Official Title:	**A Phase 2, Open-Label, Multi-Dose, Dose Escalation Trial to Evaluate the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of Intravenous Infusions of ALN-TTR02 in Patients with TTR Amyloidosis**
NCT Number:	**NCT01617967**
Document Date:	**Clinical Study Protocol, Version 2.1, 16 January 2013**

CLINICAL STUDY PROTOCOL – CONFIDENTIAL

ALN-TTR02-002

A Phase 2, Open-Label, Multi-Dose, Dose Escalation Trial to Evaluate the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of Intravenous Infusions of ALN-TTR02 in Patients with TTR Amyloidosis

Original Protocol	03 February 2012
Amendment 1:	19 June 2012
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EudraCT Number:	2012-000467-24
Sponsor:	Alnylam Pharmaceuticals, Inc 300 Third Street Cambridge, MA 02142 USA
Sponsor Contact:	[redacted]

CONFIDENTIAL

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 _____

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PROTOCOL SYNOPSIS

Protocol Title:	A Phase 2, Open-Label, Multi-Dose, Dose Escalation Trial to Evaluate the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of Intravenous Infusions of ALN-TTR02 in Patients with TTR Amyloidosis
Indication:	Treatment of patients with transthyretin (TTR)-mediated amyloidosis (ATTR)
Protocol Number:	ALN-TTR02-002
Phase of Development:	2
Design:	Multi-center, multi-national, open-label, multi-dose, dose escalation study. Consented patients meeting the eligibility criteria will be enrolled into sequential cohorts of increasing doses of ALN-TTR02, the investigational drug. Each cohort will comprise 3 patients, who are to receive 2 intravenous (IV) infusions of ALN-TTR02, 4 weeks apart. A Safety Review Committee (SRC) will evaluate safety in the study and determine if it remains acceptable to continue dosing per their safety review charter. To ensure timely safety information exchange across the participating study centers, the SRC will be comprised of all Principal Investigators (PIs) participating in the study or their designee, the Alnylam Medical Monitor, and the Contract Research Organization (CRO) Medical Monitor. The dosing of cohorts will be as follows: • Dosing within a cohort: The first patient will receive their first dose and if the dose is well tolerated, per Section 5.7.3 (Dose-limiting Toxicity), Patients 2 and 3 will receive their first dose at that same dose level no sooner than 48 hours apart, with Patient 2 receiving a dose of study drug no sooner than 48 hours after the dosing of Patient 1. Similar to the first dose, the second dose will be administered to patients 4 weeks after the first dose in a sequential manner with at least 48 hours separating dosing of each patient. • Dosing of the next cohort: Collective cohort safety and tolerability data on Patients 1 to 3 through at least

	96 hours post-first dose will be reviewed by the SRC; if the administered dose is found to be safe and well tolerated, dosing for the next cohort will begin no sooner than 96 hours after Patient 3 had safely received dose 1 from the previous cohort. Patients in the cohort would be dosed and reviewed for safety and tolerability following the same procedures as used for other cohorts (as stated above). For patients on all dose levels other than the starting dose level of 10 μg/kg, prior to receiving the second dose, the SRC will review the cumulative safety and tolerability data of at least 2 patients from the previous dose level(s) with at least 96 hours of follow-up after receiving a second dose of study drug. The SRC will also be convened in the event of any potential dose-limiting toxicity (DLT) to determine whether stopping rules have been met.
Study Sites	This study is planned to be conducted at up to 10 study centers worldwide.
Investigational Drug:	ALN-TTR02 is comprised of drug substance ALN-18328 (small interfering ribonucleic acid [siRNA] targeting wild type TTR messenger ribonucleic acid [mRNA] as well as all TTR mutations) formulated in a lipid nanoparticle (LNP) containing the following lipid excipients: DLin-MC3-DMA, PEG_{2000}-C-DMG, DSPC, and cholesterol.
Control Drug:	Not applicable.
Dosage, Route of Administration and Duration of Treatment of Investigational Drug:	Four cohorts are planned to be enrolled to evaluate 2 consecutive doses, 4 weeks apart of the following dose levels: 10, 50, 150, and 300 μg/kg ALN-TTR02. Based on the interim evaluation of safety data, it may also be decided by the SRC that an optional cohort may be utilized which will include 3 additional patients. Dosing in these optional cohorts may occur at a previously tested dose that was found to be safe and where stopping rules were not met or at an intermediate dose level. Patients in the optional cohort would be dosed and reviewed for safety and tolerability following the same procedures as used for other cohorts. Optional cohort(s) would be included to further confirm the safety and/or pharmacodynamic (PD) effect of previously evaluated dose levels, which may include evaluation of the original dosing regimen (once every 4 weeks), an alternative dosing regimen (once every 3 weeks), and/or an alternative

	premedication regimen and infusion rate. The dosing regimen for the first optional cohort will be once every 4 weeks, and the dosing regimen for the remaining 4 optional cohorts will be once every 3 or 4 weeks. It is anticipated that the first 2 optional cohorts will use the original premedication regimen, and the remaining 3 optional cohorts may be used to explore the alternative premedication regimen and infusion rate. The SRC will review all available safety data and make recommendations on dosing and premedication regimens prior to dosing of any of the optional cohorts. The Independent Ethics Committees (IECs) will be informed of the implementation of optional cohorts. Up to 5 optional cohorts are permitted in this study. Patients receiving the original premedication regimen will be administered the following prior to each dose of ALN-TTR02: • Oral (PO) dexamethasone (8 mg) or equivalent administered the evening before dosing and 20 mg 30 to 60 minutes prior to start of infusion of ALN-TTR02; • Oral paracetamol (500 mg) or equivalent the evening before dosing and 30 to 60 minutes prior to the start of infusion of ALN-TTR02; • Oral H2 blocker (e.g., ranitidine 150 mg or famotidine 20 mg or equivalent other H2 blocker dose) the evening before dosing and 30 to 60 minutes prior to start of infusion of ALN-TTR02; and • Oral H1 blocker, 10 mg cetirizine or equivalent (hydroxyzine 25 mg or fexofenadine may be substituted if patient does not tolerate cetirizine) the evening before dosing and 30 to 60 minutes prior to start of infusion of ALN-TTR02. The alternative premedication regimen that can be used in select optional cohorts, as agreed upon by the SRC, will be administered prior to each dose of ALN-TTR02 and includes the following: • Intravenous (IV) dexamethasone (10 mg) or equivalent, administered at least 60 minutes prior to the start of infusion of ALN-TTR02;

	• Oral paracetamol (500 mg) or equivalent at least 60 minutes prior to the start of 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 start of infusion of ALN-TTR02; and • Intravenous H1 blocker: diphenhydramine 50 mg IV (or equivalent other IV H1 blocker available at the study site) at least 60 minutes prior to start of 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. For patients receiving the original premedication regimen and infusion rate, doses of ALN-TTR02 will be administered IV over 60 minutes by a controlled infusion device at a flow rate of approximately 3.3 mL/min. For patients enrolled in the optional cohorts evaluating the alternative premedication regimen and infusion rate, the flow rate of ALN-TTR02 will be approximately 1.1 mL/min during the first 15 minutes (1/3rd the original flow rate) with the remainder of the infusion administered at a flow rate of 3.3 mL/min for a total infusion time of approximately 70 minutes. 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). Patients will remain hospitalized at the study site for at least 24 hours following completion of dosing on Days 0 and 28, for safety assessments and pharmacokinetic (PK) sampling.

Time on Study	The duration of patient participation in this study is approximately 36 weeks (screening [up to 45 days prior to study drug administration] through Day 208). An open-label extension study at the recommended Phase 3 dose and regimen (as derived from Study ALN-TTR02-002) is planned, which will enable the patients who enrolled in Study ALN-TTR02-002 to receive additional, long-term dosing and safety follow-up. For some of the patients, this may preclude the completion of all of the follow-up period assessments of Study ALN-TTR02-002 if they are deemed eligible to participate in the extension study prior to completion of the full follow up through Day 208. Such patients will be followed up for a minimum of 28 days after their last dose in the current ALN-TTR02-002 study prior to being enrolled in the extension study. The extension study will be implemented only after dose escalation is completed in Study ALN-TTR02-002, with post second dose follow-up through Day 208 in at least 1 cohort at the highest dose. Note: A patient will be followed every 2 weeks after Day 56 until TTR levels have recovered to at least 80% of baseline. Evaluations will be performed as indicated in the schedule of assessments.
Primary Objective:	• To evaluate the safety and tolerability of multiple doses of ALN-TTR02.
Secondary Objectives:	• To characterize the plasma and urine PK of ALN-TTR02. • To assess preliminary evidence of the PD effect of ALN-TTR02 on serum total TTR levels.
Sample Size:	Based on the entry criteria and the proposed dose escalation scheme, up to 27 patients are expected to be enrolled. Three patients are to be enrolled at each dose level and up to 5 optional cohorts of 3 patients each will be permitted.
Inclusion and Exclusion Criteria:	Each patient must meet the following criteria to be enrolled: 1. Male or female aged 18 years or older. 2. Patients has a biopsy-proven diagnosis of TTR amyloidosis with documented signs/symptoms of the disease (e.g., sensory, motor, or autonomic neuropathy) that are at least mild to moderate in severity. 3. Body mass index (BMI) of 17–33 kg/m^2.

	4. Karnofsky performance status of 60% or greater. 5. Absolute neutrophil count (ANC) ≥1500 cells/mm³, platelet count ≥100,000 cells/mm³, and hemoglobin ≥10 g/dL. 6. Adequate liver function, demonstrated by an aspartate transaminase (AST) and alanine transaminase (ALT) ≤2.5 × the upper limit of normal (ULN), total bilirubin within normal limits, albumin >3 g/dL, international normalized ratio (INR) ≤1.2. 7. Adequate renal function: serum creatinine ≤1.5 × ULN. 8. Seronegative for hepatitis B virus (HBV) and hepatitis C virus (HCV). 9. Women of child-bearing potential must have a negative pregnancy test, cannot be breast feeding, and must be using two highly effective methods of contraception prior to screening, throughout study participation, and for 1 month after ending study participation. 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 (IUD) in conjunction with spermicide or condom; or surgical sterilization of partner in conjunction with spermicide, condom, or diaphragm. 10. Males agree to use appropriate contraception throughout study participation and for 1 month after ending study participation. 11. Patient is willing and able to comply with protocol-required visit schedule and visit requirements and provide written informed consent.

