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| **Official Title:** | **A Phase 2, Multicenter, Open-Label, Extension Study to Evaluate the Long-Term Safety, Clinical Activity, and Pharmacokinetics of ALN-TTR02 in Patients With Familial Amyloidotic Polyneuropathy Who Have Previously Received ALN-TTR02** |
| **NCT Number:** | **NCT01961921** |
| **Document Date:** | **Protocol Version 2.0, 15 June 2015** |

# CLINICAL STUDY PROTOCOL – CONFIDENTIAL
# ALN-TTR02-003

**A Phase 2, Multicenter, Open-Label, Extension Study to Evaluate the Long-Term Safety, Clinical Activity, and Pharmacokinetics of ALN-TTR02 in Patients With Familial Amyloidotic Polyneuropathy Who Have Previously Received ALN-TTR02**

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| Protocol Version | Version 2.0 (Incorporating Amendment 1) |
| Protocol Date | 15 June 2015 |
| EudraCT Number | 2013-001644-65 |
| Sponsor: | Alnylam Pharmaceuticals, Inc<br>300 Third Street<br>Cambridge, MA 02142 USA |
| Sponsor Contact: | [redacted] |
| Sponsor Signature | [redacted] Date 15 Jun 2015 |
| Name (print) | [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 trial 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) _____

**Version History:**

| Version Number | Date | Comment |
|---|---|---|
| 1.0 (Original) | 26 April 2013 | Initial Release |
| 2.0 | 15 June 2015 | Incorporating Global Amendment 1 |

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# PROTOCOL SYNOPSIS

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| **Protocol Title:** | A Phase 2, Multicenter, Open-Label, Extension Study to Evaluate the Long-Term Safety, Clinical Activity, and Pharmacokinetics of ALN-TTR02 in Patients With Familial Amyloidotic Polyneuropathy Who Have Previously Received ALN-TTR02 |
| **Indication:** | Treatment of patients with familial amyloidotic polyneuropathy (FAP) |
| **Protocol Number:** | ALN-TTR02-003 |
| **Phase of Development:** | 2 |
| **Design:** | This study is a multicenter, multinational, open-label, extension study that provides a mechanism for continued administration of ALN-TTR02 to FAP patients who participated in the ALN-TTR02-002 study.<br><br>Consented eligible patients will be enrolled and receive 0.3 mg/kg ALN-TTR02 once every 3 or 4 weeks for approximately 2 years, based on results from the ongoing ALN-TTR02-002 study where the safety and pharmacodynamics (PD) of these 2 dosing schedules are being explored.<br><br>Safety will be assessed by collection of adverse events (AEs), including serious adverse events (SAEs); clinical laboratory tests, including hematology, clinical chemistry, thyroid function parameters, coagulation parameters, and urinalysis; electrocardiograms (ECGs); vital signs; physical examination findings; and ophthalmology examinations.<br><br>Pharmacodynamic (PD) evaluation will include serial measurement of serum levels of transthyretin (TTR).<br><br>Serum levels of secondary PD biomarkers, including retinol binding protein (RBP) and vitamin A, will also be evaluated.<br><br>Clinical activity will be assessed through clinical examinations and electrophysiologic testing of neurologic impairment, patient reported outcomes for quality of life and disability, evaluation of motor functions with impact on activities of daily living, assessment of nutritional status, autonomic symptom assessment, and pathologic evaluation of sensory and autonomic innervation. All evaluations of |

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| | clinical activity will be conducted at Screening/Baseline and approximately once every 6 months.<br><br>Plasma and urine pharmacokinetic (PK) samples will be collected at specified time points up through 56 days post last dose.<br><br>A subset of the FAP patients will undergo additional blood draws for assessment of PK/PD (PK/PD subgroup). In addition, any patients with pre-existing cardiac amyloid involvement (Cardiac subgroup), will undergo additional testing including echocardiograms, troponin I, N-terminal prohormone of B-type natriuretic peptide (NT-proBNP) assessments to evaluate cardiac structure and function.<br><br>Disease burden and healthcare utilization will be assessed using a patient reported pharmacoeconomics questionnaire.<br><br>Blood will also be collected to evaluate exploratory biomarkers and anti-drug antibodies (ADA). |
| **Study Sites** | This study will be conducted at the same study sites that enrolled patients to Study ALN-TTR02-002. |
| **Investigational Drug:** | ALN-TTR02 is comprised of a small interfering ribonucleic acid (siRNA) targeting wild type TTR messenger RNA (mRNA), as well as all known TTR mutations, formulated in a lipid nanoparticle (LNP) containing the following lipid excipients: 1,2-dilinoleyloxy-N,N-dimethylpropylamine (DLin-MC3-DMA), 3-N-[(ω-methoxy poly(ethylene glycol)2000) carbamoyl]-1,2-dimyristyloxy-propylamine ($PEG_{2000}$-C-DMG), 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), and cholesterol. |
| **Dosage, Route of Administration and Duration of Treatment of Investigational Drug:** | Patients will receive 0.3 mg/kg ALN-TTR02 once every 3 or 4 weeks, based on results from the ongoing ALN-TTR02-002 study where the safety and PD of 2 dosing schedules are being evaluated. Doses of ALN-TTR02 will be administered as a 70-minute intravenous (IV) infusion (or at a more prolonged infusion rate if required due to prior infusion related reaction [IRR]) by a controlled infusion device.<br><br>Prior to ALN-TTR02, patients will receive the following premedications:<br><br>• Intravenous dexamethasone (10 mg) or equivalent, administered at least 60 minutes prior to the start of the infusion of ALN-TTR02; |

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| | • Oral paracetamol (500 mg) or equivalent at least 60 minutes prior to the start of the infusion of ALN-TTR02;<br>• 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 ALN-TTR02; and<br>• 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 ALN-TTR02. Hydroxyzine or fexofenadine 25 mg per os (orally; PO) or cetirizine 10 mg PO may be substituted for any patient who does not tolerate IV diphenhydramine or other IV H1 blocker.<br><br>Patients will remain at the study site for at least 1 hour following completion of dosing for safety assessments. The PK/PD subgroup will remain at the site for 6 hours following the first dose and the doses given at 8 and 24 months. |
| **Time on Study** | The duration of patient participation in this study is approximately 2 years and 4 months (Screening/Baseline [up to 28 days prior to study drug administration] through the 56-day Follow-up visit.) |
| **Primary Objective:** | To evaluate the safety and tolerability of long-term dosing with ALN-TTR02. |
| **Secondary Objectives:** | To assess the PD effect associated with long-term dosing of ALN-TTR02 on serum TTR.<br><br>To assess changes from baseline in:<br>• Neurologic impairment using the modified Neuropathy Impairment Score (mNIS) +7 composite score<br>• Quality of life (EQ5D) and disability (Rasch-built Overall Disability Scale [R-ODS])<br>• Motor function impacting activities of daily living, including a 10-meter walk test and test of grip strength<br>• Nutritional status (modified body mass index [mBMI]) |

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| **Tertiary Objectives:** | To further characterize the plasma and urine PK profile of ALN-TTR02.<br><br>To assess changes in:<br><br>• Secondary PD biomarkers (RBP and vitamin A)<br>• Sensory and autonomic innervation (skin punch biopsies for intraepidermal nerve fiber density [IENFD] and sweat gland nerve fiber density [SGNFD])<br>• Neuropathy Impairment Score (NIS) and vibration detection threshold (VDT)<br>• Heart rate response to deep breathing (HRdb)<br>• Ambulation using FAP Stage and Polyneuropathy Disability (PND) Score<br>• Patient reported autonomic neuropathy symptoms using Composite Autonomic Symptom Score (COMPASS 31)<br>• Healthcare utilization using a pharmacoeconomics questionnaire<br>• Cardiac structure/function through echocardiograms and serum levels of troponin I and NT-proBNP in patients with evidence of pre-existing cardiac amyloid involvement |
| **Sample Size:** | Up to 28 patients will be enrolled in this study. Of these patients, up to 25 will be enrolled in the PK/PD subgroup, and additionally, any patients with pre-existing cardiac amyloid involvement will be enrolled in the Cardiac subgroup. |
| **Inclusion and Exclusion Criteria:** | To be enrolled in the study, each patient must meet the following criteria at Screening/Baseline:<br><br>1. Previously received and tolerated ALN-TTR02 in Study ALN-TTR02-002.<br>2. Karnofsky performance status of 60% or greater.<br>3. Absolute neutrophil count (ANC) ≥1500 cells/mm$^3$, platelet count ≥100,000 cells/mm$^3$, and hemoglobin ≥10 g/dL.<br>4. Adequate liver function, demonstrated by an aspartate transaminase (AST) and alanine transaminase (ALT) level ≤2.5 × the upper limit of normal (ULN), total bilirubin within normal limits, albumin >3 g/dL, and international normalized ratio (INR) ≤1.2.<br>5. Adequate renal function, demonstrated by serum creatinine ≤1.5 × ULN.<br>6. Women of child-bearing potential must have a negative pregnancy test, cannot be breast feeding, and must be using 2 highly effective methods of contraception prior |

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| | to screening, throughout study participation, and for 75 days after the last dose of study medication.<br>7. Males agree to use appropriate contraception throughout study participation and for 75 days after the last dose of study medication.<br>8. Patient is willing and able to comply with protocol-required visit schedule and visit requirements and provide written informed consent.<br><br>Additional inclusion criteria for patients participating in the PK/PD subgroup:<br>9. At the time of enrollment, at least 6 months has elapsed since their last dose of ALN-TTR02.<br><br>Additional inclusion criteria for patients participating in the Cardiac subgroup:<br>10. Have a left ventricular wall thickness of ≥13 mm on transthoracic echocardiogram<br>11. Be normotensive or have hypertension that is well-controlled<br>12. No aortic valve disease<br><br>Patients will be excluded if they meet any of the following criteria at the time of Screening/Baseline:<br>1. Pregnant or nursing.<br>2. Has had a liver transplant.<br>3. Has a known or suspected systemic bacterial, viral, parasitic, or fungal infection.<br>4. Received an investigational agent, other than tafamidis or diflunisal, within 30 days prior to study drug administration.<br>5. Has a New York Heart Association heart failure classification >2.<br>6. Has unstable angina.<br>7. Has uncontrolled clinically significant cardiac arrhythmia.<br>8. Is considered unfit for the study by the Principal Investigator. |

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| **Safety Assessments** | Safety evaluations will include assessment of AEs, ECGs, vital signs (including blood pressure, pulse rate, oral body temperature, and respiration rate), clinical laboratory safety tests (including hematology, serum chemistry, thyroid function parameters, coagulation parameters, and urinalysis), and ophthalmology and physical examinations |
| **Pharmacodynamic Assessments** | The pharmacodynamic endpoint consists of serial measurement of serum levels of TTR. Secondary PD biomarkers to be assessed include RBP and vitamin A. |
| **Clinical Activity Assessments** | Neurologic impairment will be assessed using the mNIS+ 7 composite score. The mNIS + 7 includes the modified NIS (weakness and reflexes only), nerve conduction studies (NCS) 5 attributes (Σ5), quantitative sensory testing (QST) by body surface area including touch pressure (TP) and heat pain (HP), as well as autonomic assessment through postural blood pressure. The NIS composite score will be assessed, as well as determining the vibration detection threshold (VDT) of patients.<br><br>Patient reported quality of life will be evaluated using the EQ5D. Disability will be reported by patients using the R-ODS.<br><br>Autonomic neuropathy symptoms will be assessed using the COMPASS 31 questionnaire.<br><br>Additional motor function assessments to be evaluated include a timed 10-meter walk test and test of grip strength, as well as evaluation of PND Score and FAP Stage.<br><br>Autonomic function will be further assessed using HRdb.<br><br>Nutritional status will be assessed using mBMI.<br><br>Pathologic evaluation of sensory and autonomic innervation will be evaluated by IENFD analysis and quantitation of dermal SGNFD via 3 mm skin punch biopsies taken from the lower extremities at Baseline and approximately once every 6 months.<br><br>For any patient with pre-existing cardiac amyloid involvement, cardiac structure and function will be assessed through serial echocardiograms as well as measurement of serum levels of NT-proBNP and troponin I. |

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| **Pharmacokinetic Assessments** | The PK evaluation will include plasma-concentration time profiles for siRNA and the novel lipid components DLin-MC3-DMA and polyethylene glycol $(PEG)_{2000}$-C-DMG. The siRNA, DLin-MC3-DMA, and $PEG_{2000}$-C-DMG concentration will be determined at specific time points pre- and post-dose. For the PK/PD subgroup, PK profiles will be evaluated for the first dose, and at Months 8 and 24. In addition, population PK from all subjects will be evaluated whenever possible from sparse samples collected at pre-dose and post-dose at specified dosing period, and from various samples collected at specified time points at first dose, up through and including the 3 month dose, and once every 3 months during the study period. PK/PD relationship will be evaluated whenever possible for all samples collected<br><br>Urine will be collected with void volume recorded at specified time points to determine the siRNA and 4-dimethylaminodibutyric acid (the metabolite of DLin-MC3-DMA) renal clearance ($CL_R$) after dosing with ALN-TTR02. |
| **Other Assessments** | Other effects of ALN-TTR02 will be evaluated by:<br>• Measurement of other hepatocyte-derived proteins and ADA.<br>• Determination of pharmacoeconomics outcomes through disease burden and healthcare utilization questionnaire. |
| **Statistical Methods:** | Statistical analyses will be primarily descriptive in nature. Adverse event summaries will include tabulations of all treatment-emergent AEs (TEAEs), treatment-related AEs, SAEs, discontinuations due to AEs, and AEs of various grading severity. By-patient listings will be provided for deaths, SAEs, and AEs leading to discontinuation of treatment.<br><br>Descriptive statistics will be provided for clinical laboratory data, vital signs data, and ECG interval data, presented as both actual values and changes from baseline relative to each on-study evaluation. Laboratory shift tables from baseline to worst values will be presented.<br><br>Summary tables and graphical displays of observed values and changes from baseline in serum TTR, RBP, and vitamin A will be used to assess the durability of suppression over the course of the study. As part of PK/PD analysis, additional PD baseline analysis on serum TTR, RBP, and vitamin A may be conducted to include the PD data obtained during the recovery period post last dose. |

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| | Pharmacokinetic analyses will be conducted using noncompartmental and/or compartmental evaluation. The PK parameters of siRNA, DLin-MC3-DMA (lipid), and $PEG_{2000}$-C-DMG (lipid) in plasma will be evaluated using a validated version of WinNonlin® Enterprise (Version 5.2 or higher) with NCA Model 200. Summary tables and figures and inferential statistics will be provided.<br><br>Population PK analyses will be performed on available siRNA and possibly DLin-MC3-DMA from sparse samples collected that may include sparse samples obtained from the PK/PD subgroups at various time points during the duration of the study. Population PK modeling of siRNA and possibly DLin-MC3-DMA in plasma will be performed with 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.<br><br>The PK/PD analysis will be performed whenever possible using WinNonLin or Phoenix NLME (Version 1.1 or later) or similar software. Summary tables and figures and inferential statistics of PD and PK/PD parameters, correlation between siRNA, DLin-MC3-DMA or $PEG_{2000}$CDMG exposure versus TTR, RBP, or vitamin A, and between TTR versus RBP and vitamin A will be provided.<br><br>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 (i.e., the NIS weakness and reflex scores, the Σ5 NC, and QST values). The full NIS (i.e., the sum of cranial nerve, muscle weakness, reflex and sensation scores) will be summarized also. Values of the individual components of the mNIS weakness and reflex scores will be depicted graphically by presenting the mean (±SE) values over time for each location (hip, knee, etc.).<br><br>Patient reported quality of life and disability will be assessed by summary statistics for the EQ5D 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.<br><br>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), |

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| | sensory and autonomic innervation (IENFD and SGNFD), VDT, and ambulation (FAP stage and PND score).<br><br>Descriptive statistics will be provided for actual values and changes from baseline in serum levels of troponin I and NT-proBNP in the subset of patients with evidence of pre-existing cardiac amyloid involvement. Results of echocardiograms will be summarized. |

