharmacoeconomics questionnaire. This assessment will be performed at a CAS.

7.6.2 Suicidality Questionnaire

The Columbia–Suicide Severity Rating Scale (C-SSRS) will be used to assess patient's mental status as it relates to suicidal ideation and behavior.⁵⁰ This questionnaire will be administered to the patient by trained study personnel.

7.6.3 Phone Contact for Health Status Update and FAP Treatment

Patients following the Modified Schedule of Assessments will be called on the telephone by study personnel at the time points specified in [Table 1-3](#). Patients will be asked about their general health status and any treatments they may have received for FAP.

8 REPORTING ADVERSE EVENTS

8.1 Adverse Event Definition

An AE is any untoward medical occurrence in a patient or clinical investigational patient administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment.

An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Disease progression (including worsening of neuropathy impairment) will not be considered an AE.

All IRRs will be recorded as AEs.

For patients who meet the criteria for rapid disease progression at Month 9 and receive their last dose of study drug on Day 252 but remain on the study, AEs will be followed through Day 294. Following Day 294, only study-procedure-related AEs will be collected (e.g., skin-biopsy-related AE, venipuncture-related AE).

8.2 Serious Adverse Event Definition

An SAE is any untoward medical occurrence that at any dose:

- Results in death;
- Is life-threatening (an event which places the patient at immediate risk of death from the event as it occurred. It does not include an event that had it occurred in a more severe form might have caused death);
- Requires inpatient hospitalization or prolongation of existing hospitalization;
- Results in persistent or significant disability/incapacity;
- Is a congenital anomaly or birth defect;
- An important medical event that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above (e.g., such events include allergic bronchospasm, blood dyscrasias or convulsions, or the development of drug dependency or abuse).

8.3 Eliciting Adverse Event Information

The patient should be asked about medically relevant changes in his/her health since the last visit. The patient should also be asked if he/she has been hospitalized, had any accidents, used any new medications, or changed concomitant medication routines (both prescription and OTC).

In addition to patient observations, AEs will be documented from any clinically relevant laboratory findings, physical examination findings, ECG changes, or other findings.

8.4 Adverse Event Reporting

The Investigator is responsible for reporting all AEs that are observed or reported after the first dose of study drug regardless of their relationship to study drug through until the end of the reporting periods defined in [Section 8.5](#). For patients who meet the criteria for Rapid Disease Progression at Month 9 and receive their last dose of study drug on Day 252 but remain on the study, AEs will be followed through Day 294. Following Day 294, only study-procedure-related AEs will be collected (e.g., skin-biopsy-related AE, venipuncture-related AE).

Any medical condition that is present when a patient is screened and does not deteriorate should not be reported as an AE. However, if it does deteriorate at any time during the study, it should be reported as an AE.

All AEs must be fully recorded in the site's source records and in the patients' CRF, whether or not they are considered to be drug-related. Each AE should be described in detail: onset time and date, description of event, severity, relationship to investigational product, action taken, and outcome (including time and date of resolution, if applicable).

Adverse events should be followed through until the end of the reporting periods defined in [Section 8.5](#) or until recovery to the normal state has been achieved, whichever occurs first. In the event of a patient not returning to the clinical unit, the outcome of this event will be recorded as lost at follow-up.

For patients who withdraw from the study early, ongoing AEs will be followed until resolution or 28 days from last dose, whichever occurs first.

8.5 Adverse Event Reporting Period

AEs and SAEs will be reported according to the following timeframes:

- For patients who complete the study, AEs will be assessed through the End of Study visit on Day 567 or the Follow-up visit on Day 602 (depending whether or not the patient plans to roll over to the open-label extension study). All AEs that occur after the start of study drug administration on Day 0 must be reported in detail on the appropriate CRF page and followed to satisfactory resolution, or through the end of study visit on Day 567 or Day 602 (depending whether or not the patient plans to roll over to the open label extension study) after the last dose of study drug administration. SAEs will be followed through the end of study visit on Day 567 or Day 602 (depending whether or not the patient plans to roll over to the open label extension study), or until satisfactory resolution, or until the SAE is considered by the Investigator to be chronic or the patient is stable, whichever occurs first.
- For patients who meet the criteria for Rapid Disease Progression at Month 9 and receive their last dose of study drug on Day 252 but remain on the study, AEs will be followed through Day 294. Following Day 294, only study-procedure-related AEs will be collected (e.g., skin-biopsy-related AE, venipuncture-related AE). SAEs will be followed by the Investigator until satisfactory resolution or the Investigator deems the SAE to be chronic or stable.

- For patients who withdraw from the study early, ongoing AEs will be followed until resolution or 28 days from last dose, whichever occurs first. SAEs will be followed by the Investigator until satisfactory resolution or the Investigator deems the SAE to be chronic or stable.

8.6 Assessment of Causality

Causal relationship assessment to drug treatments is required for purposes of reporting AEs. To promote consistency, the following guidelines should be taken into consideration along with good clinical and scientific judgment when determining the relationship of drug treatments to an AE:

Definitely Related: A clinical event, including laboratory test abnormality, occurring in a plausible time relationship to the medication administration, and which cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug should be clinically plausible.

Possibly Related: A clinical event, including laboratory test abnormality, with a reasonable time sequence to the medication administration, but which could also be explained by concurrent disease or other drugs or chemicals. Information on the drug withdrawal may be lacking or unclear.

Unlikely Related: A clinical event, including laboratory test abnormality, with little or no temporal relationship to medication administration, and which other drugs, chemicals, or underlying disease provide plausible explanations.

Not Related: A clinical event, including laboratory test abnormality, that has no temporal relationship to the medication or has more likely alternative etiology.

8.7 Assessment of Severity

Adverse events are to be graded according to the categories detailed below.

Mild: Mild events are those which are easily tolerated with no disruption of normal daily activity.

Moderate: Moderate events are those which cause sufficient discomfort to interfere with daily activity.

Severe: Severe events are those which incapacitate and prevent usual activity.

Changes in severity should be documented in the medical record to allow assessment of the duration of the event at each level of severity. Adverse events characterized as intermittent require documentation of the start and stop of each incidence. When changes

in the severity of an AE occur more frequently than once a day, the maximum severity for the experience that day should be noted. If the severity category changes over a number of days, then those changes should be recorded separately (with distinct onset dates).

8.8 Action Taken for Adverse Event

Action taken in regards to study drug will be defined as:

- None;
- Infusion interrupted and restarted at a later time;
- Infusion stopped and was not restarted at a later time;
- Infusion cycle delayed.

8.9 Outcome of Adverse Event

Outcome will be defined as:

- Resolved (with or without sequelae);
- Ongoing;
- Lost to follow-up.

8.10 Coding of Adverse Events

The Medical Dictionary for Regulatory Activities (MedDRA[®]) will be used to code AEs.

8.11 Serious Adverse Event Reporting

An assessment of the seriousness of each AE will be made by the Investigator. Any AE and laboratory abnormality that meets the above seriousness criteria (Section 8.2) must be reported immediately to the CRO, always within 24 hours from the time that site personnel first learn of the event. All SAEs must be reported regardless of the relationship to study drug.

The initial report should include at least the following information:

- Patient's study number;
- Description and date of onset of the event;
- Criterion for serious;
- Preliminary assignment of causality to study drug.

SAE reporting will be via electronic data capture (EDC).

To report the SAE, complete the SAE form electronically in the electronic data capture (EDC) system for the study. When the form is completed, [REDACTED] Safety personnel will be notified electronically and will retrieve the form. If the event meets serious criteria and it is not possible to access the EDC system, send an email to [REDACTED] [REDACTED] [REDACTED] or call the [REDACTED] SAE hotline (phone number will be provided in the Study Manual), and fax the completed back-up paper SAE form to [REDACTED] (fax number will be provided in the Study Manual) within

24 hours of awareness. When the EDC system becomes available, the SAE information must be entered into the EDC system within 24 hours of the system becoming available.

██████████ Safety personnel will be available for SAE reporting on a 24-hour basis. Incoming reports will be reviewed during normal business hours.

Within 24 hours of receipt of follow-up information, the investigator must update the SAE form electronically in the EDC system for the study and submit any supporting documentation (e.g., patient discharge summary or autopsy reports) to ██████████ Safety personnel via fax or e-mail. If it is not possible to access the EDC system, refer to the procedures outlined above for initial reporting of SAEs.

Appropriate remedial measures should be taken by the Investigator using his/her best medical judgment to treat the SAE. These measures and the patient's response to these measures should be recorded. All SAEs, regardless of relationship to study drug, will be followed by the Investigator until satisfactory resolution or the Investigator deems the SAE to be chronic or stable. Clinical, laboratory, and diagnostic measures should be employed by the Investigator as needed to adequately determine the etiology of the event.

The Investigator will be responsible for reporting all SAEs to the local independent ethics committee (IEC) or Institutional Review Board (IRB) when required by national regulations.

Alnylam or its representative will be responsible for the reporting of all relevant events to the concerned regulatory authorities according to all applicable regulations.

In Europe, in accordance with the Directive 2001/20/EC, the Competent Authorities and the Ethics Committees in the concerned Member States will be notified of fatal and life-threatening Suspected Unexpected Serious Adverse Reactions (SUSARs) as soon as possible but no later than 7 calendar days after Alnylam or its representative has first knowledge of the minimum criteria for expedited reporting. Non-fatal and non-life-threatening SUSARs should be reported no later than 15 calendar days after Alnylam or its representative has first knowledge of them.

The Investigator may be informed by Alnylam or its representative of SAEs from other Investigators or clinical studies which may have relevance to this clinical study. These SAEs should also be reported promptly to the IEC/IRB that approved the study. All SAE reports should be transmitted to the IEC/IRB with a cover letter or transmittal form, and a copy of that transmittal should be maintained in the Investigator's files and forwarded to Alnylam as part of the TMF on study completion.

8.12 Pregnancy Reporting

A female patient with a positive pregnancy test at Screening is ineligible for this study. If a female patient is found to be pregnant during the course of the study or during the first month after receiving the last dose of study drug, the Investigator should report the pregnancy to the CRO within 24 hours of being notified of the pregnancy. Details of the pregnancy will be recorded on the pregnancy reporting form. The patient should receive any necessary counseling regarding the risks of continuing the pregnancy and the possible effects on the fetus.

The pregnancy should be followed by the Investigator until completion. At the completion of the pregnancy, the Investigator will document the outcome of the pregnancy. If the outcome of the pregnancy meets the criteria for an SAE (i.e., postpartum complication, spontaneous abortion, stillbirth, neonatal death, or congenital anomaly), then the Investigator should follow the procedures for reporting an SAE outlined above.

Pregnancy occurring in the partner of a patient participating in the study should also be reported to the Investigator, who will then report this to the CRO and follow to outcome as described above.

9 STATISTICAL METHODS

9.1 Sample Size

Approximately 200 patients will be enrolled in this study. Of those 200 patients, no more than 40 patients will have a NIS range of 101 to 130. An mNIS+7 progression rate (primary endpoint) in the placebo group of 24 ± 16 points in 18 months was estimated using natural history data from FAP patients. A sample of 154 patients provides 90% power for a 2-sided test with an 8.95 point (37.5%) mean difference between treatment groups in the primary endpoint at 2-sided alpha = 0.05. Assuming a 25% random premature discontinuation rate (due to liver transplantation or other factors), the sample size for this study is approximately 200. Additional patients may be enrolled based on a recommendation to increase the sample size in the interim analysis.

9.2 Statistical Methodology

A full statistical analysis plan will be finalized prior to database lock.

9.2.1 Populations to be Analyzed

The following patient populations (i.e., analysis sets) may be evaluated and used for presentation of the data:

- Modified ITT (mITT) population: All patients who were randomized and received at least 1 dose of study drug;
- Per protocol (PP) population: All patients who completed the 18-month efficacy assessment visit and did not have any major protocol violations;
- Safety population: All patients who received at least 1 dose of study drug (analyzed as treated, not as randomized).

The primary population for efficacy analyses will be the mITT population; key efficacy results will also be analyzed secondarily for the PP population. For efficacy analyses, patients will be grouped according to the treatment to which they were randomized. The primary population for safety analysis will be the safety population. Patients will be grouped according to treatment received for summaries of safety.

9.2.2 Baseline Evaluations

Demographic and baseline disease characteristic data will be summarized. Data to be tabulated will include sex, age, and race, as well as disease-specific information.

9.2.3 Efficacy Analyses

9.2.3.1 Primary Efficacy Endpoint

The primary analysis will compare change in mNIS+7 from baseline between treatment groups of the mITT population. An analysis of covariance (ANCOVA) model, with baseline mNIS+7 value and age as continuous covariates and genotype (V30M vs Non-V30M), prior tetramer stabilizer (tafamidis or diflunisal) use (Yes vs No), and treatment group (patisiran [ALN-TTR02] vs Placebo) as factors will be employed to analyze the primary mNIS+7 endpoint. The mNIS+7 change from baseline will be assessed at 78 weeks. Primary endpoint data that are missing will be inferred using multiple

imputation (MI) (see [Section 9.2.10](#)). Analyses will be conducted using PROC MI and PROC MIANALYZE in SAS 9.2 (or later). The efficacy of patisiran will have been established if the estimate of the parameter associated with treatment variable demonstrates that patisiran improves mNIS+7 relative to placebo with a p-value (2-sided) less than or equal to 0.05, based on the MI methodology.

Sensitivity analyses will assess the robustness of the primary analysis in the mITT and PP populations for different methods of handling missing data, including mixed model repeated measures (MMRM), complete cases (CC), and last observation carried forward (LOCF).

9.2.3.2 Secondary Efficacy Endpoints

Secondary efficacy endpoints will be analyzed using methods similar to those employed for the primary analysis, e.g. ANCOVA with multiple imputations, as appropriate.

Type I error control for secondary endpoints will be achieved by a hierarchical ordering procedure. Endpoints will be tested in the following prespecified hierarchy:

- Norfolk QOL-DN questionnaire;
- NIS-W score;
- mBMI;
- Timed 10-meter walk test;
- COMPASS-31.

If and only if a comparison is significant at $p < 0.05$, the next endpoint in the hierarchy may be tested.

9.2.3.3 Exploratory Efficacy Endpoints

Continuous exploratory efficacy variables, including those closely related to the mNIS+7 primary endpoint, may be compared using methods similar to those employed for the primary analysis, e.g. ANCOVA with multiple imputations, as appropriate. Binary secondary endpoints will be assessed by the Cochran-Mantel-Haenszel test, stratified by the randomization stratification factors.

9.2.4 Safety Analyses

A summary of study drug exposure, including the durations of the infusions and doses, and the proportions of patients with modifications in the durations of infusions will be produced.

Adverse events will be summarized by MedDRA system organ class and preferred term. Separate tabulations will be produced for all treatment emergent AEs, treatment-related AEs (those considered by the Investigator as at least possibly drug related), SAEs, and discontinuations due to AEs. By-patient listings will be provided for deaths, SAEs, and events leading to discontinuation of treatment.

Descriptive statistics will be provided for clinical laboratory data and vital signs data, presented as both actual values and changes from baseline relative to each on-study evaluation and to the last evaluation on study.

Descriptive statistics will be provided for ECG interval data and presented as both actual values and changes from baseline relative to each on-study evaluation and to the last evaluation on study. Details of any abnormalities will be included in patient listings.

9.2.5 Pharmacodynamics

Summary tables and graphical displays of observed values and changes from baseline in serum TTR will be used to assess the durability of suppression over the course of the study. Similar analyses will be performed for the secondary PD biomarkers (RBP and vitamin A).

9.2.6 Pharmacokinetics

Pharmacokinetic analyses will be conducted using non-compartmental and/or compartmental evaluation. Whenever possible, the PK parameters of siRNA, DLin-MC3-DMA, and PEG₂₀₀₀-DMG (lipid) in sparse plasma samples collected from all patients. PK parameters will be calculated using a validated version of WinNonlin® Enterprise (Version 5.2 or higher) with NCA Model 200.

Population PK analyses, will be performed whenever possible, on available siRNA, DLin-MC3-DMA, and PEG₂₀₀₀-C-DMG from sparse plasma samples obtained from all patients during the duration of the study using Phoenix NLME (Version 1.1 or later). Summary tables and figures and inferential statistics will be generated with Phoenix NLME (Version 1.1 or later) or similar software.

Pharmacokinetic/PD analysis will be conducted whenever possible, of sparse plasma samples collected during the study period. The analysis will include, but not limited to, the determination of the relationship between exposure to siRNA, DLin-MC3-DMA, and PEG₂₀₀₀-C-DMG and the extent of suppression of TTR, RBP, and vitamin A and their correlation will be evaluated. Correlation between TTR versus RBP and vitamin A will also be performed. The strength of the relationship will be assessed using statistical estimators. As part of the PK/PD analysis, additional PD baseline analysis on serum TTR, RBP, and vitamin A may be conducted to include the PD data obtained post-PD recovery period. The PD and PK/PD analysis will be performed with WinNonLin or Phoenix NLME (Version 1.1 or later) or similar software. The PD and PK/PD parameters summary tables and figures and inferential statistics will be generated and will not be limited to descriptive statistics.

9.2.7 Summary of Efficacy Assessments

Summary statistics of observed values and changes from baseline will be provided for the mNIS+7 composite score. Summaries will also be provided for the components of the composite score (e.g., the NIS weakness and reflex scores, the NCS Σ 5, and QST values). The NIS+7 score, including its components (e.g. full NIS, HRdb, VDT) will also be summarized.

Patient reported quality of life and disability will be assessed by summary statistics for the Norfolk QOL-DN, EQ-5D, and R-ODS. Summary statistics will be provided for observed values and changes from baseline. Patient reported autonomic neuropathy symptoms will be assessed by descriptive statistics for the COMPASS-31.

Descriptive statistics will also be provided for observed values and changes from baseline in motor function (10-meter walk test and test of grip strength), nutritional status (mBMI), sensory and autonomic innervation (IENFD and SGNFD), and ambulation (FAP stage and PND score). Descriptive statistics will also be provided for proportions of patients in each group that meet the rapid progression definition at 9 months and those that meet the responder criterion at 18 months.

9.2.8 Other Assessments

The observed values and changes from baseline in burden of disease and healthcare utilization will be evaluated and summarized using descriptive statistics. Data on suicidality will be summarized by treatment group using descriptive statistics.

9.2.9 Interim Analysis

Because the sample size estimates are based on limited data for variance and disease progression, it is intended that an interim analysis will be conducted by an independent committee when approximately 50% of patients have completed their 9-month mNIS+7 assessment. This interim analysis will be blinded and will only estimate the overall variance observed in the primary endpoint. Since the interim analysis examines only the pooled variance of all patients in a blinded fashion, it does not require an adjustment to the overall alpha level for the test of the primary endpoint. Based on the results of the interim analysis, the committee can recommend either increasing the study size or making no adjustment to the sample size. Details regarding the Interim Analysis Committee are provided in [Section 10.3.3](#).

9.2.10 Missing Data

Patients who discontinue due to rapid disease progression will have their 78-week mNIS+7 change from baseline value imputed using a stepwise regression approach for identification of explanatory variables (e.g., demographics, stratifying variables, and baseline/9-month mNIS+7 data when available); treatment assignment will not be included in the imputation. Imputed datasets will then be analyzed as complete cases via the ANCOVA model specified for the primary analysis, and then combined to produce inferential results. Further details on imputations and sensitivity analyses will be included in the SAP that will be finalized before database lock.

10 STUDY MANAGEMENT

The Investigator is accountable for the conduct of the study. If any responsibilities are delegated, the Investigator should maintain a list of appropriately qualified staff to whom he/she has delegated specified significant trial related duties.

10.1 Data Handling and Quality Assurance

10.1.1 Case Report Forms

The Investigator and designees agree to maintain accurate CRFs and source documentation as part of these case histories. Source documents are the originals of any documents used by the Investigator or hospital/institution that allow verification of the existence of the patient and substantiate the integrity of the data collected during the trial.

Alnylam will supply CRFs for each patient. Case report forms must be completed only by persons designated by the Investigator. Corrections must be made so as not to obliterate original data and must be identified and dated by the person who made the correction. All data entered into the CRF must also be available in the source documents. The Investigator will allow designated Alnylam representatives and regulatory bodies to have direct access to the source documents to verify the data reported in the CRFs.

Each completed CRF must be reviewed and signed by the Investigator or designee in a timely manner. The completed CRF will be the records maintained by Alnylam. A copy of the CRF will remain in the Investigator's files.

10.1.2 Monitoring

The clinical monitor, as a representative of Alnylam, has an obligation to follow the study closely. In doing so, the monitor will visit the Investigators and sites periodically as well as maintain frequent telephone and written contact. The monitor will maintain current personal knowledge of the study through observation, review of study records and source documentation and discussion of the conduct of the study with the Investigator and staff.

All aspects of the study will be carefully monitored by Alnylam or its designee for compliance with applicable government regulations in respect to Good Clinical Practice (GCP) and current standard operating procedures.

10.1.3 Inspections

The Investigator will permit trial-related monitoring, audits and review by the IEC or IRB and/or Regulatory Authorities, providing direct access to source data/documents. The study may be subject to audit by Alnylam or its representatives or by regulatory authorities. If such an audit occurs, the Investigator must agree to allow access to the required patient records. In the event of an audit, the Investigator agrees to allow Alnylam, representatives from Alnylam, or regulatory agencies access to all study records.

10.2 Regulatory Guidelines

This study will be performed in accordance with the clinical trial agreement, the protocol, all applicable government laws, regulations, and guidances where the study is being conducted including policies with foundations in the World Health Organization (WHO)

Declaration of Helsinki, the International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use, the Health Insurance Portability and Accountability Act of 1996 (“HIPAA”), and all other applicable medical privacy laws and regulations.

10.2.1 Institutional Review Board/Independent Ethics Committee

National regulations and ICH require that approval be obtained from an IRB or an IEC prior to participation of patients in research studies. Prior to the study onset, the protocol, any protocol amendments, ICFs, advertisements to be used for patient recruitment, and any other written information regarding this study to be provided to a patient or patient’s legal guardian must be approved by the IRB or IEC.

All IRB and IEC approvals must be dated and contain IRB/IEC Chairman or designee authorization and must identify the IRB/IEC (e.g., name and address), the clinical protocol by title and/or protocol number, and the date of approval or favorable opinion was granted for the clinical research.

No drug will be released to the site to dose a patient until written IRB/IEC authorization has been received by Alnylam or designee.

The Investigator is responsible for obtaining continuing review of the clinical research at least annually or more often if specified by the IRB or IEC. The Investigator must supply Alnylam with written documentation of the approval of the continued clinical research.

The Investigator will make all attempts to ensure that the IRB or IEC is constituted and operates in accordance with Federal and ICH GCP and any local regulations.

10.2.2 Regulatory Authorities

Regulatory authorities will receive the protocol, amendments, reports on SAEs, and the Integrated Clinical Trial Report according to national and any local regulations.

10.2.3 Modification of the Protocol

Major changes in this research activity, except those to remove an apparent immediate hazard to the patient, must be reviewed and approved by Alnylam and the IRB or IEC that approved the study. Amendments to the protocol must be submitted in writing to the Investigator’s IRB or IEC and the Regulatory Authority for approval prior to patients being enrolled under the amended protocol.

10.2.4 Informed Consent Form

Written informed consent in compliance with 21 Code of Federal Regulations (CFR) § 50 and ICH will be obtained from each patient prior to undergoing any protocol-specific tests or procedures that are not part of routine care.

Alnylam or the CRO designee will provide an ICF template to the Investigator for use in developing a site-specific ICF. Prior to submission of the site-specific ICF to the IRB or IEC, the site-specific ICF must be reviewed and approved by Alnylam or designee. Any changes requested by the IRB or IEC must also be agreed upon. The final IRB/IEC approved ICF must be provided to Alnylam. Revisions to the ICF required during the study must be agreed upon, and a copy of the revised ICF provided to Alnylam.

At the time of recruitment, each prospective patient (or legal guardian) will be given a full explanation of the study and be allowed to read the ICF. Once the Investigator is assured that the patient/legal guardian understands the commitments of participating in the study, the patient/legal guardian will be asked to sign and date the ICF. A copy of the fully signed and dated ICF will be given to the patient. The original will be maintained in the patient's medical record at the site. All active patients will sign an updated ICF if revisions are made to the ICF during the course of the study.

10.2.5 Study Reporting Requirements

The Investigator will submit reports of SAEs as outlined in this protocol. In addition, the Investigator agrees to submit progress reports to his/her IRB or IEC per their local reporting requirements, or at least annually and at the conclusion of the study. The reports will be made available to AInylam or designee.

Deviations from the protocol necessary to protect patient safety should be reported to the CRO within 24 hours of knowledge of the event.