	12. Patients will be excluded if they meet any of the following criteria: 13. Pregnant or nursing. 14. Has had a liver transplant. 15. Has a known surgery planned during any point of the study period. 16. Has known human immunodeficiency virus (HIV) positive status. 17. Has a known or suspected systemic bacterial, viral, parasitic, or fungal infection. 18. Received an investigational agent, other than tafamidis or diflunisal, within 30 days prior to study drug administration. 19. Has a New York Heart Association heart failure classification >2. 20. Has unstable angina. 21. Has uncontrolled clinically significant cardiac arrhythmia. 22. Is considered unfit for the study by the Principal Investigator. 23. Had a prior severe reaction to a liposomal product. 24. Has known hypersensitivity to oligonucleotides. 25. Is an employee or family member of Alnylam or the clinical study site personnel.
Pharmacodynamic Assessments:	The PD evaluation will include assessment of effects of ALN-TTR02 on serum total TTR levels.

Pharmacokinetic Assessments:	The PK evaluation will include plasma-concentration time profiles for ALN-18328 and the novel lipid components DLin-MC3-DMA and polyethylene glycol $(PEG)_{2000}$-C-DMG. ALN-18328 concentration will be determined at specified time points post-dose. DLin-MC3-DMA and PEG_{2000}-C-DMG concentrations will be determined at specified time points up to 180 days post- second dose. An assessment of plasma concentrations of free and encapsulated siRNA will be determined at specified time points. If the infusion of study drug is stopped and the site is considering restarting, a PK blood sample will be taken when the infusion is stopped. Urine will be collected to determine ALN-18328 excreted in urine and renal clearance (CL_R) of ALN-18328 after dosing with ALN-TTR02. In addition urine samples will be collected to quantify analytes that may include DLin-MC3-DMA and/or its metabolites at specified time points.
Safety Assessments:	26. Safety evaluations will include assessment of adverse events (AEs), electrocardiograms (ECGs), cardiac monitoring (telemetry), arterial oxygen saturation (SaO_2) using pulse oximetry, vital signs (blood pressure, pulse rate, oral body temperature, and respiration rate), clinical laboratory safety tests (hematology, serum chemistry, liver function tests, thyroid function parameters, serology, coagulation parameters, urinalysis, cytokines, C-reactive protein, complement factors), and physical examinations.
Other Assessments:	27. Exploratory effects of ALN-TTR02 will be evaluated by: 28. Serial measurement of circulating mutant and wild type TTR levels 29. Serial measurement of circulating vitamin A and retinol binding protein (RBP) levels • Blood collections to explore the expression of hepatocyte derived proteins to further characterize the biological effects of siRNA LNPs.

Dose-Limiting Toxicities and Stopping Criteria:	Dose-limiting toxicities will include any of the following: 1. Any life-threatening toxicity. 2. ALT and AST ≥5 × ULN or total bilirubin >2.0 mg/dL. 3. An infusion reaction that requires hospitalization, despite premedication. 4. Any other toxicity which in the opinion of the SRC precludes further dosing. **Stopping Rules:** For any DLT, accrual to that dose level will stop, all dosing will be discontinued, and dose escalation will end, pending further evaluation of all available safety data by the SRC.
Statistical Methods:	Statistical analyses will be primarily descriptive in nature. Adverse event summaries will include tabulations of all treatment-emergent AEs, treatment-related AEs, serious adverse events (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. Pharmacokinetic analyses will be conducted using non-compartmental and/or compartmental evaluation of ALN-18328 (siRNA), DLin-MC3-DMA (lipid) and PEG_{2000}-C-DMG (lipid) plasma concentration-time data to determine PK parameter estimates using a validated software program, [redacted]. PK and PD data will be assessed descriptively whenever possible and by exploratory statistical comparisons.

Table 1-1: Schedule of Assessments for Cohorts Administered ALN-TTR02 Once Every Four Weeks

	Screening	Pre-Dosing		Dosing Cycles							Follow-Up		
Procedures	**D -45 to D -3**	**D -1 / D 27**	**D 0 / D 28 (+2 D)**	**D 0 / D 28 (+2 D)**	**D 1 / D 29**	**D 2 / D 30**	**D 7 (±1 D) / D 35 (±1 D)**	**D 10 (±2 D) / D 38 (±2 D)**	**D 14 (±3 D) / D 42 (±3 D)**	**D 21 (±3 D) / D 49 (±3 D)**	**D 56[a] (±3 D)**	**D 112 (±10 D)**	**D 208[a] (±2 W)**
Informed Consent	X												
Demographics	X												
Medical History	X		X[b]										
Inclusion/Exclusion Criteria	X		X										
Physical Examination, excluding weight	X		X[c]		X[c]	X[c]			X[c]		X		
Weight	X		X[d]										
Height	X												
Body Mass Index (BMI)	X		X										
Vital Signs[e]	X					X			X		X		
Vital Signs (Serial)[f]			X	X	X								
Echocardiogram[g]	X												
12-Lead ECG	X										X		
ECG (Serial)[h]			X	X	X								
Inpatient at Study Site			X	X	X[i]								
Cardiac Monitoring (Telemetry)[j]			X	X	X								
Pulse Oximetry (Serial)[f]			X	X	X								
Serum Pregnancy Test (females only)	X										X		
Urine Pregnancy Test (females only)[k]			X										
Hepatitis B/C Status[l]	X												
Serum Chemistry, Hematology, Urinalysis	X		X[m]		X				X		X		

Procedures	Screening	Pre-Dosing		Dosing Cycles							Follow-Up		
	D -45 to D -3	D -1 / D 27	D 0 / D 28 (+2 D)	D 0 / D 28 (+2 D)	D 1 / D 29	D 2 / D 30	D 7 (±1 D) / D 35 (±1 D)	D 10 (±2 D) / D 38 (±2 D)	D 14 (±3 D) / D 42 (±3 D)	D 21 (±3 D) / D 49 (±3 D)	D 56[a] (±3 D)	D 112 (±10 D)	D 208[a] (±2 W)
Liver Function Tests[n]	X		X[m]		X	X	X		X		X		
Coagulation Studies[o]	X		X[m]		X								
Lipid Panel[p]			X										
TTR protein, Vitamin A, and RBP in serum	X		X[q]		X	X	X	X	X	X	X[r]	X	X
TTR mRNA in serum	X		X		X	X							
Thyroid Function Tests[s]	X		X[m]						X		X		
Complement Bb[t]			X	X	X								
If infusion reaction: Tryptase and C3a[u]				X	X								
Premedication[v]		X	X										
Premedication reminder[w]		X											
Study Drug Administration				X[x]									
Anti-PEG Antibody Testing (IgG, IgM)	X		X				X				X		X
Cytokines and CRP[y]			X	X	X								
Plasma PK Sampling[z]			X	X	X	X	X		X	X	X	X	X
Urine PK Sampling[aa]			X	X	X	X	X		X	X	X	X	X
Exploratory Biomarkers	X		X[bb]		X	X	X	X	X	X	X		
Concomitant Medications	X			Continuous Monitoring									
Review/Record AEs													
Study Completion											X		

Footnotes on following pages.

Note: The schedule of assessments for optional cohorts administered ALN-TTR02 once every 3 weeks is provided in Appendix 4.

a Early termination procedures: if a patient withdraws prior to Day 56, then the Days 56 and 208 visits should be performed. If a patient is withdrawn/withdraws after Day 56 and prior to Day 208, then the Day 208 visit should be performed.

b Interval medical history.

c Focused physical examination (includes head/ears/eyes/nose/throat [HEENT], cardiovascular, respiratory, abdominal, and hepatic assessments). If the screening physical examination was performed within 72 hours of Day 0, then the pre-dose (Day 0) physical examination does not need to be repeated; however, the patient's weight will be obtained.

d Weight measured on Day 0 and Day 28 will be used for calculating first and second dose, respectively.

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 Serial measures (vital signs and pulse oximetry) are to be measured within 30 minutes pre-dose; at the end of infusion (EOI); and 30 (±5) minutes; 1, 2, and 3 (±15 minutes) hours; 6, 12, and 18 (±30 minutes) hours; and 24 (+30 minutes) hours post-infusion.

g Not needed if a normal echocardiogram has been obtained within the past 90 days.

h Serial electrocardiograms (ECGs) will be collected in 3 replicates within 30 minutes pre-dose, EOI, and 30 (±5) minutes, 2 and 4 (±15 minutes) hours, and 24 (+30 minutes) hours post-infusion.

i Patients will be hospitalized at the study site for at least 24 hours after the end of infusion of study drug. Patients may be discharged upon completion of review by the Investigator of ECG, sodium, potassium, creatinine, albumin, calcium, glucose, phosphate, and LFTs results obtained at 24-hours post-infusion, if results are deemed not clinically significant. Any clinically significant findings must be discussed with the medical monitor to determine whether patient can be discharged and to formulate plans for patient follow-up.

j Continuous cardiac monitoring will be performed via telemetry starting no later than 30 minutes prior to dosing and continuing through 24 hours (+1 hour) post-dose.

k Day 0 pre-dose only, not performed on Day 28.

l Serologies include hepatitis B surface antibody (HbsAb), hepatitis B surface antigen (HbsAg), and anti–hepatitis C virus antibody (anti-HCV Ab).

m If the parameter was assessed and meets eligibility requirements within 72 hours of Days 0 and 28, then it does not need to be repeated pre-dose (Days 0 and 28, respectively).

n Liver function tests include aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase, and bilirubin (total and direct).

o Coagulation studies include prothrombin time (PT), activated partial thromboplastin time (aPTT), and international normalized ratio (INR).

p Lipid panel (non-fasting) includes total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), and triglycerides will be collected on Day 0 only.

q Pre-dose samples for TTR protein, vitamin A, and RBP measurements will be drawn immediately (within 10 minutes) prior to the premedications and immediately prior to dosing.

r A patient will be followed approximately every 2 weeks after Day 56 if their TTR level continues to recover but is not found to have returned to within at least 80% of the baseline value. If this occurs, the patient will be followed and discussed at each SRC meeting until the TTR level returns to within at least 80% of the baseline value.

s Thyroid function tests include thyroid stimulating hormone (TSH), thyroxine (T4), and triiodothyronine (T3).

t A blood sample will be collected for the assessment of complement Bb immediately (within 10 minutes) pre-dose, and 30 (±5) minutes, and 2 (± 15 minutes) and 24 hours (±120 minutes) post infusion. If the patient experiences an infusion reaction, a blood sample for analysis of complement factors should be obtained within 1 hour of the start of the reaction.

u A blood sample for the assessment of tryptase and C3a is to be collected only in the event of an acute infusion reaction: at time of event or as soon as possible after onset, 1 hour, and 24 hours after the event.

v Premedications include 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) will be self-administered per os (PO) the evening before study drug administration. Thirty to 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. Patients enrolled in an optional cohort evaluating the use of an alternative premedication regimen, as agreed upon by the SRC, will receive the following medications at least 60 minutes prior to the start of 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). These patients will not receive any premedications the evening prior to ALN-TTR02 dosing.

w Site personnel are to call patients the day before dosing to remind them to take premedications that evening (the day before dosing). This reminder will not be needed for patients enrolled in optional cohorts evaluating the alternative premedication regimen.