**Table 1-1: Schedule of Assessments for Year 1 — ALN-TTR02 Administered Once Every 3 Weeks**

| Procedure | Visit Type | Screening/ Baseline[a] | Dosing | | | | | | | | | Mid-year/Annual Efficacy Assessment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Study Week | NA | 1 & 28 | 4 & 31 | 7 & 34 | 10 & 37 | 13 & 40 | 16 & 43 | 19 & 46 | 22 & 49 | 25 & 52 | 27 & 54 |
| | Study Day | Day -28 to -0 | D0 & D189 | D21 & D210 | D42 & D231 | D63 & D252 | D84 & D273 | D105 & D294 | D126 & D315 | D147 & D336 | D168 & D357 | D182 & D371 |
| | Window | NA | ±3D[b] | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±5D |
| Informed Consent | | X | | | | | | | | | | |
| Demographics | | X | | | | | | | | | | |
| Medical History[c] | | X | | | | | | | | | | |
| Inclusion/Exclusion Criteria | | X | X[d] | | | | | | | | | |
| Physical Examination | | X | | | | | | | | | | X |
| Weight[e] | | X | X | X | X | X | X | X | X | X | X | X |
| Height | | X | | | | | | | | | | |
| Vital Signs[f] | | X | X | X | X | X | X | X | X | X | X | X |
| Echocardiogram | | X | | | | | | | | | | |
| 12-Lead ECG[g] | | X | | | | | | | | | | X |
| Urine Pregnancy Test (females only)[h] | | X | | | | | | | | | | |
| Hematology[i] | | X | X[d] | | | | | | | | X | |
| Serum Chemistry and Urinalysis[i] | | X | X[d] | | | | X | | | | X | |
| Thyroid Function Tests and Coagulation Studies[i] | | X | X[d] | | | | | | | | X | |
| Ophthalmology Exam | | X | | | | | | | | | | X |
| **PD Assessments:** | | | | | | | | | | | | |
| TTR protein (ELISA and turbidimetric) [j] | | X | X[d] | | | | X | | | | X | X |
| RBP and Vitamin A[j] | | X | X[d] | | | | X | | | | X | X |

| Procedure | Visit Type | Screening/ Baseline[a] | Dosing | | | | | | | | | Mid-year/Annual Efficacy Assessment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Study Week | NA | 1 & 28 | 4 & 31 | 7 & 34 | 10 & 37 | 13 & 40 | 16 & 43 | 19 & 46 | 22 & 49 | 25 & 52 | 27 & 54 |
| | Study Day | Day -28 to -0 | D0 & D189 | D21 & D210 | D42 & D231 | D63 & D252 | D84 & D273 | D105 & D294 | D126 & D315 | D147 & D336 | D168 & D357 | D182 & D371 |
| | Window | NA | ±3D[b] | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±5D |
| **Other Assessments:** | | | | | | | | | | | | |
| Exploratory Biomarkers[k] | | X | X[d] | | | | X | | | | X | |
| Anti-drug Antibodies[k] | | X | X[d] | X[l] | | | X[l] | | | | X | |
| Pharmacoeconomics Questionnaire | | X | | | | | | | | | | X |
| **Clinical Activity Assessments:** | | | | | | | | | | | | |
| mNIS + 7[m,n] | | X | | | | | | | | | | X |
| VDT[n] | | X | | | | | | | | | | X |
| HRdb[n] | | X | | | | | | | | | | X |
| Grip Strength[n,o] | | X | | | | | | | | | | X |
| 10-Meter Walk Test[n,p] | | X | | | | | | | | | | X |
| NIS[n] | | X | | | | | | | | | | X |
| Skin Punch Biopsy (IENFD & SGNFD)[q] | | X | | | | | | | | | | X |
| mBMI | | X | | | | | | | | | | X |
| FAP Stage and PND Score | | X | | | | | | | | | | X |
| COMPASS 31 | | X | | | | | | | | | | X |
| QOL and Disability Questionnaires[r] | | X | | | | | | | | | | X |
| Premedication Administration[s] | | | X | X | X | X | X | X | X | X | X | |
| Study Drug Administration[t] | | | X | X | X | X | X | X | X | X | X | |
| Plasma PK Sampling[u] | | | X[v] | X[v] | X[v] | X[v] | X | | | | X | X[w] |
| Urine PK Sampling[x] | | | X[v] | X[v] | X[v] | X[v] | X | | | | X | X[w] |

| Procedure | Visit Type | Screening/ Baseline[a] | Dosing | | | | | | | | | Mid-year/Annual Efficacy Assessment |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Study Week | NA | 1 & 28 | 4 & 31 | 7 & 34 | 10 & 37 | 13 & 40 | 16 & 43 | 19 & 46 | 22 & 49 | 25 & 52 | 27 & 54 |
| | Study Day | Day -28 to -0 | D0 & D189 | D21 & D210 | D42 & D231 | D63 & D252 | D84 & D273 | D105 & D294 | D126 & D315 | D147 & D336 | D168 & D357 | D182 & D371 |
| | Window | NA | ±3D[b] | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±5D |
| **PK/PD Subgroup Only** | | | | | | | | | | | | |
| TTR (ELISA and turbidimetric)[y] | | | X | | X | | | | | | | |
| RBP and Vitamin A[y] | | | X | | X | | | | | | | |
| Plasma PK Sampling | | | X[z] | | X[z] | | | | | | | |
| Urine PK Sampling | | | X[aa] | | X[aa] | | | | | | | |
| **Cardiac Subgroup Only:** | | | | | | | | | | | | |
| Echocardiogram | | | | | | | | | | | | X |
| Troponin I and NT-proBNP | | | X[d] | | | | X | | | | X | |
| Concomitant Medications | | X | X | | | | | | | | | |
| Adverse Events | | | X[bb] | | | | | | | | | |

Note: The schedule of assessments for patients administered ALN-TTR02 once every 4 weeks is provided in Appendix 2.

a Assessments performed during the ALN-TTR02-002 study that occur within the Screening/Baseline timeframe will not need to be repeated at the Screening/Baseline visit.
b Window does not apply to Day 0.
c Any AEs from the previous study (ALN-TTR02-002) that are ongoing on Day 0 are to be recorded on the Medical History case report form for the current study (ALN-TTR02-003) and followed until resolution.
d To be performed only on Day 0, not on Day 189.
e On dosing days, weight will be measured predose.
f On dosing days, vital signs will be measured predose. 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.
g Triplicate recordings will be obtained.
h A pregnancy test will be performed on all females of child-bearing potential at Screening/Baseline and any time pregnancy is suspected.
i To be performed before administration of premedications and dosing with ALN-TTR02.
j Blood samples for TTR, RBP, and vitamin A will be collected on Day 0 at 2 separate time points: 1) prior (within 10 minutes) to administration of premedications and 2) after administration of premedications but immediately prior (within 10 minutes) to dosing of ALN-TTR02. At all other dosing visits, a blood sample will be collected only once; prior to administration of premedications. At the Mid-year and Annual visits, one sample should be collected on each day. Additionally, aliquots of serum samples will be taken and frozen, to permit testing of additional proteins related to FAP.
k Blood samples for exploratory biomarkers and ADA will be collected prior to dosing with ALN-TTR02.

l To be collected only on Days 21 and 84, not on Days 210 and 273.

m The mNIS + 7 consists of the modified NIS tool (or aspects of the tool; weakness and reflexes only), nerve conduction studies (NCS), quantitative sensory testing (QST) by body surface area including touch pressure (TP) and heat pain (HP), and postural blood pressure. Every effort will be made to use the same devices for a patient throughout the duration of the study.

n Two independent assessments will be performed on separate days at Screening/Baseline and then approximately every 6 months. The Screening/Baseline assessments must be performed within 14 days prior to the first dose of study drug (Day 0). The second (repeat) assessment must be conducted at least 24 hours (approximately) after the first assessment, but not more than 7 days apart.

o Hand 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. At each time point, 2 independent assessments (each assessment performed in triplicate) will be performed.

p Patient will walk without assistance for 10 meters in order to complete the 10-meter walk test. The time required for the patient to complete 2, 8, and 10 meters will be recorded.

q Two 3-mm skin punch biopsies are to be obtained. One will be obtained from the distal lower leg, when a patient's clinical status allows, and one from the distal thigh.

r Quality of life and disability assessments will include the EQ5D and R-ODS questionnaires.

s The following premedications will be administered at least 60 minutes prior to the start of the infusion of ALN-TTR02: dexamethasone (10 mg IV, or equivalent), paracetamol (PO 500 mg; or equivalent), IV H2 blocker (e.g., ranitidine 50 mg, famotidine 20 mg, or equivalent other H2 blocker dose), and IV H1 blocker (e.g., diphenhydramine 50 mg or equivalent; 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 blockers).

t The infusion site will be assessed for any localized reaction pre-dose, during the infusion, at the end of the infusion (EOI), and for 30 minutes after the infusion.

u Plasma PK samples will be collected pre-dose (within 1 hour of planned dosing start; -5 minutes), EOI (+5 minutes), and 1 hour post-dose (±5 min), except on Days 0 and 231 for subjects in the PK/PD Subgroup. The EOI will usually occur at 70 minutes after the start of the infusion, except in patients where the infusion has been prolonged. All post-infusion times are relative to the EOI, regardless of the duration of the infusion. In addition, if the infusion is stopped and the site is considering restarting the infusion, a PK blood sample will be taken while the infusion is stopped.

v Samples to be collected on Day 0, 21, 42 and 63 only.

w Samples will be collected once on each day of the 2-day study visit.

x For each dose, urine PK samples will be collected pre-dose (within 1 hour of planned dosing start; -5 minutes) and 1 hour (±5 minutes) after dosing with ALN-TTR02.

y For patients in the PK/PD subgroup, blood samples for TTR, RBP, and vitamin A will be collected on Days 0 and 231 immediately prior (within 10 minutes) to administration of premedications. In addition, a TTR, RBP, and vitamin A sample will also be collected 24 hours (±120 minutes) post-dose, and 3, 7, and 17 days (±1 day) after dosing with ALN-TTR02 for the study visits noted (blood samples for TTR will not be collected on Days 42 and 189).

z On Days 0 and 231, patients in the PK/PD subgroup will have PK samples taken pre-dose (within 1 hour of planned dosing start; -5 minutes), EOI (+5 minutes), and 1, 2, 4, 6, and 24 hours postinfusion. In addition, a plasma PK sample will also be collected 3, 7, and 17 days (±1 day) after dosing with ALN-TTR02 for the study visits noted. The windows for sampling are ±5 minutes for the 1, 2, 4, and 6 hour sampling periods; and ±120 minutes for the 24 hour sampling period.

aa On Days 0 and 231, patients in the PK/PD subgroup will have a urine sample for PK analysis taken pre-dose (within 1 hour of planned dosing start; -5 minutes), EOI (+5 minutes), and 6 and 24 hours postinfusion. In addition, a urine PK sample will also be collected 3, 7, and 17 days (±1 day) after dosing with ALN-TTR02 for the study visits noted. The windows for sampling are ±5 minutes for the 1, 2, 4, and 6 hour sampling periods; and ±120 minutes for the 24-hour sampling period.

bb Adverse events are to be documented beginning with the initiation of the first infusion.

**Table 1-2: Schedule of Assessments for Year 2 — ALN-TTR02 Administered Once Every 3 Weeks**

| Procedure | Visit Type | Dosing | | | | | | | | | Mid-year/ Annual Efficacy Assess-ment | 21- and 56-day Follow-up[a] | | Early Termi-nation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Study Week | 55 & 82 | 58 & 85 | 61 & 88 | 64 & 91 | 67 & 94 | 70 & 97 | 73 & 100 | 76 & 103 | 79 & 106 | 81 & 108 | 109 | 114 | ET |
| | Study Day | D378 & D567 | D399 & D588 | D420 & D609 | D441 & D630 | D462 & D651 | D483 & D672 | D504 & D693 | D525 & D714 | D546 & D735 | D560 & D749 | D756 | D791 | N/A |
| | Window | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±5D | ±5D | ±5D | 2D to 7D |
| Physical Examination | | | | | | | | | | | X | X | | X |
| Weight[b] | | X | X | X | X | X | X | X | X | X | X | X | | X |
| Vital Signs[c] | | X | X | X | X | X | X | X | X | X | X | X | | X |
| 12-lead ECG[d] | | | | | | | | | | | X | X | | X |
| Hematology[e] | | | | | | | | | | X | | X | | X |
| Serum Chemistry and Urinalysis[e] | | | | | | X | | | | X | | X | | X |
| Thyroid Function Tests and Coagulation Studies[e] | | | | | | | | | | X | | X | | X |
| Ophthalmology Exam | | | | | | | | | | | X | | | X |
| **PD Assessments:** | | | | | | | | | | | | | | |
| TTR protein (ELISA and turbidimetric)[f] | | X | X | X | X | X | X | X | X | X | X | X | X | X |
| RBP and Vitamin A[f] | | | | | | X | | | | X | X | X | X | X |
| **Other Assessments:** | | | | | | | | | | | | | | |
| Exploratory Biomarkers[g] | | | | | | X | | | | X | | | | |
| Anti-drug Antibodies | | | | | | | | | | X[h] | | | | |
| Pharmacoeconomics Questionnaire | | | | | | | | | | | X | | | X[i] |

| Procedure | Visit Type | Dosing | | | | | | | | | Mid-year/ Annual Efficacy Assess-ment | 21- and 56-day Follow-up[a] | | Early Termi-nation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Study Week | 55 & 82 | 58 & 85 | 61 & 88 | 64 & 91 | 67 & 94 | 70 & 97 | 73 & 100 | 76 & 103 | 79 & 106 | 81 & 108 | 109 | 114 | ET |
| | Study Day | D378 & D567 | D399 & D588 | D420 & D609 | D441 & D630 | D462 & D651 | D483 & D672 | D504 & D693 | D525 & D714 | D546 & D735 | D560 & D749 | D756 | D791 | N/A |
| | Window | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±5D | ±5D | ±5D | 2D to 7D |
| **Clinical Activity Assessments:** | | | | | | | | | | | | | | |
| mNIS + 7[j,k] | | | | | | | | | | | X | | | X[i] |
| VDT[k] | | | | | | | | | | | X | | | X[i] |
| HRdb[k] | | | | | | | | | | | X | | | X[i] |
| Grip Strength[k,l] | | | | | | | | | | | X | | | X[i] |
| 10-Meter Walk Test[k,m] | | | | | | | | | | | X | | | X[i] |
| NIS[k] | | | | | | | | | | | X | | | X[i] |
| Skin Punch Biopsy (IENFD & SGFND)[n] | | | | | | | | | | | X | | | X[i] |
| mBMI | | | | | | | | | | | X | | | X[i] |
| FAP Stage and PND Score | | | | | | | | | | | X | | | X[i] |
| COMPASS 31 | | | | | | | | | | | X | | | X[i] |
| QOL and Disability Questionnaires[o] | | | | | | | | | | | X | | | X[i] |
| Premedication Administration[p] | | X | X | X | X | X | X | X | X | X | | | | |
| Study Drug Administration[q] | | X | X | X | X | X | X | X | X | X | | | | |
| Plasma PK Sampling[r] | | | | | | X | | | | X | X | X | X | X |
| Urine PK Sampling[s] | | | | | | X | | | | X | X | | | X |

| Procedure | Visit Type | Dosing | | | | | | | | | Mid-year/ Annual Efficacy Assess-ment | 21- and 56-day Follow-up[a] | | Early Termi-nation |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Study Week | 55 & 82 | 58 & 85 | 61 & 88 | 64 & 91 | 67 & 94 | 70 & 97 | 73 & 100 | 76 & 103 | 79 & 106 | 81 & 108 | 109 | 114 | ET |
| | Study Day | D378 & D567 | D399 & D588 | D420 & D609 | D441 & D630 | D462 & D651 | D483 & D672 | D504 & D693 | D525 & D714 | D546 & D735 | D560 & D749 | D756 | D791 | N/A |
| | Window | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±3D | ±5D | ±5D | ±5D | 2D to 7D |
| **PK/PD Subgroup Only:** | | | | | | | | | | | | | | |
| TTR (ELISA and turbidimetric) | | | | | | | | | | X[t] | | | | |
| RBP and Vitamin A | | | | | | | | | | X[t] | | | | |
| Plasma PK Sampling | | | | | | | | | | X[u] | | | | |
| Urine PK Sampling | | | | | | | | | | X[v] | | | | |
| **Cardiac Subgroup Only:** | | | | | | | | | | | | | | |
| Echocardiogram | | | | | | | | | | | X | | | X |
| Troponin I and NT-proBNP | | | | | | X | | | | X | | | | X |
| Concomitant Medications | | X[w] | | | | | | | | | | | | |
| Adverse Events | | X[x] | | | | | | | | | | | | |

Note: The schedule of assessments for patients administered ALN-TTR02 once every 4 weeks is provided in Appendix 2.

a If a patient enrolls in the global extension study, the patient will only have to complete the 21-day follow-up assessments (Day 756) and not the 56-day follow-up assessments (Day 791). Patients who do not enroll in the extension study will need to complete both Follow-up visits (Days 756 and 791).
b On dosing days, weight will be measured predose.
c On dosing days, vital signs will be measured predose. 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.
d Triplicate recordings will be obtained.
e On dosing days, to be performed before administration of premedications and dosing with ALN-TTR02.
f Blood samples for TTR, RBP, and vitamin A will be collected on Day 0 at 2 separate time points: 1) prior to administration of premedications and 2) after administration of premedications but before dosing with ALN-TTR02. At all other dosing visits, a blood sample will be collected only once; prior to administration of premedications. At the

Mid-year and Annual visits, one sample should be collected on each day. Additionally, aliquots of serum samples will be taken and frozen, to permit testing of additional proteins related to FAP.

g Blood samples for exploratory biomarkers will be collected prior to dosing with ALN-TTR02.

h Blood samples for ADA will be collected only at the Day 735 visit (approximately 24 months), prior to dosing with ALN-TTR02.

i To be performed only in patients who have received at least 4 doses of study drug and who withdraw >3 months after the last clinical activity assessments were performed. These tests will not be repeated.

j The mNIS + 7 consists of the modified NIS tool (or aspects of the tool; weakness and reflexes only), NCS, QST by body surface area including TP and HP, and postural blood pressure. Every effort will be made to use the same devices for a patient throughout the duration of the study.

k Two independent assessments will be performed at Screening/Baseline and at the Mid-year and Annual visits. The second (repeat) assessment must be conducted at least 24 hours (approximately) after the first assessment, but not more than 7 days apart.

l Hand 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. At each time point, 2 independent assessments (each assessment performed in triplicate) will be performed.

m Patient will walk without assistance for 10 meters in order to complete the 10-meter walk test. The time required for the patient to complete 2, 8, and 10 meters will be recorded.

n Two 3-mm skin punch biopsies are to be obtained. One will be obtained from the distal lower leg, when a patient's clinical status allows, and one from the distal thigh.

o Quality of life and disability assessments will include the EQ5D and R-ODS questionnaires.

p The following premedications will be administered at least 60 minutes prior to the start of the infusion of ALN-TTR02: dexamethasone (10 mg IV, or equivalent), paracetamol (PO 500 mg; or equivalent), IV H2 blocker (e.g., ranitidine 50 mg, famotidine 20 mg, or equivalent other H2 blocker dose), and IV H1 blocker (e.g., diphenhydramine 50 mg or equivalent; 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 blockers).

q The infusion site will be assessed for any localized reaction pre-dose, during the infusion, at the end of the infusion and for 30 minutes after the infusion.

r Plasma PK samples will be collected pre-dose (within 1 hour of planned dosing start; -5 minutes) and at EOI (+5 minutes). In addition, if the infusion is stopped and the site is considering restarting the infusion, a PK blood sample will be taken while the infusion is stopped. In addition, plasma samples will be collected once on each day of the Mid-year and Annual visits.

s For each dose, urine PK samples will be collected 1 hour postdose (±5 minutes).

t For patients in the PK/PD subgroup, blood samples for TTR, RBP, and vitamin A will be collected on Day 735 immediately prior (within 10 minutes) to administration of premedications. In addition, a TTR, RBP, and vitamin A sample will also be collected 24 hours (±120 minutes) post-dose, and 3, 7, and 17 days (±1 day) after dosing with ALN-TTR02 for the study visit noted.

u On Day 735, patients in the PK/PD subgroup will have PK samples taken pre-dose (within 1 hour of planned dosing start; -5 minutes), EOI (+5 minutes), and 1, 2, 4, 6, and 24 hours post-infusion. In addition, a plasma PK sample will also be collected 3, 7, and 17 days (±1 day) after dosing with ALN-TTR02 for the study visit noted. The windows for sampling are ±5 minutes for the 1, 2, 4, and 6 hour sampling periods; and ±120 minutes for the 24 hour sampling period.

v On Day 735, patients in the PK/PD subgroup will have a urine sample for PK analysis taken pre-dose (within 1 hour of planned dosing start; -5 minutes), EOI (+5 minutes), and 6 and 24 hours post-infusion. In addition, a urine PK sample will also be collected 3, 7, and 17 days (±1 day) after dosing with ALN-TTR02 for the study visit noted. The windows for sampling are ±5 minutes for the 1, 2, 4, and 6 hour sampling periods; and ±120 minutes for the 24-hour sampling period.

w Use of all concomitant medications during Screening/Baseline, predose, and postdose will be recorded on the patient's CRF up to the 21-day or 56-day Follow-up visit (depending on whether or not the patient plans to roll over to the open-label global extension study).

x Adverse events are to be documented beginning with the initiation of the first infusion through the 21-day or 56-day Follow-up visit (depending on whether or not the patient plans to roll over to the open-label global extension study).