Any communications from regulatory agencies in regard to inspections, other studies that impact this protocol or the qualifications of study personnel should be promptly reported to the CRO.

10.2.6 Financial Disclosure Reporting Obligations

Each Investigator (including principal and any sub-investigators) directly involved in the treatment or evaluation of study patients is required to provide financial disclosure information according to all applicable legal requirements. In addition, Investigators must commit to promptly updating this information if any relevant changes occur during the study and for a period of one year after the completion of the study.

10.3 Study Committees

10.3.1 Data Monitoring Committee

A Data Monitoring Committee (DMC) will be involved in the conduct of this study. The DMC has the responsibility for monitoring the progress of the clinical study and the safety of the study participants. The DMC will perform periodic reviews of data and study conduct during the course of the clinical trial, as defined in the DMC Charter for this clinical trial. The membership of the DMC and reporting structure are defined in the DMC Charter.

10.3.2 Clinical Adjudication Committee

An independent clinical adjudication committee will perform a blinded adjudication of the results of those patients who have clinical evidence of rapid disease progression (defined as ≥ 24 point increase in mNIS+7 from baseline [based on an average of 2 measurements] and FAP stage progression relative to baseline) at 9 months. Each review will follow procedures detailed in the committee's charter.

10.3.3 Interim Analysis Committee

An Interim Analysis Committee (IAC), comprised of 2 statisticians (1 blinded and 1 unblinded) independent of the conduct of the study, will be responsible for the

implementation of the interim analysis and for the calculations and recommendations surrounding whether an adjustment to the sample size is warranted, and if so, the appropriate adjustment, based on the study's primary endpoint data from the interim analysis. The Sponsor, the CROs, and all other parties conducting the study will remain blinded to all interim analyses until study completion. The IAC will follow the procedure outlined in the committee's charter.

10.4 Ancillary Research

Research ancillary to this main protocol may not be performed by individual study sites without prior discussion and approval by Alnylam.

10.5 Study Record Retention

Essential documents should be retained for the period of time required by applicable local law. The essential documents include the signed and dated final protocol, signed and dated amendments(s), if applicable, signed and dated Curriculum Vitae (CVs) of the Investigators, copies of the completed CRFs, signed ICFs, IEC/IRB approval and all related correspondence, financial agreements, regulatory approval, drug accountability, study correspondence, and patient identification codes. Records will not be destroyed without informing Alnylam in writing and giving Alnylam the opportunity to store the records for a longer period of time at Alnylam's expense.

The International Conference on Harmonization requires that patient identification codes be retained for at least 15 years after the completion or discontinuation of the study.

10.6 Discontinuation of the Study by Alnylam

Alnylam reserves the right to discontinue the study for clinical or administrative reasons at any time. If the site does not recruit at a reasonable rate, the study may be discontinued at that site. Should the study be terminated and/or the site closed for whatever reason, all documentation and study drug pertaining to the study must be returned to Alnylam or its representative, and the Investigators, IEC/IRB and Regulatory Authorities will be promptly informed of the termination and the reason for the decision. The Investigator should promptly inform the patients and assure appropriate therapy and follow-up.

10.7 Study Documentation

Prior to beginning the study, the Investigator will be asked to comply with ICH E6 and 21 CFR by providing at least the following essential documents:

- An original signed Investigator agreement page of the protocol and any amendments;
- An IEC/IRB and Alnylam approved ICF;
- IEC/IRB approval of the protocol, and any amendments;
- Completed and signed FDA form 1572;
- Curriculum vitae for the Investigator signed and dated by the Investigator indicating that it is current;
- Financial disclosure information (if applicable);

- Other documents which the Investigator should provide before study start include:
 - Curriculum vitae for all Sub-investigators; these should be signed and dated by the Sub-investigators indicating that they are current;
 - Financial disclosure information for all Sub-investigators (if applicable);
 - Advertisements for patient recruitment and any other written information to be given to patients, family members or legal guardians and IEC/IRB approval of any advertisements and any other written information;
 - IEC/IRB composition: If the Investigator or any of the Sub-investigators is a member of the IEC/IRB, assurance that he/she refrained from voting should be provided;
 - Laboratory accreditation and reference ranges for any laboratory values for local laboratories.

10.8 Confidentiality

The Investigator must ensure that the patients' anonymity will be maintained. On the CRFs or other documents submitted to Alnylam or designees, patients should not be identified by their names, but by the assigned patient number and initials. If patient names are included on copies of documents submitted to Alnylam or designees, the names (except for initials) will be obliterated and the assigned patient number added to the document. Documents not for submission to Alnylam (e.g. signed ICFs) should be maintained by the Investigator in strict confidence.

Following the principles of the Good Clinical Practice, if local regulations specify a patient's number and initials will be used to identify the patient on their study records. Laboratory samples may be labeled with an independent numbering code, and the label will not contain any other personal identification information. The numbering code associated with these labels will be held by the study CRO and Alnylam, thereby allowing no unwarranted access to the information. When reporting results for interim safety assessment, the interim analysis, and at the end of the study, the code will be shared per standard operating procedures with the responsible member of the Biostatistical and Data Management Departments of the CRO. The numbering code will also be held for samples in storage until marketing approval of patisiran (ALN-TTR02) in the countries where this study was conducted, or until clinical development of patisiran is halted. Throughout sample collection, storage (limited, staff only access area containing locked sample storage, and limited access sample tracking) and processing, the samples will only be handled by appropriate personnel per the laboratory's standard operating procedures.

The Investigator must treat all of the information related to the study and the compiled data as confidential, whose use is for the purpose of conducting the study. Alnylam must approve any transfer of information not directly involved in the study.

10.9 Publications/Reports

Following completion of the study, the data may be considered for publication in a scientific journal or for reporting at a scientific meeting. A copy of the manuscript must be provided and confirmed received at Alnylam at least 30 days prior to its submission.

No submission of a manuscript may be made until the results from all of the study sites have been received and analyzed by Alnylam, or the study has been terminated at all centers. A separate, individual publication of the results of the study will be delayed until initial publication of the results of the multicenter study, or a decision not to publish is made. If an initial draft is not produced within 18 months of completion of the study at all centers, or the timeframe for publication is not satisfactory, the Investigator may disclose the results after providing a copy and Alnylam confirms receipt of the manuscript 30 days prior to submission.

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12 APPENDICES

Appendix 1: Karnofsky Scale

| | | |
|---|-----|---|
| Able to carry on normal activity and to work; no special care needed. | 100 | Normal no complaints; no evidence of disease. |
| | 90 | Able to carry on normal activity; minor signs or symptoms of disease. |
| | 80 | Normal activity with effort; some signs or symptoms of disease. |
| Unable to work; able to live at home and care for most personal needs; varying amount of assistance needed. | 70 | Cares for self; unable to carry on normal activity or to do active work. |
| | 60 | Requires occasional assistance, but is able to care for most of his personal needs. |
| | 50 | Requires considerable assistance and frequent medical care. |
| Unable to care for self; requires equivalent of institutional or hospital care; disease may be progressing rapidly. | 40 | Disabled; requires special care and assistance. |
| | 30 | Severely disabled; hospital admission is indicated although death not imminent. |
| | 20 | Very sick; hospital admission necessary; active supportive treatment necessary. |
| | 10 | Moribund; fatal processes progressing rapidly. |
| | 0 | Dead |

Appendix 2: New York Heart Association Classification of Heart Failure

| Class | Symptomatology |
|--------------|---|
| I | No symptoms. Ordinary physical activity such as walking and climbing stairs does not cause fatigue or dyspnea. |
| II | Symptoms with ordinary physical activity. Walking or climbing stairs rapidly, walking uphill, walking or stair climbing after meals, in cold weather, in wind or when under emotional stress causes undue fatigue or dyspnea. |
| III | Symptoms with less than ordinary physical activity. Walking one to two blocks on the level and climbing more than one flight of stairs in normal conditions causes undue fatigue or dyspnea. |
| IV | Symptoms at rest. Inability to carry on any physical activity without fatigue or dyspnea. |

Appendix 3: Categorization of Infusion-Related Reactions

Signs and symptoms of an infusion-related reaction (IRR) usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Signs/symptoms may include: allergic reaction/hypersensitivity (including drug fever), arthralgia (joint pain), bronchospasm, cough, dizziness, dyspnea (shortness of breath), fatigue (asthenia, lethargy, malaise), headache, hypertension, hypotension, myalgia (muscle pain), nausea, pruritus/itching, rash/desquamation, rigors/chills, sweating (diaphoresis), tachycardia, urticaria (hives, welts, wheals), vomiting.

Categorization of IRRs is as follows:

| Categorization | Description |
|----------------|---|
| Mild | Mild reaction: infusion may be continued; if intervention is indicated it is minimal and additional treatment (other than paracetamol for delayed reactions) is not required. |
| Moderate | Moderate reaction: requires treatment including more intensive therapy (e.g., IV fluids, nonsteroidal anti-inflammatory [NSAIDs]) in addition to infusion interruption but responds promptly to medication. Treatment is indicated for ≤ 24 hours. |
| Severe | More than moderate reaction: not rapidly responsive to medication or to interruption of infusion; and/ or prolonged (treatment is indicated for > 24 hours); recurrence of severe symptoms following initial improvement. |

Appendix 4: Neuropathy Scores and Their Components

| Assessment Tool | Total Points | Components (points) |
|-----------------|--------------|--|
| NIS+7 | 270 | <ul style="list-style-type: none"> • Neurologic exam of lower limbs, upper limbs and cranial nerves (NIS^a) <ul style="list-style-type: none"> • Weakness (192) • Sensation (32) • Reflexes (20) • Nerve conduction studies $\sum 5$ (18.6)^a <ul style="list-style-type: none"> • Sural SNAP, tibial motor n. distal latency, peroneal SNAP/motor n. conduction velocity/motor n. distal latency • Vibration detection threshold (3.7) • Heart rate response to deep breathing (3.7) |
| Modified NIS+7 | 304 | <ul style="list-style-type: none"> • Neurologic exam of lower limbs, upper limbs and cranial nerves (mNIS^a) <ul style="list-style-type: none"> • Weakness (192) • Reflexes (20) • Nerve conduction studies $\sum 5$ (10)^a <ul style="list-style-type: none"> • Ulnar CMAP and SNAP, sural SNAP, tibial CMAP, peroneal CMAP • Quantitative sensory testing: QST-BSA_{TP+HP5} (80) • Postural blood pressure (2) |

^a Components that are shared between the mNIS+7 and NIS+7 (including NIS and NCS) will be performed once at each assessment.

Appendix 5: Polyneuropathy Disability Score

| Stage | Description |
|--------------|---|
| 0 | No symptoms |
| I | Sensory disturbances but preserved walking capability |
| II | Impaired walking capacity but ability to walk without a stick or crutches |
| IIIA | Walking with the help of one stick or crutch. |
| IIIB | Walking with the help of two sticks or crutches. |
| IV | Confined to a wheelchair or bedridden. |

Appendix 6: Familial Amyloidotic Polyneuropathy Stage

| Stage | Description |
|--------------|---|
| 0 | No symptoms |
| I | Unimpaired ambulation; mostly mild sensory, motor, and autonomic neuropathy in the lower limbs |
| II | Assistance with ambulation required, mostly moderate impairment progression to the lower limbs, upper limbs, and trunk. |
| III | Wheelchair-bound or bedridden; severe sensory, motor, and autonomic involvement of all limbs. |

ALN-TTR02-004
APOLLO Global Protocol Amendment Summary
Version 4.0 – 30 January 2017

| Protocol Version No. | Date | Summary of Changes |
|-----------------------|-----------------|---|
| 1.0 (Original) | 15 August 2013 | N/A |
| 2.0 | 18 October 2013 | <ul style="list-style-type: none"> • Secondary endpoints reordered and methods of analysis were modified <ul style="list-style-type: none"> ○ EuroQOL questionnaire was made an exploratory endpoint ○ Removal of the analysis by families • Clarification that study personnel performing the efficacy assessments will remain blinded to the results of any previous assessment until the study has been completed • Change of the schedule of assessments in tables 1-1,1-2, and 1-3 and Sections 6.1.1, 6.1.2, and 6.1.3 • Inclusion criteria modified to allow an INR of ≤ 3 only for patients on warfarin • Definition of “highly effective birth control” was clarified in Section 4.7 • Premedication regimen was modified to reflect those used in earlier trials of ALN-TTR02 • Addition that either Bazett’s or Friderica’s correction may be used in the case that an ECG machine does not calculate QTc • Description of the pharmacoeconomics questionnaire was corrected to reflect the inclusion of 21, rather than 16, questions |
| 3.0 | 21 March 2014 | <ul style="list-style-type: none"> • Additional clarification that entry criteria, besides Inclusion Criteria #3 [Have a NIS of 10 to 100 (inclusive)] and #4 [REDACTED], will be assessed at the Screening Visit only • Inclusion Criterion #1 [Male or female of 18 to 80 years of age (inclusive)] modified to allow for enrollment of subjects up to 85 years of age (inclusive) • Lower limit of the Neuropathy Impairment Score (NIS) from 10 to 5 in Inclusion Criterion #3 [Have a NIS of 10 to 100 (inclusive)] • Inclusion Criterion #4 [REDACTED] • Inclusion Criterion #6 [Have an absolute neutrophil count (ANC) ≥ 1500 cells/mm³, a platelet count $\geq 100,000$ cells/mm³, and hemoglobin ≥ 10 g/dL (or ≥ 100 g/L)] modified from a platelet count of $\geq 100,000$ cells/mm³ to $\geq 50,000$ cells/mm³ • Inclusion Criterion #7 [Have aspartate transaminase (AST) and alanine transaminase (ALT) levels $\leq 2.5 \times$ the upper limit of normal (ULN), total bilirubin within normal limits, albumin > 3 g/dL (or > 30 g/L), international normalized ratio (INR) ≤ 1.2 (patients on warfarin with an INR of ≤ 3 will be allowed)] modified to increase INR value from ≤ 3 to ≤ 3.5 • Exclusion Criterion #1 [Has vitamin A levels below the lower limit of normal (LLN)] was clarified to exclude patients with vitamin A levels consistent with vitamin A deficiency (ie, $< 20 \mu\text{g/dL}$) • Exclusion Criterion #18 was removed ([Participated in a clinical trial with an antisense oligonucleotide for more than 3 months; if in a clinical trial with antisense oligonucleotide for ≤ 3 months, must have completed a 3-month wash-out prior to start of study drug administration in this study]) • Diflusalal was removed from Exclusion Criterion #19 [Is currently taking diflusalal, tafamidis, doxycycline, or tauroursodeoxycholic acid; if previously on any of these agents, must have completed a 14-day wash-out prior to start of study drug administration in this study] and added as new Exclusion Criterion #20 [Is currently taking diflusalal; if previously on this agent, must have at least a 3-day wash-out prior to start of study drug administration in this study] to clarify that a 3-day washout period prior to start of study drug for this particular agent is sufficient for that agent |

| Protocol Version No. | Date | Summary of Changes |
|----------------------|----------------|---|
| 4.0 | 24 April 2014 | <ul style="list-style-type: none"> The screening window was expanded from 28 days to 42 days to make it less restrictive for traveling patients. <i>Corrected typographical errors identified in the SOA in version 3.0</i> |
| 5.0 | 04 August 2014 | <ul style="list-style-type: none"> Inclusion Criterion #3 [Have an NIS of 5 to 100 (inclusive) (Note: This criterion must be met at the Screening/Baseline Visit)] changed to the upper limit NIS from 100 to 130 and added requirement for a Polyneuropathy Disability (PND) score of $\leq 3b$. Inclusion Criterion #7 [Have aspartate transaminase (AST) and alanine transaminase (ALT) levels $\leq 2.5 \times$ the upper limit of normal (ULN), total bilirubin within normal limits, albumin >3 g/dL (or >30 g/L), international normalized ratio (INR) ≤ 1.2 (patients on anticoagulant therapy with an INR of ≤ 3.5 will be allowed)] modified to remove albumin criterion and to increase INR criterion from ≤ 1.2 to ≤ 2.0 Inclusion Criterion #8 [Have a serum creatinine $\leq 1.5 \times$ ULN] modified from serum creatinine ≤ 1.5 to $\leq 2 \times$ ULN Inclusion Criterion #9 [Have negative serology for hepatitis B virus (HBV) and hepatitis C virus (HCV)] clarified to exclude only patients with an active hepatitis B or hepatitis C infection Inclusion Criterion #10 [Women of child-bearing potential must have a negative pregnancy test, cannot be breastfeeding, and must be using 2 highly effective methods of contraception prior to screening, throughout study participation, and for 1 month after last dose of study drug. Highly effective methods of birth control are defined in Section 4.7] modified to extend the period from 1 month to 75 days after last dose of study drug for WOCBP Inclusion Criterion #11 [Males with partners of child-bearing potential, must agree to use 1 barrier method (e.g., condom) and 1 additional method (e.g., spermicide) of contraception throughout study participation and for 1 month after the last dose of study drug; males must also abstain from sperm donation after the first dose of study drug through study participation and for 1 month after last dose of study drug] modified to extend the period that males with partners of child-bearing potential must use 1 barrier method and 1 additional method of contraception from 1 month to 75 days after the last dose of study drug Exclusion Criterion #1 [Has vitamin A levels consistent with vitamin A deficiency (<20 $\mu\text{g}/\text{dL}$)] removed Exclusion Criterion #16 [Has a known history of alcohol abuse or daily heavy alcohol consumption (females: more than 14 units of alcohol per week; males: more than 21 units of alcohol per week [unit: 1 glass of wine [125 mL] = 1 measure of spirits = $\frac{1}{2}$ pint of beer])] clarified to patients with a history of alcohol abuse within the past 2 years or daily heavy alcohol consumption Exclusion Criterion #17 [Participated in a clinical trial with antisense oligonucleotide, must have completed a 3-month wash-out prior to start of the study drug administration in this study] added to exclude patients who participated in a clinical trial with antisense oligonucleotide unless there is a 3 month wash-out period Exclusion Criterion #24 [Is under legal protection] modified to define "under legal protection" The protocol has been modified to permit select changes to the premedication regimen for an individual having difficulty tolerating the premedication regimen only after consultation with the study medical monitors. The sample size was modified to limit the number of patients with a Neuropathy Impairment Score (NIS) in the range of 101 to 130 to no more than 40 of the 200 planned patients to prevent overenrolling patients with more severe impairment. |

| Protocol Version No. | Date | Summary of Changes |
|----------------------|-------------------|---|
| 6.0 | 08 September 2015 | <ul style="list-style-type: none"> • Implemented a reduced dose of dexamethasone for the protocol-specified premedication regimen • Included that specified patients who are intolerant of 10 mg IV dexamethasone or equivalent on the day of infusion may be considered for further step-wise reduction in dexamethasone or equivalent after consultation with the medical monitor • The risk benefit assessment has been updated to reflect liver function test abnormalities and risk for osteoporosis • Because patients may be at risk for osteoporosis, it has been added that, if appropriate, study participants should receive therapy for the prevention and early treatment of osteoporosis • Inclusion #4 [Redacted] Note: This criterion must be met at the Screening/Baseline visit]] added ulnar SNAP and ulnar CAP measurements to the qualifying NCS • Inclusion #7 [Have aspartate transaminase (AST) and alanine transaminase (ALT) levels $\leq 2.5 \times$ the upper limit of normal (ULN), total bilirubin within normal limits, international normalized ratio (INR) ≤ 2.0 (patients on anticoagulant therapy with an INR of ≤ 3.5 will be allowed))] changed to permit patients with a total bilirubin level elevation to $\leq 2 \times$ upper limit of normal to enroll • Exclusion #14 [Has uncontrolled clinically significant cardiac arrhythmia or unstable angina] changed to include clarification that patients with any uncontrolled cardiac arrhythmia or unstable angina are not permitted to enroll in the study • Included the option for patients to permanently discontinue study treatment and remain on-study • Provided clarification about local personnel responsible for reading ECGs to include that ECGs will be read locally by a cardiologist or qualified physician |

STATISTICAL ANALYSIS PLAN: PROTOCOL ALN-TTR02-004

Patisiran

APOLLO: A Phase 3 Multicenter, Multinational, Randomized, Double-blind, Placebo-controlled Study to Evaluate the Efficacy and Safety of Patisiran (ALN-TTR02) in Transthyretin (TTR)-Mediated Polyneuropathy (Familial Amyloidotic Polyneuropathy-FAP)

Protocol Number: ALN-TTR02-004

Protocol Version and Date: Amendment 4: 4 August 2014
Amendment 3: 24 April 2014
Amendment 2: 21 March 2014
Amendment 1: 18 October 2013
Original: 15 August 2013

Name of Test Drug: Patisiran (ALN-TTR02)

Phase: Phase 3

Methodology: Randomized, double-blind, placebo-controlled study

Sponsor: Alnylam Pharmaceuticals, Inc.
300 Third Street
Cambridge, MA 02142
[REDACTED]

Sponsor Representative: [REDACTED]
[REDACTED]

Analysis Plan Date: 31 March 2015

Analysis Plan Version: Final Version 1.0

Confidentiality Statement

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

| Abbreviation | Definition |
|---------------------|--|
| Σ5 | 5 Attributes |
| AA | Amyloid A |
| AE | Adverse Event |
| ANCOVA | Analysis of Covariance |
| Anti-HCV Ab | Anti-hepatitis C virus antibody |
| ATC | Anatomic Therapeutic Class |
| ATTR | Transthyretin-Mediated Amyloidosis |
| BMI | Body mass index |
| CC | Complete Cases |
| CL _R | Renal Clearance |
| CMAP | Compound Muscle Action Potential |
| COMPASS-31 | Autonomic Symptoms Questionnaire (Composite Autonomic Symptom Score) |
| CRO | Contract Research Organization |
| CSR | Clinical Study Report |
| DMC | Data Monitoring Committee |
| ECG | Electrocardiogram |
| eCRF | Electronic Case Report Form |
| EQ-5D | Euro Quality of Life- 5 Dimensions |
| EU | European Union |
| FAC | Familial Amyloidotic cardiomyopathy |
| FAP | Familial Amyloidotic Polyneuropathy |
| HbsAb | Hepatitis B surface antibody |
| HbsAg | Hepatitis B surface antigen |
| HP | Heat pain |
| HRdb | Heart Rate Response to Deep Breathing |
| IAC | Interim analysis Committee |
| ICH | International Conference on Harmonisation |
| IENFD | Intraepidermal Nerve Fiber Density |
| IFE | Immunofixation Electrophoresis |
| INN | International Nonproprietary Name |
| IRS | Interactive response system |
| IV | Intravenous |
| LNP | Lipid Nanoparticle |
| LOCF | Last Observation Carried Forward |
| LS | Least Squares |
| mBMI | Modified Body Mass Index |

| Abbreviation | Definition |
|---------------------|---|
| MedDRA | Medical Dictionary for Regulatory Activities |
| MI | Multiple Imputation |
| mITT | Modified Intent-to-Treat |
| MMRM | Mixed Model Repeated Measures |
| mNIS | Modified Neuropathy Impairment Score |
| NCS | Nerve Conduction Studies |
| NIS | Neuropathy Impairment Score |
| NIS-W | Neuropathy Impairment Score-Weakness Score |
| Norfolk QOL-DN | Norfolk Quality of Life-Diabetic Neuropathy Questionnaire |
| NSAID | Nonsteroidal Anti-Inflammatory Drug |
| NT-proBNP | N Terminal Prohormone of B-Type Natriuretic Peptide |
| PD | Pharmacodynamic |
| PEG | Polyethylene glycol |
| PK | Pharmacokinetic |
| PND | Polyneuropathy Disability |
| PP | Per-Protocol |
| QST | Quantitative Sensory Testing |
| RBP | Retinol Binding Protein |
| RNAi | RNA interference |
| R-ODS | Rasch-Built Overall Disability Scale |
| SAE | Serious Adverse Event |
| SAP | Statistical Analysis Plan |
| SD | Standard Deviation |
| SGNFD | Sweat Gland Nerve Fiber Density |
| siRNA | Small Interfering Ribonucleic Acid |
| SNAP | Sensory Nerve Action Potential |
| SOC | System Organ Class |
| SUSAR | Suspected Unexpected Serious Adverse Reaction |
| T4 | Thyroxine |
| TEAE | Treatment-Emergent Adverse Event |
| TP | Touch pressure |
| TTR | Transthyretin |
| V30M | Val30Met |
| VAS | Visual Analog Scale |
| VDT | Vibration Detection Threshold |
| WHO | World Health Organization Drug Dictionary |
| WT | Wild-Type |

1. INFORMATION FROM THE STUDY PROTOCOL

1.1. Introduction and Objectives

1.1.1. Introduction

Transthyretin-mediated amyloidosis (ATTR) is an inherited, autosomal dominant, systemic disease caused by mutations in the transthyretin (TTR) gene.[1] Transthyretin is a tetrameric 127 amino acid protein that is secreted predominantly (>95%) by hepatocytes, with a smaller fraction produced by the choroid plexus and retina.[2] Physiologically, TTR is a major serum carrier for retinol binding protein (RBP) and a minor carrier of thyroxine (T4). Mutations in the TTR protein lead to destabilization of the tetrameric form and dissociation into dimers and monomers. Misfolding of mutated monomers from the α -helical to the β -pleated sheet structure, results in tissue deposition of amyloid fibrils.[3] Amyloid deposits typically contain both mutant and wild-type (WT) TTR. The particular TTR mutation and site of amyloid deposition determines the clinical manifestations of the disease, which include sensory and motor neuropathy, autonomic neuropathy, and/or cardiomyopathy. ATTR is a progressive disease associated with severe morbidity, with a life expectancy limited to 5 to 15 years from symptom onset.[4] There are over 100 reported TTR mutations which are associated with 2 clinical syndromes: familial amyloidotic polyneuropathy (FAP) and familial amyloidotic cardiomyopathy (FAC).[5,6,7]

‘Patisiran’ (the International Nonproprietary Name [INN] name for the drug product previously referred to as ALN-TTR02) is being developed for the treatment of ATTR patients with symptomatic FAP.