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

y A blood sample for the assessment of cytokines and C-reactive protein (CRP) will be collected immediately (within 10 minutes) pre-dose, and 2 (±15 minutes), 6 (±15 minutes), and 24 hours (±120 minutes) post infusion. If the patient experiences an infusion reaction, a blood sample for analysis of cytokines should be obtained within 1 hour of the start of the reaction.

z For each dose, plasma PK samples (siRNA and lipids) will be collected pre-dose (within 1 hour of planned dosing start), EOI, and then 5, 10, and 30 minutes, and 1, 2, 4, 6, 24 and 48 hours post infusion. Samples will also be collected on Days 7, 14, 21, 35, 42, 49, 56, 112, and 208. Plasma PK on Day 0 at EOI and then 2 hours post-infusion will be analyzed for both free and encapsulated siRNA for cohort 3 and onward, including any optional cohorts. For each post dose PK blood draw, the following sampling windows are allowed: ±1 minute for the 5- and 10-minute draws; ±2 minutes for the 30-minute draws; ±5 minutes for the 1-, 2-, 4-, and 6-hour draws; and ±120 minutes for the 24- and 48-hour draws.

aa For each dose, urine PK samples will be collected pre-dose (within 1 hour of planned dosing start), and from 0-6 hours post-infusion (pooled). Samples will also be collected on Days 7, 14, 21, 35, 42, 49, 56, 112, and 208.

bb Pre-dose samples should be collected prior to infusion, but after premedications have been administered.

ABBREVIATIONS

Abbreviation	Definition
λ_z	Elimination rate constant
AE	Adverse event
ALT	Alanine transaminase
ANC	Absolute neutrophil count
anti-HCV Ab	Anti–hepatitis C virus antibody
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
Bb	Activation fragment of complement fragment B
BMI	Body mass index
BUN	Blood urea nitrogen
C3a	Complement component 3a
CEC	Clinical Events Committee
CFR	Code of Federal Regulations
CL	Systemic clearance
CL_R	Renal clearance
C_{max}	Observed maximum plasma concentration
CPK	Creatine phosphokinase
CPK-MB	Myocardial band of enzymes of creatine phosphokinase
CRF	Case Report Form

Abbreviation	Definition
CRO	Contract research organization
CRP	C-reactive protein
CV	Curriculum vitae
DEHP	di(2-ethylhexyl)phthalate
DLin-MC3-DMA	1,2-Dilinoleyloxy-N,N-dimethylpropylamine
DLT	Dose-limiting toxicity
EC_{50}	50% effective concentration
ECG	Electrocardiogram
EOI	End of infusion
ET	Early termination
EU	European Union
FAC	Familial amyloidotic cardiomyopathy
FAP	Familial amyloidotic polyneuropathy
G-CSF	Granulocyte-colony stimulating factor
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
H1/H2 blocker	Histamine H1/H2 receptor antagonist
HbsAb	Hepatitis B surface antibody
HbsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HDL	High density lipoprotein
HEENT	Head/ears/eyes/nose/throat
HIPAA	Health Insurance Portability and Accountability Act of 1996
HIV	Human immunodeficiency virus
ICF	Informed consent form
ICH	International Conference on Harmonization

Abbreviation	Definition
IEC	Independent Ethics Committee
IFN-α	Interferon-alpha
IFN-γ	Interferon-gamma
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL-1β	Interleukin-1 beta
IL-1 RA	IL-1 receptor antagonist
IL-12	Interleukin-12
IL-6	Interleukin-6
INR	International normalized ratio
IP-10	Interferon-inducible protein-10
IRR	Infusion-related reaction
ITT	Intent-to-treat
IV	Intravenous(ly)
LC/MS/MS	Liquid chromatography/mass spectrometry
LDH	Lactate dehydrogenase
LDL	Low density lipoprotein
LFT	Liver function test
LNP	Lipid nanoparticles
MedDRA®	Medical Dictionary for Regulatory Activities
mRNA	Messenger ribonucleic acid
NHP	Non-human primate (cynomolgus monkey)
NOAEL	No observed adverse effect limit
NOEL	No observable effect level
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

Abbreviation	Definition
PK	Pharmacokinetic(s)
PO	Per os (orally)
PP	Per-protocol
PT	Prothrombin time
PVC	Polyvinyl chloride
QTc	QT interval corrected for heart rate
RBC	Red blood cell
RBP	Retinol binding protein
RNAi	RNA interference
RSV	Respiratory syncytial virus
SAE	Serious adverse event
SaO_2	Arterial oxygen saturation
siRNA	Small interfering ribonucleic acid
SNALP	Stable nucleic acid lipid particles
SRC	Safety Review Committee
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
t_{max}	Time of observed maximum plasma concentration
TNF-α	Tumor necrosis factor-alpha
TSH	Thyroid stimulating hormone
TTR	Transthyretin
ULN	Upper limit of normal
US/USA	United States
USP/EP	United States Pharmacopeia/European Pharmacopoeia

Abbreviation	Definition
VLDL	Very low density lipoprotein
V_{ss}	Volume of distribution at steady state
V_z	Volume of distribution based on the terminal phase
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 AEs in TTR amyloidosis patients treated

with ALN-TTR01 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: familial amyloidotic polyneuropathy (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 (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] 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 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 and non-human primates (NHPs), 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 (ALN-18328) targeting the TTR mRNA.

Unformulated synthetic siRNAs administered by IV injection are rapidly eliminated and, thus, do not achieve significant tissue distribution.[37] Since 95% of TTR is produced in the liver, it was necessary to develop a formulation that could deliver ALN-18328 to the liver. One approach that has been used successfully employs IV delivery of siRNA in liposomal formulations. Recent preclinical studies have demonstrated targeted organ distribution, leading to significantly improved efficacy with lipid particle formulated siRNAs directed against liver-expressed genes.[38,39] Following IV administration, the lipid particles enter the circulation and extravasate into the interstitial fluid. The lipid particles accumulate preferentially in the liver due to their ability to pass through the fenestrated capillaries in that organ. Subsequent uptake of the lipid particles is believed to result from endocytosis of the intact particles, followed by fusion with the endosomal membrane,[40] leading to release of the siRNA into the cytoplasm. The siRNA is then available to interact with the endogenous cellular RNAi machinery to bring about targeted degradation of the mRNA and subsequent reductions in the target protein levels.

ALN-TTR02 consists of ALN-18328, an siRNA designed to suppress production of both mutant and WT TTR, formulated in an 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. The proposed indication for ALN-TTR02 is for the treatment of ATTR. ALN-TTR02 is intended for administration as an IV infusion over 1 hour.

ALN-TTR02, a second generation siRNA LNP formulation termed AF-011, employs the same siRNA (ALN-18328) as ALN-TTR01, which utilizes the first generation LNP (termed stable nucleic acid lipid particles [SNALP]). Both ALN-TTR01 and ALN-TTR02 are intended for the treatment of patients with ATTR; the AF-011 formulation, however, has been optimized to be more potent such that mRNA and protein reduction effects are observed at significantly lower doses with AF-011 than with the SNALP formulation. Furthermore, ALN-PCS02 employs the same AF-011 formulation as ALN-TTR02, but has a siRNA targeting a different liver derived protein, that of PCSK9. Since the overall nonclinical toxicology findings are attributable primarily to the lipid formulation regardless of the encapsulated siRNA, previous non-clinical evaluation (including pharmacology, pharmacokinetics [PK], metabolism, and safety/toxicity), and clinical evaluations of ALN-PCS02 and ALN-TTR01 can provide important data regarding the anticipated safety of ALN-TTR02 (see Section 1.3).

1.2 Summary of ALN-TTR02 Non-Clinical Data

[redacted]

[redacted]

[redacted]

Further information can be found in the ALN-TTR02 Investigator's Brochure and its Expedited Safety Report addendum dated 7 June 2012.

1.3 Summary of Clinical Data with siRNA-LNPs

Clinical trials with multiple different RNAi medicinal products using local or systemic administration in indications including age-related macular degeneration, respiratory syncytial virus (RSV) infection, oncology, and renal failure have been conducted.[28] Alnylam currently has experience with 4 systemic delivery programs in the clinic evaluating the safety and efficacy of different siRNAs in 2 different LNP formulations, including 1 program which employs the same AF-011 formulation as ALN-TTR02. An overview of the safety and pharmacological clinical data with these siRNA-LNPs is included in the ALN-TTR02 Investigator's Brochure (IB), Edition 3, dated 19 October 2012. [redacted]

Importantly, a Phase 1 study with ALN-TTR02 was recently completed. Study ALN-TTR02-001 was a multicenter, randomized, placebo-controlled, single-blind, single-ascending dose clinical study conducted in the UK to evaluate the safety, tolerability, PK, and pharmacodynamic (PD) in healthy volunteers (EudraCT # 2011 005291-42). ALN-TTR02 was administered as a single 60-minute IV infusion to healthy volunteers at the following doses: 10, 50, 150, 300, and 500 μg/kg (4 patients per dose level; 3 receiving ALN-TTR02 and 1 receiving placebo). Patients were premedicated with dexamethasone,

H1 and H2 blockers, and paracetamol prior to dosing to minimize the risk of infusion reaction. The data show that ALN-TTR02 was safe and well-tolerated and exhibited robust effects on serum TTR levels at doses ≥0.15 mg/kg. Lowering of TTR levels was reversible following administration of a single dose, and there were no AEs associated with lowering TTR by >90%. Further details on this study can be found in the ALN-TTR02 IB, Edition 3.

1.4 Study Hypothesis and Rationale

ALN-TTR02 is a novel drug to treat ATTR and consists of a single LNP-formulated siRNA (ALN-18328) 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 FAP, it also underscores the potential advantage of a drug that can lower *both* mutant and WT TTR for treating both the cardiac and neuropathic complications of ATTR.

The ALN-TTR02 nonclinical pharmacology data described in Section 1.2 demonstrate that administration of ALN-TTR02 results in significant suppression of mutant and WT TTR mRNA as well as protein, and that this suppression has the potential to prevent the deposition of TTR in key tissues. 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 a multiple doses of ALN-TTR02 administered to patients with ATTR. Secondary objectives include the characterization of plasma and urine PK for ALN-TTR02 as well as to assess preliminary evidence of the PD effect of ALN-TTR02 on serum total TTR.

1.5 Dose Selection and Rationale

Two consecutive ALN-TTR02 doses, separated by a 4-week period (or a 3-week period in select optional cohorts), will be administered to patients. Study drug will be administered as a 60-minute IV infusion with the following proposed doses for each of the original cohorts: 10, 50, 150, and 300 µg/kg ALN-TTR02. For those patients enrolled in an optional cohort evaluating the alternative premedication regimen and infusion rate, the study drug will be administered IV over approximately 70 minutes.