## ABBREVIATIONS

| Abbreviation | Definition |
|---|---|
| Σ5 | 5 attributes |
| $\lambda_z$ | Elimination rate constant |
| ADA | Anti-drug antibodies |
| AE | Adverse event |
| ALT | Alanine transaminase |
| ANC | Absolute neutrophil count |
| aPTT | Activated partial thromboplastin time |
| AST | Aspartate transaminase |
| ATTR | Transthyretin-mediated amyloidosis |
| AUC | Area under the plasma concentration-time curve |
| $AUC_{0-\infty}$ | Area under the plasma concentration-time curve extrapolated to infinity |
| $AUC_{0-last}$ | Area under the plasma concentration-time curve from zero to the last measurable time point |
| $AUC_{0-t}$ | Area under the plasma concentration-time curve to the last measurable concentration |
| $AUC_p$ | Partial area under the plasma concentration-time curve |
| BUN | Blood urea nitrogen |
| CDT | Cooling detection threshold |
| CEC | Clinical Events Committee |
| CFR | Code of Federal Regulations |
| CL | Systemic clearance |
| $CL_R$ | Renal clearance |
| $C_{max}$ | Observed maximum plasma concentration |
| COMPASS 31 | Composite Autonomic Symptom Score |
| CRF | Case Report Form |
| CRO | Contract research organization |
| CV | Curriculum vitae |
| 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 |
| EOI | End of infusion |

| Abbreviation | Definition |
|---|---|
| ET | Early termination |
| EU | European Union |
| FAC | Familial amyloidotic cardiomyopathy |
| FAP | Familial amyloidotic polyneuropathy |
| GCP | Good Clinical Practice |
| GMP | Good Manufacturing Practice |
| H1/H2 blocker | Histamine H1/H2 receptor antagonist |
| HIPAA | Health Insurance Portability and Accountability Act of 1996 |
| 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 |
| INR | International normalized ratio |
| IRR | Infusion-related reaction |
| IV | Intravenous(ly) |
| LNP | Lipid nanoparticles |
| mBMI | Modified body mass index |
| MedDRA® | Medical Dictionary for Regulatory Activities |
| mNIS | Modified Neuropathy Impairment Score |
| mRNA | Messenger ribonucleic acid |
| NCS | Nerve conduction studies |
| NHP | Non-human primate (cynomolgus monkey) |
| NOAEL | No observed adverse effect limit |
| NSAID | Nonsteroidal anti-inflammatory |
| NT-proBNP | N-terminal prohormone of B-type natriuretic peptide |
| OTC | Over-the-counter |
| 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) |
| 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 | Rasch-built Overall Disability Scale |
| SAE | Serious adverse event |
| SGNFD | Sweat gland nerve fiber density |
| siRNA | Small interfering ribonucleic acid |
| SUSAR | Suspected unexpected serious adverse reaction |
| $t_{1/2}$ | Terminal elimination half-life |
| $t_{1/2\alpha}$ | Alpha half-life |
| $t_{1/2\beta}$ | Beta half-life |
| T3 | Triiodothyronine |
| T4 | Thyroxine |
| TBG | Thyroxine-binding globulin |
| TEAE | Treatment-emergent adverse event |
| $t_{max}$ | Time of observed maximum plasma concentration |
| 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 |
| $V_{ss}$ | Volume of distribution at steady state |
| $V_z$ | Volume of distribution based on the terminal phase |
| 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 Transthyretin Biology and Impact of Transthyretin Lowering

Transthyretin (TTR), also known as prealbumin, is a tetramer protein produced predominantly by hepatocytes (>95% of TTR is liver-derived), with a small fraction produced in the choroid plexus and retina.[1] The primary physiological role of TTR is to serve as a carrier of retinol (also known as vitamin A); it also plays a minor role as a carrier for thyroxine (T4).

In humans, approximately 15% of T4 circulating in the plasma is bound to TTR; the remainder is predominantly bound to thyroxine-binding globulin (TBG). In the mouse, where TTR plays a greater role as a carrier for T4, the absence of TTR reduces circulating levels of T4 but does not adversely affect the concentration of the hormone in peripheral tissues.[2,3]

Vitamin A circulates through the plasma primarily bound to retinol binding protein (RBP).[1] The clearance of RBP from the circulation is greatly reduced through its binding to TTR. Because vitamin A is lipid-soluble, it can diffuse across the membranes of cells and thus, most tissues receive adequate levels of vitamin A obtained normally from the diet without its being bound to RBP. Studies performed in TTR-knockout mice have demonstrated that in the absence of TTR, circulating levels of both RBP and vitamin A are dramatically reduced (e.g., 5% of vitamin A seen in wild type [WT] mice); however, the concentration of vitamin A in the tissues is comparable with that seen in WT mice.[4,5] In addition, the TTR-knockout mice did not demonstrate any signs of vitamin A deficiency. In humans, individuals with mutations in the RBP gene leading to complete loss of circulating RBP and very low concentrations of circulating vitamin A have not shown any significant signs of vitamin A deficiency other than modest retinal dystrophy and decrease in night vision.[6] This confirmed the finding in mice that vitamin A uptake by most tissues continues in the absence of RBP. In women with breast cancer treated for an average of at least 30 months with the retinoid fenretinide, which causes a 75% reduction in circulating vitamin A levels, there were no reports of night blindness or any other signs of vitamin A deficiency, and only subtle changes in retinal function were seen on electroretinograms in women older than 50 years of age who were on fenretinide for 30 months or longer.[7,8] The safety of lowering vitamin A through TTR suppression has now been further confirmed by the absence of any vitamin A deficiency-related AEs in non-human primates treated with ALN-TTR02 at doses up to 2 mg/kg every 3 weeks for 9 months, and in both healthy volunteers and familial amyloidotic polyneuropathy (FAP) patients treated with ALN-TTR02 who experienced substantial lowering of both TTR and vitamin A (see Section 1.3).

### 1.1.2 Disease Overview

Mutations in the TTR gene can lead to destabilization of the tetrameric protein and disassociation of the TTR subunits into dimers and individual monomers. These misfolded TTR monomers (both mutant and WT) can then self-assemble into amyloid fibrils.[9] The amyloid fibrils are deposited into the extracellular space of various tissues where they form amyloid plaques, with the peripheral nervous system, gastrointestinal tract, and heart being major sites of deposition.

There are over 100 reported TTR genetic mutations. These mutations are phenotypically expressed as a spectrum of disease which is collectively referred to as TTR-mediated amyloidosis (ATTR).[10] There is a range of clinical manifestations of ATTR; the most common manifestations include some form of cardiac and/or neurologic involvement (e.g., cardiomyopathy, autonomic neuropathy, and sensory and motor neuropathy) that depends, in part, upon the particular TTR mutation and the site of amyloid deposition. Transthyretin amyloidosis is associated with severe morbidity and mortality, with a life expectancy limited to approximately 5 to 15 years from symptom onset.[11]

Two significant clinical syndromes of ATTR have been described: FAP and familial amyloidotic cardiomyopathy (FAC), both of which are characterized by amyloid deposits of both mutant and WT TTR.[12]

Familial amyloidotic polyneuropathy, caused predominantly by the V30M mutation, occurs primarily in families with heritage from Portugal, Sweden, and Japan, has an earlier onset (age 30-50 years), and is characterized initially by peripheral neuropathy leading to sensory and motor deficits, as well as profound autonomic dysfunction that produces disabling gastrointestinal pathology, orthostatic hypotension, and bladder dysfunction.[13,14] Amyloid infiltration of the sinus node and atrioventricular conduction system in the heart is also common in FAP. Sudden death is not uncommon in FAP, and is believed to result from heart block or tachyarrhythmias.[15,16]

Familial amyloidotic cardiomyopathy, caused primarily by the TTR variant V122I, is a late-onset (age >60 years) syndrome in which amyloid deposition is largely restricted to the heart and manifests as conduction defects, arrhythmias, congestive heart failure, and death; neuropathy is uncommon.[17] The V122I mutation is found in up to 4% of African Americans and in over 5% of West African populations, although rates of disease penetrance are low.[18]

It is estimated that 45,000 – 50,000 individuals have FAP or FAC. In both FAP and FAC, quality of life is severely impacted following the onset of symptoms, and the disease proceeds inexorably to death.[19,20]

Because the liver is the primary source of mutant TTR, liver transplantation has been used over the past 20 years in an attempt to treat ATTR.[1] However, the procedure is only effective in halting or slowing the progression of disease in patients with an early age of onset,[21] especially for those with the V30M mutation and short disease duration prior to transplant; consequently almost two-thirds of ATTR patients are not transplant-eligible. When performed early in the course of the disease, liver transplantation can stabilize and slow progression of neuropathy in patients with FAP due to V30M. However, in FAC patients and FAP patients with evidence of cardiac involvement, liver transplantation is contraindicated since it does not halt the progression of cardiac disease in these patients,[22,23,24,25] and may actually accelerate the course of cardiomyopathy due to further deposition of WT TTR (originating from the transplanted liver) in the heart.[26]

Tafamidis, a TTR tetramer stabilizer, was approved in November 2011 in the European Union (EU) for the treatment of ATTR in adult patients with stage 1 symptomatic polyneuropathy to delay peripheral neurologic impairment.[27] Diflunisal, another TTR tetramer stabilizer, is currently being evaluated in a Phase 3 trial in FAP. However, the large majority of ATTR patients do not qualify for either liver transplantation or tafamidis. In these patients, the disease is primarily managed with palliative care.

### 1.1.3 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).[28] 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.[29] 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.

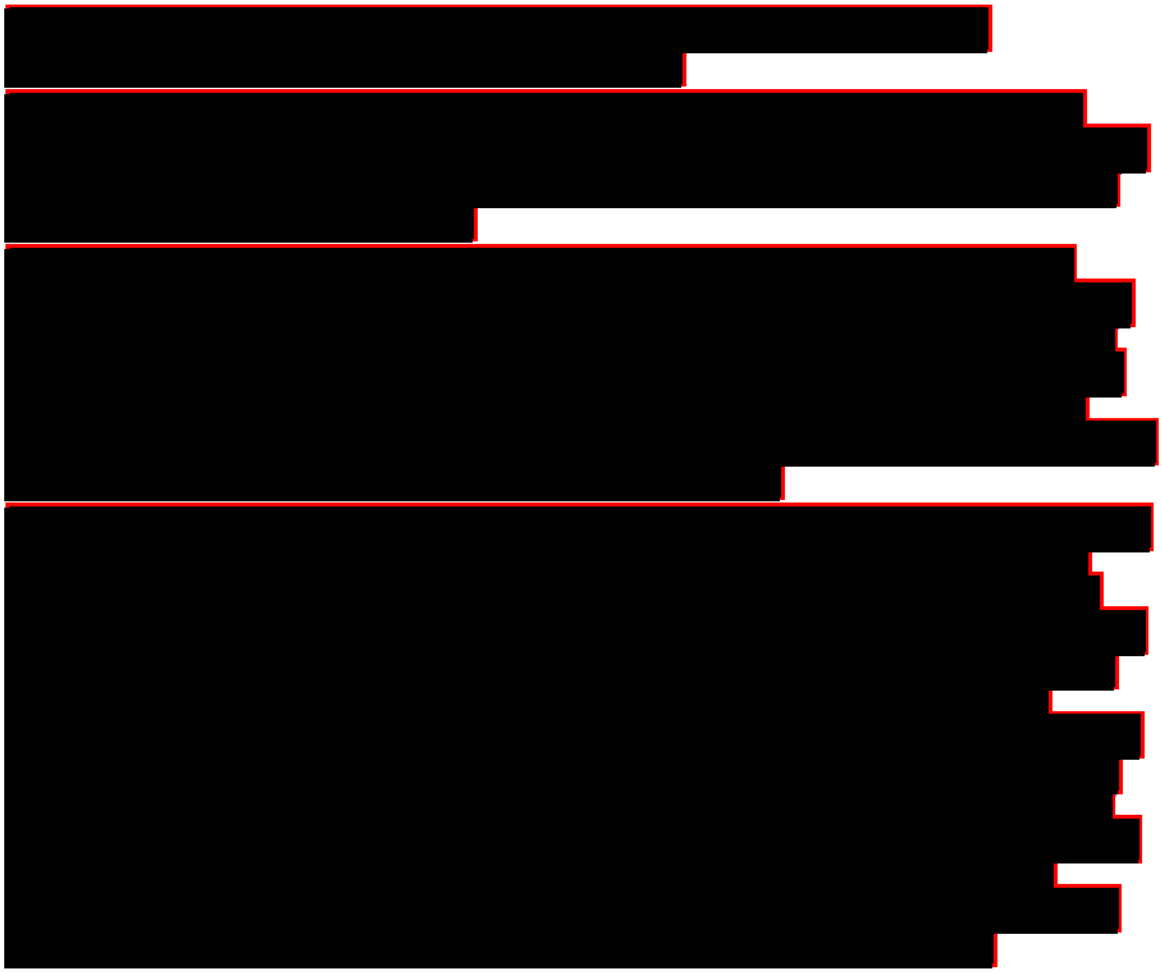
Unformulated siRNAs, and those without chemical modification, are rapidly degraded and eliminated upon systemic administration, and thus do not achieve significant tissue distribution.[30] As a result, various formulations are used to target siRNA distribution to tissues, and to facilitate their uptake into the relevant cell type. One approach that has been used successfully *in vivo*, including in rodents, non-human primates (NHPs), and humans, employs intravenous (IV) delivery of siRNAs in lipid nanoparticles (LNPs).[31,32,33,34] 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 to cause targeted degradation of the mRNA, and a consequent reduction in target protein levels. [35,36]

### 1.1.4 ALN-TTR02

Alnylam Pharmaceuticals is developing ALN-TTR02 Solution for Injection (hereafter referred to as ALN-TTR02), a synthetic investigational RNAi therapeutic comprising an siRNA designed to suppress production of both mutant and WT TTR, formulated in a second generation LNP termed AF-011. The LNP enables delivery of the siRNA primarily to the liver upon systemic administration, resulting in the down-regulation of hepatic TTR expression, and in turn, reducing serum mutant and WT TTR levels. ALN-TTR02 is intended for administration as an IV infusion over 70 minutes.

## 1.2 Summary of ALN-TTR02 Non-Clinical Data

[redacted]



Further information can be found in the ALN-TTR02 Investigator's Brochure (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 across doses of 10 to 500 µg/kg. Patients were premedicated with dexamethasone, H1 and H2 blockers, and paracetamol 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 21-28, with return 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) is currently enrolling patients to determine the safety and tolerability, pharmacokinetic (PK), and pharmacodynamic (PD) of a 60-70 minute IV infusion of ALN-TTR02 administered once every 3 -4 weeks for 2 doses. There have been no discontinuations or dose-limiting toxicities, and dosing is currently ongoing with the 0.3 mg/kg ALN-TTR02 dose. The preliminary PD effects on TTR are consistent with the data observed in the Phase 1 ALN-TTR02-001 study. It is anticipated that the

ALN-TTR02 dose and regimen for long-term dosing will be determined based on Study ALN-TTR02-002.

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

### 1.4 Study Hypothesis and Rationale

ALN-TTR02 is a novel drug to treat ATTR and consists of a single LNP-formulated TTR siRNA targeting both WT and all known mutant forms of TTR. Since TTR amyloid deposits consist of both mutant and WT TTR, it is desirable to be able to lower the production of both WT and mutant TTR with a single drug in order to treat the different variants of ATTR. While liver transplantation has shown both the advantages as well as the limitations of eliminating mutant TTR in ATTR, it also underscores the potential advantage of a drug that can lower *both* mutant and WT TTR for treating both the neuropathic and cardiac complications of ATTR.

The ALN-TTR02 nonclinical pharmacology data described in the IB demonstrate that administration of ALN-TTR02 results in significant sustained suppression of TTR. It is anticipated that lowering of mutant and WT TTR protein in humans will result in a decrease in the deposition of amyloid fibrils, thereby potentially slowing down or reversing the course of the disease.

The primary objective of this study is to evaluate the safety and tolerability of long term ALN-TTR02 dosing administered to patients with FAP. Secondary objectives include assessment of the PD effect of ALN-TTR02 on serum TTR, and preliminary evaluation of the clinical activity of ALN-TTR02 including neurologic impairment, motor functioning impacting activities of daily living, nutritional status, quality of life, and disability. Tertiary objectives include the characterization of plasma and urine PK for ALN-TTR02, and other measures of clinical activity including pathologic changes in sensory and autonomic innervation, neurologic impairment, autonomic function, ambulation, autonomic neuropathy symptoms, and cardiac structure and function (in patients with pre-existing cardiac amyloid involvement).