The estimated worldwide prevalence of FAP is 5,000 to 10,000, with the majority of cases in Portugal, Sweden, France, Japan, Brazil, and the United States.[8,9] The most common causative mutation of FAP is TTR Val30Met (V30M), with the onset of symptoms typically occurring between 30 and 55 years of age.[10] Amyloid deposition occurs largely in the peripheral nerves, starting as a nerve length-dependent sensory polyneuropathy in the feet causing numbness and pain and progressing to painful dysesthesias. Disabling motor neuropathy follows, characterized by leg weakness and eventually the inability to walk. Autonomic neuropathy is another common feature of the disease, resulting in severe gastrointestinal pathology (including diarrhea or constipation and malabsorption, leading to severe malnutrition), orthostatic hypotension, and bladder dysfunction with recurring urinary tract infections.[11,12,13,14] For several mutations, cardiac pathology also occurs due to amyloid infiltration of the sinus node, atrioventricular conduction system, and infiltration of the myocardium.[15,16] Involvement of the conduction system can lead to sudden death due to dysrhythmias, and myocardial infiltration can lead to diastolic dysfunction and right-sided heart failure.[17] Cardiomyopathy then proceeds inexorably, leading to death typically within 10 years.[18]

There are multiple lines of evidence demonstrating that reduction of circulating TTR improves outcomes in patients with ATTR. Because the liver is the primary source of WT and mutant TTR, orthotopic liver transplantation has been used since 1990 in an attempt to treat FAP,[19] and is the current standard of care in patients who are eligible for transplant (patients with minimal neuropathy symptoms and no cardiac involvement). When liver transplantation is performed early in the course of the disease, it can stabilize and slow the course of neuropathic disease in patients with FAP due to V30M, but is less effective in patients with other TTR

mutations.[20] However, it is less effective in patients with more advanced disease, especially those with heart involvement, due to the continued production and deposition of WT TTR in tissues with pre-existing amyloid.[21,22,23]

It is estimated that approximately two-thirds of FAP patients are not transplant-eligible. Furthermore, liver transplant poses risks from the surgical procedure and from life-threatening complications due to graft rejection or infections. The 1-year mortality rate post-transplant is 10%.[24]

Nonsurgical options that are used for the treatment of FAP (depending on geographic location) include tafamidis (Vyndaqel®) and diflunisal. Tafamidis is a small molecule TTR stabilizer that binds to the thyroxine binding sites of the TTR tetramer, thus preventing its dissociation to monomers and potentially preventing fibril formation. While tafamidis is approved in the European Union (EU) for the treatment of ATTR in adult patients with Stage 1 symptomatic polyneuropathy to delay peripheral neurologic impairment, the pivotal trial data were primarily from FAP patients with the V30M mutation; furthermore, tafamidis is not considered the standard of care throughout the EU and it has not been approved for use in the US.[25]

Diflunisal is a generic, nonsteroidal anti-inflammatory drug (NSAID) that is also a tetramer stabilizer and binds to TTR in a similar manner as tafamidis. An NIH-sponsored multicenter, placebo-controlled Phase 3 study in FAP patients was completed in 2012; data suggest an effect of diflunisal on neuropathic score NIS+7, the primary endpoint of the study.[26] Due to the restricted use of liver transplantation and tafamidis in patients with early stage of disease, and the non-standard use of diflunisal among practitioners, there remains an unmet medical need for a potent and effective therapy for FAP that will have an impact on patients across a broad range of neurologic impairment, regardless of their mutation (V30M or non-V30M).

Patisiran comprises a small interfering ribonucleic acid (siRNA) which is specific for TTR, and is formulated in a hepatotropic lipid nanoparticle (LNP) for intravenous (IV) administration.[27] This TTR siRNA has a target region within the 3'UTR region of the TTR gene to ensure and confirm homology with WT TTR as well as all reported TTR mutations. Following LNP-mediated delivery to the liver, the siRNA targets TTR mRNA for degradation, resulting in the potent and sustained reduction of mutant and WT TTR protein via the RNA interference (RNAi) mechanism.

Since circulating TTR is almost exclusively synthesized in the liver, the IV administration of patisiran is postulated to reduce the level of precursors that lead to amyloid fibril deposition, resulting in clinical benefit to patients with FAP.

The therapeutic hypothesis that systemic amyloidoses can be managed by reduction in circulating levels of amyloidogenic protein has been validated in other acquired (e.g., immunoglobulin light chain systemic [AL], or amyloid A [AA]) and hereditary (e.g., Fibrinogen A α -chain, ApoA1) amyloidosis. The experience from these systemic amyloidotic disorders,[28,29,30,31] as well as the liver transplant data in FAP, suggest that lowering of the circulating amyloidogenic protein by at least 50% is required to impact the clinical course of the disease, with reductions in protein beyond 50% providing further incremental improvements in outcomes. It is therefore postulated that the >80% suppression in both WT and mutant TTR observed upon administration of 0.3 mg/kg patisiran once every 21 days will result in clinical benefit in FAP patients with mild to moderate polyneuropathy. This hypothesis is further supported by evidence from tafamidis suggesting that reduction in free TTR monomer can slow neuropathy progression in early-stage V30M patients with FAP.[10]

Importantly, preliminary data from an ongoing Phase 2 open-label extension study with patisiran (ALN-TTR02-003) in 27 FAP patients showed a mean sustained TTR reduction of ~80% accompanied with stabilization of neuropathic impairment scores at 6 months, which compared favorably to the anticipated increase in scores based on natural history and other datasets [32]

Patisiran is currently being investigated in APOLLO, a Phase 3 pivotal study (ALN-TTR02-004) evaluating the efficacy and safety of patisiran in ATTR patients with FAP. This protocol was discussed with the Agency at the end-of-Phase 2 meeting on 23 September, 2013 (FDA ref ID # 3394206 for meeting minutes). This statistical analysis plan (SAP) provides the plan for analysis of the data from this study.

1.1.2. Document and Study Objectives

This SAP is designed to outline the methods to be used in the analysis of study data in order to address the study objectives of Study ALN-TTR02-004. Populations for analysis, data handling rules, statistical methods, and formats for data presentation are provided. The statistical analyses and summary tabulations described in this SAP will provide the basis for the results sections of the clinical study report (CSR) for this trial.

This SAP will also outline differences, if any, in the currently planned analytical objectives relative to those planned in the study protocol.

1.1.2.1. Primary Objective

The primary objective of the study is to determine the efficacy of patisiran by evaluating the difference between the patisiran and placebo groups in the change from baseline of Modified Neuropathy Impairment Score (mNIS+7) score at 18 months.

1.1.2.2. Secondary Objectives

The secondary objectives of the study are to determine the effect of patisiran on various clinical parameters by assessing the difference between patisiran and placebo in the change from baseline in the following measurements at 18 months:

- Norfolk Quality of Life-Diabetic Neuropathy (Norfolk QOL-DN) Total QoL Score.
- Neuropathy Impairment Score-weakness (NIS-W) score.
- Modified body mass index (mBMI), calculated by multiplying the BMI by serum albumin level.
- Timed 10-meter walk test.
- Autonomic symptoms questionnaire (Composite Autonomic Symptom Score [COMPASS-31]).

1.1.2.3. Exploratory Objectives

The exploratory objectives of the study are:

- To determine the difference between the patisiran and placebo groups in the change from baseline in the following measurements at 18 months:
 - NIS+7 score.
 - Individual components of the NIS (sensation and reflexes), nerve conduction studies 5 attributes ($\Sigma 5$ NCS [sural nerve sensory nerve action potential {SNAP}, peroneal nerve compound muscle action potential {CMAP}, peroneal motor nerve conduction velocity, peroneal motor nerve distal latency, tibial motor nerve distal latency]), vibration detection threshold (VDT), heart rate response to deep breathing (HRdb).
 - Grip strength.
 - Euro Quality of Life-5 Dimensions (EQ-5D) index value.
 - EuroQol visual analog scale (EQ VAS)
 - Level of disability (Rasch-built Overall Disability Scale [R-ODS]).
 - Individual components of the mNIS+7, including $\Sigma 5$ NCS (ulnar nerve CMAP, ulnar nerve SNAP, sural nerve SNAP, tibial nerve CMAP, peroneal nerve CMAP), quantitative sensory testing (QST) by body surface area including touch pressure and heat pain, and postural blood pressure.
 - Pathologic evaluation of sensory and autonomic innervation through voluntary skin punch biopsies and analysis of intraepidermal nerve fiber density (IENFD) and sweat gland nerve fiber density (SGNFD).
 - Assessment of sensory disturbance and ambulation through FAP stage and Polyneuropathy Disability (PND) score.
 - Norfolk QOL-DN subscales.
 - COMPASS-31 dimension scores.
 - Cardiac assessment through echocardiogram, troponin I, and N-terminal prohormone of B-type natriuretic peptide (NT-proBNP) levels.
 - Pharmacodynamic (PD) biomarkers (TTR, RBP, vitamin A).
- To compare the proportion of patients in the patisiran and placebo groups who meet the pre-defined criterion for rapid disease progression (defined as ≥ 24 point increase in mNIS+7 from baseline [based on an average of 2 measurements] and FAP stage progression relative to baseline) at 9 months.
- To assess the relationship between treatment (patisiran or placebo) and ordinal responses to each of the five EQ-5D domains at 18 months.
- To compare the proportions of patients in the patisiran and placebo groups exhibiting a clinical response at 9 months and 18 months, defined as a less than 2-point increase from baseline in mNIS+7.

- To compare the proportions of patients in the patisiran and placebo groups exhibiting a clinical response at 9 months and 18 months, defined as no increase from baseline in PND score.

1.2. Study Design

1.2.1. Synopsis of Study Design

This is a multicenter, multinational, randomized, double-blind study comparing patisiran to placebo in ATTR patients with symptomatic FAP.

Consented eligible patients will be randomized to receive either 0.3 mg/kg patisiran or placebo in a 2:1 ratio (patisiran to placebo) in a blinded manner. Treatment arms will be balanced at entry for Neuropathy Impairment Score (NIS; < 50 vs. ≥ 50), early onset V30M (< 50 years of age at onset) vs. all other mutations (including late onset V30M), and previous tetramer stabilizer use (tafamidis or diflunisal) vs. no previous tetramer stabilizer use. Patients will receive patisiran or placebo once every 21 days for 78 weeks (18 months).

Patients will have efficacy assessments at Screening/Baseline, 9 months, and 18 months. Study personnel performing assessments related to the efficacy endpoints will be different from the Investigator and other personnel managing the patient, and all of these study personnel will be blinded to any clinical laboratory results that could potentially unblind them (e.g., TTR levels, vitamin A levels, thyroid function tests). In addition, the study personnel performing assessments related to the efficacy endpoints will also be blinded to the results of any previous assessments (e.g., Screening/Baseline, Baseline, or 9-month assessments). Whenever possible, the same site personnel (individual) will conduct efficacy assessments for a given patient across timepoints.

At the 9-month time point, if the clinical adjudication committee determines that a patient is exhibiting rapid disease progression (defined as ≥ 24 point increase in mNIS+7 from baseline [based on an average of 2 measurements] and FAP stage progression relative to baseline), the patient's treating physician will provide the patient with the option of discontinuing study drug and receiving local standard of care treatment for FAP. Patients who discontinue study drug will remain on study, following a modified schedule of visits, through completion of the 18-month efficacy assessments and blinding will be maintained throughout.

Patients who complete the 18-month efficacy assessments can elect to participate in an extension study in which patients would receive open-label administration of 0.3 mg/kg patisiran once every 21 days.

A Data Monitoring Committee (DMC) will be implemented for the study and will operate under a pre-specified charter.

1.2.2. Randomization Methodology

Patients will be randomly assigned in a 2:1 ratio to receive either 0.3 mg/kg patisiran or placebo (normal saline 0.9%).

Patients will be randomized via an interactive response system (IRS). Either designated site personnel or the pharmacist may request randomization for the patient, but only the pharmacist or pre-identified unblinded personnel will be allowed to receive the randomized treatment code. The treatment code will be delivered to the unblinded personnel or the pharmacist to prepare the necessary number of vials for that patient based on their weight.

As described above in Section 1.2.1, treatment arms will be balanced at entry for NIS (< 50 vs. ≥ 50), early onset V30M (<50 years of age at onset) vs. all other mutations (including late onset V30M), and previous tetramer stabilizer use (tafamidis or diflunisal) vs. no previous tetramer stabilizer use.

1.2.3. Unblinding

Unblinding is only to occur in the case of patient emergencies or when necessary from a regulatory reporting perspective (e.g., Suspected Unexpected Serious Adverse Reaction [SUSAR] occurring in the EU), and at the conclusion of the study.

Patients who discontinue study drug at 9 months due to rapid disease progression will remain blinded throughout the remainder of the study.

In the event that the Investigator requests to know a patient's study treatment assignment, the Investigator is instructed to contact the Contract Research Organization (CRO) Medical Monitor to discuss the need for unblinding. In case of an emergency, the treatment allocation for each patient will be available from the unblinded site personnel, pharmacist, or the IRS system.

If a patient becomes pregnant or seriously ill during the study, the blind should be broken only if knowledge of the treatment administered will affect treatment options available to the patient. Before breaking the blind, the Principal or Sub-investigator should attempt to contact the CRO Medical Monitor. If the Medical Monitor is immediately unreachable, the Principal or Sub-investigator should break the blind as necessary using the code breaking information provided and contact the CRO Medical Monitor as soon as possible. A record will be kept of when the blind was broken, who broke it, and why.

1.2.4. Study Procedures

The schedule of assessments is described in the study protocol.

1.2.5. Efficacy, Pharmacokinetic, Pharmacodynamic, and Safety Parameters

1.2.5.1. Efficacy Parameters

Efficacy parameters will include the following (all evaluations will be conducted at baseline and at 9 and 18 months).

Primary Endpoint

- Neurologic impairment will be assessed using the mNIS+7 composite score (maximum of 270 points). The mNIS+7 includes the modified NIS (weakness and reflexes), Σ 5 NCS, QST, as well as autonomic assessment through postural blood pressure. Two assessments will be performed at each visit; each component contributing to the composite score is the average of the two assessments. A scoring algorithm, including methods for handling missing components of the mNIS+7, is included in Appendix 7.1.

Secondary Endpoints

Secondary endpoints will be analyzed in the following order, as discussed in Section 3.7.

- Patient reported QOL will be evaluated using the Norfolk QOL-DN (maximum of 136 points). A scoring algorithm for this instrument is included in Appendix 7.2.
- Motor function will be evaluated using NIS-W, calculated as the average of the two assessments performed at each visit (maximum score of 192).
- Nutritional status will be assessed using mBMI, calculated as $BMI \times \text{serum albumin}$.
- Motor function will additionally be assessed by the timed 10-meter walk test.
- Autonomic symptoms will be assessed using the COMPASS-31 total score (maximum of 100). A scoring algorithm for the COMPASS-31 total score and domain scores is included in Appendix 7.5.

Exploratory Endpoints

Exploratory endpoints are detailed in Section 1.1.2.3.

1.2.5.2. Pharmacokinetic Parameters

Blood samples for determination of patisiran pharmacokinetics (PK) will be collected as outlined in the schedule of assessments. Pharmacokinetic parameters to be determined include plasma-concentration time profiles for siRNA and the novel lipid components in patisiran: DLin-MC3-DMA and polyethylene glycol (PEG)₂₀₀₀-C-DMG. The siRNA, DLin-MC3-DMA, and PEG₂₀₀₀-C-DMG concentration will be determined for all patients.

Urine will be collected with void volume recorded for all patients at time points specified in the schedule of assessments to determine renal clearance (CL_R) of siRNA and 4-dimethylaminodibutyric acid (the metabolite of DLin-MC3-DMA) after dosing with study drug.

Pharmacokinetic parameter estimates using the concentration time data will be performed by Alnylam. All tabulations of pharmacokinetic data will be presented in a PK and PK/PD report separate from the CSR.

1.2.5.3. Pharmacodynamic Parameters

Pharmacodynamic markers assessed serially will include serum TTR, vitamin A, and RBP. Additional blood samples will be collected for exploratory biomarkers related to FAP.

The PD assessment for use in the PK/PD analysis will be similar to that described above, but may include additional PD analyses which will be used for the purpose of PK/PD analysis. The methods for the PK/PD analysis will be described in the PK and PK/PD analysis plans and the analysis result will be reported in the separate PK and PK/PD report which will be appended to the CSR.

1.2.5.4. Safety Parameters

Safety evaluations to be performed during the study include physical examinations, measurement of vital signs, 12-lead ECGs, clinical laboratory evaluations including hematology, clinical chemistry (including liver function tests), thyroid function parameters, and urinalysis, measurement of anti-drug antibodies, ophthalmology examinations, and monitoring of adverse events and concomitant medications.

2. PATIENT POPULATION

2.1. Population Definitions

The following patient populations will be evaluated and used for presentation and analysis of the data:

- Modified ITT (mITT) population: All patients who were randomized and received at least 1 dose of patisiran or placebo.
- Per-protocol (PP) population: All patients who were randomized, received at least 1 dose of patisiran or placebo, completed the 9-month or 18-month mNIS+7 and Norfolk QOL assessments, and did not experience any major protocol violations.
- Safety population: All patients who received at least 1 dose of study drug.

The primary population for efficacy analyses will be the mITT population; the primary endpoint and the first secondary endpoint (Norfolk QOL) will also be analyzed using the PP population. The remaining secondary endpoints will be analyzed using the mITT population. For efficacy analyses, patients will be analyzed according to the treatment to which they were randomized. The primary population for safety analyses will be the safety population. In the safety population, patients will be analyzed according to treatment received.

2.2. Protocol Violations

Patients who experience one or more major protocol violations, as determined by a review of the data prior to unblinding of the study results, will be excluded from the PP population. The Sponsor or designee will be responsible for producing the final protocol violation file (formatted as a Microsoft Excel file). This file will include a description of the protocol violation and clearly identify whether or not this violation warrants exclusion from the PP population, based on the potential impact on the efficacy results according to the judgment of the sponsor. This file will be finalized prior to database lock and unblinding of treatment assignments for all patients.

All protocol violations, and major protocol violations, will be presented in separate data listings.

Minimally, major protocol violations will include:

1. Failure to meet 1 or more key inclusion/exclusion criteria (listed below);
2. Any use of any potentially effective drug/tetramer stabilizer (as determined by the study medical monitor) other than study drug from the time the patient is given the 1st dose through completion of the 18 month efficacy assessment;
3. Receiving the wrong study medication for ≥ 1 dose;
4. Receiving a surgical procedure that would alter FAP disease course (*e.g.* liver transplant).

The following key inclusion/exclusion criteria must be met at the Screening or Screening/Baseline visits as specified in the protocol, in order to be included in the Per Protocol population:

Inclusion:

1. Have a diagnosis of FAP with documented TTR mutation;
2. Have an NIS of 5 to 130 (inclusive) and a PND score of $\leq 3b$;
3. Have an NCS sum of the sural sensory nerve action potential (SNAP), tibial compound muscle action potential (CMAP), ulnar sensory nerve Action potential (USAW) and peroneal CMAP of ≥ 2 points;
4. Have a Karnofsky performance status of $\geq 60\%$.

Exclusion:

1. Had a prior liver transplant or is planning to undergo liver transplant during the study period;
2. Has other known causes of sensorimotor or autonomic neuropathy (e.g., autoimmune disease, monoclonal gammopathy, etc.);
3. Has known primary amyloidosis or leptomeningeal amyloidosis;
4. Has known type I diabetes;
5. Has had type II diabetes mellitus for ≥ 5 years;
6. Has vitamin B12 levels below the lower limit of normal (LLN);
7. Received an investigational agent or device within 30 days of anticipated study drug administration or 5 half-lives of the investigational drug, whichever is longer;
8. Participated in a clinical trial with antisense oligonucleotide, must have completed a 3-month wash-out prior to start of the study drug administration in this study.

3. GENERAL STATISTICAL METHODS

3.1. Sample Size Justification

Approximately 200 patients will be enrolled in this study. For the estimation of sample size, a mean (\pm SD) mNIS+7 progression rate (primary endpoint) in the placebo group of 24 ± 16 points over 18 months was estimated using natural history data[33] from FAP patients. A sample of 154 patients provides 90% power for a 2-sided t-test with an 8.95 point (37.5%) mean difference between treatment groups in the primary endpoint at 2-sided $\alpha = 0.05$. Assuming a 25% random premature discontinuation rate (due to liver transplantation or other factors), the required sample size for this study is approximately 200. Additional patients may be enrolled based on the recommendation of the Interim Analysis Committee (IAC) to increase the sample size at the time of the pre-planned interim analysis and sample size re-estimation.

3.2. General Methods

All data listings that contain an evaluation date will contain a relative study day (Rel Day). Pre-treatment and on-treatment study days will be numbered relative to the day of the first dose of study medication, which is designated as Day 0. The preceding day is Day -1, the day before that is Day -2, etc. The last day of study medication is designated with an "L" (e.g., Day 214L). Post-treatment study days will be numbered relative to the last dose and will be designated as Day 1P, Day 2P, etc.

All output will be incorporated into Microsoft Word files, sorted and labeled according to the International Conference on Harmonisation (ICH) recommendations, and formatted to the appropriate page size(s).

For categorical variables, summary tabulations of the number and percentage of patients within each category (with a category for missing data) of the parameter will be presented. For continuous variables, the number of patients, mean, median, standard deviation (SD), minimum, and maximum values will be presented.

Laboratory data (including vitamin A and RBP) collected and recorded as below the limit of detection will be set equal to the lower limit of detection for the calculation of summary statistics.

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 level of significance. Secondary endpoints will be tested in a prespecified hierarchy (described below). Summary statistics will be presented, as well as 2-sided 95% confidence intervals on selected parameters, as described in the sections below.

3.3. Computing Environment

All descriptive statistical analyses will be performed using SAS statistical software Version 9.3 (or later), unless otherwise noted. Medical history and adverse events will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) Version 16.1 (or later). Concomitant medications will be coded using the World Health Organization (WHO) Drug Dictionary Version Q12013 (or later).

3.4. Baseline Definitions

Unless noted otherwise, baseline will be defined as the measurement closest to and prior to the first dose of study medication. For PD parameters (TTR, RBP, Vitamin A), baseline will be defined as the average of all pre-dose values (i.e., all Screening and Day 0 pre-dose values, as well as any values from unscheduled visits that occur prior to dosing on Day 0).

3.5. Methods of Pooling Data

Pooling of data is not applicable to this study.

3.6. Adjustments for Covariates

Analysis of continuous efficacy variables will be performed using an analysis of covariance (ANCOVA) model. For the primary efficacy analysis of mNIS+7 and the analysis of the secondary endpoint NIS-W, the model will be used to test if the changes in the parameter from baseline to the time point of interest differs between the treatment groups (patisiran vs. placebo) while adjusting for baseline value of the parameter being modeled, genotype class (early onset V30M vs. other), and prior tetramer stabilizer (tafamidis or diflunisal) use (Yes vs. No). For analysis of the remaining continuous secondary endpoints other than NIS-W, ANCOVA models will also include baseline NIS (<50 vs. \geq 50) as a factor (ANCOVA for NIS-W will not include baseline NIS). Categorical efficacy endpoints will be compared between treatment groups by the Cochran-Mantel-Haenszel test, stratified by the randomization stratification factors. Note that the stratum assignment used in analyses 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 IVR.