Based on the preclinical and clinical experience with siRNA-LNPs to date, it appears that the translation of data from animals to humans occurs based on a mg/kg basis. However, taking a more conservative approach, exposures based on allometric scaling as well as body surface area have been estimated, and safety margins were calculated (see Investigator's Brochure). Based on allometric scaling, a 2-fold and 44-fold safety margin was determined for the starting dose in humans (10 μg/kg) [redacted] a similar margin was obtained based on body surface area. Based on body weight, the margin for the proposed human starting dose is 10-fold and 100-fold [redacted]. The top dose proposed in the study (300 μg/kg) is [redacted]. Preliminary safety data are available from ongoing Phase 1 trials with ALN-TTR02 and ALN-PCS02. In the ALN-TTR02-001 Phase 1 trial in healthy volunteers, these same dose levels were found to be safe and well-tolerated. Furthermore, ALN-PCS02, which uses the same second generation LNP formulation as ALN-TTR02, was safe and well-tolerated at doses up to 400 μg/kg in healthy volunteers with elevated cholesterol. See the ALN-TTR02 Investigators Brochure and its Expedited Safety Report addendum dated 7 June 2012 for updated information on the ALN-TTR02 and ALN-PCS02 Phase 1 trials.

Additionally, a conservative dosing approach is being taken. In each cohort, patients will be dosed in a sequential fashion with a minimum of 48 hours elapsing between patients. Collective cohort safety and tolerability data on the 3 patients from the prior cohort through at least 96 hours post-dose will be reviewed by the Safety Review Committee (SRC) prior to approving dosing for the next dose level. In addition, cumulative safety and tolerability data observed in at least 2 patients through at least 96 hours post-second dose of the previous cohort will be reviewed by the SRC prior to administration of the second dose of study drug.

Based on the interim evaluation of safety data, it may also be decided by the SRC that an optional cohort may be utilized which will include 3 additional patients. Dosing in these cohorts may occur at a previously tested dose that was found to be safe and where stopping rules were not met or at an intermediate dose level. Patients in the optional cohort would be dosed and reviewed for safety and tolerability following the same procedures as used for other cohorts. The optional cohort(s) would be included to further confirm the safety and/or PD effect of previously evaluated dose levels, which may include evaluation of the original dosing regimen (once every 4 weeks), an alternative dosing regimen (once every 3 weeks), and/or an alternative premedication regimen and

infusion rate. The dosing regimen for the first optional cohort will be once every 4 weeks, and the dosing regimen for the remaining 4 optional cohorts will be once every 3 or 4 weeks. It is anticipated that the first 2 optional cohorts will use the original premedication regimen, and the remaining 3 optional cohorts may be used to explore the alternative premedication regimen and infusion rate. The SRC will review all available safety data and make recommendations on dosing and premedication regimens prior to dosing of any of the optional cohorts. If this occurs, the Independent Ethics Committees (IECs) will be informed of the implementation of optional cohorts. Up to 5 optional cohorts of 3 patients each are permitted in this study.

The optional cohorts will follow the same treatment schema as cohorts 1 to 4, as outlined in Section 5.6.

1.6 Risk-Benefit Assessment

No benefit is expected from the transient lowering of TTR during this study. Please see Investigator's Brochure for expanded risk/benefit assessment.

1.6.1 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 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 IRRs.[41,42] 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 and ALN-TTR01 clinical studies that employed siRNAs in LNP formulations (Section 1.3).

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 will include dexamethasone (or equivalent), paracetamol (or equivalent), and H1 and H2 blockers (as described in Section 5.5). The infusion rate of 1 hour or longer will also help to reduce the potential for acute IRRs. The suggested clinical course for managing IRRs is provided in Section 5.11 and in Appendix 1 and Appendix 2.

1.6.2 Hepatotoxicity

Alnylam's previous clinical experience with LNPs, either second generation AF-011 or first generation SNALP, in Phase 1 studies suggests that the risk for hepatotoxicity is low. No clinically significant changes in LFTs have been seen to date in the ongoing ALN-VSP02, ALN-TTR01, or ALN-PCS02 Phase 1 trials. In the ALN-TTR02 study, the risk of liver toxicity is lowered by requiring patients to have adequate liver function, which will then be followed closely through serial measurements after dosing.

2 STUDY OBJECTIVES

2.1 Primary Objective

- To evaluate the safety and tolerability of multiple doses of ALN-TTR02.

2.2 Secondary Objectives

- To characterize the plasma and urine PK of ALN-TTR02.
- To assess preliminary evidence of the PD effect of ALN-TTR02 on serum total TTR.

3 STUDY PLAN

3.1 Overall Design

Protocol ALN-TTR02-002 is a multi-national, multi-center, Phase 2, open-label, multi-dose, dose escalation study designed to determine the safety, tolerability, PK, and PD of 2 consecutive doses (separated by approximately 4 weeks) of ALN-TTR02 in patients with ATTR.

Patients of any mutant TTR genotype with a biopsy-proven diagnosis of ATTR who exhibit documented signs/symptoms of the disease (e.g., sensory, motor, or autonomic neuropathy) that are at least mild to moderate in severity will be eligible for the study, provided they have a body mass index (BMI) of 17-33 kg/m^2, an adequate performance status (Karnofsky performance status of 60% or greater; see Appendix 3), adequate hepatic and renal function, no active infection or inflammatory disorder, stable cardiac status, and have not had a liver transplant.

Two doses, separated by 4 weeks, of ALN-TTR02 (10, 50, 150, and 300 µg/kg) will be investigated in 4 sequential cohorts comprised of 3 patients each. No patients will be a member of more than 1 treatment group. An alternative dosing regimen of 2 doses of ALN-TTR02 (at a dose previously determined by the SRC to be safe and tolerable) separated by 3 weeks may be evaluated in the optional cohort(s).

Within each of the cohorts, the first patient will receive their first dose and if the dose is well tolerated (per Section 5.7.3, Dose-limiting Toxicity), Patients 2 and 3 will receive their first dose at that same dose level no sooner than 48 hours apart, with Patient 2 receiving a dose of study drug no sooner than 48 hours after the dosing of Patient 1. Similar to the first dose, the second dose will be administered to patients 4 weeks after the first dose in a sequential manner with at least 48 hours separating dosing of each patient. If after the first dose, ALT and AST are > 2.5 x ULN, that patient would not receive their second dose.

Dose escalation to the next cohort will proceed after the collective safety and tolerability data through at least 96 hours post-first dose from the 3 patients in the previous cohort has been reviewed by the SRC. If the administered dose is found to be safe and well tolerated, dosing for the next cohort will begin no sooner than 96 hours after Patient 3 has safely received dose 1 from the previous cohort. Patients in the cohort would be dosed

and reviewed for safety and tolerability following the same procedures as used for other cohorts (as stated above).

For patients on all dose levels other than the starting dose level of 10 μg/kg, prior to receiving the second dose the SRC will review the cumulative safety and tolerability data of at least 2 patients from the previous dose level(s) with at least 96 hours of follow-up after receiving a second dose of study drug.

For any DLT, accrual to that dose level will stop, all dosing will be discontinued, and dose escalation will end, pending further evaluation of all available safety data by the SRC (see Section 5.7.4). The SRC may be convened earlier at the discretion of Alnylam if important safety issues arise requiring the attention of the committee (e.g., new safety information attained in other ongoing studies with ALN-TTR02).

The duration of patient participation in this study is approximately 36 weeks. Patients will be screened from -45 to -3 days prior to dose administration. Eligible patients will undergo further pre-treatment assessments (performed on Day 0). Patients receiving the original premedication regimen will receive oral premedication with dexamethasone (or equivalent), paracetamol (or equivalent), and H1 and H2 blockers the night before and 30 to 60 minutes prior to each dose of ALN-TTR02 to reduce the potential of an IRR (see Section 5.5). Those patients in an optional cohort evaluating the alternative premedication regimen, as agreed upon by the SRC, will receive IV dexamethasone (or equivalent), oral paracetamol (or equivalent), and IV H1 and H2 blockers at least 60 minutes prior to ALN-TTR02 dosing; no premedication will be administered the evening prior to dosing. On Days 0 and 28, patients will receive a single dose of ALN-TTR02 administered as a 60-minute IV infusion (for cohorts with the original premedication regimen and infusion rate), or as an approximate 70-minute IV infusion, for those patients in an optional cohort evaluating the alternative premedication regimen and infusion rate. 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). Details on the study drug administration are provided in Section 5.6. Patients will be hospitalized at the study site for at least 24 hours after the end of the study drug infusion. Patients may be discharged upon Investigator review of the 24 hour ECG, LFTs, and a subset of serum chemistries (sodium, potassium, creatinine, albumin, calcium, glucose, and phosphate), if results are

deemed not clinically significant. Patients will return to the site for out-patient visits for safety, PK, and PD monitoring up to 208 days post-dose (see Section 6 for details).

The SRC will evaluate safety in the study and determine if it remains acceptable to dose escalate or administer the second dose to the next dose level per their safety review charter. To ensure timely safety information exchange across the participating study centers, the SRC will be comprised of all Principal Investigators (PIs) participating in the study or their designee, the Alnylam Medical Monitor, and the Contract Research Organization (CRO) Medical Monitor. The SRC will communicate frequently to ensure that patients are dosed according to the time intervals specified in Section 5.6, even as patients are being accrued across multiple centers.

3.2 Safety Assessments

Safety monitoring will include assessment of AEs, 12-lead ECGs, cardiac monitoring (telemetry), arterial oxygen saturation (SaO_2) using pulse oximetry, vital signs (blood pressure, pulse rate, oral body temperature, and respiratory rate), clinical laboratory safety tests (hematology, serum chemistry, LFTs, thyroid function parameters, serology, coagulation parameters, urinalysis, cytokines, CRP, and complement factors), 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 of ALN-TTR02 in patients with ATTR will be evaluated by serial measurement of serum concentrations of serum total TTR levels.

3.4 Pharmacokinetic Assessments

The PK evaluation will include plasma-concentration time profiles for siRNA ALN-18328 and the novel lipid components DLin-MC3-DMA and PEG_{2000}-C-DMG. ALN-18328 concentration will be determined at specified time points post-dose. An assessment of plasma concentrations of free and encapsulated siRNA will be determined at specified time points. PEG_{2000}-C-DMG and DLin-MC3-DMA will be determined at specified time points up to 180 days post-dose. Renal clearance (CL_R) will be determined for ALN-18328 and DLin-MC3-DMA and/or its metabolites excreted in the urine.

3.5 Other Assessments

Serum levels of mutant vs wild-type TTR protein, TTR mRNA, and vitamin A and RBP will be assessed in patients with ATTR.