### 1.5 Dose Selection and Rationale

The optimal dose and schedule for ALN-TTR02 is one that will obtain the greatest level of sustained TTR suppression with a favorable safety profile. The experience from other systemic amyloidotic disorders,[37,38,39,40] 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 course of the disease, with increases in suppression beyond 50% providing further incremental improvements in outcomes. Based on the data to date from the nonclinical and clinical studies with ALN-TTR02, >80% suppression of circulating TTR is achieved consistently at the 0.3 mg/kg dose, and is anticipated to be the dose for the Phase 3 trial. Dosing once every 3 or 4 weeks is currently being explored in Study ALN-TTR02-002; the schedule that is well tolerated and provides optimal PD effect will be employed in this study.

### 1.6 Risk-Benefit Assessment

Further details about potential benefits and risks can be found in the IB.

### 1.6.1 Potential Benefit

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,[41,42,43,44] 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.[45]

Data as 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. Neuropathy impairment scores were stable through 12 months with mean change in mNIS+7 and NIS of -2.5 and +0.4 points, respectively.

### 1.6.2 Potential Risks

#### 1.6.2.1 Infusion-Related Reactions

Infusion-related reactions can occur with systemic administration of certain biological agents such as liposomes or monoclonal antibodies. These may include acute reactions and/or delayed febrile reactions. Premedicating patients with corticosteroids and/or antihistamines, or slowing of the infusion rate, are approaches that have been taken to reduce the incidence and/or severity of infusion-related reactions (IRRs).[46,47,48] Consistent with this, a premedication regimen (dexamethasone or equivalent, paracetamol or equivalent, histamine H1/H2 receptor antagonist [H1/H2 blocker]) similar to that proposed for this study was used in the prior ALN-PCS02, ALN-VSP02, ALN-TTR01, and ALN-TTR02 clinical studies that employed siRNAs in LNP formulations (Section 1.3). In these studies, while IRRs have been observed, including with ALN-TTR02, they have been generally managed with temporarily stopping the infusion and resuming administration at a slower rate for the remainder of the dose; no subjects have discontinued dosing due to an IRR.

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. The premedication regimen is described in Section 5.5). The infusion rate of approximately 70 minutes may further reduce the potential for an acute IRR.

#### 1.6.2.2 Vitamin A Lowering

The suppression of serum levels of TTR and RBP are 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, as there are mechanisms independent of RBP by which vitamin A is transported to tissues and taken up by cells. 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. As the vitamin A content of the diet may vary between different countries and different individuals within a country, the recommended daily allowance of vitamin A will be provided to all patients on the study.

#### 1.6.2.3 Osteoporosis

Patients with FAP may be at risk for osteoporosis.[49] 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.

# 2 STUDY OBJECTIVES

## 2.1 Primary Objective

The primary objective of this study is to evaluate the safety of long-term (approximately 2 years) dosing with ALN-TTR02.

## 2.2 Secondary Objectives

Secondary objectives include:

- Assessing the PD effect of long-term dosing of ALN-TTR02 on serum TTR.
- Assessing changes from baseline in:
    - Neurologic impairment using the modified Neuropathy Impairment Score (mNIS) +7 composite score
    - Quality of life (EQ5D) and disability (Rasch-built Overall Disability Scale [R-ODS])
    - Motor function impacting activities of daily living, including a 10-meter walk test and test of grip strength
    - Nutritional status (modified body mass index [mBMI])

## 2.3 Tertiary Objectives

Tertiary objectives include:

- Further characterization of the plasma and urine PK of ALN-TTR02.
- Assessing changes in:
    - Secondary PD biomarkers (RBP and vitamin A)
    - Sensory and autonomic innervation (skin punch biopsies for intraepidermal nerve fiber density [IENFD] and sweat gland nerve fiber density [SGNFD])
    - NIS and vibration detection threshold (VDT)
    - Heart rate variability with deep breathing (HRdb)
    - Ambulation, using FAP stage and Polyneuropathy Disability (PND) score
    - Patient reported autonomic neuropathy symptoms using the Composite Autonomic Symptom Score (COMPASS 31) questionnaire
    - Healthcare utilization using a pharmacoeconomics questionnaire
    - Cardiac structure/function through echocardiograms and serum levels of troponin I and N-terminal prohormone of B-type natriuretic peptide (NT-proBNP) in patients with evidence of pre-existing cardiac amyloid involvement

# 3 STUDY PLAN

## 3.1 Overall Design

Protocol ALN-TTR02-003 is a multinational, multicenter, Phase 2, open-label, extension study designed to evaluate the safety and tolerability of long term ALN-TTR02 dosing administered to patients with FAP; additional information to be evaluated include PK, PD, and clinical activity.

Patients eligible for inclusion in this study had to have previously received and tolerated ALN-TTR02 in Study ALN-TTR02-002. In addition, they must have an adequate performance status (Karnofsky performance status of 60% or greater; see Appendix 1), and adequate hepatic and renal function.

Patients will be eligible for inclusion in the PK/PD subgroup if their last dose of ALN-TTR02 was administered at least 6 months prior to the first dose in this study. Patients who have pre-existing cardiac amyloid involvement will be eligible for inclusion in the Cardiac subgroup.

The duration of patient participation in this study is approximately 2 years and 4 months. Patients will be screened within 28 days prior to administration of study medication. In order to reduce the potential of an IRR, patients will receive the following premedications at least 60 minutes prior to the start of infusion of ALN-TTR02: IV dexamethasone (or equivalent), oral paracetamol (or equivalent), and IV H1 and H2 blockers. ALN-TTR02 will be administered as a 70-minute IV infusion once every 3 or 4 weeks (based on results from the ongoing ALN-TTR02-002 study where the safety and PD of these 2 dosing schedules is being evaluated). 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). Details on the study drug administration are provided in Section 5.6. Patients will remain at the clinic from 1-6 hours after the end of the study drug infusion. In addition to returning to the site for dosing of study medication, patients will also return for out-patient visits approximately every 6 months for safety, PK, PD, and clinical activity monitoring (see Section 6 for details).

## 3.2 Safety Assessments

Safety monitoring will include assessment of AEs, 12-lead ECGs, vital signs (blood pressure, pulse rate, oral body temperature, and respiratory rate), clinical laboratory safety tests (hematology, serum chemistry, thyroid function parameters, coagulation parameters, and urinalysis), and ophthalmology and physical examinations. Patients will be closely monitored for both acute and delayed IRRs (see Section 5.11 for clinical guidance). All IRRs will be recorded as AEs.

## 3.3 Pharmacodynamic Assessments

The PD associated with long-term dosing of ALN-TTR02 in patients with FAP will be evaluated by serial measurement of serum TTR. Secondary PD biomarkers to be assessed include RBP and vitamin A.

## 3.4 Pharmacokinetic Assessments

Pharmacokinetic analyses will be conducted using noncompartmental and/or compartmental evaluation. The PK parameters of siRNA, 1,2-dilinoleyloxy-N,N-dimethylpropylamine (DLin-MC3-DMA) and polyethylene glycol 3-N-[(ω-methoxy

poly(ethylene glycol)2000) carbamoyl]-1,2-dimyristyloxy-propylamine ($PEG_{2000}$-C-DMG) (lipid) in plasma obtained from the PK/PD subgroup will be evaluated with concentration-time profiles obtained at Day 0 and Months 8 and 24 with blood samples that will be collected pre- and postdose at specified time points up to receiving the next subsequent dose. Pharmacokinetic 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 on available siRNA and possibly DLin-MC3-DMA on samples obtained from the PK/PD subgroup including the sparse samples collected during the study period. Population PK modeling of siRNA and possibly DLin-MC3-DMA in plasma will be performed with 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 of samples from the PK/PD subgroup, including sparse 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 $PEG_{2000}$-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. Whenever possible 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.

Urine samples will be collected from the PK/PD subgroup subjects including sparse samples collected during the study period. Renal clearance ($CL_R$) will be determined for siRNA and 4-dimethylaminobutyric acid, metabolite of DLin-MC3-DMA, excreted in the urine. Urine samples and urine volume void will be obtained at specified time points.

### 3.5 Clinical Activity Assessments

The clinical activity of ALN-TTR02 will be evaluated through changes in polyneuropathy measures. Neurologic impairment will be assessed using the mNIS + 7 composite score. Quality of life and disability will be assessed through the EQ5D and R-ODS, respectively. The influence of changes in motor function impacting activities of daily living will be assessed using a 10-meter walk test and a grip strength test. Patient reported autonomic neuropathy symptoms will be assessed using COMPASS 31. The nutritional status of the patients will be monitored through mBMI.

Other measures of the clinical activity of ALN-TTR02 will be evaluated through IENFD, SGNFD, NIS, VDT, HRdb, FAP stage, and PND score.

### 3.6 Additional Assessments for the Pharmacokinetic/Pharmacodynamic Subgroup

Patients enrolled in the PK/PD subgroup will have additional samples taken for evaluation of PK and PD (TTR and secondary PD biomarkers [RBP and vitamin A]).

### 3.7 Additional Clinical Activity Assessments for the Cardiac Subgroup

Patients enrolled in the Cardiac subgroup will complete additional clinical activity assessments to evaluate cardiac structure/function. This will be achieved through echocardiograms and monitoring of NT-proBNP and troponin I.

### 3.8 Other Assessments

Disease burden and healthcare utilization will be assessed using a patient-reported pharmacoeconomics questionnaire. Blood will also be collected to evaluate anti-drug bodies and exploratory biomarkers.

# 4 PATIENT POPULATION

## 4.1 Eligibility of Patients

Up to 28 patients are expected to be enrolled. All centers have been selected on the basis of their extensive experience with treatment of patients with FAP, their participation in Study ALN-TTR02-002, and have the equipment necessary to treat patients in case of an emergency.

## 4.2 Inclusion Criteria

Each patient must meet all of the following criteria within the Screening/Baseline period to be enrolled in the study:

1. Patients have previously received and tolerated ALN-TTR02 in Study ALN-TTR02-002.
2. Karnofsky performance status of 60% or greater.
3. Absolute neutrophil count (ANC) ≥1500 cells/mm$^3$, platelet count ≥100,000 cells/mm$^3$, and hemoglobin ≥10 g/dL.
4. Adequate liver function, demonstrated by an aspartate transaminase (AST) and alanine transaminase (ALT) levels ≤2.5 × the upper limit of normal (ULN), total bilirubin level within normal limits, albumin >3 g/dL, international normalized ratio (INR) ≤1.2.
5. Adequate renal function, demonstrated by serum creatinine level ≤1.5 × ULN.
6. Women of child-bearing potential must have a negative pregnancy test, cannot be breast feeding, 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 medication. Highly effective methods of birth control are defined as: hormonal - oral, implantable, injectable, or transdermal contraceptives in conjunction with spermicide, condom, or diaphragm; mechanical - 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.
7. Males agree to use appropriate contraception throughout study participation and for 75 days after the last dose of study medication.
8. Patient is willing and able to comply with protocol-required visit schedule and visit requirements and provide written informed consent.

Additional inclusion criteria for patients participating in the PK/PD subgroup include:

9. At the time of enrollment, at least 6 months have elapsed since the patient's last dose of ALN-TTR02.

Additional inclusion criteria for patients participating in the Cardiac subgroup:

10. Have a left ventricular wall thickness of ≥13 mm on transthoracic echocardiogram.

11. Be normotensive or have hypertension that is well-controlled.
12. Absence of aortic valve disease.

### 4.3 Exclusion Criteria

Patients meeting any of the following criteria within the Screening/Baseline period (Day -28 to Day 0) will be excluded from the study:

1. Patient is pregnant or nursing.
2. Patient has had a liver transplant.
3. Has a known or suspected systemic bacterial, viral, parasitic, or fungal infection.
4. Patient received an investigational agent, other than tafamidis or diflunisal, within 30 days prior to first dose study drug administration.
5. Patient has a New York Heart Association heart failure classification >2 (see Appendix 3).
6. Patient has unstable angina.
7. Patient has uncontrolled clinically significant cardiac arrhythmia.
8. Patient is considered unfit for the study by the Principal Investigator (PI).

### 4.4 Blinding Procedure

This is an open-label, single-arm study; all patients will receive 0.3 mg/kg ALN-TTR02. No blinding procedures will be needed.

### 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. A patient will be considered to have completed the study if the patient completes protocol-specified procedures up through the 21-day Follow-up visit for patients continuing into the global extension study, and up through the 56-day Follow-up visit for Year 2 for patients not continuing into the global extension study. For patients who withdraw from the study early, every effort should be made to complete the Early Termination (ET) 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)
- Develops conditions listed in the exclusion criteria during the course of the study
- Becomes pregnant
- Requires a prohibited medication (see Section 5.10)
- Requests to be withdrawn from the study
- Is found to be considerably non-compliant with the protocol required ALN-TTR02 dosing visits

A patient may 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 medication will not necessarily result in the patient being withdrawn from the study. However, if a patient misses 3 consecutive doses of ALN-TTR02, the Investigator at the site and the Medical Monitor will determine whether the patient will be withdrawn from the study. If the patient is withdrawn from the study, the reason will be noted on the case report form (CRF).

### 4.5.2 Handling of Withdrawals

In the event a patient is withdrawn from the study, the contract research organization (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.

Patients who voluntarily withdraw are termed dropouts. Dropouts will not be replaced.

If a patient is withdrawn/withdraws every effort should be made to conduct the ET 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. The site will follow-up by telephone at least twice and send a registered letter to any patient who fails to return for the final evaluation.

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.

# 5 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 LNPs. The ALN-TTR02 drug product is a sterile formulation of TTR siRNA with lipid excipients (DLin-MC3-DMA, DSPC, cholesterol, and $PEG_{2000}$-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.

## 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 will prepare the study drug under aseptic conditions. The amount (in mg) of ALN-TTR02 to be administered will be determined based on the patient's weight (kg). For each dose administered, the weight obtained during the previous dosing day should 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 pre-dose 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.

A total of 200 mL will be infused into patients. 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.

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

## 5.3 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 medication will be stored upright and refrigerated at approximately 5 ± 3°C. Any deviation from the recommended storage conditions should 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. Alnylam will be permitted, upon request, to audit the supplies, storage, dispensing procedures, and records.

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

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

## 5.4 Labeling and Packaging of Study Drug

All packaging and labeling as well as the preparation of ALN-TTR02 IV 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.

### 5.5 Premedication Plan

Prior to ALN-TTR02, 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 ALN-TTR02;
- Oral paracetamol (500 mg) or equivalent at least 60 minutes prior to the start of the infusion of ALN-TTR02;
- 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 ALN-TTR02; 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 ALN-TTR02. 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.

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 5.11.

Further details can be found in the Study Reference Manual.

### 5.6 Dose, Route, and Schedule of Study Drug Administration

Patients meeting the eligibility criteria will receive 0.3 mg/kg ALN-TTR02 once every 3 or 4 weeks, based on results from the ongoing ALN-TTR02-002 study where the safety and PD of 2 dosing schedules are being evaluated.

Study drug will be administered under the supervision of the Investigator or designee, as a 70-minute IV infusion (or at a more prolonged infusion rate if required due to prior IRR). ALN-TTR02 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 containing polyvinyl chloride, di(2-ethylhexyl)phthalate (PVC, DEHP) must NOT be used.

- 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 patisiran administration will not be resumed until the case is discussed with the Medical Monitor (see Section 5.11).
- If the infusion time for a patient was already extended on the parent study due to an IRR, that modified infusion duration may be continued on this 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 patisiran 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.

Additional details can be found in the Pharmacy Manual.

In addition, patients will receive an oral daily supplemental dose of the recommended daily allowance of vitamin A that should be taken after blood draws on study visit days.

### 5.7 Criteria for Dose Modification or Discontinuation of Study Drug

If a study drug related AE occurs in a patient that the Investigator judges as presenting a potential risk to the patient for further dosing, the Medical Monitors and Investigator will review all available safety data for that patient and for all other patients enrolled in the study. Based on this review it may be decided to discontinue further administration of ALN-TTR02, or, if the Investigator concurs, the patient may resume ALN-TTR02 as before or any of the following modifications may be instituted for that patient: reduce the dose of ALN-TTR02 to 0.15 mg/kg, modify the premedication regimen, and/or increase the number of weeks between dosing.

## 5.8 Measurement of Patient Compliance

Patient compliance with study drug administration is dependent on the proper preparation and administration of IV infusions by study site personnel. Treatment compliance will be verified by study staff observation.

Patients will be permitted to miss an occasional dose of study medication. However, if a patient misses 3 consecutive doses of ALN-TTR02, the PI, in consultation with the Medical Monitor, will determine whether the patient will be able to continue on the study.

## 5.9 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 an Alnylam Monitor or designee. 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 Pharmacy Manual.

## 5.10 Concomitant Medication / Treatment

Use of the following medications/treatments is prohibited during study participation:

- Any investigational agent other than ALN-TTR02.
- Concurrent tafamidis or diflunisal (permitted only if started prior to study entry).
- 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.

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

Use of all concomitant medications during Screening/Baseline, pre-dose, and post-dose up to end of participation in the study will be recorded on the patient's CRF. This will include all prescription drugs, herbal preparations, over-the-counter (OTC) medications, vitamins, and minerals. Any changes in medications during the study will also be recorded on the CRF.

Any concomitant medication 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 are recorded on the CRF, and coded using an internationally recognized and accepted coding dictionary.

### 5.11 Suggested Guidelines for Management of Infusion Reactions

In the event of an acute infusion reaction, the infusion of study drug will be stopped and the patient closely monitored until resolution of the reaction.

- In the event of an IRR, the infusion of patisiran 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 patisiran 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 patisiran 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 patisiran. 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.
- Patisiran 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.

After dosing, patients will remain at the study site for at least 1 hour for observation and PK assessments. Subjects in the PK/PD subgroup will remain at the study site for 6 hours for additional PK assessments following the first dose and the doses given at 8 and 24 months. Patients will be provided directions on self-administration of medications as needed to ameliorate reactions experienced outside of the study site to study drug infusion, such as fever, chills, and myalgia and to contact the Investigator if they experience any of these symptoms. Medications may include, but are not limited to: antipyretics, antihistamines, and oral corticosteroids. All concomitant medications, including self-administered medications, are to be listed on the CRF.