3.7. Multiple Comparisons/Multiplicity

Type I error control for secondary endpoints will be achieved by a hierarchical ordering procedure. Endpoints will be tested in the following pre-specified hierarchy:

1. Norfolk QOL-DN questionnaire [Total Score]
2. NIS-W score
3. mBMI
4. Timed 10-meter walk test
5. COMPASS-31 (overall summary score)

If and only if a comparison is significant at a 2 sided 0.05 significance level, the next endpoint in the hierarchy may be formally tested; if a given comparison is not significant at a 2-sided 0.05 significance level, the subsequent tests will be performed and the results summarized but statistical significance will not be inferred.

3.8. Subpopulations

Subgroup analyses will be performed for the following subgroups, defined based on baseline values:

- Age [>65 at randomization; ≤ 65 at randomization]
- Sex [M; F]

- Race [White; Non-White]
- Region [Asia; Europe; North America; Central & South America (including Mexico)]
- NIS [< 50 ; ≥ 50]
- Genotype Class [Early-onset V30M; Other]
- Previous Tetramer Use [Yes; No]
- Genotype [V30M; non-V30M]
- PND Score [I, II, IIIa, IIIb, IV]
- FAP Stage [1, 2, 3]

For subgroup analyses involving >2 levels (*e.g.* PND Score), levels may be collapsed into multilevel categories prior to unblinding if sample sizes (within level) are very small. Decisions to collapse will be made, justified, and documented prior to unblinding.

The significance of each factor above will be tested by including the treatment group by factor interaction term in the ANCOVA model described in Section 3.6. If the interaction term is statistically significant at the 0.05 level, then the statistical significance of the subgroup analysis will be interpreted for that factor. The subgroup analysis will be performed using simplified ANCOVA models including baseline value of the variable being analyzed as a covariate and treatment as a factor. Subgroup analyses will be performed using the 10 imputed datasets created for the primary analysis (see Section 3.10).

Subgroup analyses will be performed for the primary endpoint (mNIS+7) and Norfolk QOL using the mITT population.

3.9. Withdrawals, Dropouts, Loss to Follow-up

Patients will be free to withdraw from the study at any time and for any reason, without penalty to their continuing medical care. For those patients who withdraw early, every effort will be made to determine their reason for dropping out, and to complete both the Early Withdrawal and 18-month efficacy assessment visits. Patients who fail to return for final evaluations will be contacted by the site in an attempt to have them comply with the protocol.

A patient will be considered to have completed the study if the patient completes the 18-month efficacy assessment visit.

Patients who withdraw early from the study will not be replaced.

3.10. Missing Data

3.10.1. Completely Missing Primary and Secondary Efficacy Endpoints

Patients who do not complete their 18-month efficacy assessment will have primary and secondary efficacy endpoints imputed using a multiple imputation approach. Instead of filling in a single value for each missing endpoint (*e.g.*, last observation carried forward [LOCF]), multiple imputation will be used to replace each missing value with a set of plausible values that represent the uncertainty about the right value to impute. Single imputation does not reflect the uncertainty about the predictions of the unknown missing values, and the resulting estimated variances of the parameter estimates are biased toward zero. Missing values will be imputed separately for each treatment group using a regression procedure, with randomization

stratification factors, baseline age, baseline mBMI, disease duration at baseline, and 9-month efficacy assessment value (when available) as explanatory variables. For subjects completely missing the 9-month assessment but for whom the 18-month assessment is available, the 9-month assessment will be imputed as the treatment group mean. The proportion of non-monotone missing data is expected to be small. Ten imputed datasets (per group) will be generated from the regression procedure. The 10 sets of imputed datasets will then be analyzed as complete cases via the ANCOVA model specified for the primary analysis, and the resulting estimates (least squares [LS] means and standard errors) combined using SAS PROC MIANALYZE to produce inferential results (difference in LS means, 95% CI for the difference, and the p-value from the test that the difference is zero). Point estimates (LS means and differences) will be calculated as the average of the 10 complete-data estimates. A total variance estimate will be calculated as a weighted sum of within-imputation variance, which is the average of the complete-data variance estimates, and a between-imputation variance term. Complete details may be found in the SAS documentation for the MIANALYZE procedure (see Combining Inferences from Imputed Data Sets under Details).

<http://support.sas.com/documentation/onlinedoc/stat/131/mianalyze.pdf>

Multiple imputation usually assumes that the data are missing at random (MAR). Since the MAR assumption cannot be verified, sensitivity analyses will be performed using different methods of handling missing data. Details of these sensitivity analyses are in Section 4.3.1.

The patterns of missing data within study arm will be summarized by:

- 1) The percentage of missing data at each visit (baseline, 9-month, and 18-month);
- 2) The reasons for early withdrawals;
- 3) Time to withdrawal;
- 4) The 9-month efficacy outcome comparing patients who do, and do not have missing 18-month assessments.

Sample SAS code for the imputation model and the combined analysis for inference can be found in Appendix 8.

3.10.2. Partially Missing Primary and Secondary Efficacy Endpoints

Missing subcomponents of the primary mNIS+7 endpoint (per patient, per visit) will be imputed whenever possible according to the algorithm specified in Appendix 7.1. When this “partial imputation” is successful (*i.e.* complete mNIS+7 values are produced), these values will be used in all analyses. When partial imputation is unsuccessful, mNIS+7 will be scored as missing and imputed as above (Section 3.10.1).

Missing subcomponents of secondary endpoints will be imputed whenever possible according to the algorithms specified in Appendices 7.2-7.5. When partial imputation is successful, these secondary endpoint values will be used in all analyses and TLFs. When partial imputation is unsuccessful, secondary endpoints will be scored as missing and imputed as above (Section 3.10.1).

The frequency and types of partial imputations will be summarized for all endpoints.

3.11. Visit Windows

It is expected that all visits should occur according to 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.

Efficacy assessments collected at discontinuation visits will be grouped with the 9-month or 18-month assessments, if the discontinuation assessments are performed within 2 months of the scheduled assessment.

In data listings, the relative day of all dates will be presented.

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 will be considered for baseline values, as discussed in Section 3.4, and for inclusion in any categorical shift summaries (e.g., shift from baseline to “worst” post-baseline value). In addition, summaries of PD parameters (serum TTR, vitamin A, and RBP) will include separate tables and figures using All Data and Scheduled Visits Only.

3.12. Interim Analyses

Because the sample size estimates are based on limited data for variance and disease progression based on mNIS+7 change from baseline, it is intended that an interim analysis will be conducted by an independent IAC when approximately 50% of patients have completed their 9-month mNIS+7 assessment. This interim analysis will be blinded and will only estimate the overall variance observed in the primary endpoint. Since the interim analysis examines only the pooled variance of all patients in a blinded fashion, it does not require an adjustment to the overall alpha level for the test of the primary endpoint. Based on the results of the interim analysis, the IAC can recommend either increasing the study sample size or making no adjustment to the sample size. The IAC will follow the procedures outlined in the committee’s charter. The IA charter will be sent to the FDA prior to implementation of the IA.

4. STUDY ANALYSES

4.1. Patient Disposition

Patient disposition will be tabulated and will include the following parameters: number of patients enrolled (i.e., signed the informed consent form), the number of patients randomized, the number of patients dosed, the number of patients in each analysis population, the number of patients completing the study, the number of patients meeting the protocol specified criteria for rapid disease progression, and the number of patients who withdrew prior to completing the study and reasons for premature withdrawal. Patient disposition will be presented by randomized treatment group (patisiran and placebo) and overall.

A by-patient data listing of study completion information including the reason for premature study withdrawal, if applicable, will be presented.

4.2. Demographics and Baseline Characteristics

Demographics, baseline characteristics, and medical history information will be summarized for the mITT population and presented by randomized treatment group and overall. No formal statistical comparisons will be performed.

Age, height, weight, body mass index (BMI), albumin, and mBMI will be summarized using descriptive statistics (number of patients, mean, SD, median, minimum, and maximum). Sex, race, and ethnicity will be summarized by presenting the numbers and percentages of patients in each category.

Baseline disease characteristics will be summarized by presenting the numbers and percentages of patients with or without the V30M mutation. Randomization stratification factors (Neuropathy Impairment Score [NIS; < 50 vs. \geq 50], early onset V30M [$<$ 50 years of age at onset] vs. all other mutations [including late onset V30M], and previous tetramer stabilizer use [tafamidis or diflunisal] vs. no previous tetramer stabilizer use) will be summarized similarly. Time in years since diagnosis with ATTR will be summarized using descriptive statistics. Screening Karnofsky Performance Status will be summarized using descriptive statistics. Screening New York Heart Classification will be summarized by presenting the numbers and percentages of patients in each category.

All demographic and baseline data for each patient will be provided in data listings.

Screening vitamin B12 results and paraprotein typing by immunofixation electrophoresis (IFE) will be included in data listings. Results of serology testing [hepatitis B surface antibody (HbsAb), hepatitis B surface antigen [HbsAg], and anti-hepatitis C virus antibody [anti-HCV Ab)] will be included in a data listing.

Medical history and prior surgeries will be presented in a data listing. Pregnancy test results will be presented in data listings.

Any data from former neurological test scores will be presented in a data listing.

4.3. Efficacy Evaluation

Efficacy analyses will be conducted primarily using the mITT population. In addition, analyses of the primary endpoint and the first secondary endpoint of Norfolk QoL will also be evaluated for the PP population.

4.3.1. Primary Efficacy Evaluations

The primary analysis will compare change in mNIS+7 from baseline to Month 18 between treatment arms of the mITT population. An ANCOVA model, with baseline mNIS+7 value as covariate and genotype class (early onset V30M *vs.* other), prior tetramer stabilizer (tafamidis or diflunisal) use (Yes *vs.* No), and treatment group (patisiran *vs.* placebo) as factors will be employed to analyze the primary mNIS+7 endpoint. Primary endpoint data that are missing will be multiply imputed as described in Section 3.10. Analyses will be conducted using PROC MI and PROC MIANALYZE in SAS 9.3 (or later). The efficacy of patisiran will have been established if the estimate of the differences in mNIS+7 LS means, after adjusting for the terms in the ANCOVA model, demonstrates a treatment effect of patisiran relative to placebo with a p-value (2-sided) less than or equal to 0.05, based on the MI methodology.

This mNIS+7 analysis will be repeated for the PP population.

Sensitivity analyses will assess the robustness of the primary analysis in the mITT population for different methods of handling missing data, including mixed model for repeated measures (MMRM), complete cases (CC), and last observation carried forward (LOCF). The MMRM outcome variable will be change from baseline in mNIS+7. The regression model will include baseline mNIS+7 value as a covariate, patient as a random effect, and fixed effect terms including treatment group, visit, treatment group-by-visit interaction, early onset V30M [<50 years of age at onset] *vs.* all other mutations [including late onset V30M], and previous tetramer stabilizer use (yes *vs.* no). It will also include visit time point as a random effect. The covariance structure will be selected by minimizing the Akaike Information Criterion (AIC) across models fit with the independent, unstructured, autoregressive (1), and compound symmetry covariance structures. Additional covariance structures may also be evaluated. All change in mNIS+7 assessments recorded post-baseline may be included; however the primary comparison will be based on the change in mNIS+7 from baseline to 18 months.

Complete cases (CC) are defined as all patients with non-missing 18 month mNIS+7 assessments. The CC analysis will be performed using ANCOVA, with the model mirroring that described for the primary efficacy analysis; however only complete cases will be included in this analysis. Note that this commonly used approach loses power and bias may well be introduced if the data are not missing at random.

A complementary CC on study drug analysis will examine only those patients who received $\geq 80\%$ of scheduled doses. Analysis will proceed as above.

The LOCF analysis will be performed for any patient who drops out prior to 18 months or is otherwise missing the 18 month mNIS+7 assessment. For this analysis, the last recorded mNIS+7 value will be carried forward and used as the 18 month assessment value. The ANCOVA model will otherwise mirror that of the primary efficacy analysis. For this study, given the progressive nature of the disease, LOCF will favor early discontinuations. With equal drop-out rates in patisiran and placebo groups, LOCF will generally underestimate the treatment effect; but with unequal drop-out rates, bias could be much larger and in either direction.

Least Squares Means and 95% CIs for change from baseline in mNIS+7 will be plotted over time. P-values from the differences of LS Means will be included in the plot. Estimates of LS Means, associated CIs and p-values for the 18 month time point will be based on the MI methodology, whereas the 9 month time point will be based on observed data (i.e., complete cases).

4.3.2. Secondary Efficacy Evaluations

Change from baseline at Month 18 in the secondary efficacy endpoints will be analyzed using methods similar to those employed for the primary analysis, i.e., ANCOVA with multiple imputations, as appropriate. All secondary endpoints will be analyzed using the mITT population; analysis of Norfolk QOL-DN Total QoL Score will also be conducted using the PP population.

Type I error control for secondary endpoints will be achieved by a hierarchical ordering procedure. Endpoints will be tested in the following pre-specified hierarchy:

1. Norfolk QOL-DN Total QoL Score
2. NIS-W score
3. mBMI
4. Timed 10-meter walk test
5. COMPASS-31

If and only if a comparison is significant at two-sided $p \leq 0.05$, the next endpoint in the hierarchy may be formally tested; if a given comparison is not significant at two-sided $p \leq 0.05$, the subsequent tests will be performed and the results summarized but statistical significance will not be inferred.

Plots of LS Means and 95% CIs will be provided for each secondary endpoint, as described above for the primary endpoint.

4.3.3. Exploratory Efficacy Evaluations

An analysis of mNIS+7 change from baseline at 9 months using complete cases will be performed as an exploratory analysis. An exploratory, complementary CC on study drug analysis of 9 month efficacy using only patients who received $\geq 80\%$ of doses (through 9 months) will also be conducted.

Additional exploratory analyses will include comparisons of the proportion of patients in each arm that progress by ≥ 24 mNIS+7 points at 18 months, those that progress by ≥ 1 FAP Stage, and those that meet both criteria, via Fisher's Exact Test.

The additional continuous exploratory efficacy variables (see Section 1.1.2.3), including those closely related to the mNIS+7 primary endpoint, will be compared using ANCOVA methods similar to those employed for the primary analysis; however no imputation of missing data will be performed. Binary exploratory endpoints, including a responder analysis of patients exhibiting neurological stability (< 2 -point increase in mNIS+7 composite score from baseline to Month 18), will be assessed by the Cochran-Mantel-Haenszel test, stratified by the randomization stratification factors. A categorical summary of the numbers and percentages of patients reporting each ordinal response within each EQ-5D domain will be presented. The association between treatment and ordinal response will be assessed by the Pearson chi-square test.

Mixed models may also be used to explore the heterogeneity of the treatment effect across sites. These models consider site and treatment-by-site effects to be random, and are especially relevant when the number of sites is large.

4.3.4. Exploratory Analysis of Association between Pharmacodynamic and Clinical Activity

Associations between TTR knockdown (as indicated below) and clinical activity parameters will be explored via graphical presentation and linear regression.

- Serum TTR protein area under the curve
- Average trough percent TTR level relative to baseline (excluding pre-first dose)
- Average trough TTR concentration ($\mu\text{g/mL}$; excluding pre-first dose)

Additionally, patients in the patisiran study arm will be placed into percentile-based groupings (e.g. tertiles, based on the 33rd and 67th percentiles), according to the above measures.

At the 9 and 18-month timepoints, clinical activity parameters listed above in sections 4.3.1-4.3.2 (both actual data and change from baseline) will be summarized in the above PD groups and presented graphically and via descriptive statistics.

Additional exploratory analyses will summarize clinical activity parameters listed above in sections 4.3.1-4.3.3 (both actual data and change from baseline) in groups of patients with < or \geq 80% average predose TTR relative to baseline, as defined above.

4.4. Pharmacokinetic and Pharmacodynamic Evaluations

All tabulations of PK data will be presented separately from the summaries and analyses discussed in this SAP.

Summary tables and graphical displays of observed values and changes from baseline in serum TTR will be used to assess the durability of suppression over the course of the study. Similar summaries will be provided for the secondary PD biomarkers (RBP and vitamin A).

All PD data will be displayed in data listings.

4.5. Safety Analyses

Safety analyses will be conducted using the Safety population.

4.5.1. Study Drug Exposure

Exposure to study drug will be characterized by presenting descriptive statistics for duration of infusion (per infusion) and amount of study drug received (per infusion and in total). Descriptive statistics (both continuous and categorical) will also be presented for the number of doses received. The number of patients who experienced dose interruptions for any reason will be tabulated, as well as the number of patients with dose interruptions due to an acute infusion reaction.

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

4.5.2. Adverse Events

All AEs will be coded using the MedDRA coding system (version 16.1 or later) and displayed in tables and data listings using system organ class (SOC) and preferred term.

Summaries of AEs will be performed for those events that are considered treatment-emergent, where treatment-emergent is defined per protocol as any adverse event with onset after the first administration of study medication through 28 days after the last dose, or any event that was

present at baseline but worsened in intensity or was subsequently considered drug-related by the Investigator. 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 medication.

AEs will be summarized by the numbers and percentages of patients reporting a given AE. AEs Therefore, in any tabulation, a patient contributes only once to the count for a given AE (overall, by SOC, by preferred term). Overall event counts and frequencies may also be summarized.

An overall summary of AEs will include the number and percentage of patients with any treatment-emergent adverse event (TEAE), with any TEAE assessed by the Investigator as related to treatment (definite or possible relationship), with any severe TEAE, with a severe TEAE related to treatment, with any treatment-emergent serious adverse event (SAE), and with any TEAE leading to discontinuation will be summarized by treatment group and overall.

Tabulations by SOC and preferred term will be produced for all TEAEs, for all TEAEs related to study medication, for all severe TEAEs, for all TEAEs leading to study discontinuation, and for all treatment-emergent SAEs. The most commonly occurring TEAEs, defined as those events experienced by at least 5% of patients in either treatment group, will be tabulated by preferred term in decreasing order in frequency. AEs will also be tabulated by severity.

Infusion reactions (a particular class of TEAE) will be similarly summarized.

Separate tables will present TEAE incidence rates by maximum relationship to study drug and by maximum severity. Patients who report multiple occurrences of the same TEAE (preferred term) will be classified according to the most related or most severe occurrence, respectively.

No formal hypothesis-testing analysis of AE incidence rates will be performed.

All AEs occurring on-study will be listed in patient data listings.

By-patient listings will also be provided for the following: all patient deaths, all SAEs, and TEAEs leading to study discontinuation.

4.5.3. Laboratory Data

Clinical laboratory values will be expressed in SI units.

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

For each continuous laboratory parameter, results will be categorized as low, normal, or high based on the laboratory normal ranges. Shift tables will be employed to summarize the baseline category versus the “worst” post-baseline category, where the “worst” post-baseline category will be based on the maximum difference (in absolute value) from the upper or lower limits of the normal range. All out-of-range and clinically significant laboratory results will be identified in patient data listings.

All laboratory data will be provided in data listings. Laboratory values outside of the normal ranges will be listed separately, together with comments as to their clinical significance.

4.5.4. Vital Signs and Physical Examination

Descriptive statistics will be provided for vital signs, including blood pressure, pulse rate, oral body temperature and respiration rate.

Vital sign measurements will be presented for each patient in a data listing.

All physical examination findings will be presented in a by-patient data listing. Abnormal physical examination findings will be presented in an additional, separate listing.

4.5.5. Electrocardiogram

Electrocardiogram (ECG) results will be summarized descriptively, including the number and percentage of patients with normal, abnormal, and clinically significant abnormal results at baseline and each study visit. Descriptive statistics will be provided for ECG interval data.

The numbers and percentages of patients with QTc interval values (corrected according to Fridericia's formula) or changes from baseline meeting the following criteria at any time post baseline will be presented:

- QTc interval > 450 msec
- QTc interval > 480 msec
- QTc interval > 500 msec
- QTc interval increases from baseline > 30 msec
- QTc interval increases from baseline > 60 msec

Electrocardiogram data for each patient will be provided in a data listing.

4.5.6. Concomitant Medications

Concomitant medications will be coded using the WHO Drug Dictionary (Q12013 or later). Results will be tabulated by anatomic therapeutic class (ATC) and preferred term.

Concomitant medications will be tabulated by treatment group, where any medications that did not end prior to first dose will be included. If an end date is missing or the medication is ongoing, the medication will be included.

The use of concomitant medications will be included in a by-patient data listing.

5. CHANGES TO PLANNED ANALYSES

The primary efficacy analysis, as described in this document, differs from that described in the current clinical protocol (Amendment 4: 4 August 2014) in the explanatory covariates included in the (complete data) ANCOVA and in the multiple imputation method. These differences also cascade to supportive analyses of mNIS+7 and analyses of other endpoints.

This document specifies a complete data ANCOVA with baseline mNIS+7 value as a continuous covariate, and genotype class (early onset V30M vs. other), prior tetramer stabilizer (tafamidis or diflunisal) use (Yes vs. No), and treatment group (patisiran [ALN-TTR02] vs. Placebo) as factors. The clinical protocol specifies an ANCOVA in which age and genotype appear independently. This change is made to let the analysis better reflect the stratification employed in randomization.

The multiple imputation (for completely missing mNIS+7 assessments) described in this document differs from that in the clinical protocol in that 1) stepwise variable selection has been replaced with a pre-specified set of covariates to be included; 2) imputation is done separately by treatment arm; and 3) 10 imputation datasets are to be created per endpoint. The rationale for these changes is outlined below.

Stepwise variable selection was removed in order to simplify the analysis.

This document specifies that imputation is done separately by treatment arm; the current protocol specifies says that treatment assignment will not be included in the imputation. This change is motivated by simulations [results not shown] demonstrating a non-negligible loss in power when a single imputation model is employed and treatment is not included in the model; this loss in power is due to “cross-contamination” of imputation models between randomized arms.[34]

The number of imputed datasets per endpoint has been reduced from 100 to 10, consistent with the observation that 3-5 imputations are often sufficient.[35]

The protocol will be updated in a future amendment to reflect these updates to the primary efficacy analysis.

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7. QUESTIONNAIRE/SCORING APPENDICES

7.1. Neuropathy Impairment Score (NIS+7) and Modified Neuropathy Impairment Score (mNIS+7)

Note: the mNIS+7 and NIS+7 measurements are conducted in duplicate per timepoint. The average of two complete duplicate values will be reported, except in cases of missing or partially missing data as described below.

| Assessment Tool | Total Points | Components (maximum points) |
|-----------------|--------------|---|
| NIS+7 | 270 | <ul style="list-style-type: none"> • Neurologic exam of lower limbs, upper limbs and cranial nerves (NIS) <ul style="list-style-type: none"> • NIS-W: Weakness (192) • NIS-S: Sensation (32) • NIS-R: Reflexes (20) • Nerve conduction studies $\sum 5$ (18.6)^a <ul style="list-style-type: none"> • Sural SNAP, tibial motor n. distal latency, peroneal CMAP/motor n. conduction velocity/motor n. distal latency • Vibration detection threshold (3.7) • Heart rate response to deep breathing (3.7) |
| Modified NIS+7 | 304 | <ul style="list-style-type: none"> • Neurologic exam of lower limbs, upper limbs and cranial nerves (mNIS) <ul style="list-style-type: none"> • NIS-W: Weakness (192) • NIS-R: Reflexes (20) • Nerve conduction studies $\sum 5$ (10) <ul style="list-style-type: none"> • Ulnar CMAP and SNAP, sural SNAP, tibial CMAP, peroneal CMAP • Quantitative sensory testing: QST-BSA_{TP+HP5} (80) • Postural blood pressure (2) |

NIS + 7 Scoring Method

1. Sum the point values of NIS-W, NIS-R and NIS-S.
- 2.



[REDACTED]

- [REDACTED]
- [REDACTED]
- [REDACTED]
- [REDACTED]
- [REDACTED]
- [REDACTED]
- [REDACTED]

MODIFIED NIS + 7 (mNIS+7) Scoring Method

If values at a given timepoint and (replicate) assessments are non-missing for each component, do the following:

1. [REDACTED]
- [REDACTED]
- [REDACTED]
- [REDACTED]
 - [REDACTED]
 - [REDACTED]
 - [REDACTED]

- 

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

Part I: Symptoms

[REDACTED]

Part II: Activities of Daily Life

[REDACTED]

Subscales and Scoring Algorithm

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

- Total QOL
- Physical Functioning/Large Fiber
- Activities of Daily Living (ADLs)
- Symptoms
- Small Fiber
- Autonomic

[REDACTED]

Missing Values

[REDACTED]

- [REDACTED]
- [REDACTED]
- [REDACTED]

7.3. EuroQOL-5-Dimension 5-Level (EQ-5D-5L)

Each of the 5 dimensions (Mobility, Self-Care, Usual Activities, Pain/Discomfort, Anxiety/Depression) is scored on a 5-point Likert scale from 1 (“I have no problems/pain/anxiety”) to 5 (“I am unable to...”, “I have extreme anxiety/depression”).