4 PATIENT POPULATION

4.1 Eligibility of Patients

Based on the planned dose escalation scheme (see Section 5.7.1), up to 27 patients are expected to be enrolled. All centers have been selected on the basis of their extensive experience with treatment of patients with ATTR, 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 period (Day -45 to Day -3) to be enrolled in the study:

5. Male or female aged 18 years or older.
6. Patient has biopsy-proven diagnosis of TTR amyloidosis with documented signs/symptoms of the disease (e.g., sensory, motor, or autonomic neuropathy) that are at least mild to moderate in severity.
7. Body mass index of 17–33 kg/m^2.
8. Patient has a Karnofsky performance status of 60% or greater (see Appendix 3).
9. Patient has an absolute neutrophil count (ANC) ≥1500 cells/mm^3, platelet count ≥100,000 cells/mm^3, and hemoglobin ≥10 g/dL.
10. Patient has adequate liver function, demonstrated by an aspartate transaminase (AST) and alanine transaminase (ALT) ≤2.5 × the upper limit of normal (ULN), total bilirubin within normal limits, albumin >3 g/dL, and international normalized ratio (INR) ≤1.2.
11. Patient has adequate renal function: serum creatinine ≤1.5 × ULN.
12. Patient is seronegative for hepatitis B virus (HBV) and hepatitis C virus (HCV).
13. Women of child-bearing potential must have a negative pregnancy test, cannot be breast feeding, and must be using two highly effective methods of contraception prior to screening, throughout study participation, and for 1 month after ending study participation. 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.

14. Males agree to use appropriate contraception throughout study participation and for 1 month after ending study participation.
15. Patient is willing and able to comply with protocol-required visit schedule and visit requirements and provide written informed consent.

4.3 Exclusion Criteria

Patients meeting any of the following criteria within the Screening period (Day -45 to Day -3) will be excluded from the study:

1. Patient is pregnant or nursing.
2. Patient has had a liver transplant.
3. Has a known surgery planned during any point of the study period.
4. Patient has known human immunodeficiency virus (HIV) positive status.
5. Has a known or suspected systemic bacterial, viral, parasitic, or fungal infection.
6. Patient received an investigational agent, other than tafamidis or diflunisal, within 30 days prior to first dose study drug administration.
7. Patient has a New York Heart Association heart failure classification >2 (see Appendix 5).
8. Patient has unstable angina.
9. Patient has uncontrolled clinically significant cardiac arrhythmia.
10. Patient is considered unfit for the study by the Principal Investigator.
11. Patient had a prior severe reaction to a liposomal product.
12. Patient has known hypersensitivity to oligonucleotides.
13. Patient is an employee or family member of Alnylam, the CRO, or the clinical study site personnel.

4.4 Assignment to Dose Cohort/Patient Number

4.4.1 Assignment to Dose Cohort

After confirmation of eligibility during screening, patients satisfying the entry criteria will be assigned to the cohort currently enrolling. Each cohort will comprise 3 patients. No patients will be a member of more than 1 treatment group.

A unique patient identification number will be assigned sequentially after the patient has completed all of the screening procedures and is determined to be eligible for the study. If applicable, replacement patients will receive the same treatment allocation as those whom they replace.

4.4.2 Blinding Procedures

Not applicable.

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 receives both doses of study medication and completes protocol-specified procedures up through Day 56.

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 AE (including infusion reactions).
- Experiences a DLT (see Section 5.7.3).
- 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.

The Investigator will also withdraw the patient from the study upon the request of Alnylam or the SRC, or if Alnylam terminates the study. Upon occurrence of a serious or intolerable AE, the Investigator will confer with Alnylam before discontinuing the patient, and the SRC will be notified. A patient may withdraw consent to participate in the study at any time.

4.5.2 Handling of Withdrawals

Patients are free to withdraw from the study upon request. Patient participation in the clinical study may be terminated at any time at the discretion of the Investigator or Alnylam.

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

Patients who voluntarily withdraw are termed dropouts. Dropouts may be replaced following discussion with the Investigator and Alnylam (see Section 4.5.3).

Patients who are withdrawn due to AEs during infusion of the ALN-TTR02 will not be replaced.

If a patient is withdrawn/withdraws the discharge procedures should be performed and an early termination (ET) visit scheduled as follows:

- If a patient is withdrawn/withdraws prior to Day 56 then the Day 56 assessments should be completed;
- If a patient is withdrawn/withdraws after Day 56 and prior to Day 208 then the Day 208 assessments should be completed.

If a patient is withdrawn due to an AE, appropriate medical care should be provided and the AE should be followed through 56 days post last dose. Follow-up procedures should be conducted as scheduled.

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 case report form (CRF) and all efforts will be made to complete and report the observations as thoroughly as possible.

4.5.3 Patient Replacement

Any patient who misses a dose, does not receive 80% of each ALN-TTR02 dose, or who discontinues from the study for a non-safety related issue, as determined by the SRC,

prior to completing the 56-day study period may be replaced at the advice of the SRC and the discretion of Alnylam.

5 STUDY MEDICATION

5.1 Presentation of Study Drug

ALN-TTR02 Solution for Injection is an RNAi therapeutic consisting of drug substance ALN-18328 (siRNA targeting TTR mRNA) formulated in LNPs. The ALN-TTR02 drug product is a sterile formulation of ALN-18328 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 drug substance ALN-18328.

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 assembly and labelling according to separate IMP handling instructions and allocating treatments to the patients.

The pharmacist will prepare the study drug under aseptic conditions. The amount (in μg) of ALN-TTR02 to be administered will be determined based on the patient's weight (kg) and the dose cohort they are assigned to. For the first dose, the weight obtained pre-dose on Day 0 will be used to calculate the dose of study drug. The patient's weight measured pre-dose on Day 28 will be used to calculate the second dose of study drug. Subjects who weigh 105 kg or more will receive ALN-TTR02 dosing based on an assumption of a body weight of 104 kg.

Approximately 200 mL of 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 location and may be dispensed only by the Investigator or 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, protected from light in the storage area of the investigational

site pharmacy, in a secure, temperature controlled, locked environment with restricted access. Any deviation from the recommended storage conditions should be reported to 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 preparation details are provided in the ALN-TTR02 Pharmacy Manual.

5.4 Labeling and Packaging of Study Drug

All packaging and labelling as well as the production of study medication 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 for use at study centers 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

All patients will be premedicated prior to dosing with ALN-TTR02 to reduce the potential for an infusion reaction.

Patients receiving the original premedication regimen will be administered the following:

- Oral dexamethasone (8 mg) or equivalent administered the evening before dosing and 20 mg 30 to 60 minutes prior to start of infusion of ALN-TTR02;
- Oral paracetamol (500 mg) or equivalent the evening before dosing and 30 to 60 minutes prior to the start of infusion of ALN-TTR02;
- Oral H2 blocker (e.g., ranitidine 150 mg, famotidine 20 mg, or equivalent other H2 blocker dose) the evening before dosing and 30 to 60 minutes prior to start of infusion of ALN-TTR02;
- Oral H1 blocker, 10 mg cetirizine (hydroxyzine 25 mg, fexofenadine, or equivalent may be substituted if patient does not tolerate cetirizine) the evening before dosing and 30 to 60 minutes prior to start of infusion of ALN-TTR02.

Staff at each investigative site should make every effort to contact the patient by telephone the day prior to being administered the study drug (Days -1 and 27) to remind the patient of the premedication regimen.

An alternative premedication regimen and infusion rate can be used in select optional cohorts, as agreed upon by the SRC. The dosing regimen for the first optional cohort will be once every 4 weeks, and the dosing regimen for the remaining 4 optional cohorts will be once every 3 or 4 weeks. It is anticipated that, the first 2 optional cohorts will use the original premedication regimen and the remaining 3 optional cohorts may be used to explore the alternative premedication regimen and infusion rate. Patients in these cohorts will not receive any premedications the evening prior to ALN-TTR02 dosing. Prior to each dose of ALN-TTR02, these patients will receive the following premedications:

- Intravenous (IV) dexamethasone (10 mg) or equivalent, administered at least 60 minutes prior to the start of infusion of ALN-TTR02;
- Oral paracetamol (500 mg) or equivalent at least 60 minutes prior to the start of 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 start of infusion of ALN-TTR02; and
- Intravenous H1 blocker: diphenhydramine 50 mg IV (or equivalent other IV H1 blocker available at the study site) at least 60 minutes prior to start of 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 blockers.

Further details can be found in the Study Reference Manual.

5.6 Dose, Route, and Schedule of Study Drug Administration

Consented patients meeting the eligibility criteria will be enrolled into sequential cohorts of increasing doses of ALN-TTR02. Each cohort will consist of 3 patients.

Study drug will be administered under the supervision of the Investigator or designee. Study drug doses will be administered as a 60-minute IV infusion (flow rate of approximately 3.3 mL/min) 4 weeks apart. The dosing of ALN-TTR02 in the optional cohorts will be once every 4 weeks for the first optional cohort and once every 3 or 4 weeks for the remaining 4 optional cohorts, at a dose previously determined by the SRC to be safe and tolerable. Study drug will be administered via a controlled infusion device

with an extension set containing a 1.2 micron filter supplied by Alnylam or designee. Infusion products containing polyvinyl chloride, di(2-ethylhexyl)phthalate (PVC, DEHP) must NOT be used. For patients enrolled in an optional cohort evaluating the alternative premedication regimen, the flow rate will be approximately 1.1 mL/min during the first 15 minutes (1/3rd the original flow rate) with the remainder of the infusion taking place over 55 minutes (at a flow rate of approximately 3.3 mL/min) for a total infusion time of approximately 70 minutes.

The patient's infusion site will be checked pre-dose, during infusion, and after infusion for any safety concerns (e.g., infusion-associated reactions). 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). Patients will remain hospitalized at the study site for at least 24 hours following completion of dosing on Day 0 and Day 28 to be observed for safety assessments and PK sampling.

The planned study drug doses are 10, 50, 150, and 300 μg/kg.

Subjects who weigh 105 kg or more will receive ALN-TTR02 dosing based on an assumption of a body weight of 104 kg.

Prior to administration of the second dose of ALN-TTR02, the inclusion and exclusion criteria must be reassessed to ensure that the patient still qualifies for participation in the study.

The planned study drug doses and patient cohorts are identified in Table 5-1. Dose escalation procedures are described in Section 5.7.1.

Table 5-1: Planned Dose Cohorts and Enrollment

Patient Cohort	ALN-TTR02 Dose (µg/kg)	Initial Enrollment	Total Enrollment[a]
1	10	3	Up to 27 patients
2	50	3	
3	150	3	
4	300	3	

a Up to 5 optional cohorts may be included to confirm the safety, tolerability, and/or PD effect of previously evaluated dose levels, which may include evaluation of the original dosing regimen (once every 4 weeks), an alternative dosing regimen (once every 3 weeks), and/or an alternative premedication regimen and infusion rate at a specific dose of ALN-TTR02. Dosing in these optional cohorts may occur at a previously tested dose that was found to be safe and where stopping rules were not met or at an intermediate dose level. Each optional cohort will be comprised of 3 patients.

5.7 Criteria for Dose Escalation, Dose Modification or Discontinuation of Study Drug

5.7.1 Dose Escalation Procedures

The initial dose of ALN-TTR02 is 10 µg/kg. Dose escalation to 50, 150, and 300 µg/kg is planned.