Patients will be instructed to contact the Investigator should they experience any reactions after leaving the study site.

# 6 STUDY VISITS

The duration of a patient's participation in this study is approximately 2 years and 4 months.

Screening/Baseline evaluations are to be performed within 28 days before receiving the first dose of study drug, as indicated in Table 1-1 and (Appendix 2 provides the schedule of assessments for patients administered ALN-TTR02 once every 4 weeks). Patients determined to be eligible based on Screening/Baseline assessments will receive treatment (IV infusion of study drug) once every 3 or 4 weeks; based on results from the ongoing ALN-TTR02-002 study where the safety and PD of 2 dosing schedules are being evaluated. Patients will remain at the study site for at least 1 hour following completion of dosing for observation and for PK sampling. Subjects in the PK/PD subgroup will remain at the study site for 6 hours following completion of dosing following the first dose and the doses given at 8 and 24 months for additional PK sampling. Patients will return to the study site for clinical activity assessments approximately once every 6 months.

All patients who discontinue the study before the 56-day Follow-up visit (or before the 21-day Follow-up visit for those patients continuing into the global extension study) will return to the study site for their Early Termination visit assessments.

Visiting nurses may conduct interim PK/PD visits.

## 6.1 Screening/Baseline Visit (Day -28 to 0)

Screening/Baseline evaluations will be conducted up to 28 days prior to administration of the first dose of study drug (Day 0). Table 1-1 provides an overview of the schedule of events required for Screening/Baseline.

Prior to Screening/Baseline 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.

The following activities should be performed during the Screening/Baseline visit as part of the initial screening:

- Obtain demographic information
- Obtain medical history information
- Assess study eligibility using the inclusion and exclusion criteria
- Obtain concomitant medications information
- Perform a physical examination, including weight
- Measure height
- Measure vital signs
- Perform an echocardiogram
- Perform a 12-lead ECG
- Collect blood samples for clinical laboratory tests, including:

  - Hematology
  - Serum chemistries
  - Coagulation studies
  - Thyroid function tests
- Collect blood sample for serum TTR protein, RBP, and vitamin A. Additionally, aliquots of serum samples will be taken and frozen, to permit testing of additional proteins related to FAP.
- Collect blood sample for exploratory biomarkers and ADA
- Collect urine sample for urinalysis
- Perform a urine pregnancy test (for females of child-bearing potential only)
- Once the patient is deemed eligible to participate in the study, the following clinical activity assessments should be performed:
  - mNIS + 7*
  - VDT*
  - Grip strength*
  - Ten-meter walk test*
  - mBMI
  - NIS*
  - HRdb*
  - COMPASS 31
  - EQ5D
  - R-ODS
  - Pharmacoeconomics questionnaire
  - PND score
  - FAP stage
  - Collect paired 3-mm skin punch biopsies for IENFD and SGNFD analysis

  *Must be performed within 14 days prior to the first dose of study drug.
- Perform an ophthalmology examination.

Note: Any of the above assessments that are performed during the ALN-TTR02-002 study during the Screening/Baseline timeframe for ALN-TTR02-003 will not need to be repeated at the Screening/Baseline visit.

## 6.2 Treatment Visits

### 6.2.1 Routine Study Visits

The procedures described below are to be performed for all study visits, with the exception of the Mid-Year and Annual visits occurring approximately once every 6 months during which patients undergo assessment of clinical activity parameters.

Patients who are determined to be eligible for the study, based on Screening/Baseline evaluations, will be enrolled on Day 0.

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

#### 6.2.1.1 Pre-dosing

Patients will undergo the following procedures before study drug administration:

- Obtain measurements of:
  - Weight
  - Vital signs
- Collect blood samples for serum chemistries at first dose, and during the 3, 6, 9, 12, 15, 18, 21, and 24 month dosing days:
- Collect blood samples for hematology, coagulation, and thyroid function tests at first dose and during the 6, 12, 18, and 24 month dosing days.
- Collect urine sample for urinalysis at first dose and during the 3, 6, 9, 12, 15, 18, 21 and 24 month dosing days.
- Collect blood sample for PK pre-dose (within 1 hour of planned dosing start; -5 minutes) for the first dose and every dose up through, and including, the 3 month dosing visit, and then on 6, 9, 12, 15, 18, 21, and 24 month dosing days.
- Collect urine sample for PK pre-dose (within 1 hour of planned dosing start; -5 minutes) for the first dose and every dose up through, and including, the 3 month dosing visit, and then on 6, 9, and 12 month dosing days.
- Obtain concomitant medications information.
- Collect blood samples for TTR and secondary PD biomarkers immediately (10 minutes) prior to and after administration of premedication, but before dosing of ALN-TTR02 at first dose on Day 0. A single sample will be collected during the 3, 6, 9, and 12 month dosing days prior to administration of premedication. After the 12 month dosing day, a single blood sample for TTR will be collected prior to administration of premedication for each dosing day, except for the mid-year and annual clinical visits (TTR collection for these visits is described in Section 6.2.2).
- Collect blood samples for exploratory biomarkers pre-dose at first dose, and during the 3, 6, 9, 12, 15, 18, 21, and 24 month dosing days.
- Collect blood samples for ADA at first dose, second dose, and at the 3, 6, 12, and 24 month dosing days.
- Administer premedications (see Section 5.5)

#### 6.2.1.2 Administration of Study Drug

After completion of all pre-dose evaluations and procedures, ALN-TTR02 will be administered as a 70-minute IV infusion (or at a more prolonged infusion rate if required due to prior IRR) by a controlled infusion device. The infusion site will continue to be assessed for signs of any localized reaction through 30 minutes postdose.

The infusion time may be extended up to 3 hours in the event of a mild or moderate infusion reaction (study drug administration will not be resumed for any patient following a severe infusion reaction). Suggested guidelines for treatment of infusion reactions are provided in Section 5.11. In addition, if the infusion is stopped and the site is considering restarting the infusion, a PK blood sample will be taken when the infusion was stopped. All remaining PK samples will be collected according to the schedule of assessments when the dose is completed.

#### 6.2.1.3 Post-dose

Patients will remain at the study site for at least 1 hour following completion of dosing for the following assessments:

- Collect blood samples for PK analyses at EOI and 1 hour post-dose for the first dose and every dose up through, and including, the 3 month dosing visit, and then on 6, 9, and 12 month dosing days. Collect blood samples for PK analyses at EOI (+5 minutes) on 15, 18, 21, and 24 month dosing days. If the infusion is stopped and the site is considering restarting the infusion, a PK blood sample will be taken while the infusion is stopped.
- Collect urine for PK at 1 hour post-dose for the first dose and every dose up through, and including, the 3 month dosing visit, and then every 6, 9, 12, 15, 18, 21, and 24 month dosing days.
- Document any AEs, beginning with the initiation of the first infusion.
- Obtain concomitant medications information.

### 6.2.2 Mid-Year and Annual Clinical Activity Study Visits

The clinical activity of the patients will be assessed approximately every 6 months. Patients will not receive any study medication on these days. This visit will take place over 2 days.

- Perform a physical examination, including weight.
- Measure vital signs.
- Perform a 12-lead ECG.
- Collect blood samples for serum TTR protein and secondary PD biomarker analysis. Additionally, aliquots of serum samples will be taken and frozen, to permit testing of additional proteins related to FAP.
- Collect blood samples for PK analyses
- Collect urine samples for PK analyses
- Perform clinical activity assessments
    - mNIS + 7

- VDT
- Grip strength
- Ten-meter walk test
- mBMI
- NIS
- HRdb
- COMPASS 31
- EQ5D
- R-ODS
- Pharmacoeconomics questionnaire
- PND score
- FAP stage
- Collect paired 3-mm skin punch biopsies for IENFD analysis

  At each time point, 2 independent assessments for the mNIS+7, VDT, HRdb, NIS, 10-meter walk test, and grip strength test will be performed. Each site will make every effort to have these assessments performed by the same Investigator. The second (repeat) assessment must be performed at least 24 hours (approximately), but not more than 7 days after the first assessment. During the repeat assessments (on each testing day), blood will be collected for PK, TTR, and secondary PD biomarkers (RBP and vitamin A) analyses. A urine sample will also be collected for PK analyses.

- Perform an ophthalmology examination.
- Obtain concomitant medications information.
- Document any AEs.

### 6.2.3 Additional Assessments for Pharmacokinetic/Pharmacodynamic Subgroup

- For patients who are eligible for inclusion in the PK/PD subgroup, additional blood samples will be collected for the quantification of TTR, secondary PD biomarkers (RBP and vitamin A), and PK assessments.
- At the first dosing (Day 0) and then the 8, and 24-month dosing visits, in addition to the predose, EOI, and 1 hour post-infusion, collect blood samples for PK at 2, 4, and 6 hours post-infusion. Urine samples will also be collected for PK at EOI and 6 hours post-infusion.
- In addition, at these study visits, blood and urine samples for PK analysis of TTR, RBP, and vitamin A will also be collected at 24 hours post-infusion and 3, 7, and 17 days after dosing with ALN-TTR02.

### 6.2.4 Additional Assessments for Cardiac Subgroup

For patients who are eligible for inclusion in the Cardiac subgroup:

- Perform an echocardiogram at the Mid-year and Annual study visits.

- Collect blood sample for assessment of NT-proBNP and troponin I at first dose and approximately once every 3 months.

### 6.2.5 Follow-Up Visits

Patients will return to the study site for 2 follow-up visits (approximately 21 and 56 days after receiving their last dose of ALN-TTR02). If a patient enrolls in the global extension study, the patient will not have to complete the 56-day follow-up assessments. Patients who do not enroll in the extension study will need to complete both follow-up visits.

#### 6.2.5.1 Twenty-One-Day Follow-Up

The following procedures will be performed at the 21-day Follow-up visit.

- Perform a physical examination, including weight.
- Measure vital signs.
- Perform a 12-lead ECG.
- Collect blood samples for clinical laboratory tests, including:
    - Hematology
    - Serum chemistries
    - Coagulation studies
    - Thyroid function tests
- Collect blood samples for serum TTR protein and secondary PD biomarker analysis. Additionally, aliquots of serum samples will be taken and frozen, to permit testing of additional proteins related to FAP.
- Collect blood sample for PK analyses
- Collect urine sample for urinalysis.
- Obtain concomitant medications information.
- Document any AEs.

#### 6.2.5.2 Fifty-Six-Day Follow-Up Visit

The following procedures will be performed at the 56-day Follow-up visit.

- Collect blood samples for serum TTR protein and secondary PD biomarker analysis.
- Collect blood sample for PK analyses.

A patient will continue to be followed every 4 weeks after their 56 day Follow Up visit if their TTR protein levels continue to recover but have not returned to within 50% of Screening/Baseline visit levels. During these visits, blood samples will be collected for serum TTR protein and secondary PD biomarker analysis. Additionally, aliquots of serum samples will be taken and frozen, to permit testing of additional proteins related to FAP. Blood samples will also be collected for PK analysis.

## 6.3 Early Termination

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.

- Perform a physical examination, including weight.
- Measure vital signs.
- Perform a 12-lead ECG.
- Collect blood samples for clinical laboratory tests, including:
    - Hematology
    - Serum chemistries
    - Coagulation studies
    - Thyroid function tests
- Collect blood samples for serum TTR protein and secondary PD biomarker analysis. Additionally, aliquots of serum samples will be taken and frozen, to permit testing of additional proteins related to FAP.
- Collect blood sample for PK analyses
- Collect urine sample for PK analyses
- Collect urine sample for urinalysis.
- Perform clinical activity assessments once, only in patients who withdraw >3 months after the last clinical activity assessments were performed and who received at least 4 doses of study drug.
    - mNIS + 7
    - VDT
    - Grip strength
    - Ten-meter walk test
    - mBMI
    - NIS
    - HRdb
    - COMPASS 31 survey
    - Pharmacoeconomics questionnaire
    - EQ5D
    - R-ODS
    - PND score
    - FAP stage
    - Collect paired 3-mm skin punch biopsies for IENFD analysis
- Perform an ophthalmology examination.
- For patients enrolled in the Cardiac subgroup:
    - Perform an echocardiogram

- Collect blood sample for assessment of NT-proBNP and troponin I

- Obtain concomitant medications information.
- Document any AEs.

### 6.4 Unscheduled Visits

Unscheduled visits may occur if deemed clinically significant by the Investigator.

A patient will continue to be followed every 4 weeks (±3 days) after their 56-day Follow-up Visit if their TTR protein levels continue to recover but have not returned to within 50% of Screening/Baseline visit levels. The patient will have the blood samples collected for the following clinical laboratory tests at these visits:

- Serum TTR protein and secondary PD biomarkers. Additionally, aliquots of serum samples will be taken and frozen, to permit testing of additional proteins related to ATTR.
- PK analysis

These patients will be followed until their TTR level returns to within at least 50% of the Baseline value.

# 7 STUDY ASSESSMENTS

## 7.1 Demographic Data and Medical History

Patient demographic data and a complete medical history will be obtained during Screening/Baseline. The medical history will also look to include prior neuropathy assessments, including but not limited to, Neuropathy Impairment Scales and Nerve Conduction Studies.

Any AEs from the previous study (ALN-TTR02-002) that are ongoing on Day 0 of Study ALN-TTR02-003 will be considered as part of the medical history and recorded on the Medical History case report form for the current study (ALN-TTR02-003).

## 7.2 Safety Assessments

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

### 7.2.1 Physical Examination

Physical examinations will be performed by a physician and will include the examination of the following: general appearance, head, eyes, ears, nose, throat, chest/respiratory, heart/cardiovascular, gastrointestinal/liver, musculoskeletal/extremities, dermatological/skin, thyroid/neck, lymph nodes, and neurological/psychiatric.

Body weight will be measured at each study visit, with the exception of the 56-day Follow-up study visit, prior to dosing with ALN-TTR02. The weight measured during the previous dosing day should 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 pre-dose on the dosing day can be used for dose calculation.

Height will only be measured at Screening/Baseline.

### 7.2.2 Vital Signs

Vital signs will be measured at Screening/Baseline and pre-dose at each subsequent study visit with the exception of the 56-day Follow-up visit; including systolic/diastolic blood pressure, pulse rate, respiratory rate, and oral body temperature. Vital signs 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. 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.

### 7.2.3 Electrocardiogram

Computerized 12-lead ECG recordings will be obtained. Each lead shall be recorded for at least 3 beats at a speed of 25 mm/s. Triplicate recordings will be obtained during Screening/Baseline, approximately once every 6 months, at the 21-day Follow-up visit, and at the Early Termination visit.

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).

### 7.2.4 Ophthalmology Examination

Ophthalmology examinations will be performed at Screening/Baseline and approximately once every 6 months and at the Early Termination visit.

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

### 7.2.5 Clinical Laboratory Tests

Laboratory tests of hematology, serum chemistries, pregnancy (urine), coagulation studies, thyroid function tests, and urinalysis will be collected prior to the administration of premedications and dosing with ALN-TTR02 and will be performed by each study site's local laboratory.

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
- Hemoglobin
- Red blood cell (RBC) count
- White blood cell (WBC) count
- Mean corpuscular volume
- Mean corpuscular hemoglobin
- Mean corpuscular hemoglobin concentration
- Neutrophils, absolute and %
- Lymphocytes, absolute and %
- Monocytes, absolute and %
- Eosinophils, absolute and %
- Basophils, absolute and %
- Platelet count

**Serum Chemistries**

- Aspartate transaminase (AST)
- Alanine transaminase (ALT)
- Sodium
- Potassium
- Blood urea nitrogen (BUN)
- Creatinine
- Alkaline phosphatase
- Bilirubin (total and direct)
- Glucose
- Phosphate
- Albumin
- Calcium

**Coagulation Studies**

- Prothrombin time (PT)
- Activated partial thromboplastin time (aPTT)
- International Normalized Ratio (INR)

**Thyroid Function Tests**

- Thyroid stimulating hormone (TSH)
- Thyroxine (T4)
- Triiodothyronine (T3)

**Urinalysis**

- Visual inspection for color and appearance
- pH
- Specific gravity
- Ketones
- Protein
- Glucose
- Leukocytes
- Bilirubin
- Nitrite
- Urobilinogen
- Microscopic inspection of sediment

**Other**

- β-human chorionic gonadotropin (women of child-bearing potential only)

Additional and repeat testing may be performed at the discretion of the Investigator.

#### 7.2.5.1 Hematology, Coagulation Studies, and Thyroid Function Tests

Blood samples for hematology, coagulation studies, and thyroid function tests will be collected Screening/Baseline, first dose, approximately every 6 months, at the 21-day Follow-up visit, and at the Early Termination visit. These samples are to be processed at the site's local laboratory.

#### 7.2.5.2 Serum Chemistries and Urinalysis

Blood samples for serum chemistries and urine for urinalysis will be collected at Screening/Baseline, first dose, approximately every 3 months, at the 21-day Follow-up visit, and at the Early Termination visit. These samples are to be processed at the site's local laboratory.

#### 7.2.5.3 Pregnancy Test

A urine pregnancy test will be performed for women of child-bearing potential at Screening/Baseline, and any time pregnancy is suspected, the results of which must be known prior to administration of study drug. Patients who are pregnant are not eligible for study participation. Patients determined to be pregnant while on study will be followed until pregnancy outcome is known (see Section 8.12).

## 7.3 Pharmacodynamic Assessments

Details of how samples will be collected, processed, and stored will be in the Study Laboratory Manual.

### 7.3.1 Transthyretin

Blood for serum TTR levels is to be collected at Screening/Baseline, immediately prior (within 10 minutes) to the administration of premedication and immediately prior (within 10 minutes) to first dose, and then approximately once every 3 months (prior to administration of premedications) through the 12 month dosing day, thereafter at every dosing day after the 12 month dosing day, as well as at the 21- and 56-day Follow-up visits, and at the Early Termination visit. Select clinical assessments are to be performed on 2 separate days for each of the Mid-year and Annual study visits. For those visits, blood will be collected on each day that an assessment is performed.