The five scores are concatenated together (in the order of Mobility, Self-Care, Usual Activities, Pain/Discomfort, Anxiety/Depression) to create an EQ-5D-5L profile (e.g., 11111, 55555). The profile is then used to obtain an index value using the United States value set. The index values range from -0.109 , associated with a profile of 55555, to 1.0, associated with a profile of 11111. Smaller index values indicate greater impairment.

Missing values are handled as follows:

- Any single missing item will be imputed as the maximum of the non-missing values among all other patients (within study arm).
- Missing values will only be imputed for the purpose of creating a profile value; no imputation will be performed for the analysis of ordinal responses.
- If the entire instrument is missing, the EQ-5D-5L index value will be imputed using the multiple imputation model as discussed in section 3.10.

7.4. Rasch-Built Overall Disability Scale (R-ODS)

The R-ODS consists of 25 items scored on a scale of 0 (unable to perform), 1 (able to perform, but with difficulty) or 2 (able to perform without difficulty). A total score will be calculated as the average of all non-missing items multiplied by 25.

[REDACTED]

[REDACTED]

- [REDACTED]
- [REDACTED]

[REDACTED]

The table consists of 8 rows and 2 columns. All text within the table is completely redacted with black bars. The structure of the table is as follows:

| | |
|------------|------------|
| [Redacted] | [Redacted] |
| [Redacted] | [Redacted] |
| [Redacted] | [Redacted] |
| [Redacted] | [Redacted] |
| [Redacted] | [Redacted] |
| [Redacted] | [Redacted] |
| [Redacted] | [Redacted] |
| [Redacted] | [Redacted] |

The table consists of five rows. Each row is almost entirely obscured by a thick black horizontal bar. A vertical black line runs down the left side of the table, separating the first column from the rest. The redaction covers all text and numbers within the table cells.

STATISTICAL ANALYSIS PLAN: PROTOCOL ALN-TTR02-004

Patisiran

APOLLO: A Phase 3 Multicenter, Multinational, Randomized, Double-blind, Placebo-controlled Study to Evaluate the Efficacy and Safety of Patisiran (ALN-TTR02) in Transthyretin (TTR)-Mediated Polyneuropathy (Familial Amyloidotic Polyneuropathy-FAP)

Protocol Number: ALN-TTR02-004

Global Protocol Version and Date: Version 6: 08 September 2015
Version 5: 4 August 2014
Version 4: 24 April 2014
Version 3: 21 March 2014
Version 2: 18 October 2013
Original Version 1: 15 August 2013

Name of Test Drug: Patisiran (ALN-TTR02)

Phase: Phase 3

Methodology: Randomized, double-blind, placebo-controlled study

Sponsor: Alnylam Pharmaceuticals, Inc.
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Sponsor Representative: [REDACTED]

Analysis Plan Version: Version 2.1: 23 August 2017
Version 2.0: 31 May 2017
Version 1.0: 31 March 2015

Confidentiality Statement

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[REDACTED]

[REDACTED] [REDACTED]

[REDACTED] [REDACTED]

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

| Abbreviation | Definition |
|---------------------|--|
| Σ5 | 5 Attributes |
| AA | Amyloid A |
| ADA | Anti-drug Antibody |
| AE | Adverse Event |
| ANCOVA | Analysis of Covariance |
| ATC | Anatomic Therapeutic Class |
| BMI | Body mass index |
| CMAP | Compound Muscle Action Potential |
| Cmax | Post-Infusion Peak Concentration |
| Cmax_ss | Post-Infusion Peak Concentration at Steady State |
| COMPASS-31 | Autonomic Symptoms Questionnaire (Composite Autonomic Symptom Score) |
| Cp(30min) | 30 Minutes Post-Infusion Concentration |
| Cp_ss(30min) | 30 Minutes Post-Infusion Concentration at Steady State |
| CRO | Contract Research Organization |
| CSR | Clinical Study Report |
| Ctrough | Pre-Infusion Trough Concentration |
| Ctrough_ss | Pre-Infusion Trough Concentration at Steady State |
| CV | Coefficient of Variation |
| DMC | Data Monitoring Committee |
| ECG | Electrocardiogram |
| eCRF | Electronic Case Report Form |
| EQ-5D | Euro Quality of Life- 5 Dimensions |
| EU | European Union |
| FAC | Familial Amyloidotic cardiomyopathy |
| FAP | Familial Amyloidotic Polyneuropathy |
| hATTR | Hereditary Transthyretin-Mediated Amyloidosis |
| HP | Heat pain |
| HRdb | Heart Rate Response to Deep Breathing |
| ICH | International Conference on Harmonisation |
| IENFD | Intraepidermal Nerve Fiber Density |
| INN | International Nonproprietary Name |
| IRS | Interactive response system |
| IV | Intravenous |
| LNP | Lipid Nanoparticle |
| LS | Least Squares |
| mBMI | Modified Body Mass Index |
| MedDRA | Medical Dictionary for Regulatory Activities |

| Abbreviation | Definition |
|---------------------|---|
| MI | Multiple Imputation |
| mITT | Modified Intent-to-Treat |
| MMRM | Mixed-Effects Model Repeated Measures |
| mNIS | Modified Neuropathy Impairment Score |
| NCS | Nerve Conduction Studies |
| NIS | Neuropathy Impairment Score |
| NIS-W | Neuropathy Impairment Score-Weakness Score |
| Norfolk QOL-DN | Norfolk Quality of Life-Diabetic Neuropathy Questionnaire |
| NSAID | Nonsteroidal Anti-Inflammatory Drug |
| NT-proBNP | N Terminal Prohormone of B-Type Natriuretic Peptide |
| PD | Pharmacodynamic |
| PEG | Polyethylene glycol |
| PK | Pharmacokinetic |
| PMM | Pattern Mixture Model |
| PND | Polyneuropathy Disability |
| PP | Per-Protocol |
| QST | Quantitative Sensory Testing |
| RBP | Retinol Binding Protein |
| RNAi | RNA interference |
| R-ODS | Rasch-Built Overall Disability Scale |
| SAE | Serious Adverse Event |
| SAP | Statistical Analysis Plan |
| SD | Standard Deviation |
| SEM | Standard Error of the Mean |
| SGNFD | Sweat Gland Nerve Fiber Density |
| siRNA | Small Interfering Ribonucleic Acid |
| SNAP | Sensory Nerve Action Potential |
| SOC | System Organ Class |
| SUSAR | Suspected Unexpected Serious Adverse Reaction |
| T4 | Thyroxine |
| TP | Touch pressure |
| TTR | Transthyretin |
| V30M | Val30Met Genotype |
| VAS | Visual Analog Scale |
| VDT | Vibration Detection Threshold |
| WHO | World Health Organization Drug Dictionary |
| WT | Wild-Type |

1. INFORMATION FROM THE STUDY PROTOCOL

1.1. Introduction and Objectives

1.1.1. Introduction

Hereditary transthyretin-mediated (hATTR) amyloidosis is an inherited, autosomal dominant, systemic disease caused by mutations in the transthyretin (TTR) gene [1]. Transthyretin is a tetrameric 127 amino acid protein that is secreted predominantly (> 95%) by hepatocytes, with a smaller fraction produced by the choroid plexus and retina [1]. Physiologically, TTR is a major serum carrier for retinol binding protein (RBP) and a minor carrier of thyroxine (T4). Mutations in the TTR protein lead to destabilization of the tetrameric form and dissociation into dimers and monomers. Misfolding of mutated monomers from the α -helical to the β -pleated sheet structure, results in tissue deposition of amyloid fibrils [2]. Amyloid deposits typically contain both mutant and wild-type (WT) TTR. The particular TTR mutation and site of amyloid deposition determines the clinical manifestations of the disease, which include sensory and motor neuropathy, autonomic neuropathy, and/or cardiomyopathy. hATTR is a progressive disease associated with severe morbidity, with a life expectancy limited to 5 to 15 years from symptom onset [2]. There are over 100 reported TTR mutations which are associated with 2 clinical syndromes: familial amyloidotic polyneuropathy (FAP) and familial amyloidotic cardiomyopathy (FAC) [2, 3, 4].

‘Patisiran’ (the International Nonproprietary Name [INN] name for ALN-TTR02) is being developed for the treatment of hATTR patients with symptomatic polyneuropathy.

The estimated worldwide prevalence of FAP is 5,000 to 10,000, with the majority of cases in Portugal, Sweden, France, Japan, Brazil, and the United States [2, 3]. The most common causative mutation of FAP is TTR Val30Met (V30M), with the onset of symptoms typically occurring between 30 and 55 years of age [4]. Amyloid deposition occurs largely in the peripheral nerves, starting as a nerve length-dependent sensory polyneuropathy in the feet causing numbness and pain and progressing to painful dysesthesias. Disabling motor neuropathy follows, characterized by leg weakness and eventually the inability to walk. Autonomic neuropathy is another common feature of the disease, resulting in severe gastrointestinal pathology (including diarrhea or constipation and malabsorption, leading to severe malnutrition), orthostatic hypotension, and bladder dysfunction with recurring urinary tract infections [4, 5, 6, 7]. For several mutations, cardiac pathology also occurs due to amyloid infiltration of the sinus node, atrioventricular conduction system, and infiltration of the myocardium [4, 5]. Involvement of the conduction system can lead to sudden death due to dysrhythmias, and myocardial infiltration can lead to diastolic dysfunction and right-sided heart failure [4]. Cardiomyopathy then proceeds inexorably, leading to death typically within 10 years [4].

Because the liver is the primary source of WT and mutant TTR, orthotopic liver transplantation has been used since 1990 in an attempt to treat FAP [2], and is the current standard of care in patients who are eligible for transplant (patients with minimal neuropathy symptoms and no cardiac involvement). When liver transplantation is performed early in the course of the disease, it can stabilize and slow the course of neuropathic disease in patients with FAP due to V30M, but is less effective in patients with other TTR mutations [2]. However, it is less effective in patients with more advanced disease, especially those with heart involvement, due to the continued production and deposition of WT TTR in tissues with pre-existing amyloid [2, 3, 4].

It is estimated that approximately two-thirds of FAP patients are not transplant-eligible. Furthermore, liver transplant poses risks from the surgical procedure and from life-threatening complications due to graft rejection or infections. The 1-year mortality rate post-transplant is 10% [2].

Nonsurgical options that are used for the treatment of FAP (depending on geographic location) include tafamidis (Vyndaqel®) and diflunisal. Tafamidis is a small molecule TTR stabilizer that binds to the thyroxine binding sites of the TTR tetramer, thus preventing its dissociation to monomers and potentially preventing fibril formation. While tafamidis is approved in the European Union (EU) for the treatment of hATTR in adult patients with Stage 1 symptomatic polyneuropathy to delay peripheral neurologic impairment, the pivotal trial data were primarily from FAP patients with the V30M mutation; furthermore, tafamidis is not considered the standard of care throughout the EU and it has not been approved for use in the US [2].

Diflunisal is a generic, nonsteroidal anti-inflammatory drug (NSAID) that is also a tetramer stabilizer and binds to TTR in a similar manner as tafamidis. An NIH- sponsored multicenter, placebo-controlled Phase 3 study in FAP patients was completed in 2012; data suggest an effect of diflunisal on neuropathic score NIS+7, the primary endpoint of the study [2]. Due to the restricted use of liver transplantation and tafamidis in patients with early stage of disease, and the non-standard use of diflunisal among practitioners, there remains an unmet medical need for a potent and effective therapy for FAP that will have an impact on patients across a broad range of neurologic impairment, regardless of their mutation (V30M or non-V30M).

Patisiran comprises a small interfering ribonucleic acid (siRNA) which is specific for TTR, and is formulated in a hepatotropic lipid nanoparticle (LNP) for intravenous (IV) administration [2]. This TTR siRNA has a target region within the 3'UTR region of the TTR gene to ensure and confirm homology with WT TTR as well as all reported TTR mutations. Following LNP-mediated delivery to the liver, the siRNA targets TTR mRNA for degradation, resulting in the potent and sustained reduction of mutant and WT TTR protein via the RNA interference (RNAi) mechanism.

Since circulating TTR is almost exclusively synthesized in the liver, the IV administration of patisiran is postulated to reduce the level of precursors that lead to amyloid fibril deposition, resulting in clinical benefit to patients with FAP.

The therapeutic hypothesis that systemic amyloidoses can be managed by reduction in circulating levels of amyloidogenic protein has been validated in other acquired (e.g., immunoglobulin light chain systemic [AL], or amyloid A [AA]) and hereditary (e.g., Fibrinogen A α -chain, ApoA1) amyloidosis. The experience from these systemic amyloidotic disorders [2, 3, 4, 5], as well as the liver transplant data in FAP, suggest that lowering of the circulating amyloidogenic protein by at least 50% impacts the clinical course of the disease, with reductions in protein beyond 50% providing further incremental improvements in outcomes. It is therefore postulated that the > 80% suppression in both WT and mutant TTR observed upon administration of 0.3 mg/kg patisiran once every 21 days will result in clinical benefit in hATTR patients with polyneuropathy. This hypothesis is further supported by evidence from tafamidis suggesting that reduction in free TTR monomer can slow neuropathy progression in early-stage V30M patients with FAP [8].

Importantly, data from a Phase 2 open-label extension study with patisiran (ALN-TTR02-003) in 27 FAP patients showed a mean sustained TTR reduction of ~80% accompanied with stabilization of neuropathic impairment scores at 6 months, which compared favorably to the anticipated increase in scores based on natural history and other datasets [2].

Patisiran is currently being investigated in APOLLO, a Phase 3 pivotal study (ALN-TTR02-004) evaluating the efficacy and safety of patisiran in hATTR patients with polyneuropathy. This protocol was discussed with the Agency at the end-of-Phase 2 meeting on 23 September 2013 (FDA ref ID # 3394206 for meeting minutes).

The original statistical analysis plan (SAP) Version 1.0 was submitted to the Agency on 07 April 2015 (FDA IND 117395 Serial No. 0019). The response to the Agency's review comment on multiple imputation (MI) was submitted on 31 August 2015 (FDA serial No. 0028). The SAP amendment Version 2.0 was submitted to the Agency on 01 June 2017. This SAP Version 2.1 incorporates a few minor updates for analysis of data from this study.

1.1.2. Document and Study Objectives

This SAP is designed to outline the methods to be used in the analysis of study data in order to address the study objectives of Study ALN-TTR02-004. Populations for analysis, data handling rules, statistical methods, and formats for data presentation are provided. The statistical analyses and summary tabulations described in this SAP will provide the basis for the results sections of the clinical study report (CSR) for this trial.

This SAP will also outline differences, if any, in the currently planned analytical objectives relative to those planned in the study protocol and/or in the original SAP V1.0.

The study objectives from protocol are listed below.

1.1.2.1. Primary Objective

The primary objective of the study is to determine the efficacy of patisiran by evaluating the difference between the patisiran and placebo groups in the change from baseline of Modified Neuropathy Impairment Score (mNIS+7) score at 18 months.

1.1.2.2. Secondary Objectives

The secondary objectives of the study are to determine the effect of patisiran on various clinical parameters by assessing the difference between patisiran and placebo in the change from baseline in the following measurements at 18 months:

- Norfolk Quality of Life-Diabetic Neuropathy (Norfolk QOL-DN) Total QoL Score.
- Neuropathy Impairment Score-weakness (NIS-W) score.
- Modified body mass index (mBMI), calculated by multiplying the BMI (kg/m²) by serum albumin level (g/L).
- Timed 10-meter walk test speed (meter/second), calculated as 10 meters divided by the walking time (second).
- Autonomic symptoms questionnaire (Composite Autonomic Symptom Score [COMPASS-31]).

1.1.2.3. Exploratory Objectives

The exploratory objectives of the study are:

- To determine the difference between the patisiran and placebo groups in the change from baseline in the following measurements at 18 months:
 - NIS+7 score;

- Grip strength;
- EuroQOL (EQ-5D) questionnaire;
- Level of disability (Rasch-built Overall Disability Scale [R-ODS]);
- Large vs. small nerve fiber function including nerve conduction studies (NCS) 5 attributes ($\Sigma 5$), quantitative sensory testing by body surface area including touch pressure and heat pain (QST), vibration detection threshold (VDT), heart rate response to deep breathing (HRdb), postural blood pressure;
- Pathologic evaluation of sensory and autonomic innervation through voluntary skin punch biopsies and analysis of intraepidermal nerve fiber density (IENFD) and sweat gland nerve fiber density (SGNFD))
- Assessment of ambulation through FAP stage and Polyneuropathy Disability (PND) score;
- Cardiac assessment through echocardiogram, troponin I, and N-terminal prohormone of B-type natriuretic peptide (NT-proBNP) levels;
- Pharmacodynamic (PD) biomarkers (TTR, RBP, vitamin A);
- To compare the proportion of patients in the patisiran and placebo groups who meet the pre-defined criterion for rapid disease progression (defined as ≥ 24 -point increase in mNIS+7 from baseline [based on an average of 2 measurements] and FAP stage progression relative to baseline) at 9 months;
- To serially evaluate lower limb nerve injury via voluntary magnetic resonance (MR) neurography approximately every 6 months in a subset of patients receiving either patisiran or placebo who consent to perform this assessment.

1.2. Study Design

1.2.1. Synopsis of Study Design

This is a multicenter, multinational, randomized, double-blind study comparing patisiran to placebo in hATTR patients with symptomatic polyneuropathy.

Consented eligible patients will be randomized to receive either 0.3 mg/kg patisiran or placebo in a 2:1 ratio (patisiran to placebo) in a blinded manner. Randomization will be stratified by Neuropathy Impairment Score (NIS; < 50 vs. ≥ 50), early onset V30M (< 50 years of age at onset) vs. all other mutations (including late onset V30M), and previous tetramer stabilizer use (tafamidis or diflunisal) vs. no previous tetramer stabilizer use. Patients will receive patisiran or placebo once every 21 days for 78 weeks (18 months).

Patients will have efficacy assessments at Screening/Baseline, 9 months, and 18 months. Study personnel performing assessments related to the efficacy endpoints will be different from the Investigator and other personnel managing the patient, and all of these study personnel will be blinded to any clinical laboratory results that could potentially unblind them (e.g., TTR levels, vitamin A levels, thyroid function tests). In addition, the study personnel performing assessments related to the efficacy endpoints will also be blinded to the results of any previous assessments (e.g., Screening/Baseline, Baseline, or 9-month assessments). Whenever possible, the same site personnel (individual) will conduct efficacy assessments for a given patient across time points.

At the 9-month time point, if the clinical adjudication committee determines that a patient is exhibiting rapid disease progression (defined as ≥ 24 -point increase in mNIS+7 from baseline [based on an average of 2 measurements] and FAP stage progression relative to baseline), the patient's treating physician will provide the patient with the option of discontinuing study drug and receiving local standard of care treatment for FAP. Patients who discontinue study drug will remain on study, following a modified schedule of visits, through completion of the 18-month efficacy assessments and blinding will be maintained throughout.

Patients who complete the 18-month efficacy assessments can elect to participate in an extension study in which patients would receive open-label administration of 0.3 mg/kg patisiran once every 21 days.

A Data Monitoring Committee (DMC) will be implemented for the study and will operate under a pre-specified charter.

1.2.2. Randomization Methodology

Patients will be randomly assigned in a 2:1 ratio to receive either 0.3 mg/kg patisiran or placebo (normal saline 0.9%).

Patients will be randomized via an interactive response system (IRS). Either designated site personnel or the pharmacist may request randomization for the patient, but only the pharmacist or pre-identified unblinded personnel will be allowed to receive the randomized treatment code. The treatment code will be delivered to the unblinded personnel or the pharmacist to prepare the necessary number of vials for that patient based on their weight.

As described above in Section 1.2.1, the stratification factors for randomization include NIS (< 50 vs. ≥ 50), early onset V30M (< 50 years of age at onset) vs. all other mutations (including late onset V30M), and previous tetramer stabilizer use (tafamidis or diflunisal) vs. no previous tetramer stabilizer use.

1.2.3. Rapid Disease Progression

Patients who have evidence of rapid disease progression at 9 months (defined as ≥ 24 -point increase in mNIS+7 from baseline [based on an average of 2 measurements] and FAP stage progression relative to baseline) will be given the option of discontinuing study drug and receiving local standard of care treatment for their FAP. Those who elect this option will be asked to follow a modified study visit schedule and return for their 18-month efficacy assessment (blinding will be maintained throughout).

1.2.4. Withdrawals, Dropouts, Loss to Follow-up

Patients are free to discontinue treatment or withdraw from the study at any time and for any reason, without penalty to their continuing medical care.

There are 3 ways for a patient to discontinue treatment and/or withdraw from the study:

- 1) The patient or investigator decides to discontinue study treatment, but the patient agrees to remain in the study and undergo follow-up assessments;
- 2) The patient experiences protocol-defined rapid disease progression at Month 9 and elects to discontinue study treatment but remain in the study and return for protocol-specified visits, including follow-up assessment at Month 18;
- 3) The patient decides to no longer participate in the study and withdraws consent.

A patient will be considered to have completed the study if the patient does not withdraw consent from the study and completes protocol-specified procedures up through the 18-month efficacy assessment visit.

1.2.5. Unblinding

Unblinding is only to occur in the case of patient emergencies or when necessary from a regulatory reporting perspective (e.g., Suspected Unexpected Serious Adverse Reaction [SUSAR] occurring in the EU), and at the conclusion of the study.

Patients who discontinue study drug at 9 months due to rapid disease progression will remain blinded throughout the remainder of the study.

In the event that the Investigator requests to know a patient's study treatment assignment, the Investigator is instructed to contact the Contract Research Organization (CRO) Medical Monitor to discuss the need for unblinding. In case of an emergency, the treatment allocation for each patient will be available from the unblinded site personnel, pharmacist, or the IRS system.

If a patient becomes pregnant or seriously ill during the study, the blind should be broken only if knowledge of the treatment administered will affect treatment options available to the patient. Before breaking the blind, the Principal or Sub-investigator should attempt to contact the CRO Medical Monitor. If the Medical Monitor is immediately unreachable, the Principal or Sub-investigator should break the blind as necessary using the code breaking information provided and contact the CRO Medical Monitor as soon as possible. A record will be kept of when the blind was broken, who broke it, and why.

1.2.6. Study Procedures

The schedule of assessments is described in the study protocol (Table 1-1, Table 1-2, and Table 1-3).

1.2.7. Efficacy, Pharmacokinetic, Pharmacodynamic, and Safety Parameters

1.2.7.1. Efficacy Parameters

Efficacy parameters will include the following. All evaluations will be conducted at baseline and at 9 and 18 months (except for mBMI as described below).

Primary Endpoint

- Neurologic impairment will be assessed using the mNIS+7 composite score (range: 0 to 304 points). The mNIS+7 include the modified NIS (weakness and reflexes), $\Sigma 5$ NCS, QST, as well as autonomic assessment through postural blood pressure. Two assessments will be performed at each visit; each component contributing to the composite score is the average of the 2 assessments. A scoring algorithm, including methods for handling missing components of the mNIS+7, is included in Appendix 7.2.1.

Secondary Endpoints

- Patient reported QOL will be evaluated using the Norfolk QOL-DN total score (range: -4 to 136 points). A scoring algorithm for this instrument is included in Appendix 7.2.2.
- Motor strength will be evaluated using NIS-W, calculated as the average of the 2 assessments performed at each visit (range: 0 to 192 points).

- Level of disability will be assessed using the Rasch-built Overall Disability Scale (R-ODS; range: 0 to 48). A scoring algorithm for the R-ODS is included in Appendix 7.2.4.
- Functional status will additionally be assessed by 10-meter walk gait speed.
- Nutritional status will be assessed using mBMI, calculated by multiplying the BMI (kg/m²) by serum albumin level (g/L). mBMI will be assessed at baseline, Day 84, Day 189, Day 357, Day 462 and Day 546.
- Autonomic symptoms will be assessed using the COMPASS-31 total score (range: 0 to 100 points). A scoring algorithm for the COMPASS-31 total score and domain scores is included in Appendix 7.2.5.

Exploratory Endpoints

Exploratory endpoints are detailed in Section 1.1.2.3.

In addition, the evaluation of dermal amyloid content (% Congo Red staining) using same skin punch biopsy specimens analyzed for nerve fiber density (SGNFD and IENFD) will also be assessed as an exploratory endpoint.

1.2.7.2. Pharmacokinetic Parameters

Blood samples for determination of patisiran pharmacokinetics (PK) will be collected as outlined in the schedule of assessments. Plasma siRNA, DLin-MC3-DMA, and PEG₂₀₀₀-C-DMG concentrations will be determined in all patients in order to estimate individual PK parameters such as peak and trough concentrations.