Within each of the cohorts, the first patient will receive their first dose and if the dose is well tolerated, per Section 5.7.3 (Dose-limiting Toxicity), Patients 2 and 3 will receive their first dose at that same dose level no sooner than 48 hours apart, with Patient 2 receiving a dose of study drug no sooner than 48 hours after the dosing of Patient 1. Similar to the first dose, the second dose will be administered to patients 4 weeks after the first dose in a sequential manner with at least 48 hours separating dosing of each patient. If after the first dose, ALT and AST are > 2.5 x ULN, that patient would not receive their second dose. Dose escalation to the next cohort will proceed after the collective safety and tolerability data through at least 96 hours post-first dose from the 3 patients in the previous cohort has been reviewed by the SRC. If the administered dose is found to be safe and well tolerated, dosing for the next cohort will begin no sooner than 96 hours after Patient 3 has safely received dose 1 from the previous cohort. Patients in the cohort would be dosed and reviewed for safety and tolerability following the same procedures as used for other cohorts (as stated above).

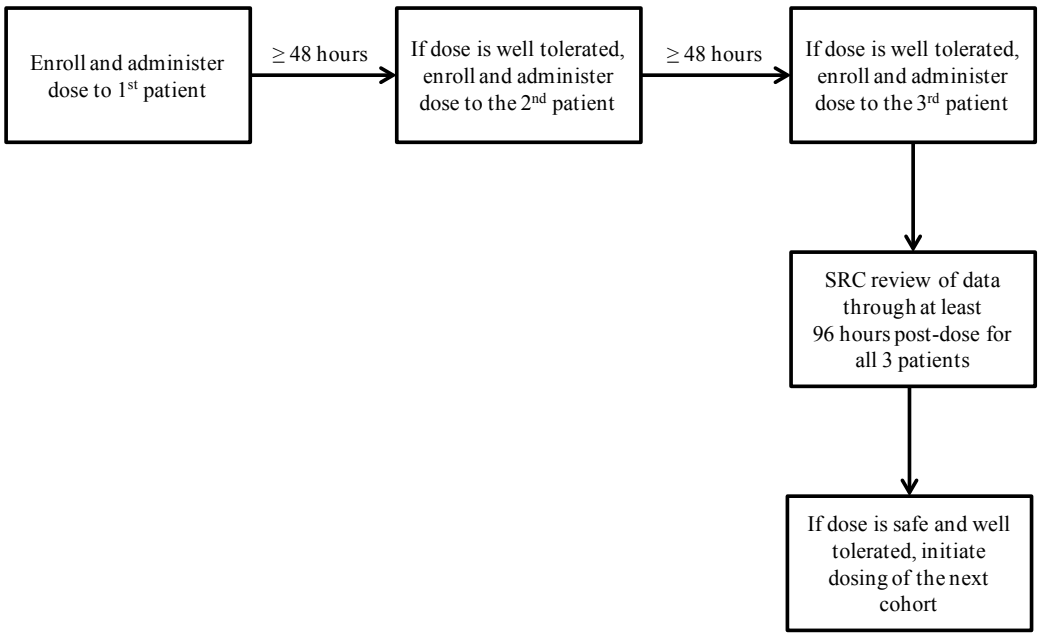
For patients on all dose levels other than the starting dose level of 10 μg/kg, prior to receiving the second dose the SRC will review the cumulative safety and tolerability data of at least 2 patients from the previous dose level(s) with at least 96 hours of follow-up after receiving a second dose of study drug.

The dosing of patients within each of the first 4 cohorts is depicted in

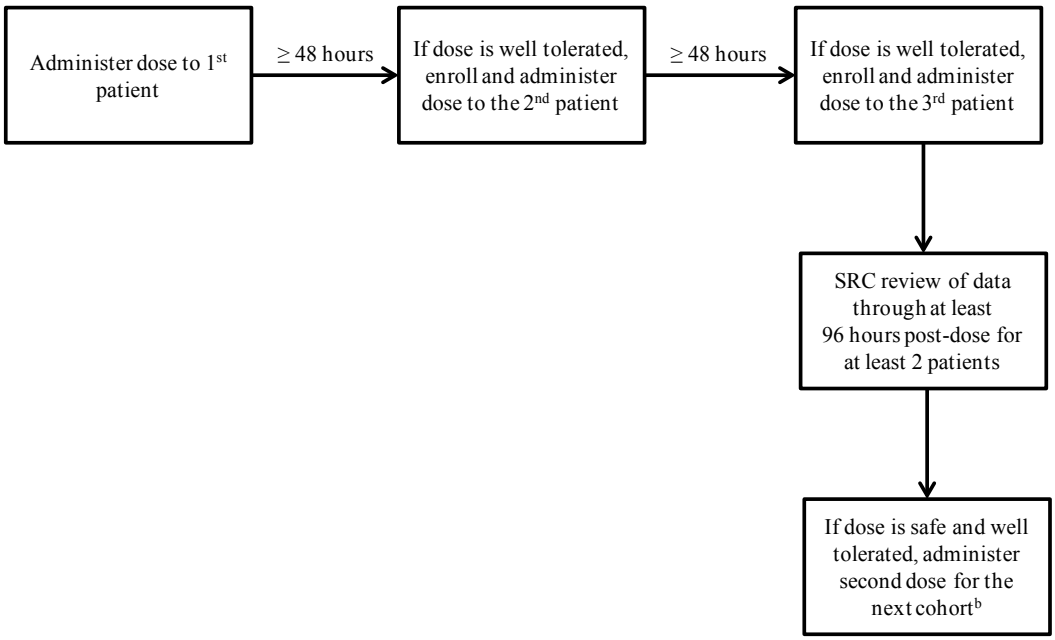
.

Figure 5-1: Scheme for Patient Enrollment and SRC Review Within and Between Each Patient Cohort

A. First Dose



B. Second Dose (4 weeks after first dose)[a]



a Some optional cohorts may have the second dose of ALN-TTR02 administered 3 weeks after the first dose.

b Dosing of next cohort will follow the same schema as outlined in A with the next higher dose level.

Dose-limiting toxicities are described further in Section 5.7.3, and study stopping rules are described in Section 5.7.4.

5.7.2 Optional Cohorts

Based on the interim evaluation of safety data, it may also be decided by the SRC that an optional cohort may be utilized which will include 3 additional patients. Dosing in these optional cohorts may occur at a previously tested dose that was found to be safe and where stopping rules were not met or at an intermediate dose level. Patients in the optional cohort would be dosed and reviewed for safety and tolerability following the same procedures as used for other cohorts. The optional cohort would be included to further confirm the safety and/or PD effect of previously evaluated dose levels, which may include evaluation of the original dosing regimen (once every 4 weeks), an alternative dosing regimen (once every 3 weeks), and/or to evaluate an alternative premedication regimen and infusion rate. The dosing regimen for the first optional cohort will be once every 4 weeks, and the dosing regimen for the remaining 4 optional cohorts will be once every 3 or 4 weeks. It is anticipated that the first 2 optional cohorts will use the original premedication regimen, and the remaining 3 optional cohorts may be used to explore the alternative premedication regimen and infusion rate. The SRC will review all available safety data and make recommendations on dosing and premedication regimens prior to dosing of any of the optional cohorts. If this occurs, the IECs will be informed of the implementation of optional cohorts. Up to 5 optional cohorts of 3 patients each are permitted in this study.

5.7.3 Dose-limiting Toxicity

A DLT is defined as:

- Any life-threatening toxicity;
- ALT and AST levels ≥5 × ULN or total bilirubin >2.0 mg/dL;
- An infusion reaction that requires hospitalization, despite premedication;
- Any other toxicity which in the opinion of the SRC would have precluded further dosing.

5.7.4 Stopping Criteria

For any DLT, accrual to that dose level will stop, all dosing will be discontinued, and dose escalation will end, pending further evaluation of all available safety data by the SRC.

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 center personnel. Because the study drug will be administered by study staff as an IV infusion, treatment compliance will be verified by observation in the clinic by study staff. Treatment will be considered completed if 80% or more of each of the doses (based on total volume of the IV solution) has been administered to the patient.

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 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 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 to a patient and returned unused must not be redispensed to a different patient.

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, tafamidis, or diflunisal;
- 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, pre-dose and post-dose up to Day 56/ET 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. A blood sample should be obtained for analysis of tryptase, complement factors (including C3a), and cytokines at the time of the event, or as soon as possible after onset, 1 hour, and 24 hours after the start of the reaction. In addition, if the infusion is stopped and the site is considering restarting, a PK blood sample will be taken when the infusion is stopped and this will be treated as the EOI sample if the infusion is restarted.

Drugs that may be used to facilitate resolution and permit resumption of study drug administration include, but are not limited to: paracetamol (or equivalent), additional H1/H2 blockers (e.g., ranitidine), non-steroidal anti-inflammatory [NSAID], adrenaline, supplemental oxygen, IV fluids, and corticosteroids. For moderate infusion reactions (see Appendix 1), resumption of study drug administration at a slower infusion rate (but not to exceed 3 hours) may be considered at the Investigator's discretion. Study drug administration will not be resumed for any patient following a severe infusion reaction. 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.

Infusion reactions may have a delayed onset following the completion of the infusion. The medical management of a delayed reaction (e.g., use of corticosteroids, etc.) would follow similar guidelines as provided above for acute infusion reactions (see Appendix 2).

After dosing, patients will be discharged from the hospital no sooner than 24 hours post-dose. Prior to discharge, the 24-hour ECG and local serum chemistries and LFTs must be reviewed by the Investigator as described in Section 3.1. Patients will be discharged with directions on self-administration of medications as needed to ameliorate potential delayed reactions to study drug infusion, such as fever, chills, and myalgia. 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.

6 STUDY VISITS

The duration of a patient's participation in this study is approximately 36 weeks. This duration may be extended should optional cohorts with dosing intervals greater than 4 weeks be included.

An open-label extension study at the recommended Phase 3 dose and regimen (as derived from Study ALN-TTR02-002) is planned, which will enable the patients who enrolled in Study ALN-TTR02-002 to receive additional, long-term dosing and safety follow-up. For some of the patients, this may preclude the completion of all of the follow-up period assessments of Study ALN-TTR02-002 if they are deemed eligible to participate in the extension study prior to completion of the full follow up through Day 208. Such patients will be followed up for a minimum of 28 days after their last dose in the current ALN-TTR02-002 study prior to being enrolled in the extension study. The extension study will be implemented only after dose escalation is completed in Study ALN-TTR02-002, with post second dose follow-up through Day 208 in at least 1 cohort at the highest dose.