In addition, for those patients enrolled in the PK/PD subgroup, blood for serum TTR assessment will be collected prior to the administration of premedications at Month 8, and 24 hours, 3, 7, and 17 days post first dose and 8 and 24 month dosing days.

Additionally, aliquots of serum samples drawn for measurement of TTR levels will be taken and frozen, to permit future testing of TTR and other types of testing related to FAP.

Serum TTR will be assessed using both ELISA and turbidimetric assays.

### 7.3.2 Secondary Pharmacodynamic Biomarkers

#### 7.3.2.1 Retinol Binding Protein

Blood for serum RBP levels is to be collected at Screening/Baseline, immediately prior (within 10 minutes) to the administration of premedication and immediately prior (within 10 minutes) to first dose, and then approximately once every 3 months (prior to administration of premedications), at the 21- and 56-day Follow-up visits, and at the Early Termination visit. Select clinical assessments are to be performed on 2 separate days for each of the Mid-year and Annual study visits. For those visits, blood will be collected on each day that an assessment is performed.

In addition, for those patients enrolled in the PK/PD subgroup, blood for RBP levels will be collected prior to the administration of premedications at Month 8, and 24 hours, 3, 7, and 17 days post first dose and 8 and 24 month dosing days.

Serum RBP will be quantified using nephelometry.

#### 7.3.2.2 Vitamin A

Blood for serum vitamin A levels is to be collected at Screening/Baseline, immediately prior (within 10 minutes) to the administration of premedication and immediately prior (within 10 minutes) to first dose, and then approximately once every 3 months (prior to administration of premedications), at the 21- and 56-day Follow-up visits, and at the Early Termination visit. Select clinical assessments are to be performed on 2 separate days for each of the Mid-year and Annual study visits. For those visits, blood will be collected on each day that an assessment is performed.

In addition, for those patients enrolled in the PK/PD subgroup, blood for vitamin A levels will be collected prior to the administration of premedications at Month 8, and 24 hours, 3, 7, and 17 days post first dose and 8 and 24 month dosing days.

### 7.4 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 selected time points (see Table 1-1).

Details of how samples will be collected, processed, and stored will be in the Study 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.

### 7.5 Pharmacokinetic Evaluations

Details of sample collection and processing for PK analyses will be included in a laboratory manual.

#### 7.5.1 Plasma Pharmacokinetics

The PK evaluation will include plasma-concentration time profiles for siRNA and the novel lipid components DLin-MC3-DMA and polyethylene glycol $(PEG)_{2000}$-C-DMG. Details of the PK evaluation and analysis will be described in the PK and PK/PD analysis plan.

Plasma and urine samples will be evaluated using a validated ATTO-Probe-HPLC assay to determine siRNA concentration and by LC/MS/MS for DLin-MC3-DMA and $PEG_{2000}$-C-DMG concentrations.

Blood sample collection times are included in the schedule of events (see Table 1-1 and Table 1-2 or Table 12-1 and Table 12-2). For all patients, plasma PK samples will be collected pre-dose (within 1 hour of planned dosing start) EOI and 1 hour post-dose at first dose and every dose up through, and including, the 3 month dosing visit, and then at 6, 9, and 12 month dosing days. Plasma PK samples will be collected pre-dose (within 1 hour of planned dosing start) and EOI at 15, 18, 21, and 24 month visits. The EOI will usually occur at 70 minutes after the start of the infusion, except in patients where the infusion has been prolonged. In addition, if the infusion is stopped and the site is considering restarting the infusion, a PK blood sample will be taken while the infusion is stopped. All PK samples will be collected according to the schedule of assessments when the dose is completed (i.e., all post-infusion times are relative to the EOI, regardless of the duration of the infusion). Plasma PK samples will also be collected at the Mid-year and Annual visits (1 sample collected on each of the 2 study visit days), the 21- and 56-day Follow-up visits, and the Early Termination visit.

For the PK/PD subgroup subjects, samples will be collected pre-dose, and at 1, 2, 4, 6, and 24 hours post-infusion; and at 3, 7 and 17 days post-dose for the first dose and the 8 and 24 month dosing days.

Pharmacokinetic analyses will consist of either a noncompartmental and/or compartmental analysis of plasma siRNA, DLin-MC3-DMA (lipid), and $PEG_{2000}$-C-

DMG (lipid). Details of the PK evaluation and analysis will be described in the PK and PK/PD analysis plan. Pharmacokinetic parameter estimates to be calculated may include, but may not be limited to:

- Observed $C_{max}$.
- Time of observed maximum concentration ($t_{max}$).
- Partial area under the plasma concentration-time curve ($AUC_p$).
- Area under the plasma concentration-time curve to the last measurable concentration ($AUC_{0-t}$).
- Area under the plasma concentration-time curve from zero to the last measurable time point ($AUC_{0-last}$).
- Area under the plasma concentration-time curve extrapolated to infinity ($AUC_{0-\infty}$).
- Terminal elimination half-life ($t_{1/2\beta}$ and $t_{1/2\alpha}$).
- Elimination rate constant ($\lambda_z$).
- Systemic clearance (CL).
- Volume of distribution at steady state ($V_{ss}$).
- Volume of distribution based on the terminal phase ($V_z$).

Other parameters may be calculated if deemed necessary.

A population PK analysis of the siRNA will be performed by integrating the PK/PD subgroup and sparse samples collected during the study. Details of the Population PK evaluation and analysis will be described in the PK and PK/PD analysis plan. A compartmental model will be developed to model the concentration-time data of plasma siRNA and possibly DLin-MC3-DMA. Model evaluation and selection will be based on standard model diagnostics and goodness-of-fit criteria (e.g., log-likelihood difference) and by looking at pertinent graphical representations of goodness-of-fit (e.g., fitted and observed concentrations versus time, weighted residuals vs. time) to provide a robust evaluation of the population PK parameters of the siRNA and possibly DLin-MC3-DMA. Other parameters may be calculated if deemed necessary. Post hoc PK parameters of siRNA and possibly DLin-MC3-DMA will be derived with the population PK models and summarized using descriptive statistics.

As part of PK/PD analysis, additional PD baseline analysis on serum TTR, RBP, and vitamin A may be conducted to include the PD data obtained during PD recovery after receiving the last dose. Details of the PD evaluation and analysis will be described in the PK and PK/PD analysis plan. A PD analysis to determine PD parameters (e.g., 50% effective dose [$ED_{50}$], 50% effective concentration [$EC_{50}$]) will be conducted. The extent of suppression of TTR, RBP, and vitamin A (e.g., AUE, % $E_{max}$ and $E_{max}$) will be evaluated using graphical display of data. Correlation between TTR versus RBP and vitamin A will also be performed (e.g., TTR concentrations, AUE, % $E_{max}$ and $E_{max}$ vs. RBP and vitamin A concentrations, AUE, % $E_{max}$ and $E_{max}$). Whenever possible the strength of the relationship correlation will be evaluated using statistical estimators

(e.g. $r^2$, slope, and p-value for a slope of 0). Other parameters may be calculated if deemed necessary.

The PK/PD analysis will include, but not be limited to, the determination of the relationship between exposure to siRNA, DLin-MC3-DMA and $PEG_{2000}$-C-DMG (e,g, $C_{max}$ and AUC) and the extent of suppression of TTR, RBP, and vitamin A (e.g., AUE, % $E_{max}$ and $E_{max}$) will be explored using graphical display of data. Whenever possible the strength of the relationship correlation will be evaluated using statistical estimators (e.g. $r^2$, slope, and p-value for a slope of 0). The PD and PK/PD parameters will include, but not limited to, descriptive statistics (e.g., mean, standard deviation, coefficient of variation, median, minimum, maximum, geometric mean, and geometric CV%) and also by statistical comparisons whenever possible. Other parameters may be calculated if deemed necessary. Details of the PK evaluation and analysis will be described in the PK and PK/PD analysis plan.

### 7.5.2 Urine Pharmacokinetics

The $CL_R$ of siRNA and 4-dimethylaminobutyric acid (metabolite of DLin-MC3-DMA) after dosing with ALN-TTR02 will be evaluated for the subjects in the PK/PD subgroups and whenever possible for other subjects in the study. Other parameters may be calculated if deemed necessary. Details of the urine PK evaluation and analysis will be described in the PK and PK/PD analysis plan. Urine sample collection times are included in the schedule of assessments (see Table 1-1 and Table 1-2 or Table 12-1 and Table 12-2).

Urine will be collected into suitable containers and kept refrigerated.

## 7.6 Clinical Activity Assessments

Assessment of clinical activity will occur approximately every 6 months. Additional and repeat testing may be performed at the discretion of the Investigator and in conjunction with the Sponsor.

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

### 7.6.1 Modified Neurological Impairment Score (mNIS) + 7

The mNIS + 7 assessment includes the modified NIS tool (weakness and reflexes only), nerve conduction studies (NCS), quantitative sensory testing (QST) by body surface area including touch pressure (TP) and heat pain (HP), and postural blood pressure.

At each time point, 2 independent assessments will be performed. 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 not more than 7 days apart. In order to limit potential intra-operator bias, results of the first assessment will not be available to the Investigator when performing the second assessment.

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

### 7.6.2 Vibration Detection Threshold

Large nerve fiber function will be further evaluated by VDT using the Computer Aided Sensory Evaluator, version IV. Tests are automated and will last approximately 3 minutes.

At each time point, 2 independent assessments will be performed. 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 not more than 7 days apart. In order to limit potential intra-operator bias, results of the first assessment will not be available to the Investigator when performing the second assessment.

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

### 7.6.3 Heart Rate Response to Deep Breathing

The HRdb test evaluates autonomic function by the cardio-vagal response. The average heart rate difference while taking eight deep breaths is measured.

At each time point, 2 independent assessments will be performed. 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 not more than 7 days apart. In order to limit potential intra-operator bias, results of the first assessment will not be available to the Investigator when performing the second assessment.

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

### 7.6.4 Hand Grip Strength

Hand grip strength will be measured by dynamometer. 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 at each time point.

At each time point, 2 independent assessments (each assessment performed in triplicate) will be performed. Assessments are to be performed at least 24 hours (approximately) apart from each other but not more than 7 days apart.

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

### 7.6.5 Ten-meter Walk Test

Patients will have to be able to walk without assistance for 10 meters in order to complete the 10-meter walk test. The time required for the patient to complete 2, 8, and 10 meters will be recorded. This will allow for any potential variation due to acceleration and deceleration. The patient will complete 1 trial.

At each time point, 2 independent assessments will be performed. Assessments are to be performed at least 24 hours (approximately) apart from each other but not more than 7 days apart.

### 7.6.6 Neurological Impairment Score

Clinical activity will be assessed by a clinical assessment that tests weakness, reflexes, sensation and cranial nerves.

At each time point, 2 independent assessments will be performed. 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 not more than 7 days apart. In order to limit potential intra-operator bias, results of the first assessment will not be available to the Investigator when performing the second assessment.

### 7.6.7 Composite Autonomic Symptom Score

To evaluate changes in autonomic symptoms, patients will complete the COMPASS 31 questionnaire approximately once every 6 months. The COMPASS 31 questionnaire consists of 31 clinically selected questions. The questions evaluate 6 autonomic domains (orthostatic intolerance, vasomotor, secretomotor, gastrointestinal, bladder, and pupillomotor).[50]

### 7.6.8 Quality of Life Questionnaire

Quality of life will be assessed through the use of the EQ5D-5L, a standardized 5 question instrument for use as a measure of health outcomes.

### 7.6.9 Rasch-built Overall Disability Scale

An assessment of the disability each patient experiences will be assessed through the R-ODS[51]. The R-ODS is comprised of a 24-item linearly weighted scale that specifically captures activity and social participation limitations in patients.

### 7.6.10 Skin Punch Biopsies for Exploratory Intraepidermal Nerve Fiber Density and Sweat Gland Nerve Fiber Density

Patients will have 2 tandem 3-mm skin punch biopsies for IENFD and SGNFD assessment obtained at Screening/Baseline and approximately once every 6 months throughout the study.

One biopsy will be taken from the distal lower leg, when a patient's clinical status allows, and one from the distal thigh at each time point.

Details of how samples will be collected, processed, and stored will be in the Study Laboratory Manual.

### 7.6.11 FAP Stage and PND Score

Changes in disease stage will be evaluated through FAP Stage and PND Score.[52,53]

### 7.6.12 Modified Body Mass Index

The nutritional status of patients will be evaluated using the mBMI; calculated as BMI × albumin.

### 7.6.13 Disease Burden and Healthcare Utilization

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

### 7.6.14 Echocardiogram

An echocardiogram will be performed in all patients at Screening/Baseline, to assess any cardiac abnormalities. In addition, for those patients enrolled in the Cardiac subgroup, an echocardiogram will be performed during approximately once every 6 months.

### 7.6.15 Biomarkers of Cardiac Function

For those patients in the Cardiac subgroup, blood samples will be collected for the quantification of biomarkers of cardiac function (troponin I and NT-proBNP) prior to their first dose of study drug and approximately once every 3 months. Quantification of these biomarkers will be performed at a central laboratory.

Details of how samples will be collected, processed, and stored will be in the Study Laboratory Manual.

# 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.

## 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 the 21 or 56-day Follow-up visit (depending on whether or not the patient plans to enroll into the open-label global extension study).

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 for the reporting periods defined earlier 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

As stated in Section 8.4, AEs will be assessed for 21 or 56 days following the last dose of study drug (depending on whether or not the patient plans to roll over to the open-label global extension study) or until recovery to the normal state has been achieved, whichever occurs first; all AEs that occur after the start of study drug administration on Day 0 (Baseline) must be reported in detail on the appropriate CRF page and followed to satisfactory resolution, or a period of 21 or 56 days after the last dose of study drug administration (depending on whether or not the patient plans to roll over to the open-label global extension study), whichever is shorter.

Serious AEs will be followed for 21 or 56 days following the last dose of study drug (depending on whether or not the patient plans to roll over to the open-label global 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.

## 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 medication 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 Alnylam, always within 24 hours from the time that site personnel first learn of the event. Every SAE must be documented by the Investigator on

the Serious Adverse Event Report form in addition to the AE form in the CRF. 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] Safety at [redacted] or call the [redacted] 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. [redacted] 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 [redacted] 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) 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 trial. These SAEs should also be reported promptly to the IEC or Clinical Events Committee (CEC) that approved the study. All SAE reports should be transmitted to the IEC/CEC 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 Trial Master File on study completion.

### 8.12 Pregnancy Reporting

A female patient with a positive pregnancy test at Screening/Baseline is ineligible for this trial. If a female patient is determined to be pregnant during the course of the study or during the 75 days after receiving the last dose of study medication, the Investigator should report the pregnancy to the CRO and Alnylam 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 Alnylam. The partner should be counseled and followed as described above.

# 9 STATISTICAL METHODS

## 9.1 Sample Size

Based on the number of patients expected to be enrolled in Study ALN-TTR02-002, up to 28 patients are expected to be enrolled in this extension study. Of these patients, up to 25 are expected to be included in the PK/PD subgroup. Any patient with pre-existing cardiac amyloid involvement will be included in the Cardiac subgroup.

## 9.2 Statistical Methodology

Statistical analyses will be primarily descriptive in nature. Adverse event summaries will include tabulations of all treatment-emergent AEs (TEAEs), treatment-related AEs, SAEs, discontinuations due to AEs, and AEs of various grading severity. By-patient listings will be provided for deaths, SAEs, and AEs leading to discontinuation of treatment.

Descriptive statistics will be provided for clinical laboratory data, vital signs data, and ECG interval data, presented as both actual values and changes from baseline relative to each on-study evaluation. Laboratory shift tables from baseline to worst values will be presented.

For categorical variables, summary tabulations of the number and percentage of patients within each category (with a category for missing data) will be presented. For continuous variables, the number of patients, mean, median, standard deviation, minimum, and maximum values will be presented.

All data will be provided in by-patient listings.

### 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:

- Full Analysis Set: All patients who were enrolled will be included in the full analysis set. The full analysis set will be the primary set for the analysis of PD data and clinical activity assessments.
- Safety Analysis Set: All patients in the full analysis set who received study drug. The safety analysis set will be used for the analysis of safety assessments.
- Pharmacokinetic (PK) Analysis Set: All patients in the Safety Analysis Set who have adequate data to determine a full PK profile.

### 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 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 TEAEs, 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 AEs 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.

### 9.2.4 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).

### 9.2.5 Pharmacokinetics

Plasma and urine samples will be evaluated using a validated ATTO-Probe-HPLC method to determine siRNA concentration and by LC/MS/MS method for DLin-MC3-DMA and $PEG_{2000}$-C-DMG concentrations. The concentration of 4-dimethylbutyric acid (metabolite of DLinMC3DMA) in urine will be evaluated by a validated LC/MS/MS method.

Pharmacokinetic analyses evaluation of siRNA, DLin-MC3-DMA, and $PEG_{2000}$-C-DMG plasma concentration-time profiles. Pharmacokinetic parameter estimates will be calculated for all subjects in the PK/PD subgroup receiving ALN-TTR02 will include, but may not be limited to: $C_{max}$; $t_{max}$; $t_{½\beta}$; $t_{1/2\alpha}$, $AUC_p$, $AUC_{0\text{-}t}$, $AUC_{0\text{-}last}$ and $AUC_{0\text{-}\infty}$; $\lambda_z$; CL, $V_{ss}$, and $V_z$. The PK profile of each patient will be characterized by noncompartmental and or compartmental analysis of siRNA, DLin-MC3-DMA and $PEG_{2000}$-C-DMG plasma concentration using validated computer software (WinNonlin® or Phoenix®, Pharsight Corp., Mountain View, California, USA). Parameters will include, but may not be limited to: $C_{max}$, $t_{max}$, AUC, CL, $V_{ss}$, and $V_z$. The AUC will be calculated using the linear trapezoidal method (linear interpolation). Other parameters may be calculated, if deemed necessary. If possible, the terminal elimination phase of the PK profile will be identified and its slope calculated using log-linear regression. The coefficient of determination of the line fitted to the terminal elimination phase will be calculated. Pharmacokinetic parameters describing the systemic exposure of the test article in the test system will be estimated from observed plasma concentration values for each patient.