Urine will be collected with void volume recorded for all patients at time points specified in the schedule of assessments to determine concentration of siRNA and 4-dimethylaminodibutyric acid (the metabolite of DLin-MC3-DMA) after dosing with study drug.

Pharmacokinetic parameters will be estimated by the Clinical Pharmacology and Pharmacometrics Department at Alnylam Pharmaceuticals.

1.2.7.3. Pharmacodynamic and Pharmacology Parameters

Pharmacodynamic markers assessed serially will include serum TTR, vitamin A, and RBP. Additional blood samples will be collected for exploratory biomarkers related to FAP. Anti-drug antibodies (ADA) data will also be collected.

1.2.7.4. Safety Parameters

Safety evaluations to be performed during the study include monitoring of adverse events (AEs) and concomitant medications, physical examinations, measurement of vital signs, 12-lead ECGs, clinical laboratory evaluations including hematology, clinical chemistry (including liver function tests), thyroid function parameters, urinalysis, and ophthalmology examinations.

Suicidal ideation and behavior will be assessed using the Columbia–Suicide Severity Rating Scale (C-SSRS) questionnaire.

2. PATIENT POPULATION

2.1. Population Definitions

The following patient populations will be evaluated and used for presentation and analysis of the data:

- **Modified Intent-to-Treat (mITT) population:** All patients who were randomized and received at least 1 dose of patisiran or placebo. Patients will be analyzed according to the treatment to which they were randomized.
- **Per-protocol (PP) population:** All randomized patients who received at least 1 dose of patisiran or placebo, completed baseline and either 9-month or 18-month mNIS+7 and Norfolk QOL assessments, and did not experience any major protocol deviations that may impact the efficacy results (Section 2.2). Patients will be analyzed according to treatment received.
- **Safety population:** All patients who received at least 1 dose of patisiran or placebo. Patients will be analyzed according to the treatment received.
- **PK population:** All patients in the Safety Population who provided at least 1 PK concentration measurement.

The primary population for efficacy analysis will be the mITT population; the primary endpoint and the first secondary endpoint (Norfolk QOL) will also be analyzed using the PP population. The remaining secondary and exploratory efficacy endpoints will be analyzed using the mITT population only. Safety analysis will be conducted in the safety population. PK analysis will be conducted in the PK population.

2.2. Protocol Deviations

A deviation is considered any departure from the procedures set forth in the protocol. Protocol deviations will be classified into major and minor by medical review and recorded prior to database lock. A major deviation is a deviation that may impact patient safety or efficacy interpretation (for example, failure to meet key inclusion and exclusion criteria). Deviations not designated as major will be considered minor.

The Sponsor or designee will be responsible for producing the final 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 deviation. In addition, each major deviation will be clearly identified as to whether or not it warrants exclusion from the Per Protocol population, based on the potential impact on the efficacy results according to the judgment of the sponsor. This file will be finalized prior to database lock and unblinding of treatment assignments for all patients.

The following are some examples of key inclusion/exclusion criteria. Failure to meet such criteria may warrant the exclusion from the Per Protocol population:

Inclusion:

1. Have a diagnosis of FAP with documented TTR mutation;
2. Have an NIS of 5 to 130 (inclusive) and a PND score of $\leq 3b$;
3. Have a Karnofsky performance status of $\geq 60\%$.

Exclusion:

1. Had a prior liver transplant or is planning to undergo liver transplant during the study period;
2. Has known primary amyloidosis or leptomeningeal amyloidosis;
3. Has known type I diabetes;
4. Has had type II diabetes mellitus for ≥ 5 years;
5. Received an investigational agent or device within 30 days of anticipated study drug administration or 5 half-lives of the investigational drug, whichever is longer;
6. Participated in a clinical trial with antisense oligonucleotide, must have completed a 3-month wash-out prior to start of the study drug administration in this study.

All protocol deviations and major protocol deviations will be presented in data listings.

3. GENERAL STATISTICAL METHODS

3.1. Sample Size Justification

For the estimation of sample size, a mean (\pm SD) mNIS+7 progression rate (primary endpoint) in the placebo group of 24 ± 16 points over 18 months was estimated using natural history data [1] from FAP patients. A sample of 154 patients provides 90% power for a 2-sided t-test with an 8.95-point (37.5%) mean difference between treatment arms in the primary endpoint at 2-sided $\alpha = 0.05$. Assuming a 25% random premature discontinuation rate (due to liver transplantation or other factors), the required sample size for this study is approximately 200.

3.2. General Methods

All data listings that contain an evaluation date will contain a study day relative to the day of the first dose of study drug, which is designated as Day 1. On-treatment study days will be calculated as evaluation date – first dose date +1 and pre-treatment days will be calculated as evaluation date – first dose date. For example, the day prior to study drug administration will be Day -1, the first dose day of study drug will be Day 1 and the day after the first dose of study drug will be Day 2, etc.

All output will be incorporated into Microsoft Word files, sorted and labeled according to the International Conference on Harmonisation (ICH) recommendations, and formatted to the appropriate page size(s).

For categorical variables, summary tabulations of the number and percentage of patients within each category (with a category for missing data) of the parameter will be presented. For continuous variables, the number of patients, mean, median, standard deviation (SD), minimum, and maximum values will be presented.

Laboratory data (including vitamin A and RBP) collected and recorded as below the limit of detection will be set equal to the lower limit of detection for the calculation of summary statistics.

For assessments that are repeated multiple times on the study day (e.g., 10-meter walk tests, IENFD, SGNFD, etc.), the average will be calculated unless otherwise noted.

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 level of significance. Secondary endpoints will be tested in a prespecified hierarchy (Section 3.6). Summary statistics will be presented, as well as 2-sided 95% confidence intervals on selected parameters, as described in the sections below.

All summaries will be presented by treatment arm. All data recorded on the CRF will be included in data listings.

3.3. Computing Environment

All descriptive statistical analyses will be performed using SAS statistical software Version 9.3 (or later), unless otherwise noted. Medical history and AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) Version 18.0 (or later). Concomitant medications will be coded using the World Health Organization (WHO) Drug Dictionary Version March 2015 (or later).

3.4. Baseline Definitions

For the mNIS+7/NIS+7 individual components, total scores and related endpoints, the Screening/Baseline and Baseline visits were performed on separate days. Baseline will be calculated as the average of 2 replicate measures each assessed at the Screening/Baseline and Baseline visits. For grip strength and 10m walk test, if replicate measures are obtained at the Screening/Baseline and Baseline visits, baseline will also be calculated as the average of the 2 replicates.

For PD parameters (TTR, RBP, Vitamin A), baseline will be defined as the average of all records, including those from any unscheduled visits, prior to the date and time of first dose.

For all other parameters, unless noted otherwise, baseline will be defined as the last non-missing measurement on or prior to the first dose of study treatment.

3.5. Randomization Stratification Factors

Stratification factors for randomization include Neuropathy Impairment Score (NIS; < 50 vs. ≥ 50), early onset V30M (< 50 years of age at onset) vs. all other mutations (including late onset V30M), and previous tetramer stabilizer use (tafamidis or diflunisal) vs. no previous tetramer stabilizer use.

Stratification factors are recorded in both the IVR 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 IVR.

3.6. Multiple Comparisons/Multiplicity

Type I error control for secondary endpoints will be achieved by a hierarchical ordering procedure. Endpoints will be tested in the following pre-specified hierarchy:

1. Norfolk QOL-DN questionnaire [Total Score]
2. NIS-W score
3. R-ODS
4. 10-meter walk test speed
5. mBMI
6. COMPASS-31 total score

Only if a comparison is significant at a 2-sided 0.05 significance level, the next endpoint in the hierarchy may be formally tested; if a given comparison is not significant at a 2-sided 0.05 significance level, the subsequent tests will be performed and the results summarized, but statistical significance will not be inferred.

3.7. Initiation of Alternative Treatment for FAP

Rapid progression patients have the option to discontinue study drug, receive alternative FAP treatment, and return for their 18-month efficacy assessment. Non-rapid progressors may also initiate alternative FAP treatment during study, although this would be a major protocol violation. Alternative FAP treatment may confound the efficacy outcome. For the primary analysis of mNIS+7, Norfolk QOL-DN and NIS-W, the assessments collected after alternative FAP treatment (liver transplant or use of tafamidis or diflunisal for more than 14 days) will be

treated as missing. The data post alternative FAP treatment will be included in efficacy listings with a footnote and will also be used in sensitivity analyses as specified.

For all other efficacy endpoints, data collected post alternative FAP treatment will be included in analyses.

A separate listing will be provided for patients who initiate alternative treatment for FAP while on study.

3.8. Missing Data with Efficacy Endpoints

All efficacy data collected during study, regardless of whether before or after treatment discontinuation, will be included for analyses, with the exception of mNIS+7, Norfolk QOL, and NIS-W assessments collected post alternative FAP treatment (discussed in Section 3.7).

3.8.1. Missing Subcomponents within Primary and Secondary Efficacy Endpoints

For each patient, missing subcomponents within the primary mNIS+7 endpoint and secondary efficacy endpoints will be imputed whenever possible according to the algorithm specified in Appendix 7.2.1 through Appendix 7.2.5. When this “partial imputation” is successful (i.e., complete mNIS+7 values are produced), these values will be used in all statistical analyses. When partial imputation is unsuccessful, the efficacy endpoint will be treated as completely missing.

3.8.2. Summary of Missing Data

For each of the primary and secondary efficacy endpoints, the number and percentage of missing data (completely missing) at each visit (baseline, 9-month, and 18-month) will be summarized by study arm.

Time to treatment discontinuation will be estimated descriptively using Kaplan-Meier method by treatment arm. Patients completing study treatment will be censored at the last dose of study drug.

Spaghetti plots will be presented to display the trajectories over time for individual patient’s change from baseline in mNIS+7 and Norfolk QOL-DN for patients who have missing 18-month assessments.

3.8.3. Handling of Missing Data

For the primary and secondary 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 [11]. In this primary analysis, missing data will not be imputed and are assumed to be missing-at-random (MAR).

For the primary endpoint mNIS+7 and the first secondary endpoint Norfolk QOL, sensitivity analyses will be conducted to assess the impact of missing data as discussed in Section 4.3.

3.9. Visit Windows

It is expected that all visits should occur according to 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 efficacy assessments, if the scheduled 9-month or 18-month visits are not performed, the unscheduled and/or discontinuation visits will be grouped with the 9-month or 18-month assessments if they are performed within 3 months of the scheduled assessment. 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 3.4) and for inclusion in any categorical shift summaries (e.g., shift from baseline to “worst” post-baseline value).

3.10. Interim Analyses

No interim analysis was conducted for this study.

3.11. Final Analyses

After the last patient completes the 18-month efficacy assessment or the 18-month efficacy visit window per protocol has elapsed, the sponsor will prepare for the final analysis. The study will then be unblinded and the final analysis will be conducted. If there is additional safety data collected after the final analysis, the data will be presented in listings only.

4. STUDY ANALYSES

4.1. Patient Disposition

Patient disposition will be tabulated and will include the following parameters: the number of patients in each analysis population, the number of patients randomized, the number of patients treated, the number of patients completing treatment, the number of patients completing study, the number of patients who discontinued treatment and primary reasons for treatment discontinuation, the number of patients who withdrew prior to completing the study and primary reasons for withdrawal, the number of patients meeting the protocol specified criteria for rapid disease progression, the number of patients who discontinued treatment but completed the study, and the number of patients who completed treatment but withdrew prior to completing the study. Patient disposition will be presented by randomized treatment arm (patisiran and placebo) and overall.

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

Data listings of treatment/study completion information including the reason for treatment discontinuation and/or study withdrawal will be presented.

4.2. 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, height, weight, and body mass index (BMI) will be summarized using descriptive statistics (number of patients, mean, SD, median, minimum, and maximum). Sex, race, ethnicity, and region will be summarized by presenting the numbers 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:

- Age at hATTR Symptom onset [< 50 ; ≥ 50]
- Neuropathy Impairment Score (NIS) [< 50 ; ≥ 50 & < 100 ; ≥ 100]
- Genotype [V30M; non-V30M]
- Early onset V30M [< 50 years of age at onset] vs. all other mutations [including late onset V30M]
- Previous tetramer stabilizer use [tafamidis or diflunisal] vs. no previous tetramer stabilizer use
- Karnofsky Performance Status (KPS) [60; 70-80; 90-100]
- Cardiac Subpopulation (defined below)
- New York Heart Association (NYHA) Classification [I; II; III; IV]

The cardiac subpopulation will be comprised of patients with pre-existing cardiac amyloid involvement, defined as patients with baseline left ventricular (LV) wall thickness ≥ 1.3 cm and no aortic valve disease or hypertension in medical history.

Time in years since diagnosis with hATTR 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. The number and percent of patients with each genotype will be summarized by country and treatment group.

Continuous efficacy parameters will be summarized using descriptive summary statistics. The number and percent of patients in each category for PND score (I, II, IIIA, IIIB, IV) and FAP stage (I, II, III) will also be summarized.

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

All demographic and baseline data for each patient will be provided in data listings. Medical history data including prior neurological test scores, prior surgeries, and pregnancy test results will be presented in a data listing. Screening test results will also be presented in data listings.

4.3. Efficacy Evaluation

The primary efficacy analyses will be conducted using the mITT population. The primary endpoint mNIS+7 and the first secondary endpoint Norfolk QOL will also be evaluated for the PP population.

4.3.1. Primary Efficacy Evaluations

The primary efficacy endpoint is to compare change in mNIS+7 from baseline to Month 18 between treatment arms for the mITT population.

4.3.1.1. Primary Analysis using MMRM Method for the mITT Population

The primary analysis will be performed using a restricted maximum likelihood (REML) based MMRM approach. The outcome variable is change from baseline in mNIS+7. The model includes baseline mNIS+7 score as a continuous covariate and fixed effect terms including treatment arm, visit (Month 9 or Month 18), treatment-by-visit interaction, genotype (V30M vs. non-V30M), age at hATTR Symptom onset (< 50 ; ≥ 50), region (North America, Western Europe, and Rest of World), and previous tetramer stabilizer use (yes vs. no). An unstructured covariance structure will be used to model the within-patient errors. 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 groups at 18 months. Analysis will be implemented with SAS PROC MIXED.

4.3.1.2. Analysis using MMRM Method for the PP Population

The analysis of the primary endpoint using MMRM method will also be conducted for the Per Protocol (PP) population (defined in Section 2.1).

4.3.1.3. 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.

Multiple Imputation/ANCOVA Method

Multiple Imputation is a broadly applicable technique for handling missing data. Missing data are imputed multiple times using a regression method. Each imputed data set is analyzed by analysis of covariance (ANCOVA) model, and the point estimates and standard errors are combined to provide inferences that reflect the uncertainty about the missing values. MI also makes assumption of missing at random (MAR) mechanism.

Primary endpoint data that are missing will be multiply imputed separately for each treatment arm using a regression procedure, with baseline information including baseline score, genotype, age at ATTR onset, prior tetramer stabilizer use, region, KPS, FAP stage (I vs. II/III), cardiac subpopulation and gender, as well as 9-month efficacy assessment value (when available) as covariates. MI assumes monotone missingness. For non-monotone missing data, e.g., patients completely missing the 9-month assessment but for whom the 18-month assessment is available, the 9-month assessment will be imputed as the treatment group mean. The proportion of non-monotone missing data is expected to be very small.

After imputation, the complete dataset will be analyzed using the ANCOVA model. In the model, baseline mNIS+7 will be used as covariate and treatment arm (patisiran vs. placebo), genotype (V30M vs. non-V30M), age at ATTR onset (Before age 50 vs. After age 50), prior tetramer stabilizer use (Yes vs. No), and region (Western EU, North America, and Rest of World) as factors.

One hundred imputed datasets (per treatment arm) will be generated from the MI regression procedure. Each of the imputed datasets will then be analyzed via the ANCOVA model and the resulting estimates (LS means and standard errors) combined using SAS PROC MIANALYZE to produce inferential results (difference in LS means, 95% CI for the difference, and the p-value from the test that the difference is zero). Point estimates (LS means and differences) will be calculated as the average of the 100 complete-data estimates. A total variance estimate will be calculated as a weighted sum of within-imputation variance, which is the average of the complete-data variance estimates, and a between-imputation variance term. Complete details may be found in the SAS documentation for the MIANALYZE procedure (see Combining Inferences from Imputed Data Sets under Details).

<http://support.sas.com/documentation/onlinedoc/stat/131/mianalyze.pdf>.

Analyses will be conducted using PROC MI and PROC MIANALYZE in SAS 9.3 (or later).

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 is missing not at random (MNAR). The model will be based on the following assumptions:

1. Patients who have missing data and are alive before Month 18:
 - a. Placebo patients who have missing data (either Month 9 or 18): The missing data are considered MAR and will be imputed using 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 (either Month 9 or 18) 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 (either Month 9 or 18) after stopping their study treatment: Patients will no longer benefit from treatment in the future and will have trajectory similar to placebo patients. 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.
2. Patients who die before Month 18 and have missing data: Assuming that deaths observed in the study will likely be related to worsening of disease, the missing data at Month 18 will be imputed by taking random samples from the worst 10% mNIS+7 change scores in the entire population. The imputation will be done for patients from both patisiran and placebo arms.

Missing values will be imputed 100 times to generate 100 complete datasets using procedures as described above. An ANCOVA model will be fit to each “complete” dataset for the change from baseline in mNIS+7 at Month 18. The ANCOVA model will include baseline mNIS+7 as a continuous covariate and treatment arm (patisiran vs. placebo), genotype (V30M vs. non-V30M), age at ATTR onset (Before age 50 vs. After age 50), previous tetramer stabilizer use (Yes vs. No), and region (Western Europe, North America, and Rest of World) as factors. The resulting estimates from the 100 analyses will be combined using Rubin’s formulae, and the 95% confidence interval will be constructed, similar to the procedure described in the MI/ANCOVA section.

More details on the implementation of PMM are discussed in Appendix 7.1.

Including Data Post Alternative Treatment for FAP

The primary analysis will not include mNIS+7 assessments performed after the initiation of alternative treatment for FAP (Section 3.7). Sensitivity analysis including data post alternative treatment for FAP will be conducted using the MMRM model. In this sensitivity analysis, all assessments will be used in the analysis regardless of whether an assessment occurs before or after alternative treatment.

Revised mNIS+7 Total Score Using a Different Algorithm to Handle Missing Components

In the primary derivation of mNIS+7 total score, the “within treatment arm” imputation algorithm will be used for the imputation of missing component (Appendix 7.2.1). At each visit, if a patient has a missing component for mNIS+7, the value will be imputed using data from other patients who are on the same treatment arm and who had non-missing data for that component at that visit. In a sensitivity analysis, any such missing value will be imputed as the mean value for the component at the visit from all patients (combining placebo and patisiran arms). The analysis of this revised mNIS+7 derived scores will be conducted using the MMRM model.

4.3.1.4. Binary Analyses

The number and percentage of patients with < 10-point increase in mNIS+7 composite score from baseline to Month 18 will be calculated for each treatment arm and compared between 2 arms using the Cochran-Mantel-Haenszel test, stratified by genotype (V30M vs. non-V30M). The percentage will be based on the mITT population. Patients with missing 18-month data will be counted in the denominators.

In addition, the number of percentage of patients with a decrease (change from baseline < 0-point) in mNIS+7 total score from baseline to Month 18 will also be calculated and analyzed similar as above.

The planned analyses of the primary endpoint mNIS+7 are summarized in [Table 1](#).

Table 1 Analysis of mNIS+7

| Statistical Method | Analysis Population |
|--|----------------------------|
| Primary analysis: MMRM | mITT |
| Sensitivity analysis: <ul style="list-style-type: none"> • MI/ANCOVA • PMM • MMRM - including Data Post Alternative Treatment for FAP • MMRM - revised mNIS+7 Total Score Using a Different Algorithm to Handle Missing Components | mITT |
| Other analysis: MMRM | PP |
| Other analysis: Binary analysis using stratified CMH | mITT |

4.3.2. Secondary Efficacy Evaluations

Secondary efficacy endpoints include Norfolk QOL-DN total score, NIS-W, R-ODS, 10-meter walk test speed, mBMI, and COMPASS-31. To control overall type I error, these endpoints will be tested in a hierarchical order as described in Section 3.6.

All the secondary endpoints are assessed at baseline, Month 9, and Month 18 with the exception of mBMI. mBMI is assessed at baseline, Day 84, Day 189, Day 357, Day 462 and Day 546. Day 546 will be used as Month 18 assessment.

For 10-meter walk test, the walk speed for patients unable to perform the walk will be imputed as 0. The change from baseline will then be calculated as 0 – baseline walk speed.

Change from baseline at Month 18 in the secondary efficacy endpoints will be analyzed for the mITT population, using an MMRM model similar to the model described for the primary analysis of mNIS+7 while adjusting for baseline value of the endpoint being modeled. For these secondary endpoints (except NIS-W), MMRM model will also include baseline NIS (< 50 vs. ≥ 50) as a factor. The MMRM model for NIS-W will not include baseline NIS since baseline NIS-W will be included as a covariate in the model.

For the first secondary endpoint Norfolk QOL-DN Total Score, the MMRM method will also be conducted for the PP population. In addition, sensitivity analyses will be conducted using ANCOVA/MI and including data post alternative treatment for FAP similar as described for mNIS+7 (see Section 4.3.1.3).

Month 18 change from baseline in mBMI will be estimated from the MMRM model using assessment results from all time points.

The planned analyses of the secondary endpoints are summarized in [Table 2](#).

Table 2 Analysis of Secondary Endpoints

| Endpoint | Statistical Method | Analysis Population | Special Notes |
|----------------------------|---|---------------------|---|
| Norfolk QOL-DN total score | Primary analysis: MMRM | mITT | |
| | Sensitivity analysis: <ul style="list-style-type: none"> • MI/ANCOVA • MMRM - including Data Post Alternative Treatment for FAP | mITT | |
| | Other analysis: MMRM | PP | |
| NIS-W | MMRM | mITT | Excludes data post alternative treatment for FAP |
| R-ODS | MMRM | mITT | |
| 10-meter walk test speed | MMRM | mITT | For patients unable to perform the walk, walk speed imputed as 0 |
| mBMI | MMRM | mITT | Measured at baseline, Day 84, Day 189, Day 357, Day 462 and Day 546 |
| COMPASS-31 | MMRM | mITT | |

4.3.3. Exploratory Efficacy Evaluations

The continuous exploratory endpoints including grip strength, SGNFD, IENFD, dermal amyloid content, EQ-5D-5L index, EQ VAS, original NIS+7 and small/large fiber function (defined in Appendix 7.2.1) will be analyzed using an MMRM model similar to those employed for the primary analysis.

The categorical exploratory endpoints PND score and FAP stage will be descriptively summarized by presenting the number and percentage of patients in each category for each visit. The number of percentage of patients with improving, no change, and worsening in PND/FAP at each visit will also be summarized.

For EQ-5D-5L, a categorical summary of the numbers and percentages of patients reporting each ordinal response within each EQ-5D domain will be presented.

Cardiac structure and function will be assessed for all patients through echocardiograms. Cardiac stress and injury will be measured using serum levels of the cardiac biomarkers NT-proBNP and troponin I. Quantification of these biomarkers will be performed at a central laboratory. Descriptive statistics will be provided for actual values, changes, and percentage changes from baseline in echocardiogram parameters, serum levels of troponin I and NT-proBNP by treatment arm.

For cardiac subpopulation (defined in Section 4.2), the change from baseline to Month 18 in LV wall thickness, LV mass, LVEF, LV longitudinal strain, NT-proBNP and troponin I will be compared between the 2 treatment arms using MMRM. The model will include baseline value

as covariate and fixed effect terms including treatment arm, visit, and treatment-by-visit interaction.

All echocardiogram and cardiac function biomarkers data will be presented in data listings.

MR neurography data will be presented in a data listing.

4.3.4. Subgroup Analyses

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

- Age [≥ 65 ; < 65 at randomization]
- Sex [Male; Female]
- Race [White; Non-White]
- Region [North America; Western Europe; Rest of World]
- NIS [< 50 ; ≥ 50]
- Genotype Class [Early-onset V30M; Other]
- Previous Tetramer Use [Yes; No]
- Genotype [V30M; non-V30M]
- FAP Stage [I; II & III]

Subgroup analyses will be performed for the primary endpoint mNIS+7 and Norfolk QOL-DN using MMRM models with baseline mNIS+7 score as a continuous covariate and genotype (V30M vs. non-V30M) as a factor. A forest plot will be generated to illustrate the estimated treatment effect along with 95% CI within each subgroup.