Screening evaluations are to be performed within 45 days before receiving the first dose of study drug, as indicated in Table 1-1 (Appendix 4 provides the schedule of assessments for the optional cohort(s) administered ALN-TTR02 once every 3 weeks). Patients determined to be eligible based on screening assessments will receive treatment (IV infusion of study drug) on Days 0 (Baseline) and 28 (or date of second dose administration in optional cohorts with dosing intervals greater than 4 weeks); for patients in optional cohorts evaluating the alternative dosing regimen (once every 3 weeks), ALN-TTR02 will be administered on Days 0 (Baseline) and 21. Patients will remain hospitalized at the study site for at least 24 hours following completion of dosing to be observed for safety assessments and PK sampling. Patients will return to the study site on Days 2, 7, 10, 14, and 21 for follow-up assessments. In addition, follow-up visits after the second dose of study drug (Day 28) will occur on Days 30, 35, 38, 42, 49, and 56 (end-of-study); patients in optional cohorts evaluating the alternative dosing regimen (once every 3 weeks) will have follow-up visits on Days 21, 22, 23, 28, 31, 35, 42, 49, and 56. Patients will also return to the site for a follow-up visit on Days 112 and 208.

If necessary, visiting nurses will be allowed to conduct visits after Day 2 (but not Day 208) that do not require physical examinations. All patients who discontinue the study before Day 56 will return to the study site for their Early Termination visit for Day 56 assessments. All patients who discontinue the study after Day 56 and prior to

Day 208 will return to the study site for their Early Termination visit for Day 208 assessments.

6.1 Screening Visit (Day -45 to -3)

Screening evaluations will be conducted 3 to 45 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.

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

The following activities should be performed during the Screening visit:

- Obtain demographic information.
- Obtain medical detailed history information.
- Assess study eligibility using the inclusion and exclusion criteria (e.g., calculate BMI).
- Obtain concomitant medications information.
- Perform a physical examination.
- Obtain measurements of weight and height.
- Measure vital signs.
- Perform an echocardiogram if a normal echocardiogram has not been obtained within the past 90 days.
- Perform a 12-lead ECG.
- Collect blood samples for clinical laboratory tests, including:
    - Hematology.
    - Serum chemistries.
    - Liver function tests.
    - Coagulation studies.
    - Anti-PEG antibodies (IgG and IgM; immunoglobulin G and immunoglobulin M, respectively).

- Hepatitis B/C status (serologies include hepatitis B surface antibody [HbsAb], hepatitis B surface antigen [HbsAg], and anti–hepatitis C virus antibody [anti-HCV Ab]).
- Serum TTR protein, vitamin A, and RBP. Additionally, aliquots of serum samples will be taken and frozen, to permit testing of additional proteins related to ATTR.
- TTR mRNA.
- Thyroid function tests.
- Serum pregnancy test (women of child-bearing potential only).

- Collect urine sample for urinalysis.

6.2 Pre-Dosing (Day -1 or Day 27); Day -1 or Day 20 for optional cohorts dosed once every 3 weeks

For those patients receiving the original premedication regimen, on the day prior to administration of study drug (Days -1 and 27), study site personnel will contact the patient by phone to discuss the pretreatment medication they are to take that evening.

On the evening before each dosing, patients are to self-administer the following medications PO: 8 mg dexamethasone or equivalent, 500 mg paracetamol or equivalent, an H1 blocker (10 mg cetirizine hydroxyzine [25 mg, fexofenadine, or equivalent may be substituted if patient does not tolerate cetirizine]), and an oral H2 blocker (e.g., ranitidine 150 mg, famotidine 20 mg, or equivalent other H2 blocker).

Patients in an optional cohort evaluating the alternative premedication regimen and infusion rate will not receive any premedication the evening prior to ALN-TTR02 dosing.

6.3 Treatment Visits

6.3.1 Day 0 or Day 28 (+2 days); Day 0 or Day 21 for optional cohorts dosed once every 3 weeks

6.3.1.1 Pre-dose

Patients who are determined to be eligible for the study, based on screening evaluations, will be enrolled on Day 0 and assigned to the appropriate dose cohort (see Section 4.4.1).

Patients will undergo the following procedures before study drug administration on Day 0 or Day 28 (Day 0 or Day 21 for optional cohorts dosed once every 3 weeks):

- Reassess study eligibility using the inclusion and exclusion criteria.
- Obtain updated medical history information.

- Conduct a focused physical examination (includes head/ears/eyes/nose/throat [HEENT], cardiovascular, respiratory, abdominal and hepatic assessments), including weight. If the screening physical examination was performed within 72 hours of Day 0, then it does not need to be repeated; however, the patient's weight will need to be obtained. For the first dose of study drug, this weight assessment (Day 0) will be used by the Pharmacy in the preparation of the patient's first dose.
  - Calculate BMI.
  - Measure height.
- Perform a urine pregnancy test (women of child-bearing potential only), the results of which must be known prior to the administration of study drug.
- Obtain measurements of:
  - Vital signs
  - Twelve-lead ECG.
  - Pulse oximetry.
- Collect blood samples for clinical laboratory tests (if the screening studies were performed within 72 hours of Day 0, then these laboratory tests need not be repeated), including:
  - Hematology.*
  - Serum chemistries.*
  - Liver function tests.*
  - Coagulation studies.*
  - Anti-PEG antibodies (IgG and IgM).
  - Thyroid function tests.*
  - Lipid panel (Day 0 only).
  - Complement Bb (within 10 minutes predose).
  - Cytokines (within 10 minutes predose).
  - CRP (within 10 minutes predose).

  Note: those parameters marked with an asterisk do not have to be reassessed on Days 0 or 28 (Days 0 or 21 for optional cohorts dosed once every 3 weeks) if measures are obtained within 72 hours prior to dosing and meets eligibility criteria.
- Collect urine sample for urinalysis.*
- Collect blood sample for PK pre-dose (within 1 hour of planned dosing start).

- Collect urine sample for PK pre-dose (within 1 hour of planned dosing start).
- Obtain concomitant medications information.
- Collect blood samples for TTR, Vitamin A, and RBP immediately (10 minutes) prior to and after administration of oral premedications.
- Collect blood sample for TTR mRNA.
- For patients receiving the original premedication regimen, premedicate the patient 30 to 60 minutes prior to the start of study drug infusion with the following oral medications (or equivalent[s]): 20 mg dexamethasone, 500 mg paracetamol, 10 mg cetirizine, and an H2 blocker (e.g. 150 mg ranitidine, 20 mg famotidine). Patients in select cohorts evaluating the alternative premedication regimen and infusion rate will receive the following medications (or equivalent[s]) at least 60 minutes prior to the start of ALN-TTR02 infusion: IV dexamethasone 10 mg, PO paracetamol 500 mg, 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 other IV H1 blocker available at the study site; or hydroxyzine or fexofenadine 25 mg PO or cetirizine 10 mg PO for any patient who does not tolerate IV diphenhydramine or other IV H1 blockers).
- Aliquots of serum samples for testing of exploratory biomarkers should be collected prior to infusion, but after premedications have been administered.
- Assessment of the infusion site.
- Initiate cardiac monitoring (telemetry) 30 minute pre-dose.

6.3.1.2 Administration of Study Drug

After completion of all pre-dose evaluations and procedures, administer study drug as a 60-minute IV infusion (or an approximate 70-minute IV infusion for patients in an optional cohort evaluating the alternative premedication regimen and infusion rate) via a controlled infusion device. Continue to assess the infusion site for signs of any localized reaction through 30 minutes post-infusion.

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, 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.

Prior to administration of second dose of study drug, patients must be reassessed to confirm they continue to meet eligibility criteria.

6.3.1.3 Post-dose

Patients will undergo the following procedures after study drug administration on Day 0 and Day 28 (+2 days); or Day 0 and Day 21 for optional cohorts dosed once every 3 weeks. Patients will remain hospitalized at the study site for at least 24 hours following completion of dosing, to be observed for safety assessments and PK sampling. Unless otherwise specified, for non-PK assessments performed 30 minutes post-infusion, a window of ±5 minutes is allowed, a window of ±15 minutes is allowed for assessments performed between 1 and 3 hours, and a window of ±30 minutes for 6 through 18 hours post-infusion.

- Obtain serial measurement of vital signs at the end of infusion (EOI) and at 30 minutes and 1, 2, 3, 6, 12, and 18 hours post-infusion.
- Perform serial 12-lead ECGs in triplicate at EOI and at 30 minutes, and 2 and 4 (±15 minutes) hours post-infusion.
- Continue cardiac monitoring (telemetry) through 24 (+1) hour post-dose.
- Perform serial pulse oximetry at EOI and at 30 minutes and 1, 2, 3, 6, 12, and 18 hours post-infusion.
- Collect blood samples for clinical laboratory tests, including:
  - Complement Bb at 30 minutes and 2 (±15) hours post-infusion.
  - CRP at 2 and 6 (±15 minutes) hours post-infusion.
  - Cytokines at 2 and 6 (±15 minutes) hours post-infusion.
  - Pharmacokinetic analyses; EOI, 5, 10, and 30 minutes; and 1, 2, 4, and 6 hours post-infusion. The following windows are allowed for PK sampling: ±1 minute for the 5- and 10-minute draws; ±2 minutes for the 30-minute draw; ±5 minutes for the 1-, 2-, 4-, and 6-hour draws.
- Collect urine for PK from 0-6 hours post-infusion.
- If an infusion reaction occurs, collect a blood sample for tryptase, complement factors (including C3a), and cytokine testing at the time of the event, or as soon as possible after onset and 1 hour after the start of the reaction.
- Document any AEs, beginning with the initiation of the infusion.
- Obtain concomitant medications information.

Patients will remain in the hospital for at least 24 hours.

6.3.2 Day 1 or Day 29; Day 1 or Day 22 for optional cohorts dosed once every 3 weeks

Patients will undergo the following procedures on the day following dosing with study drug (Days 1 or 29); or Days 1 or 22 for optional cohorts dosed once every 3 weeks:

- Perform focused physical examination.
- Measure serial vital signs 24 (+30 minutes) hours post-infusion.
- Perform serial pulse oximetry at 24 (+30 minutes) hours post-infusion.
- Perform serial 12-lead ECGs in triplicate at 24 (+30 minutes) hours post-infusion.
- Conclude cardiac monitoring (telemetry) at 24 (+1) hours post-infusion.
- Collect blood samples for clinical laboratory tests, including:
  - Hematology.
  - Serum chemistries.
  - Liver function tests.
  - Coagulation studies.
- Collect blood samples for the following assessments:
  - Complement Bb, 24-hour (±120 minutes) post-infusion.
  - CRP, 24-hour (±120 minutes) post-infusion.
  - Cytokines, 24-hour (±120 minutes) post-infusion.
  - 24-hour (±120 minutes) PK sample.
  - Serum TTR protein, TTR mRNA, vitamin A, and RBP. Additionally, aliquots of serum samples will be taken and frozen, to permit testing of additional proteins related to ATTR.
  - For patients who experience an infusion reaction, collect a blood sample for tryptase, complement factors (including C3a), and cytokine testing 24 hours after the start of the reaction.
- Collect urine sample for urinalysis.
- Collect urine sample for 24-hour PK analysis.
- Document any AEs.
- Obtain concomitant medications information.