Population PK analysis of siRNA and possibly DLin-MC3-DMA will be performed by integrating PK/PD and sparse samples collected during the study. A compartmental model will be developed to model the concentration-time data of plasma siRNA and possibly DLin-MC3-DMA. Pharmacokinetic parameters of siRNA and possibly DLin-MC3-DMA will be derived with the population PK models and summarized with descriptive statistics. Other parameters may be calculated if deemed necessary.

A PK/PD analysis will be performed whenever possible. As part of PK/PD analysis, additional PD baseline analysis on serum TTR, RBP, and vitamin A may be conducted to include the PD data obtained during PD recovery after receiving the last dose. A PD analysis to determine PD parameters will be conducted. The extent of suppression of TTR, RBP, and vitamin A will be evaluated and their correlation between TTR versus RBP and vitamin A will also be performed. The PK/PD analysis will include, but not be limited to, the determination of the relationship between exposure to siRNA, DLin-MC3-DMA and $PEG_{2000}$-C-DMG to the extent of suppression of TTR, RBP, and vitamin A will be evaluated, including their correlation relationship. The PD and PK/PD parameters will include, but not be limited to, descriptive statistics. Other parameters may be calculated if deemed necessary.

The $CL_R$ of siRNA and 4-dimethylaminobutyric acid (metabolite of DLin-MC3-DMA) after dosing with ALN-TTR02 will be determined for the subjects in the PK/PD subgroups and whenever possible for the other subjects in the study. Other parameters may be calculated if deemed necessary.

### 9.2.6 Summary of Clinical Activity 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 (i.e., the NIS weakness and reflex scores, the Σ5 NC, and QST values). The full NIS (i.e., the sum of cranial nerve, muscle weakness, reflex and sensation scores) will be summarized also. Values of the individual components of the mNIS weakness and reflex scores will be depicted graphically by presenting the mean (±SE) values over time for each location (hip, knee, etc.).

Patient reported quality of life and disease burden will be assessed by summary statistics for the EQ5D 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), VDT, and ambulation (FAP stage and PND score).

### 9.2.7 Healthcare Utilization Assessments

The observed values and changes from baseline in healthcare utilization will be evaluated and summarized using descriptive statistics.

### 9.2.8 Pharmacokinetic/Pharmacodynamic and Cardiac Subgroup Analyses

Descriptive statistics will be provided for actual values and changes from baseline in serum levels of troponin I and NT-proBNP in the subset of patients with evidence of pre-existing cardiac amyloid involvement. Results of echocardiograms will be summarized.

As part of PK/PD analysis, additional PD baseline analysis on serum TTR, RBP, and vitamin A may be conducted to include the PD data obtained during PD recovery after receiving the last dose.

### 9.2.9 Interim Analysis

There is no formal interim analysis planned for this study. Interim data examinations may be performed, but these will be of a descriptive nature and will not involve any formal hypothesis testing.

# 10 STUDY MANAGEMENT

The Investigator is accountable for the conduct of the trial. 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 either paper or electronic 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 collected by Alnylam monitor as soon as practical after completion. 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 Investigator and site periodically as well as maintain frequent telephone and letter 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 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 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 Independent Ethics Committee

National regulations and ICH require that approval be obtained from 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 IEC.

All IEC approvals must be dated and signed by the IEC Chairman or designee and must identify the IEC by 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 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 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 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 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 IEC that approved the study. Amendments to the protocol must be submitted in writing to the Investigator's IEC and the Competent 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 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 IEC, the site-specific ICF must be reviewed and approved by Alnylam or designee. Any changes requested by the IEC must also be approved by Alnylam. The final IEC approved ICF must be provided to Alnylam. Revisions to the ICF required during the study must be approved by Alnylam, and a copy of the revised ICF provided to Alnylam.

Before recruitment and enrollment, 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 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 and Alnylam 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 Alnylam.

### 10.2.6 Financial Disclosure Reporting Obligations

Each Investigator (including principal and/or any subinvestigators) 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 Ancillary Research

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

## 10.4 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 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.

## 10.5 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. Should the study be terminated and/or the site closed for whatever reason, all documentation and study medication pertaining to the study must be returned to Alnylam or its representative, and the Investigators, IEC 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.

### 10.6 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 and Alnylam approved ICF;
- Independent Ethics Committee approval of the protocol, and any amendments;
- 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 or shortly thereafter include:
- Curriculum vitaes 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 or legal guardians and IEC approval of any advertisements and any other written information;
- Independent Ethics Committee composition: If the Investigator or any of the Sub-investigators is a member of the IEC, assurance that he/she refrained from voting should be provided;
- Laboratory accreditation and reference ranges for any laboratory values for local laboratories.

### 10.7 Confidentiality

The Investigator must ensure that the patients' anonymity will be maintained. On the CRFs or other documents submitted to Alnylam, 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, 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 and the Portuguese Protection of Personal Data Directive 95/46/EC, only a patient's number and initials will be used to identify the patient on their study records. Laboratory samples will 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 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.

### 10.8 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 to 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 multi-center 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 of the manuscript to Alnylam 30 days prior to submission.

# 11 REFERENCES

1 Liz MA, Mar FM, Franquinho F, Soursa MM. Aboard transthyretin: from transport to cleavage. IUBMB Life. 2010;62:429-435.

2 Palha, JA, Episkopou V, Maeda S, et al. Thyroid hormone metabolism in a transthyretin-null mouse strain. J Biol Chem. 1994;269:33135-33139.

3 Palha JA, Hays MT, Morreale de Escobar G, et al. Transthyretin is not essential for thyroxine to reach the brain and other tissues in transthyretin-null mice. Am J Physiol. 1997;272:E485-493.

4 Episkopou V, Maeda S, Nishiguchi S, et al. Disruption of the transthyretin gene results in mice with depressed levels of plasma retinol and thyroid hormone. Proc Natl Acad Sci USA. 1993;90:2375-2379.

5 Van Bennekum AM, Wei S, Gamble MV, et al. Biochemical basis for depressed serum retinol levels in transthyretin-deficient mice. J Biol Chem. 2001;276:1107-1113.

6 Biesalski HK, Frank J, Beck SC, et al. Biochemical but not clinical vitamin A deficiency results from mutations in the gene for retinol binding protein. Am J Clin Nutr. 1999;69:931-936.

7 Decensi A, Fontana V, Fioretto M, et al. Long-term effects of fenretinide on retinal function. Eur J Cancer. 1997;33:80-84.

8 Torrisi R, Parodi S, Fontana V, et al. Factors affecting plasma retinol decline during long-term administration of the synthetic retinoid fenretinide in breast cancer patients. Cancer Epidemiol Biomarkers Prev. 1994;3:507-10.

9 Hou X, Aguilar MI, Small DH. Transthyretin and familial amyloidotic polymeutopathy. Recent progress in understanding the molecular mechanism of neurodegeneration. FEBS J. 2007;274:1637-1650.

10 Connors LH, Lim A, Prokaeva T, Roskens VA, Costello CE. Tabulation of human transthyretin (TTR) variants. Amyloid. 2003;10:160-184.

11 Suhr OB, Herlenius G, Friman S, Ericzon BG. Liver transplantation for hereditary transthyretin amyloidosis. Liver Transpl. 2000;6:263-276.

12 Yazaki M, Tokuda T, Nakamura A, et al. Cardiac amyloid in patients with familial amyloid polyneuropathy consists of abundant wild-type transthyretin. Biochem Biophys Res Commun. 2000;274:702-706.

13 Carvalho M, Alves M, Luis ML. Octreotide--a new treatment for diarrhoea in familial amyloidotic polyneuropathy. J Neurol Neurosurg Psychiatry. 1992;55:860-861.

14 Soares M., Coelho T, Sousa A, et al. Susceptibility and modifier genes in Portuguese transthyretin V30M amyloid polyneuropathy: complexity in a single-gene disease. Hum Mol Genet. 2005;14:543-553.

15 Eriksson P, Olofsson BO. Pacemaker treatment in familial amyloidosis with polyneuropathy. Pacing Clin Electrophysiol. 1984;7:702-706.

16 Delahaye N, Dinanian S, Slama MS, et al.Cardiac sympathetic denervation in familial amyloid polyneuropathy assessed by iodine-123 metaiodobenzylguanidine scintigraphy and heart rate variability. Eur J Nucl Med. 1999;26:416-424.

17 Buxbaum JN. Transthyretin and the transthyretin amyloidoses. In: Uversky VN, ed. *Protein Reviews: Protein Misfolding, Aggregation, and Conformational Diseases*. New York, NY: Springer Publishing Co; 2007:259-283.

18 Jacobson DR, Pastore RD, Yaghoubian R, et al. Variant-sequence transthyretin (isoleucine 122) in late-onset cardiac amyloidosis in black Americans. N Engl J Med. 1997;336:466-473.

19 Araki S. [Amyloidosis and amyloid protein]. Tanpakushitsu Kakusan Koso. 1984;29:1770-1782.

20 Benson MD. Familial amyloidotic polyneuropathy. Trends Neurosci. 1989;12:88-92.

21 Okamoto S, Wixner J, Obayashi K, et al. Liver transplantation for familial amyloidotic polyneuropathy: impact on Swedish patients' survival. Liver Transpl. 2009;15:1229-1235.

22 Adams D., Samuel D., Goulon-Goeau C, et al. The course and prognostic factors of familial amyloid polyneuropathy after liver transplantation. Brain. 2000;123:1495-1504.

23 Sharma, P., Rakela, J., 2005a, Management of pre-liver transplantation patient--part 2. Liver Transpl 11, 249-260.

24 Sharma P, Rakela J. Management of pre-liver transplantation patients--part 1. Liver Transpl. 2005;11:124-133.

25 Suhr OB, Friman S, Ericzon BG. Early liver transplantation improves familial amyloidotic polyneuropathy patients' survival. Amyloid. 2005:12:233-238

26 Yazaki M, Mitsuhashi S, Tokuda T, et al. Progressive wild-type transthyretin deposition after liver transplantation preferentially occurs onto myocardium in FAP patients. Am J Transplant. 2007;7:235-242.

27 European Medicines Agency. Vyndaqel: EPAR-Product Information. 2011. Available at: http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/002294/human_med_001498.jsp&mid=WC0b01ac058001d124. Last accessed on 8 December 2011.

28 Vaishnaw AK, Gollob J, Gamba-Vitalo C, et al. A status report on RNAi therapeutics. Silence. 2010;1(1):14.

29 Elbashir SM, Lendeckel W, Tuschi T. RNA interference is mediated by 21- and 22-nucleotide RNAs. Genes Dev. 2001;15:188-200.

30 Soutschek J, Akinc A, Bramlage B, et al. Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs. Nature. 2004;432:173-178.

31 Geisbert TW, Hensley LE, Kagan E, et al. Postexposure protection of guinea pigs against a lethal ebola virus challenge is conferred by RNA interference. J Infect Dis. 2006;193:1650-1657.

32 Judge AD, Bola G, Lee AC, MacLachlan I. Design of noninflammatory synthetic siRNA mediating potent gene silencing in vivo. Mol Ther. 2006;13:494-505.

33 Morrissey DV, Lockridge JA, Shaw L, et al. Potent and persistent in vivo anti-HBV activity of chemically modified siRNAs. Nat Biotechnol. 2005;23:1002-1007.

34 Zimmermann TS, Lee AC, Akinc A, et al. RNAi-mediated gene silencing in non-human primates. Nature. 2006;441(7089):111-4.

35 Maurer N, Mori A, Palmer L, et al. Lipid-based systems for the intracellular delivery of genetic drugs. Mol Membr Biol. 1999;16:129-140.

36 Akinc A, Querbes W, De S, Qin J, et al. Targeted delivery of RNAi therapeutics with endogenous and exogenous ligand-based mechanisms. Mol Ther. 2010;18(7):1357-1364.

37 Lachmann HJ, et al. Outcome in systemic AL amyloidosis in relation to changes in concentration of circulating free immunoglobulin light chains following chemotherapy. Br J Haematol, 2003;122:78-84.

38 Lachmann HJ, et al. Natural history and outcome in systemic AA amyloidosis. N Engl J Med. 2007;356:2361-71.

39 Gillmore JD, et al. Organ transplantation in hereditary apolipoprotein AI amyloidosis. Am J Transplant. 2006;6:2342-7.

40 Stangou AJ, Banner NR, Hendry BM, et al. Hereditary fibrinogen A alpha-chain amyloidosis: phenotypic characterization of a systemic disease and the role of liver transplantation. Blood. 2010;115:2998-3007.

41 Lachmann HJ, et al. Outcome in systemic AL amyloidosis in relation to changes in concentration of circulating free immunoglobulin light chains following chemotherapy. Br J Haematol, 2003;122:78-84.

42 Lachmann HJ, et al. Natural history and outcome in systemic AA amyloidosis. N Engl J Med. 2007;356:2361-71.

43 Gillmore JD, et al. Organ transplantation in hereditary apolipoprotein AI amyloidosis. Am J Transplant. 2006;6:2342-7.

44 Stangou AJ, Banner NR, Hendry BM, et al. Hereditary fibrinogen A alpha-chain amyloidosis: phenotypic characterization of a systemic disease and the role of liver transplantation. Blood. 2010;115:2998-3007.

45 Coelho T, Adams D, Silva A, et al. Safety and Efficacy of an RNAi Therapeutic Targeting Transthyretin (TTR) for TTR Amyloidosis. NEJM. 2013, accepted for publication.

46 Gomes ER, Demoly P. Epidemiology of hypersensitivity drug reactions. Current Opin Allergy Clin Immunol. 2005;5:309-316.

47 Solensky R. Drug hypersensitivity. Med Clin North Am. 2006;90:233-260.

48 Tabernero J, Shapiro GI, Lorusso PM, , et al. First-in-humans trial of an RNA interference therapeutic targeting VEGF and KSP in cancer patients with liver involvement. Cancer Discov. 2013:406-417.

49 Conceiciao IM, Miranda LC, et al. Bone mineral density in familial amyloid polyneuropathy and in other neuromuscular disorders. Eur J Neurol. 2005;12(6):480-2.

50 Sletten DM, Suarez GA, Low PA, Mandrekar J, Singer W. COMPASS 31: a tefined and abbreviated composite autonomic symptom score. Mayo Clin Proc. 2012;87:1196-1201.

51 van Nes SI, Vanhoutte EK, van Doorn PA, et al. Rasch-built Overall Disability Scale (R-ODS) for immune-mediated peripheral neuropathies. Neurology. 2011;76:337-45.

52 Yamamoto S, Wilczek HE, Nowak G, et al. Liver transplantation for familial amyloidotic polyneuropathy (FAP): a single-center experience over 16 years. Am J Transplant. 2007;7:2597-604.

53 Coutinho P, DeSilva AM, Lima JL, Barbosa AR. Forty years of experience with type I amyloid neuropathy: review of 483 cases. In: Glenner GG, Pinho P, Costa E, Falcao de Freitas A, Eds. *Amyloid and Amyloidosis*.. Amsterdam: Excerpta Medica; 1980:88-98.

# 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: Schedule of Assessments for Patients Administered ALN-TTR02 Once Every Four Weeks

Patients may receive ALN-TTR02 once every 4 weeks, based on results from the ongoing ALN-TTR02-002 study where the safety and PD of 2 dosing schedules are being evaluated. The schedule of assessments for patients receiving ALN-TTR02 once every 3 weeks is provided in Table 12-1.