4.3.5. Component Analyses

Component analyses will be conducted to assess the consistency of treatment effect on the change from baseline at Month 18 for each of the component of mNIS+7 including NIS-W, NIS-R, QST, $\sum 5$ NCS, and postural blood pressure. The analyses will be performed using MMRM models similar to those employed for the primary analysis. A forest plot will be generated to illustrate the estimated treatment effect along with 95% CI for each component.

4.4. Pharmacodynamic Analyses

The PD parameters include serum TTR (ELISA), serum TTR (turbidimetric assay), RBP, and vitamin A. All summary tables and figures will be based on assessments within 21 days of last dose of study drug. Assessments more than 21 days after last dose will be presented in listings and individual patient plots only.

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

For serum TTR (ELISA), the maximum percentage reduction and mean percentage reduction over 18 months will be summarized using descriptive statistics. Subgroup analysis will be provided for age (≥ 65 vs. < 65), sex (male vs. female), genotype (V30M vs. Non-V30M), and previous tetramer stabilizer use (yes vs. no).

For vitamin A and RBP, the mean percentage reduction over 18 months will be summarized using descriptive statistics.

The correlation coefficient and p-value based on mixed model will be calculated to assess the correlation between the changes from baseline in ELISA TTR versus Turbidimetric TTR. A scatterplot will be provided to visualize the data.

The therapeutic hypothesis for patisiran is that TTR reduction will result in clinical benefit in hATTR patients. The inter-patient variability in the degree of TTR reduction provides an opportunity to examine the relationship of TTR reduction to clinical endpoints. TTR reduction 22 days after the first dose of Patisiran as the first TTR assessment timepoint and prior to the second dose, will be used for analysis of the correlation between TTR reduction and change in mNIS+7 because this time point reduces the impact of missed doses or missed TTR assessments over 18 months of dosing. Pearson correlation coefficients and 95% confidence intervals will be calculated for Day 22 TTR % reduction and mNIS+7 total score change from baseline at the 9- and 18-month time points, for patisiran arm and placebo arm separately. Scatterplots will be provided to visualize the data.

All PD data will be displayed in data listings.

4.5. Pharmacokinetic Analyses

4.5.1. Study Variables

4.5.1.1. Concentration Data

Urinary concentrations of ALN-18328 (siRNA) and 4-dimethylaminobutyric acid, as well as plasma concentrations of ALN-18328, DLin-MC3-DMA and PEG₂₀₀₀-C-DMG will be obtained. Concentration values that are below the limit of quantification (LLOQ or BLQ) will be set to zero for analysis.

4.5.1.2. Plasma ALN-TTR02 Pharmacokinetic Parameters

Model independent PK parameters to be calculated by study visit include:

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

In addition, steady-state C_{max} (C_{max_ss}), steady-state C_{trough} (C_{trough_ss}) and steady-state C_p(30 min) (C_{p_ss}(30min)) will be calculated. C_{trough_ss} is the average C_{trough} on Days 253, 400 and 547. C_{p_ss}(30 min) is the average C_p(30 min) on Days 253 and 547. C_{max_ss} is the C_{max} on Day 400.

4.5.2. Statistical Methods

Descriptive statistics for plasma PK parameters will include the number of patients, mean, SD, coefficient of variation (CV), median, minimum, maximum, geometric mean and geometric CV%.

The plasma C_{max}, C_p (30min) and C_{trough} of ALN-18328 (siRNA), DLin-MC3-DMA and PEG₂₀₀₀-C-DMG will be summarized by nominal sampling day. Urinary amounts of ALN-18328 and 4-dimethylaminobutyric acid recovered will be estimated as urine concentration

times the associated urine volume and will be summarized by nominal sampling day. Mean concentrations (+/- SD) will be plotted versus nominal sampling time.

Steady-state PK parameters for ALN-18328 (siRNA), DLin-MC3-DMA and PEG₂₀₀₀-C-DMG will be summarized overall and by subgroup (gender, anti-drug antibody status).

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 TTR protein, vitamin A and RBP will be explored graphically.

The PK exposure-response relationships for the primary endpoint (mNIS+7), TTR, and incidence of relevant AEs may also be explored. These may be summarized by ALN-18328, DLin-MC3-DMA and PEG₂₀₀₀-C-DMG PK exposure quartiles at 18-months (Day 546).

Population PK and exposure-response modeling will be reported separately.

4.6. Safety Analyses

Safety analyses will be conducted using the Safety population. All safety summaries will be descriptive and will be presented by treatment arm.

4.6.1. Study Drug Exposure

Duration of drug exposure will be defined as (the last dose of study drug – the first dose of study drug + 21)/30.44 months. Duration of drug exposure, the total number of doses received, duration of infusion (per infusion) and amount of study drug received (per infusion and in total) will be summarized by descriptive statistics. Summaries of the numbers and percentages of patients with missing dose, and the number of missing doses per patient will also be provided. The total amount of drug received and the total volume infused will also be summarized.

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 acute infusion reaction.

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

4.6.2. Adverse Events

All AEs will be coded using the MedDRA coding system (version 18.0 or later) and displayed in tables and data listings using SOC and preferred term.

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 preferred term). 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 (definite or possible relationship), any severe AE, any severe AE related to treatment, any serious AE (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 preferred term will be produced for the following: all AEs; AEs related to treatment; severe AEs; AEs leading to infusion interruption; AEs leading to drug delay; AEs leading to treatment discontinuation; AEs leading to study withdrawal; and SAEs. Separate tables will be provided summarizing signs and symptoms of IRRs (overall and by premedication regimen) and AEs related to premedication (overall and by premedication regimen) by SOC and preferred term. The incidence and frequency of AEs and IRRs over time will also be summarized by SOC and preferred term. Adverse events and AEs related to treatment will also be tabulated by preferred term in decreasing order in frequency in the patisiran arm. Adverse events and SAEs will also be summarized by SOC and preferred term for the cardiac subpopulation.

Separate tables will present AE incidence rates by maximum relationship to study drug and by maximum severity. Patients who report multiple occurrences of the same AE (preferred term) will be classified according to the most related or most severe occurrence, respectively.

AEs mapping to the standardized MedDRA query (SMQ) Depression and Suicide/Self-injury will be summarized by preferred term. Adverse events mapping to the SMQ Drug Related Hepatic Disorder will be summarized by SOC and preferred term. Adverse events mapping to the SMQ Malignant or Unspecified Tumors will be summarized by high level term and preferred term. 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, AEs leading to study withdrawal, IRRs, AEs related to premedications, AEs related to study procedures, and AEs mapping to the SMQ as described above. A listing of patients who underwent liver transplant will also be provided.

4.6.3. Laboratory Data

Clinical laboratory values will be expressed in SI units.

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

For each continuous laboratory parameter, results will be categorized as low, normal, or high based on the laboratory normal ranges. Shift tables will be employed to summarize the baseline category versus the “worst” post-baseline category, where the “worst” post-baseline category will be based on the maximum difference (in absolute value) from the upper or lower limits of the normal range.

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

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.

A shift table from baseline to worst post-baseline for ALT, AST, and total bilirubin will also be provided. 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 blood chemistry, summary tables of potentially clinically significant abnormalities will be provided. The results may also be graded according to the NCI CTCAE Version 4.0 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 m²) will be categorized as below: ≥ 90; 60-89; 30-59; 15-29 and < 15. A shift summary of baseline to worst post-baseline eGFR category will be presented.

All laboratory data will be provided in data listings. Out-of-range laboratory results will be identified in the listings.

4.6.4. Vital Signs and Physical Examination

Descriptive statistics will be provided for vital signs, including blood pressure, pulse rate, oral body temperature and respiration rate.

Vital sign measurements will be presented for each patient in a data listing.

All physical examination findings will be presented in a by-patient data listing. Abnormal physical examination findings will be presented in a separate listing.

4.6.5. Electrocardiogram

Electrocardiogram (ECG) findings will include rhythm, ventricular rate, PR interval, QRS duration, QT interval, and QTc interval. For each time point, the results will be the average of measurements from the triplicate ECGs for each patient recorded at that time point. Descriptive statistics will be provided for each measure over time. Change from pre-dose to each post-dose assessment will also be summarized. The number and percentage of patients with normal, abnormal, and clinically significant abnormal results at baseline and each study visit will also be summarized.

Corrected QT interval (QTc) will be calculated using both Fridericia's and Bazett's correction formula. Categorical analyses of the QTcF/QTcB data will be conducted and summarized as follows:

- The number and percentage of patients with maximum increase from baseline in QTcF/QTcB (< 30, 30 – 60, > 60 ms)
- The number and percentage of patients with maximum post-baseline QTcF/QTcB (< 450, 450 – < 480, 480 – 500, > 500 ms)

All ECG data for each patient will be provided in a data listing. A separate listing will be provided for patients with any post-baseline value > 500ms or an increase from baseline > 60 ms.

4.6.6. Premedication

All patients received premedication in order to reduce the potential of an IRR. The original premedication regimen as outlined below was used at the start of the study. A subset of patients experienced AEs suspected to be related to steroids (e.g., flushing) and were transitioned to a modified premedication regimen, with a reduced dose of corticosteroid to mitigate these events, as sanctioned in the protocol. After observing that the subset of patients tolerated the lower corticosteroid dose with no increase in IRRs, the protocol was amended (Amendment 5.0) to transition the rest of the patients to the modified premedication regimen (see below).

The following original premedication regimen was used prior to protocol amendment 5.0:

- Dexamethasone 8 mg PO or equivalent administered the evening before dosing and 20 mg PO at least 60 minutes prior to the start of the infusion of patisiran;
- Paracetamol 500 mg PO or equivalent administered the evening before dosing and at least 60 minutes prior to the start of the infusion of patisiran;
- H2 blocker PO (e.g., ranitidine 150 mg or famotidine 20 mg or equivalent other H2 blocker dose) administered the evening before dosing and at least 60 minutes prior to start of the infusion of patisiran; and
- H1 blocker PO, 10 mg cetirizine or equivalent (hydroxyzine 25 mg or fexofenadine could be substituted if patient did not tolerate cetirizine) administered the evening before dosing and at least 60 minutes prior to start of the infusion of patisiran.

The following reduced premedication regimen was instituted for all patients with protocol amendment 5.0:

- Dexamethasone 10 mg IV or equivalent, administered at least 60 minutes prior to the start of the infusion of patisiran;
- Paracetamol 500 mg PO or equivalent at least 60 minutes prior to the start of the infusion of patisiran;
- H2 blocker IV (e.g., ranitidine 50 mg, famotidine 20 mg, or equivalent other H2 blocker dose) at least 60 minutes prior to the start of the infusion of patisiran; and
- H1 blocker IV, diphenhydramine 50 mg (or equivalent other IV H1 blocker available at the study site) at least 60 minutes prior to the start of the infusion of patisiran. Hydroxyzine 25 mg PO or fexofenadine 30 or 60 mg PO or cetirizine 10 mg PO could be substituted for any patient who did not tolerate IV diphenhydramine or other IV H1 blocker.

A drug exposure table summarizing the treatment duration under “original” and “reduced” regimens will be provided. A patient who switches from “original” to “reduced” regimen during treatment will be counted in both categories.

Premedications will be coded using the WHO Drug Dictionary (March 2015 or later). Results will be tabulated by anatomic therapeutic class (ATC) and preferred term.

Premedication data will be listed. In addition, a listing will be provided to present the durations of study drug exposure under original regimen and reduced regimen for each patient.

4.6.7. Prior and Concomitant Medications

Prior and concomitant medications will be coded using the WHO Drug Dictionary (March 2015 or later). 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 of alternative FAP treatment (tafamidis/diflunisal). 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.

4.6.8. Ophthalmology Examinations

Ophthalmology examinations include Visual Acuity, Visual Field, Slit Lamp Biomicroscopy, Intraocular Pressure, Dilated Indirect Ophthalmoscopy, and Fundus Photography.

Visual Acuity, Visual Field, and Intraocular Pressure Results: The actual value and change from baseline results will be summarized.

Biomicroscopy (Slit Lamp) and Dilated Indirect Ophthalmoscopy Exam Results: For the baseline results, the number and percentage of patients falling into each category of the examination status (normal, abnormal/not clinically significant, abnormal/clinically significant) will be summarized for each eye structure. For post-baseline results, the number and percentage of patients falling into each category of the examination status (new findings/worsening of finding, no change, improvement of finding, etc.) will be summarized for each eye structure.

Abnormal fundus findings at baseline will be recorded in the medical history log. Treatment-emergent abnormal fundus findings that are considered clinically significant will be recorded in the AE log.

Data for each of these assessments will be provided in a data listing.

4.6.9. Suicidality Questionnaire

The number and percentage of patients experiencing the suicidal ideation, suicidal behavior, or self-injurious behavior composite outcomes (and individual components) will be summarized by visit. A shift table will be employed to summarize the baseline C-SSRS category versus the worst post-baseline C-SSRS category; the categories are defined as 1) no suicidal ideation or behavior, 2) suicidal ideation, and 3) suicidal behavior. Patients experiencing both suicidal ideation and suicidal behavior are included in the suicidal behavior category.

Data from the C-SSRS questionnaire will be provided in a data listing.

4.7. Anti-Drug Antibody

The number and percentage of patients with confirmed positive ADA assay results at any time point 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.

For patients with confirmed positive ADA results, spaghetti plots for the serum TTR (ELISA) over time and the plasma concentration of ALN-18328, DLin-MC3-DMA and PEG2000-C-DMG over time will be presented. Effect of positive ADAs on efficacy and safety (e.g., IRRs) may also be explored.

Anti-drug antibody (ADA) data and patients with confirmed positive ADA results will be presented in data listings.

5. CHANGES TO PLANNED ANALYSES

The SAP version 2.0 includes more details previously not discussed in the original SAP version 1.0. The following major changes will be implemented in the SAP version 2.0 that are different from the planned analysis specified in the SAP version 1.0 and/or the protocol version 6.0.

This SAP Version 2.1 incorporates an additional change to censor data post alternative treatment for the analysis of NIS-W, which is documented in [Section 5.6](#).

5.1. Primary Analysis Method for Efficacy Endpoints

In the study protocol and the original SAP V1.0, the primary analysis method specified for the primary and secondary efficacy endpoints was the MI/ANCOVA. The original SAP V1.0 was submitted for FDA review (IND Serial # 0019). Following FDA's comment received on 02July2015 and the sponsor's responses on 31August2015, the primary analysis method for all efficacy endpoints will be changed to MMRM method. The history and rationales for the change of primary analysis method are described below. The change is aligned with the CHMP guideline on missing data in confirmatory clinical trials (EMA/CPMP/EWP/1776/99 Rev.1).

Mixed-effects model repeated measures and MI are 2 common methods for handling missing data in analysis of continuous, longitudinal endpoints. For this study, ANCOVA/MI was originally selected as the primary analysis, with MMRM as a key sensitivity analysis.

In the literature, MMRM and MI have been compared in several simulation studies. [Collins et al. \(2001\)](#) noted that the 2 methods yielded similar inference. However, [Barnes et al. \(2008\)](#) found a larger average standard error for MI relative to MMRM, suggesting that MI controls type I error conservatively and loses power to detect true treatment differences. [Siddiqui \(2011\)](#) compared MI/ ANCOVA and MMRM approaches using both simulated datasets and 25 New Drug Application (NDA) datasets of neuropsychiatric drug products, and concluded that MMRM appears to be a better choice in maintaining statistical properties relative to the MI approach when dealing with ignorable missing data.

For the ALN-TTR02-004 trial setting, we compared the properties of these 2 methods via simulation. Our results demonstrated that, while both methods control type I error, MMRM appears to be more powerful in detecting true treatment effect compared with ANCOVA/MI.

Moreover, [White et al. \(2012\)](#) argued that excessive focus on including all randomized patients with missing outcomes can lead to a choice of analysis that rests on implausible or unnecessarily complex imputation. The authors proposed that the main focus in choosing the primary analysis should be the plausibility of its assumptions, while inclusion of all randomized individuals is a requirement only for sensitivity analyses. The MI approach, which includes all randomized patients, is often designated as a sensitivity analysis.

Given these findings – and consideration of the complexity inherent when using the multistep MI approach, the primary analysis method will be changed to MMRM for the primary and secondary efficacy endpoints. MI/ANCOVA will be performed as the key sensitivity analysis for the primary endpoint and for the first secondary endpoint (Norfolk QOL).

5.2. Multiple Imputation/ANCOVA Method

The MI/ANCOVA method, as described in this document and in SAP V1.0, differs from that described in the current clinical protocol (Version 6: September 2015) in the MI method and in the explanatory covariates included in the (complete data) ANCOVA (discussed in [Section 5.4](#)).

The MI (for completely missing mNIS+7 assessments) described in the SAP differs from that in the clinical protocol in that 1) stepwise variable selection has been replaced with a pre-specified set of covariates to be included; 2) imputation is done separately by treatment arm. The rationale for these changes is outlined below.

- Stepwise variable selection was removed in order to simplify the analysis.
- The SAP specifies that imputation is done separately by study arm; the current protocol specifies that treatment assignment will not be included in the imputation. This change is motivated by simulations demonstrating a non-negligible loss in power when a single imputation model is employed and treatment is not included in the model; this loss in power is due to “cross-contamination” of imputation models between randomized arms [9].

In the protocol, the number of imputed datasets was specified as 100. In SAP V1.0, the number of imputed datasets was reduced to 10 following the conclusion that 3-5 imputations are often sufficient [10], however, in this SAP V2.0, the number of imputed datasets is increased to 100 to have more precise estimates.

5.3. Hierarchical Testing Order of Secondary Endpoints

In the protocol and SAP version 1.0, it was specified that secondary endpoints would be tested in the following order for the control of overall type I error: Norfolk QOL, NIS-W score, mBMI, 10-meter walk test, and COMPASS-31.

In this SAP amendment, R-ODS has been added as a secondary endpoint for controlled hypothesis testing, and 10-meter walk test has been moved up in the hierarchy. The revised list and order of secondary endpoints is the following: Norfolk QOL, NIS-W, R-ODS, 10-meter walk test, mBMI and COMPASS-31.

These changes were based on the Sponsor’s re-assessment of the clinical importance of these endpoints in hATTR amyloidosis patients.

5.4. Adjustment for Covariates in Statistical Models

The table below summarizes the history of changes in baseline covariates used in the statistical models.

| Baseline Covariates | Protocol | SAP V1.0 | SAP V2.0 |
|---|----------|----------|-------------|
| | ANCOVA | ANCOVA | MMRM/ANCOVA |
| Baseline mNIS+7 | X | X | X |
| Genotype (V30M vs. Non-V30M) | X | | X |
| Genotype class (early onset V30M vs. Other) | | X | |
| Age at hATTR symptom onset (< 50 vs. ≥ 50) | | | X |
| Previous tetramer stabilizer use (Yes vs. No) | X | X | X |
| Region (Western EU, North America, Rest of World) | | | X |
| Age at study entry as continuous variable | X | | |

Genotype class (early onset V30M vs. other) is one of the stratification factors at randomization. The early onset V30M subgroup includes patients who have the V30M genotype and were younger than 50 years old at hATTR amyloidosis symptom onset. At the time of this SAP amendment, the enrollment was complete and there were only 23 early onset V30M patients. Given the small sample size, the composite covariate “genotype class” is decomposed into 2 separate covariates “genotype” and “age at hATTR amyloidosis symptom onset.”

Region is added as a covariate because different rates of attrition and disease worsening were observed for different regions during blinded data monitoring. In addition, adding region may potentially address the influence of different non-V30M types observed across regions.

5.5. Binary Analysis of mNIS+7

In SAP V1.0, binary analysis of mNIS+7 included an analysis of patients with < 2-point increase in mNIS+7 change from baseline at Month 18. The 2-point threshold was originally used in the tafamidis Phase 3 trial in hATTR amyloidosis patients with polyneuropathy to define responders for the NIS-LL co-primary endpoint. That trial enrolled patients with very early stage neuropathy and mean baseline NIS-LL of approximately 8-11 points, in whom a 2-point worsening of NIS-LL was considered to be clinically meaningful. However, the patients enrolled onto this study have more advanced disease, with a mean baseline NIS of approximately 60 points, and the mNIS+7 primary endpoint is very different from NIS-LL, with a maximum score of 304 points compared to 88 points for NIS-LL. Therefore, a 2-point threshold was not deemed relevant for mNIS+7 change in this patient population.

In this SAP V2.0, 2 thresholds, < 10-point increase and any decrease (< 0-point change from baseline) will be employed to conduct the binary analysis of mNIS+7. These thresholds of < 10-point and < 0-point were chosen based on the 18-month estimated mNIS+7 progression rate observed in a natural history study of hATTR amyloidosis patients and the observed effect of patisiran on mNIS+7 at 18 months in the Phase 2 OLE study, respectively.

5.6. Censoring Data Post Alternative FAP Treatment

In this SAP V2.0, for the primary analysis of mNIS+7 and Norfolk QoL, the data post alternative FAP treatment will be censored. This censoring rule was not previously specified in the protocol or SAP V1.0. The rationale of adding this censoring rule is to eliminate the potential confounding effect caused by alternative FAP treatment. Sensitivity analyses for mNIS+7 and Norfolk QoL will be conducted including data post alternative FAP treatment.

In SAP V2.1, an update was made to apply the censoring rule to NIS-W as well so that the results will be consistent with the component analysis of mNIS+7.

For all other efficacy endpoints, the data will not be censored since no sensitivity analyses are planned.

5.7. Other Changes

Additional changes are listed in the table below.

| Change from Protocol and/or SAP V1.0 | Detailed Description/Rationale |
|--|---|
| In the protocol and original SAP V1.0, the first day of drug administration was designated as Day 0. In this SAP amendment and the TLF outputs, the first dose day will be defined as Day 1. | The change is made to follow CDISC convention. Each calculated study day after dosing is 1 day plus the reported study day following the protocol defined Schedule of Assessments. |
| In the protocol and original SAP V1.0, it was intended that an interim analysis for sample size re-estimation would be conducted; however, no interim analysis was conducted during the study. | In 2013, Berk et al. published results of a randomized placebo-controlled trial of diflunisal in FAP. This trial demonstrated that efficacy using a composite neurologic impairment score primary endpoint (NIS+7) could be established in a similar population with a similar study design and smaller study size (N=130). The sponsor believed that an interim analysis for sample size reassessment was therefore no longer necessary. |
| In SAP V2.0, dermal amyloid content is added as an exploratory endpoint. | In addition to IENFD and SGNFD, skin punch biopsies will also be assessed for dermal amyloid content, which has been added as an efficacy parameter in this SAP V2.0. |
| In SAP V2.0, the walk speed for patients unable to perform the walk will be imputed as 0. | The imputation algorithm is added to address the informative missing data for patients unable to perform the walk due to worsening of disease. |
| Renal clearance for siRNA and 4-dimethylaminobutyric acid (DMBA) will not be analyzed. | As per protocol, renal clearance for siRNA and 4-dimethylaminobutyric acid (DMBA) were to be determined whenever possible. Only sparse pre-dose urine collections (and volumes) were obtained in patients enrolled in this study on select days that patients returned to the clinic for dosing (Days 0, 21, 126, 252, 399, 546 and at time of early withdrawal). In order to estimate renal clearance, detailed serial urine and corresponding plasma collections that describe a time course of exposures in both matrices over a dosing interval within individual subjects are required. As it is not possible to reliably estimate the renal clearance for either siRNA or DMBA in individual patients using standard pharmacokinetic methods based on the limited urine and plasma sampling implemented in this outpatient study, renal clearance estimation for either analyte were not performed and cannot be reported for this study. |

| Change from Protocol and/or SAP V1.0 | Detailed Description/Rationale |
|--|---|
| In SAP V2.0, the definition for cardiac subpopulation was added. This subpopulation was not previously defined in the protocol or in SAP V1.0. | In SAP V2.0, the cardiac subpopulation was defined in Section 4.2 as the following: the cardiac subpopulation will be comprised of patients with pre-existing cardiac amyloid involvement, defined as patients with baseline left ventricular (LV) wall thickness ≥ 1.3 cm and no aortic valve disease or hypertension in medical history. |

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7. APPENDICES

7.1. Detailed Statistical Methodology for Pattern Mixture Model

In the PMM analysis, similar to the primary analysis, mNIS+7 assessments performed after the initiation of alternative treatment for FAP will be treated as missing. For non-monotone missing data, the 9-month assessment will be imputed as the treatment group mean. The non-monotone missing data is expected to be rare.

As an initial step, an intermediate mNIS+7 dataset for all mITT patients will be prepared which will include the key variables listed in the table below.

| Key Variables | Description |
|---|---|
| Patient number | Unique subject ID for each patient in mITT population |
| Treatment arm | Placebo or patisiran |
| Baseline variables | Including gender, genotype, age at ATTR onset, previous tetramer stabilizer use, region, KPS, FAP stage (I vs. II/III), and cardiac subpopulation. |
| Baseline mNIS+7 score | |
| mNIS+7 total score change from baseline at Month 9 | Set as missing if a patient does not have 9-month data; If a patient has 18-month mNIS+7 but is missing 9-month (non-monotone missingness), the 9-month value will imputed as the treatment group mean. |
| On-treatment flag for Month 9 | If 9-month assessment is non-missing: If assessment date – last dose date \leq 60 days, then flag = “yes” otherwise flag = “no” If 9-month assessment is missing: If Day 254 (scheduled 9-month visit) – last dose date \leq 60, then flag = “yes” otherwise flag = “no” |
| mNIS+7 total score change from baseline at Month 18 | Set as missing if a patient does not have 18-month data |
| On-treatment flag for Month 18 | If 18-month assessment is non-missing: If assessment date – last dose date \leq 60 days, then flag = “yes” otherwise flag = “no” If 18-month assessment is missing: If Day 554 (scheduled 18-month visit) – last dose date \leq 60, then flag = “yes” otherwise flag = “no” |
| Death flag | Flag = “yes” if a patient has missing 18-month mNIS+7 and death day \leq 561; otherwise flag = “no” |

Step 1: For patients who have missing data and are alive before Month 18: imputation of missing data for the placebo arm and for the patisiran arm during the on-treatment period.

In the first step, missing data for the placebo arm and missing data for the patisiran arm during the on-treatment period will be imputed under the MAR assumption. Multiple imputation will be conducted among patisiran and placebo patients separately using a regression procedure, including the following covariates: baseline mNIS+7 score, genotype, age at ATTR onset, prior tetramer stabilizer use, region, KPS, FAP stage (I vs. II/III), cardiac subpopulation and gender.

The input dataset DATAIN will include a subset of the above dataset with Death Flag = “no.” For patisiran patients, the mNIS+7 data collected after treatment discontinuation will be excluded (set as missing). If Treatment Arm = “patisiran”:

- i) If On-Treatment Flag for Month 9 = “no” then mNIS+7 change at Month 9 will be set as missing.
- ii) If On-Treatment Flag for Month 18 = “no” then mNIS+7 change at Month 18 will be set as missing.

Below is a sample SAS code:

```
proc mi data=DATAIN out=DATA_STEP1 seed=234 nimpute=100;
  by treatment;
  monotone method=reg;
  var baseline_variables mNIS_BASE mNIS_chg_m9 mNIS_chg_m18;
run;
```

For patisiran arm, the imputed scores during the on-treatment period will be kept while the imputed scores after treatment discontinuation will be discarded and replaced by either observed non-missing values or imputed values described in the next step. In the output dataset DATA_STEP1, if Treatment Arm = “patisiran”:

- i) If On-Treatment Flag for Month 9 = “no” then mNIS+7 change at Month 9 will be replaced by the value in DATAIN.
- ii) If On-Treatment Flag for Month 18 = “no” then mNIS+7 change at Month 18 will be replaced by the value in DATAIN.

Denote this updated dataset as DATA_STEP1_2.

Step 2: For patients who have missing data and are alive before Month 18: imputation of missing data for the patisiran arm after treatment discontinuation.

In the second step, missing data for the patisiran arm after treatment discontinuation will be imputed using the PMM approach by creating control-based pattern imputation.

Below is a sample SAS code:

```
proc mi data=DATA_STEP1_2 out=DATA_STEP2 nimpute=1 seed=xxx;
  by _imputation_;
  class treatment;
  monotone method=reg;
  mmar model (mNIS_chg_m9 mNIS_chg_m18/modelobs=(treatment='placebo'));
  var baseline_variables mNIS_BASE mNIS_chg_m9 mNIS_chg_m18;
run;
```

Step 3: Imputation of missing data for patients who died before Month 18, regardless of treatment arm.

For patients with Death Flag = “yes,” missing 18-month mNIS+7 change from baseline scores will be imputed 100 times, using a random draw from the worst 10% 18-month mNIS+7 change scores among all non-missing values in the dataset DATAIN. Concatenate these records with DATA_STEP2, and sort by dataset number and patient number.

After step 3, each of the complete dataset will be analyzed by an ANCOVA model. The resulting estimates (LS means and standard errors) will be combined using SAS PROC MIANALYZE.

7.2. Questionnaire/Scoring

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

7.2.1. Modified Neuropathy Impairment Score (mNIS+7) and Original Neuropathy Impairment Score + 7 Nerve Tests (NIS+7)

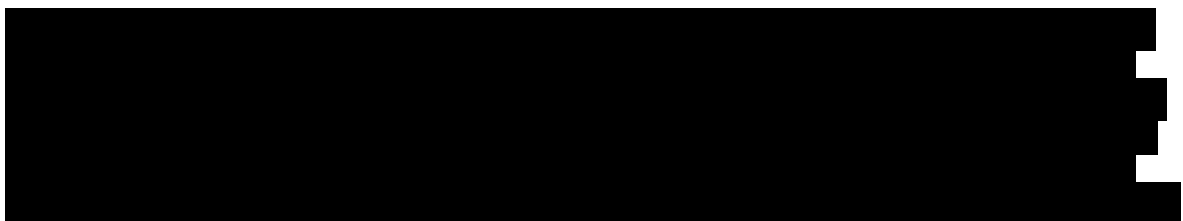
Note: the mNIS+7 and NIS+7 measurements are conducted in duplicate per time point. The average of 2 complete duplicate values will be reported, except in cases of missing or partially missing data as described in the table below.

| Assessment Tool | Total Points | Components (maximum points) |
|-----------------|--------------|---|
| Modified NIS+7 | 304 | <ul style="list-style-type: none"> • NIS-W: Weakness (192) • NIS-R: Reflexes (20) • Quantitative sensory testing by body surface area including touch pressure (TP) and heat as pain (HP): QST-BSA_{TP+HP5} (80) • $\sum 5$ nerve conduction studies (10) <ul style="list-style-type: none"> • Ulnar compound muscle action potential (ulnar CMAP) • Ulnar sensory nerve action potential (ulnar SNAP) • Sural sensory nerve action potential (sural SNAP) • Tibial compound muscle action potential (tibial CMAP) • Peroneal compound muscle action potential (peroneal CMAP) • Postural blood pressure (BP) (2) |
| NIS+7 | 270 | <ul style="list-style-type: none"> • NIS-W: Weakness (192) • NIS-R: Reflexes (20) • NIS-S: Sensation (32) • $\sum 7$ Nerve tests <ul style="list-style-type: none"> • 5 Nerve conduction studies $\sum 5$ (18.6) <ul style="list-style-type: none"> • Peroneal compound muscle action potential (peroneal CMAP) • Peroneal motor nerve conduction velocity (peroneal MNCV) • Peroneal motor nerve distal latency (peroneal MNDL) • Tibial motor DL • Sural sensory nerve action potential (sural SNAP) • Vibration detection threshold (VDT) (3.72) • Heart rate response to deep breathing (HRdb) (3.72) |

7.2.1.1. Modified Neuropathy Impairment Score (mNIS+7)

There are 5 components within mNIS+7 total score including NIS-W, NIS-R, QST, $\sum 5$ NC, and postural BP, as described in details below.

1.



[REDACTED]

- [REDACTED]
- [REDACTED]
- [REDACTED]
- [REDACTED]
- [REDACTED]

[REDACTED]

- [REDACTED]
- [REDACTED]
- [REDACTED]
- [REDACTED]

- [REDACTED]

7.2.1.2. Original Neuropathy Impairment Score (NIS+7)

The components of NIS+7 include the following:

1. NIS-W as described in previous section.
2. NIS-R as described in previous section.
3. NIS-S is the sum of the finger and toe sensation components (touch pressure, pin-prick, vibration, joint position). Assessments are performed separately for the right- and left-hand side of the body. Scoring for the sensory assessment is 0 (normal), 1 (decreased) and 2 (absent). The maximum total score for NIS-S is 32.
4. $\sum 7$ nerve tests normal deviates includes the following:
 - $\sum 5$ nerve conductions:
 - For peroneal DL and tibial DL, using normal deviate
 - For peroneal CMAP, peroneal CV and sural SNAP, using $(-1) \times$ normal deviate
 - Vibration detection threshold (VDT): using normal deviate
 - Heart rate response to deep breathing (HRdb): using $(-1) \times$ normal deviate

Each normal deviate ranges from -3.72 to +3.72. The total score is calculated as the mean normal deviates of the non-missing 7 nerve tests and multiply this value by 7. The total score ranges from -26 to +26.

Missing values will be handled as follows:

- Missing items within the 7 nerve tests are handled in step 5 above.
- If a component of NIS+7 (NIS-W, NIS-R, NIS-S, and 7 nerve tests) is missing from replicate A, then impute the component from replicate B \rightarrow recover 2 complete replicate NIS+7 measures A and B.
- If both replicates A and B are incomplete at *different* components, insert A components into B and B components into A as necessary \rightarrow recover 2 complete replicate NIS+7 measures A and B.
- If NIS-W component is missing from both replicates A & B, NIS+7 score is considered as missing. If 1 of the other NIS+7 components (NIS-R, NIS-S, and 7 nerve tests) is missing from both replicates A and B, impute this component as the average of the other patients at this time point who had non-missing data (within study arm).
- If 1 entire NIS+7 replicate is missing, use the singular (other) replicate rather than the average of the 2 in subsequent calculations.
- If no single complete NIS+7 measure can be found or recovered, NIS+7 score is considered as missing.

7.2.1.3. NIS Total Score

NIS total score is the sum of NIS-W, NIS-R, and NIS-S. The handling of missing data is similar as the steps described for mNIS+7. If NIS-W is missing from both replicates, NIS score is considered as missing.

7.2.1.4. Large Fiber Nerve Function

The large fiber nerve function is the sum of the point scores of the following:

- [REDACTED]
- [REDACTED]

[REDACTED] The maximum possible large fiber nerve function score is 52.

The handling of missing data is similar as the steps described for mNIS+7. If QST-BSA_{TP} is missing from both replicates, the large fiber nerve function score is considered as missing.

7.2.1.5. Small Fiber Nerve Function

The small fiber nerve function score is the sum of the point score of the following:

- [REDACTED]

[REDACTED]. The maximum possible small fiber nerve function score is 44.

The handling of missing data is similar as the steps described for mNIS+7. If QST-BSA_{HP5} is missing from both replicates, the small fiber nerve function score is considered as missing.

7.2.1.6. Algorithms for Setting Normal Deviates and Points

For nerve conductions and HRdb tests, raw values are provided by the Mayo Clinic. Each raw value is first converted to a z-score which is then used to set either normal deviate or point score.

[REDACTED]

6. [REDACTED]

[REDACTED]

The points for the parameters are calculated as follows:

1. [REDACTED]

[REDACTED]

Part II: Activities of Daily Life



Subscales and Scoring Algorithm

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

- Total QOL [redacted]
- Physical Functioning/Large Fiber [redacted]
- Activities of Daily Living (ADLs) [redacted]
- Symptoms [redacted]
- Small Fiber [redacted]
- Autonomic [redacted]

The total score and domain scores are calculated without weighting of any kind, and reported as the integer sum of the listed questionnaire items.

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.

7.2.3. EuroQOL-5-Dimension 5-Level (EQ-5D-5L)

Each of the 5 dimensions (Mobility, Self-Care, Usual Activities, Pain/Discomfort, Anxiety/Depression) is scored on a 5-point Likert scale from 1 (“I have no problems/pain/anxiety”) to 5 (“I am unable to...,” “I have extreme anxiety/depression”).

The 5 scores are concatenated together (in the order of Mobility, Self-Care, Usual Activities, Pain/Discomfort, Anxiety/Depression) to create an EQ-5D-5L profile (e.g., 11111, 55555). The profile is then used to obtain an index value using the United States value set. The index values range from -0.109, associated with a profile of 55555, to 1.0, associated with a profile of 11111. Smaller index values indicate greater impairment.

Missing values are handled as follows:

- Missing items are coded as “9” in creating patient profiles.
- The index value is deemed as missing when responses are missing for 1 or more of the 5 dimensions.
- If the entire instrument is missing, the EQ-5D-5L index value is considered as missing.

7.2.4. Rasch-Built Overall Disability Scale (R-ODS)

The R-ODS consists of 24 items scored on a scale of 0 (unable to perform), 1 (able to perform, but with difficulty) or 2 (able to perform without difficulty). A total score will be calculated as the average of all non-missing items multiplied by 24 if at least 90% of the items are non-missing. The total score will be deemed as missing if more than 10% of the items (3 or more items) are missing.

7.2.5. Composite Autonomic Symptom Score (COMPASS-31)

The COMPASS-31 questionnaire comprises 6 domains: Orthostatic intolerance, Vasomotor, Secretomotor, Gastrointestinal, Bladder, and Pupillomotor. Within each domain, individual questions are scored as follows: Simple yes or no questions are scored as 0 points for no and 1 point for yes. Questions about a specific site of symptoms or symptoms under specific circumstances are scored as 0 if not present and as 1 if present for each site or circumstance. All questions regarding the frequency of symptoms are scored as 0 points for rarely or never, 1 point for occasionally or sometimes, 2 points for frequently or “a lot of the time,” and 3 points for almost always or constantly. All questions regarding the severity of symptoms are scored as 1 point for mild, 2 points for moderate, and 3 points for severe. Questions assessing the time course of a symptom are scored 0 points for responses such as “gotten somewhat better,” “gotten much better,” “completely gone,” and “I have not had any of these symptoms,” 1 point for “stayed about the same,” 2 points for “gotten somewhat worse,” and 3 points for “gotten much worse.” The scores for changes in bodily functions depend on the individual question asked. For example, “I get full a lot more quickly than I used to when eating a meal” is scored 2 points and “I get full a lot less quickly than I used to” is scored 0 points, while the answer “I sweat much more than I used to” is given 1 point and “I sweat much less than I used to” is scored 2 points.

The overall scoring proceeds as follows:



[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

The table consists of 9 rows and 2 columns. All text within the table is completely redacted with black boxes. The redaction covers the entire content of each cell, leaving only the structural grid lines visible.

5. CHANGES TO PLANNED ANALYSES

The SAP version 2.0 includes more details previously not discussed in the original SAP version 1.0. The following major changes will be implemented in the SAP version 2.0 that are different from the planned analysis specified in the SAP version 1.0 and/or the protocol version 6.0.

This SAP Version 2.1 incorporates an additional change to censor data post alternative treatment for the analysis of NIS-W, which is documented in Section 5.6.

5.1. Primary Analysis Method for Efficacy Endpoints

In the study protocol and the original SAP V1.0, the primary analysis method specified for the primary and secondary efficacy endpoints was the MI/ANCOVA. The original SAP V1.0 was submitted for FDA review (IND Serial # 0019). Following FDA's comment received on 02July2015 and the sponsor's responses on 31August2015, the primary analysis method for all efficacy endpoints will be changed to MMRM method. The history and rationales for the change of primary analysis method are described below. The change is aligned with the CHMP guideline on missing data in confirmatory clinical trials (EMA/CPMP/EWP/1776/99 Rev.1).

Mixed-effects model repeated measures and MI are 2 common methods for handling missing data in analysis of continuous, longitudinal endpoints. For this study, ANCOVA/MI was originally selected as the primary analysis, with MMRM as a key sensitivity analysis.

In the literature, MMRM and MI have been compared in several simulation studies. [Collins et al. \(2001\)](#) noted that the 2 methods yielded similar inference. However, [Barnes et al. \(2008\)](#) found a larger average standard error for MI relative to MMRM, suggesting that MI controls type I error conservatively and loses power to detect true treatment differences. [Siddiqui \(2011\)](#) compared MI/ ANCOVA and MMRM approaches using both simulated datasets and 25 New Drug Application (NDA) datasets of neuropsychiatric drug products, and concluded that MMRM appears to be a better choice in maintaining statistical properties relative to the MI approach when dealing with ignorable missing data.

For the ALN-TTR02-004 trial setting, we compared the properties of these 2 methods via simulation. Our results demonstrated that, while both methods control type I error, MMRM appears to be more powerful in detecting true treatment effect compared with ANCOVA/MI.

Moreover, [White et al. \(2012\)](#) argued that excessive focus on including all randomized patients with missing outcomes can lead to a choice of analysis that rests on implausible or unnecessarily complex imputation. The authors proposed that the main focus in choosing the primary analysis should be the plausibility of its assumptions, while inclusion of all randomized individuals is a requirement only for sensitivity analyses. The MI approach, which includes all randomized patients, is often designated as a sensitivity analysis.

Given these findings – and consideration of the complexity inherent when using the multistep MI approach, the primary analysis method will be changed to MMRM for the primary and secondary efficacy endpoints. MI/ANCOVA will be performed as the key sensitivity analysis for the primary endpoint and for the first secondary endpoint (Norfolk QOL).

5.2. Multiple Imputation/ANCOVA Method

The MI/ANCOVA method, as described in this document and in SAP V1.0, differs from that described in the current clinical protocol (Version 6: September 2015) in the MI method and in the explanatory covariates included in the (complete data) ANCOVA (discussed in Section 5.4).

The MI (for completely missing mNIS+7 assessments) described in the SAP differs from that in the clinical protocol in that 1) stepwise variable selection has been replaced with a pre-specified set of covariates to be included; 2) imputation is done separately by treatment arm. The rationale for these changes is outlined below.

- Stepwise variable selection was removed in order to simplify the analysis.
- The SAP specifies that imputation is done separately by study arm; the current protocol specifies says that treatment assignment will not be included in the imputation. This change is motivated by simulations demonstrating a non-negligible loss in power when a single imputation model is employed and treatment is not included in the model; this loss in power is due to “cross-contamination” of imputation models between randomized arms [9].

In the protocol, the number of imputed datasets was specified as 100. In SAP V1.0, the number of imputed datasets was reduced to 10 following the conclusion that 3-5 imputations are often sufficient [10], however, in this SAP V2.0, the number of imputed datasets is increased to 100 to have more precise estimates.

5.3. Hierarchical Testing Order of Secondary Endpoints

In the protocol and SAP version 1.0, it was specified that secondary endpoints would be tested in the following order for the control of overall type I error: Norfolk QOL, NIS-W score, mBMI, 10-meter walk test, and COMPASS-31.

In this SAP amendment, R-ODS has been added as a secondary endpoint for controlled hypothesis testing, and 10-meter walk test has been moved up in the hierarchy. The revised list and order of secondary endpoints is the following: Norfolk QOL, NIS-W, R-ODS, 10-meter walk test, mBMI and COMPASS-31.

These changes were based on the Sponsor’s re-assessment of the clinical importance of these endpoints in hATTR amyloidosis patients.

5.4. Adjustment for Covariates in Statistical Models

The table below summarizes the history of changes in baseline covariates used in the statistical models.

| Baseline Covariates | Protocol | SAP V1.0 | SAP V2.0 |
|---|----------|----------|-------------|
| | ANCOVA | ANCOVA | MMRM/ANCOVA |
| Baseline mNIS+7 | X | X | X |
| Genotype (V30M vs. Non-V30M) | X | | X |
| Genotype class (early onset V30M vs. Other) | | X | |
| Age at hATTR symptom onset (< 50 vs. ≥ 50) | | | X |
| Previous tetramer stabilizer use (Yes vs. No) | X | X | X |
| Region (Western EU, North America, Rest of World) | | | X |
| Age at study entry as continuous variable | X | | |

Genotype class (early onset V30M vs. other) is one of the stratification factors at randomization. The early onset V30M subgroup includes patients who have the V30M genotype and were younger than 50 years old at hATTR amyloidosis symptom onset. At the time of this SAP amendment, the enrollment was complete and there were only 23 early onset V30M patients. Given the small sample size, the composite covariate “genotype class” is decomposed into 2 separate covariates “genotype” and “age at hATTR amyloidosis symptom onset.”

Region is added as a covariate because different rates of attrition and disease worsening were observed for different regions during blinded data monitoring. In addition, adding region may potentially address the influence of different non-V30M types observed across regions.

5.5. Binary Analysis of mNIS+7

In SAP V1.0, binary analysis of mNIS+7 included an analysis of patients with < 2-point increase in mNIS+7 change from baseline at Month 18. The 2-point threshold was originally used in the tafamidis Phase 3 trial in hATTR amyloidosis patients with polyneuropathy to define responders for the NIS-LL co-primary endpoint. That trial enrolled patients with very early stage neuropathy and mean baseline NIS-LL of approximately 8-11 points, in whom a 2-point worsening of NIS-LL was considered to be clinically meaningful. However, the patients enrolled onto this study have more advanced disease, with a mean baseline NIS of approximately 60 points, and the mNIS+7 primary endpoint is very different from NIS-LL, with a maximum score of 304 points compared to 88 points for NIS-LL. Therefore, a 2-point threshold was not deemed relevant for mNIS+7 change in this patient population.

In this SAP V2.0, 2 thresholds, < 10-point increase and any decrease (< 0-point change from baseline) will be employed to conduct the binary analysis of mNIS+7. These thresholds of < 10-point and < 0-point were chosen based on the 18-month estimated mNIS+7 progression rate observed in a natural history study of hATTR amyloidosis patients and the observed effect of patisiran on mNIS+7 at 18 months in the Phase 2 OLE study, respectively.

5.6. Censoring Data Post Alternative FAP Treatment

In this SAP V2.0, for the primary analysis of mNIS+7 and Norfolk QoL, the data post alternative FAP treatment will be censored. This censoring rule was not previously specified in the protocol or SAP V1.0. The rationale of adding this censoring rule is to eliminate the potential confounding effect caused by alternative FAP treatment. Sensitivity analyses for mNIS+7 and Norfolk QoL will be conducted including data post alternative FAP treatment.

In SAP V2.1, an update was made to apply the censoring rule to NIS-W as well so that the results will be consistent with the component analysis of mNIS+7.

For all other efficacy endpoints, the data will not be censored since no sensitivity analyses are planned.

5.7. Other Changes

Additional changes are listed in the table below.

| Change from Protocol and/or SAP V1.0 | Detailed Description/Rationale |
|--|---|
| In the protocol and original SAP V1.0, the first day of drug administration was designated as Day 0. In this SAP amendment and the TLF outputs, the first dose day will be defined as Day 1. | The change is made to follow CDISC convention. Each calculated study day after dosing is 1 day plus the reported study day following the protocol defined Schedule of Assessments. |
| In the protocol and original SAP V1.0, it was intended that an interim analysis for sample size re-estimation would be conducted; however, no interim analysis was conducted during the study. | In 2013, Berk et al. published results of a randomized placebo-controlled trial of diflunisal in FAP. This trial demonstrated that efficacy using a composite neurologic impairment score primary endpoint (NIS+7) could be established in a similar population with a similar study design and smaller study size (N=130). The sponsor believed that an interim analysis for sample size reassessment was therefore no longer necessary. |
| In SAP V2.0, dermal amyloid content is added as an exploratory endpoint. | In addition to IENFD and SGNFD, skin punch biopsies will also be assessed for dermal amyloid content, which has been added as an efficacy parameter in this SAP V2.0. |
| In SAP V2.0, the walk speed for patients unable to perform the walk will be imputed as 0. | The imputation algorithm is added to address the informative missing data for patients unable to perform the walk due to worsening of disease. |
| Renal clearance for siRNA and 4-dimethylaminobutyric acid (DMBA) will not be analyzed. | As per protocol, renal clearance for siRNA and 4-dimethylaminobutyric acid (DMBA) were to be determined whenever possible. Only sparse pre-dose urine collections (and volumes) were obtained in patients enrolled in this study on select days that patients returned to the clinic for dosing (Days 0, 21, 126, 252, 399, 546 and at time of early withdrawal). In order to estimate renal clearance, detailed serial urine and corresponding plasma collections that describe a time course of exposures in both matrices over a dosing interval within individual subjects are required. As it is not possible to reliably estimate the renal clearance for either siRNA or DMBA in individual patients using standard pharmacokinetic methods based on the limited urine and plasma sampling implemented in this outpatient study, renal clearance estimation for either analyte were not performed and cannot be reported for this study. |

| Change from Protocol and/or SAP V1.0 | Detailed Description/Rationale |
|--|---|
| In SAP V2.0, the definition for cardiac subpopulation was added. This subpopulation was not previously defined in the protocol or in SAP V1.0. | In SAP V2.0, the cardiac subpopulation was defined in Section 4.2 as the following: the cardiac subpopulation will be comprised of patients with pre-existing cardiac amyloid involvement, defined as patients with baseline left ventricular (LV) wall thickness ≥ 1.3 cm and no aortic valve disease or hypertension in medical history. |