**Table 12-1: Schedule of Assessment for Patients Administered ALN-TTR02 Once Every Four Weeks – Year 1**

| Procedure | Weeks | 1 & 29 | 5 & 33 | 9 & 37 | 13 & 41 | 17 & 45 | 21 & 49 | 25 & 53 | 27 & 55 |
|---|---|---|---|---|---|---|---|---|---|
| | Screening /Baseline[a] Day -28 to -0 | D0 & D196 | D28 & D224 | D56 & D252 | D84 & D280 | D112 & D308 | D140 & D336 | D168 & D364 | D182 & D378 |
| | Window | ±2D[b] | ±2D | ±2D | ±2D | ±2D | ±2D | ±2D | ±5D |
| Informed Consent | X | | | | | | | | |
| Demographics | X | | | | | | | | |
| Medical History[c] | X | | | | | | | | |
| Inclusion/Exclusion Criteria | X | X[d] | | | | | | | |
| Physical Examination | X | | | | | | | | X |
| Weight | X | X | X | X | X | X | X | X | X |
| Height | X | | | | | | | | |
| Vital Signs[e] | X | X | X | X | X | X | X | X | X |
| Echocardiogram | X | | | | | | | | |
| 12-Lead ECG | X | | | | | | | | X |
| Urine Pregnancy Test (females only) | X | | | | | | | | |
| Hematology | X | X[d] | | | | | | X | |
| Serum Chemistry and Urinalysis[f] | X | X[d] | | | X | | | X | |
| Thyroid Function Tests and Coagulation Studies | X | X[d] | | | | | | X | |
| Ophthalmology Exam | X | | | | | | | | X |
| **PD Assessments:** | | | | | | | | | |
| TTR protein (ELISA and turbidimetric)[g] | X | X[d] | | | X | | | X | X |
| RBP and Vitamin A[g] | X | X[d] | | | X | | | X | X |

| Procedure | Weeks | 1 & 29 | 5 & 33 | 9 & 37 | 13 & 41 | 17 & 45 | 21 & 49 | 25 & 53 | 27 & 55 |
|---|---|---|---|---|---|---|---|---|---|
| | Screening /Baseline[a] Day -28 to -0 | D0 & D196 | D28 & D224 | D56 & D252 | D84 & D280 | D112 & D308 | D140 & D336 | D168 & D364 | D182 & D378 |
| | Window | ±2D[b] | ±2D | ±2D | ±2D | ±2D | ±2D | ±2D | ±5D |
| **Other Assessments:** | | | | | | | | | |
| Exploratory Biomarkers | X | X[d] | | | X[g] | | | X | |
| Anti-drug Antibodies[h] | X | X[d] | X[i] | | X[i] | | | X | |
| Pharmacoeconomics Questionnaire | X | | | | | | | | X |
| **Clinical Activity Assessments:** | | | | | | | | | |
| mNIS + 7[j,k] | X | | | | | | | | X |
| VDT[k] | X | | | | | | | | X |
| HRdb[k] | X | | | | | | | | X |
| Grip Strength[k,l] | X | | | | | | | | X |
| 10-Meter Walk Test[k,m] | X | | | | | | | | X |
| NIS[k] | X | | | | | | | | X |
| Skin Punch Biopsy (IENFD & SGFND)[n] | X | | | | | | | | X |
| mBMI | X | | | | | | | | X |
| FAP Stage and PND Score | X | | | | | | | | X |
| COMPASS 31 | X | | | | | | | | X |
| QOL and Disability Questionnaires[o] | X | | | | | | | | X |
| Premedication Administration[p] | | X | X | X | X | X | X | X | |
| Study Drug Administration[q] | | X | X | X | X | X | X | X | |
| Plasma PK Sampling[r] | | X[x] | X[x] | X[x] | X | | | X | X[s] |
| Urine PK Sampling[t] | | X[x] | X[x] | X[x] | X | | | X | X[s] |

| Procedure | Weeks | 1 & 29 | 5 & 33 | 9 & 37 | 13 & 41 | 17 & 45 | 21 & 49 | 25 & 53 | 27 & 55 |
|---|---|---|---|---|---|---|---|---|---|
| | Screening /Baseline[a] Day -28 to -0 | D0 & D196 | D28 & D224 | D56 & D252 | D84 & D280 | D112 & D308 | D140 & D336 | D168 & D364 | D182 & D378 |
| | Window | ±2D[b] | ±2D | ±2D | ±2D | ±2D | ±2D | ±2D | ±5D |
| **PK/PD Subgroup Only** | | | | | | | | | |
| TTR (ELISA and turbidimetric) | | X[u] | X[u] | | | | | | |
| RBP and Vitamin A | | X[u] | X[u] | | | | | | |
| Plasma PK Sampling | | X[v] | X[v] | | | | | | |
| Urine PK Sampling[w] | | X[w] | X[w] | | | | | | |
| **Cardiac Subgroup Only:** | | | | | | | | | |
| Echocardiogram | | | | | | | | | X |
| Troponin I and NT-proBNP | | X[d] | | | X | | | X | |
| Concomitant Medications | X | X | | | | | | | |
| Adverse Events | | X | | | | | | | |

a Assessments performed during the ALN-TTR02-002 study that occur within the Screening/Baseline timeframe will not need to be repeated at the Screening/Baseline visit.
b Window does not apply to Day 0.
c Any AEs from the previous study (ALN-TTR02-002) that are ongoing on Day 0 are to be recorded on the Medical History case report form for the current study (ALN-TTR02-003) and followed until resolution.
d To be performed only on Day 0, not on Day 196
e 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.
f To be performed before dosing with ALN-TTR02.
g Blood samples for TTR, RBP, and vitamin A will be collected on Day 0 at 2 separate time points: 1) prior to administration of premedications and 2) after administration of premedications but before dosing of ALN-TTR02. On all other study visits, a blood sample will be collected only once; prior to administration of premedications.
h Blood samples for ADA will be collected prior to dosing with ALN-TTR02.
i To be collected only on Days 28 and 84, not on Days 224 and 280.
j The mNIS + 7 consists of the modified NIS tool (or aspects of the tool; weakness and reflexes only), nerve conduction studies (NCS), quantitative sensory testing (QST) by body surface area including touch pressure (TP) and heat pain (HP), and postural blood pressure. Every effort will be made to use the same devices for a patient throughout the duration of the study.
k Two assessments will be performed on separate days at Screening/Baseline and then approximately every 6 months. The Screening/Baseline assessments must be performed within 14 days prior to the first dose of study drug (Day 0). The second (repeat) assessment must be conducted at least 24 hours after the first assessment. In addition, every effort should be made to conduct the 2 assessments no greater than 7 days apart.
l Hand 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.

m Patient will walk without assistance for 10 meters.

n Two 3-mm skin punch biopsies are to be obtained. One will be obtained from the distal lower leg, when a patient's clinical status allows, and one from the distal thigh.

o Quality of life and disability assessments will include the EQ5D and R-ODS questionnaires.

p The following premedications will be administered at least 60 minutes prior to the start of the infusion of ALN-TTR02: dexamethasone (10 mg IV, or equivalent), paracetamol (PO 500 mg; or equivalent), IV H2 blocker (e.g., ranitidine 50 mg, famotidine 20 mg, or equivalent other H2 blocker dose), and IV H1 blocker (e.g., diphenhydramine 50 mg or equivalent; 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 blockers). Based on results from the ALN-TTR02-002 study, the following alternative premedication regimen can be used: dexamethasone (8 mg, or equivalent), paracetamol (500 mg, or equivalent), H2 blocker (e.g., 150 mg ranitidine, or 20 mg famotidine, or equivalent other H2 blocker dose), and H1 blocker (10 mg cetirizine, 25 mg hydroxyzine, fexofenadine or equivalent may be substituted) self-administered per os (PO) the evening before study drug administration. At least 60 minutes prior to the start of study drug infusion, dexamethasone (20 mg PO, or equivalent), paracetamol (500 mg PO, or equivalent), an H2 blocker (PO), and an H1 blocker (PO) will be administered by study site personnel.

q The infusion site will be assessed for any localized reaction pre-dose, during infusion, at the end of the infusion, for 30 minutes after the infusion.

r Plasma PK samples will be collected pre-dose (within 1 hour of planned dosing start), EOI, and 2 hrs post-dose (±5 min), except on Days 0 and 224 for subjects in the PK/PD Subgroup.

s Samples will be collected once during the study visit.

t For each dose, urine PK samples will be collected pre-dose and 2 hours after dosing with ALN-TTR02.

u For patients in the PK/PD subgroup, blood samples for TTR, RBP, and vitamin A will be collected on Days 0 and 224 prior to administration of premedications. In addition, a TTR, RBP, and vitamin A sample will also be collected 24 hours (±120 minutes) post-dose, and 3, 7, and 17 days (±1 day) after dosing with ALN-TTR02 for the study visits noted.

v On Days 0 and 224, patients in the PK/PD subgroup will have samples taken pre-dose, EOI, and 1, 2, 4, 6, and 24 hours post-infusion. In addition, a plasma PK sample will also be collected 3, 7, and 17 days (±1 day) after dosing with ALN-TTR02 for the study visits noted. The windows for sampling are ±5 minutes for the 1, 2, 4, and 6 hour sampling periods; and ±120 minutes for the 24 hour sampling period.

w On Days 0 and 224, patients in the PK/PD subgroup will have a urine sample for PK analysis taken pre-dose, and 6 and 24 hours post-infusion. In addition, a urine PK sample will also be collected 3, 7, and 17 days (±1 day) after dosing with ALN-TTR02 for the study visits noted. The windows for sampling are ±5 minutes for the 1, 2, 4, and 6 hour sampling periods; and ±120 minutes for the 24 hour sampling period.

x Samples to be collected on Day 0, Day 28, and Day 56 only

**Table 12-2: Schedule of Assessment for Patients Administered ALN-TTR02 Once Every Four Weeks – Year 2**

| | Weeks | 57 & 81 | 61 & 85 | 65 & 89 | 69 & 93 | 73 & 97 | 77 & 101 | 79 & 103 | 83 & 107 | 111 | 115 | ET |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Days | D392 & D588 | D420 & D616 | D448 & D644 | D476 & D672 | D504 & D700 | D532 & D728 | D560 & D756 | D574 & D784 | D812 | D840 | N/A |
| **Procedure** | **Window** | ±2D | ±2D | ±2D | ±2D | ±2D | ±2D | ±2D | ±5D | ±5D | ±5D | ±5D |
| Physical Examination | | | | | X | | | | X | X | | X |
| Weight | | X | X | X | X | X | X | X | X | X | | X |
| Vital Signs[a] | | X | X | X | X | X | X | X | X | X | | X |
| 12-Lead ECG | | | | | | | | | X | X | | X |
| Hematology[b] | | | | | | | | X | | X | | X |
| Serum Chemistry and Urinalysis[b] | | | | | X | | | X | | X | | X |
| Thyroid Function Tests and Coagulation Studies[b] | | | | | | | | X | | X | | X |
| Ophthalmology Exam | | | | | | | | | X | | | X |
| **PD Assessments:** | | | | | | | | | | | | |
| TTR protein (ELISA and turbidimetric) [c] | | | | | X | | | X | X | X | X | X |
| RBP and Vitamin A[c] | | | | | X | | | X | X | X | X | X |
| **Other Assessments:** | | | | | | | | | | | | |
| Exploratory Biomarkers | | | | | X | | | X | X | | | |
| Anti-drug Antibodies | | | | | | | | X[d] | | | | |
| Pharmacoeconomics Questionnaire | | | | | | | | | X | | | X[f] |
| **Clinical Activity Assessments:** | | | | | | | | | | | | |
| mNIS + 7[e,g] | | | | | | | | | X | | | X[f] |
| VDT[g] | | | | | | | | | X | | | X[f] |

| Procedure | Weeks | 57 & 81 | 61 & 85 | 65 & 89 | 69 & 93 | 73 & 97 | 77 & 101 | 79 & 103 | 83 & 107 | 111 | 115 | ET |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Days | D392 & D588 | D420 & D616 | D448 & D644 | D476 & D672 | D504 & D700 | D532 & D728 | D560 & D756 | D574 & D784 | D812 | D840 | N/A |
| | Window | ±2D | ±2D | ±2D | ±2D | ±2D | ±2D | ±2D | ±5D | ±5D | ±5D | ±5D |
| HRdb[g] | | | | | | | | | X | | | X[f] |
| Grip Strength[g,h] | | | | | | | | | X | | | X[f] |
| 10-Meter Walk Test[g,i] | | | | | | | | | X | | | X[f] |
| NIS[g] | | | | | | | | | X | | | X[f] |
| Skin Punch Biopsy (IENFD & SGFND)[j] | | | | | | | | | X | | | X[f] |
| mBMI | | | | | | | | | X | | | X[f] |
| FAP Stage and PND Score | | | | | | | | | X | | | X[f] |
| COMPASS 31 | | | | | | | | | X | | | X[f] |
| QOL and Disability Questionnaires[k] | | | | | | | | | X | | | X[f] |
| Premedication Administration[l] | | X | X | X | X | X | X | X | | | | |
| Study Drug Administration[m] | | X | X | X | X | X | X | X | | | | |
| Plasma PK Sampling[n] | | | | | X | | | X | X | X | X | X |
| Urine PK Sampling[o] | | | | | X | | | X | X | | | X |
| **PK/PD Subgroup Only:** | | | | | | | | | | | | |
| TTR (ELISA and turbidimetric)[p] | | | | | | | | X[p] | | | | |
| RBP and Vitamin A[p] | | | | | | | | X[p] | | | | |
| Plasma PK Sampling[q] | | | | | | | | X[q] | | | | |
| Urine PK Sampling[r] | | | | | | | | X[r] | | | | |
| **Cardiac Subgroup Only:** | | | | | | | | | | | | |
| Echocardiogram | | | | | | | | | X | | | |
| Troponin I and NT-proBNP | | | | | X | | | X | | | | |

| Procedure | Weeks | 57 & 81 | 61 & 85 | 65 & 89 | 69 & 93 | 73 & 97 | 77 & 101 | 79 & 103 | 83 & 107 | 111 | 115 | ET |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Days | D392 & D588 | D420 & D616 | D448 & D644 | D476 & D672 | D504 & D700 | D532 & D728 | D560 & D756 | D574 & D784 | D812 | D840 | N/A |
| | Window | ±2D | ±2D | ±2D | ±2D | ±2D | ±2D | ±2D | ±5D | ±5D | ±5D | ±5D |
| Concomitant Medications | | X | | | | | | | | | | |
| Adverse Events | | X | | | | | | | | | | |

a 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.

b To be performed before dosing with ALN-TTR02.

c Blood samples for TTR, RBP, and vitamin A will be collected on Day 0 at 2 separate time points: 1) prior to administration of premedications and 2) after administration of premedications but before dosing with ALN-TTR02. At all other study visits, a blood sample will be collected only once; prior to administration of premedications.

d Blood samples for ADA will be collected only at the Day 756 visit (approximately 24 months), prior to dosing with ALN-TTR02.

e The mNIS + 7 consists of the modified NIS tool (or aspects of the tool; weakness and reflexes only), nerve conduction studies (NCS), and quantitative sensory testing (QST) by body surface area including touch pressure (TP) and heat pain (HP), and postural blood pressure. These tests can be conducted over 2 study visits that occur no less than 24 hours from each other and no more than 7 days apart. Every effort will be made to use the same devices for a patient throughout the duration of the study.

f To be performed only in patients who have received at least 4 doses of study drug and who withdraw >3 months after the last clinical activity assessments were performed. These tests will not be repeated.

g Two assessments will be performed at Screening/Baseline and then approximately every 6 months. The Screening/Baseline assessments must be performed within 14 days prior to the first dose of study drug (Day 0). The second (repeat) assessment must be conducted at least 24 hours after the first assessment. In addition, every effort should be made to conduct the 2 assessments no greater than 7 days apart.

h Hand 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.

i Patient will walk without assistance for 10 meters.

j Two 3-mm skin punch biopsies are to be obtained. One will be obtained from the distal lower leg, when a patient's clinical status allows, and one from the distal thigh.

k Quality of life and disability assessments will include the EQ5D and R-ODS questionnaires.

l The following premedications will be administered at least 60 minutes prior to the start of the infusion of ALN-TTR02: dexamethasone (10 mg IV, or equivalent), paracetamol (PO 500 mg; or equivalent), IV H2 blocker (e.g., ranitidine 50 mg, famotidine 20 mg, or equivalent other H2 blocker dose), and IV H1 blocker (e.g., diphenhydramine 50 mg or equivalent; 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 blockers). Based on results from the ALN-TTR02-002 study, the following alternative premedication regimen can be used: dexamethasone (8 mg, or equivalent), paracetamol (500 mg, or equivalent), H2 blocker (e.g., 150 mg ranitidine, or 20 mg famotidine, or equivalent other H2 blocker dose), and H1 blocker (10 mg cetirizine, 25 mg hydroxyzine, fexofenadine or equivalent may be substituted) self-administered per os (PO) the evening before study drug administration. At least 60 minutes prior to the start of study drug infusion, dexamethasone (20 mg PO, or equivalent), paracetamol (500 mg PO, or equivalent), an H2 blocker (PO), and an H1 blocker (PO) will be administered by study site personnel.

m The infusion site will be assessed for any localized reaction pre-dose, during infusion, at the end of the infusion, for 30 minutes after the infusion.

n Plasma PK samples will be collected pre-dose (within 1 hour of planned dosing start) , EOI and 2 hours post dose.

o For each dose, urine PK samples will be collected pre-dose and 2 hours after dosing with ALN-TTR02.

p For patients in the PK/PD subgroup, blood samples for TTR, RBP, and vitamin A will be collected on Day 756: prior to administration of premedications and a blood sample will also be collected 24 hours, 3, 7 and 17 days after dosing with ALN-TTR02.

q On Day 756, patients in the PK/PD sub-group will have PK samples taken at predose, end of infusion, 1, 2, 4, 6, and 24 hours post-infusion. In addition, a plasma PK sample will also be collected 3, 7, and 17 days (±1 day) after dosing with ALN-TTR02 for the study visits noted. The windows for sampling are ±5 minutes for the 1, 2, 4, and 6 hour sampling periods; and ±120 minutes for the 24 hour sampling period.

r On Day 756, patients in the PK/PD subgroup will have a urine sample for PK analysis taken pre-dose, EOI, and 6 and 24 hours post-infusion. In addition, a urine PK sample will also be collected 3, 7, 10, and 14 days (±1 day) after dosing with ALN-TTR02 for the study visits noted. The windows for sampling are ±5 minutes for the 1, 2, 4, and 6 hour sampling periods; and ±120 minutes for the 24 hour sampling period.

## Appendix 3: 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. |

**Protocol ALN-TTR02-003**

**Amendment 1.2 Summary of Changes**

**A Phase 2, Multicenter, Open-Label, Extension Study to Evaluate the Long-Term Safety, Clinical Activity, and Pharmacokinetics of ALN-TTR02 in Patients With Familial Amyloidotic Polyneuropathy Who Have Previously Received ALN-TTR02**

Changes included in Amendment 1.2 to Protocol ALN-TTR02-003, dated 26 November 2013, are detailed below.

Text added is indicated by **bold** font.

| Section | Prior Text | New Text |
|---|---|---|
| **Protocol Synopsis – Inclusion Criteria** | 4. Adequate liver function, demonstrated by an aspartate transaminase (AST) and alanine transaminase (ALT) level ≤2.5 × the upper limit of normal (ULN), total bilirubin within normal limits, albumin >3 g/dL, and international normalized ratio (INR) ≤1.2. | 4. Adequate liver function, demonstrated by an aspartate transaminase (AST) and alanine transaminase (ALT) level ≤2.5 × the upper limit of normal (ULN), total bilirubin within normal limits, albumin >3 g/dL, and international normalized ratio (INR) ≤1.2 **(patients on warfarin with an INR of ≤3 will be allowed).** |
| **4.2 Inclusion Criteria** | 4. Adequate liver function, demonstrated by an aspartate transaminase (AST) and alanine transaminase (ALT) level ≤2.5 × the upper limit of normal (ULN), total bilirubin within normal limits, albumin >3 g/dL, and international normalized ratio (INR) ≤1.2. | 4. Adequate liver function, demonstrated by an aspartate transaminase (AST) and alanine transaminase (ALT) level ≤2.5 × the upper limit of normal (ULN), total bilirubin within normal limits, albumin >3 g/dL, and international normalized ratio (INR) ≤1.2 **(patients on warfarin with an INR of ≤3 will be allowed).** |