Patients may be discharged upon completion of review by the Investigator of ECG, sodium, potassium, creatinine, albumin, calcium, glucose, phosphate , and LFTs results obtained at 24-hours post-infusion, if results are deemed not clinically significant. Any

clinically significant findings must be discussed with the medical monitor to determine whether patient can be discharged and to formulate plans for patient follow-up.

6.3.3 Day 2 or Day 30; Day 2 or Day 23 for optional cohorts dosed once every 3 weeks

Patients will undergo the following procedures on Days 2 and 30; or Days 2 and 23 for optional cohorts dosed once every 3 weeks:

- Perform focused physical examination.
- Measure vital signs.
- Collect blood samples for the following tests:
  - Liver function tests.
  - Serum TTR protein, TTR mRNA, vitamin A, and RBP. Additionally, aliquots of serum samples will be taken and frozen, to permit testing of additional proteins related to ATTR.
  - 48-hour (±120 minutes) PK sample.
- Collect urine sample for PK analysis.
- Document any AEs.
- Obtain concomitant medications information.

6.3.4 Day 7 or Day 35 ±1 day; or Day 7 or Day 28 for optional cohorts dosed once every 3 weeks)

Patients will undergo the following procedures on the Day 7 and 35 study visits (or Day 7 and 28 for optional cohorts dosed every 3 weeks):

- Collect blood samples for the following tests:
  - Liver function tests.
  - Serum TTR protein, vitamin A, and RBP. Additionally, aliquots of serum samples will be taken and frozen, to permit testing of additional proteins related to ATTR.
  - Anti-PEG antibodies (IgG and IgM).
  - Pharmacokinetic assessments.
- Collect urine sample for PK assessments.
- Document any AEs.
- Obtain concomitant medications information.

6.3.5 Day 10 or Day 38 ±2 days; or Day 10 or Day 31 for optional cohorts dosed once every 3 weeks

Patients will undergo the following procedures the Day 10 and 38 study visits (or Days 10 and 28 for optional cohorts dosed every 3 weeks):

- Collect blood samples for the following assessments:
  - Serum TTR protein, vitamin A, and RBP. Additionally, aliquots of serum samples will be taken and frozen, to permit testing of additional proteins related to ATTR.
- Document any AEs.
- Obtain concomitant medications information.

6.3.6 Day 14 or Day 42 ±3 days; or Day 14 and Day 35 for optional cohorts dosed once every 3 weeks

Patients will undergo the following procedures on the Day 14 and 42 study visits (or Days 14 and 35 for optional cohorts dosed once every 3 weeks):

- Perform focused physical examination.
- Measure vital signs.
- Collect blood samples for the following tests:
  - Hematology.
  - Serum chemistries.
  - Liver function tests.
  - Serum TTR protein, vitamin A, and RBP. Additionally, aliquots of serum samples will be taken and frozen, to permit testing of additional proteins related to ATTR.
  - Thyroid function tests.
  - Pharmacokinetic assessments.
- Collect urine sample for urinalysis.
- Collect urine sample for PK assessments.
- Document any AEs.
- Obtain concomitant medications information.

6.3.7 Day 21 or Day 49 ±3 days; or Day 21 and Day 42 for optional cohorts dosed once every 3 weeks

Patients will undergo the following procedures on the Day 21 and 49 study visits (or Days 21 and 42 for optional cohorts dosed once every 3 weeks):

- Collect blood samples for the following assessments:
  - Serum TTR protein, vitamin A, and RBP. Additionally, aliquots of serum samples will be taken and frozen, to permit testing of additional proteins related to ATTR.
  - Pharmacokinetic assessments.
- Collect urine sample for PK assessments.
- Document any AEs.
- Obtain concomitant medications information.

6.3.8 Day 56 (±3 days; End of Study)

Patients will undergo the following procedures on the Day 56 study visit:

- Perform physical examination.
- Measure vital signs.
- Perform 12-lead ECG.
- Perform serum pregnancy test (women of child-bearing potential only).
- Collect blood samples for the following tests:
  - Hematology.
  - Serum chemistries.
  - Liver function tests.
  - Serum TTR protein, vitamin A, and RBP. Additionally, aliquots of serum samples will be taken and frozen, to permit testing of additional proteins related to ATTR.

    Note: A patient will be followed every 2 weeks after Day 56 if the TTR level continues to recover but is not found to have returned to within at least 80% of the baseline value (see Section 6.5).
  - Thyroid function tests.
  - Anti-PEG antibodies (IgG and IgM).
  - Day 56 PK sample.
- Collect urine sample for urinalysis.
- Collect urine sample for PK analysis.

- Document any AEs.
- Obtain concomitant medications information.

6.3.9 Day 112 (±10 days)

Patients will undergo the following procedures on the Day 112 study visit:

- Collect blood samples for the following tests:
  - Serum TTR protein, vitamin A, and RBP. Additionally, aliquots of serum samples will be taken and frozen, to permit testing of additional proteins related to ATTR.
  - Day 112 PK sample.
- Collect urine for PK analysis.

6.3.10 Day 208 (±2 weeks)

Patients will undergo the following procedures on the Day 208 study visit:

- Collect blood samples for the following tests:
  - Serum TTR protein, vitamin A, and RBP. Additionally, aliquots of serum samples will be taken and frozen, to permit testing of additional proteins related to ATTR.
  - Anti-PEG antibodies (IgG and IgM).
  - Day 208 PK sample.
- Collect urine for PK analysis.

6.4 Early Termination

Patients coming off study before Day 56 will return to the study site to complete their Early Termination visit which will include Day 56 assessments. Patients coming off study after Day 56 and before Day 208 will return to the study site to complete their Early Termination visit which will include Day 208 assessments.

6.5 Unscheduled Visits

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

A patient will be followed every 2 weeks (±3 days) after Day 56 if their TTR level continues to recover, but is not found to have returned to within at least 80% of the baseline value. The patient will have blood samples collected for the following clinical laboratory tests at these visits:

- Serum TTR protein, vitamin A, and RBP. Additionally, aliquots of serum samples will be taken and frozen, to permit testing of additional proteins related to ATTR.

These patients will be followed and discussed at each SRC meeting until their TTR level returns to within at least 80% of the baseline value.

6.6 Participation in an Open-label Extension Study

An open-label extension study at the recommended Phase 3 dose and regimen (as derived from Study ALN-TTR02-002) is planned, which will enable the patients who enrolled in Study ALN-TTR02-002 to receive additional, long-term dosing and safety follow-up. For some of the patients, this may preclude the completion of all of the follow-up period assessments of Study ALN-TTR02-002 if they are deemed eligible to participate in the extension study prior to completion of the full follow up through Day 208. Such patients will be followed up for a minimum of 28 days after their last dose in the current ALN-TTR02-002 study prior to being enrolled in the extension study. The extension study will be implemented only after dose escalation is completed in Study ALN-TTR02-002, with post second dose follow-up through Day 208 in at least 1 cohort at the highest dose deemed safe.

7 STUDY ASSESSMENTS

7.1 Demographic Data and Medical History

Patient demographic data will be obtained during screening, and a complete medical history will be obtained during screening and updated on Days 0 and 28 (or Days 0 and 21 for optional cohorts dosed once every 3 weeks) as needed. The medical history is to include background information confirming a diagnosis of ATTR through biopsy and ongoing documented signs/symptoms of the disease (e.g., sensory, motor, or autonomic neuropathy) that are at least mild to moderate in severity.

7.2 Safety Assessments

All safety assessment measures will be recorded in the patient's 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 Screening for assessment of eligibility, and on the Day 0 and 28 (or Days 0 and 21 for optional cohorts dosed once every 3 weeks) study visits. The weight measured pre-dose on Days 0 and 28 will be used to calculate the first and second dose of ALN-TTR02, respectively.

Height will be measured at Screening.

7.2.2 Vital Signs

Vital signs are to be measured at Screening and the Day 2, 14, 30, 42, and 56 (or Days 2, 14, 23, 35, and 56 for optional cohorts dosed once every 3 weeks; or time of early termination, if applicable) study visits and include systolic/diastolic blood pressure, pulse rate, respiration 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.

On Days 0 and 28 (or Days 0 and 21 for optional cohorts dosed once every 3 weeks), serial vital signs are to be measured within 30 minutes pre-dose, at EOI; and at 30 (±5) minutes;1, 2, and 3 (±15 minutes) hours; 6, 12, and 18 hours (±30 minutes) post-infusion. On Days 1 and 29 (or Days 1 and 22 for optional cohorts dosed once every 3 weeks), serial vital signs are to be measured at 24 hours (+30 minutes) post-infusion.

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

7.2.3 Echocardiogram

An echocardiogram is to be conducted during Screening, to assess any cardiac abnormalities as part of the screening criteria. If a normal echocardiogram has been obtained within the past 90 days, there is no need to perform an echocardiogram at Screening.

7.2.4 Electrocardiogram

Computerised 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 made at the time points indicated in Table 1-1.

Serial ECGs will be collected in triplicate 30 minutes prior to dosing (Days 0 and 28 [or Days 0 and 21 for optional cohorts dosed once every 3 weeks]), EOI, and 30 (±5) minutes, 2 and 4 (±15 minutes) hours, and on Days 1 and 29 (or Days 1 and 22 for optional cohorts dosed once every 3 weeks) at 24 (+30 minutes) hours post-infusion.

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).

The Investigator or designee is responsible for reviewing the ECG to assess whether the ECG is within normal limits and to determine the clinical significance of the results. Prior to discharge from the hospital on Days 1 and 29 (or Days 1 and 22 for optional cohorts dosed once every 3 weeks), the ECG must be reviewed by the Investigator and the results deemed not clinically significant. Any clinically significant findings must be discussed with the medical monitor to determine whether patient can be discharged and to formulate plans for patient follow-up.

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

7.2.5 Pulse Oximetry

Arterial oxygen saturation will be assessed by serial pulse oximetry on Days 0 and 28 (or Days 0 and 21 for optional cohorts dosed once every 3 weeks) within 30 (±5) minutes pre-dose; at EOI; at 30 (±5) minutes; at 1, 2, 3 hours (±15 minutes); and at 6, 12, and 18 hours (±15 minutes) post-infusion on Days 0 and 28 (or Days 0 and 21 for optional cohorts dosed once every 3 weeks); and at 24 hours (+30 minutes) post-infusion on Days 1 and 29 (or Days 1 and 22 for optional cohorts dosed once every 3 weeks).

7.2.6 Cardiac Monitoring

Continuous cardiac monitoring will be performed via telemetry starting no later than 30 minutes prior to dosing and continuing through 24 hours (+1 hour) post-dose.

7.2.7 Clinical Laboratory Tests

Laboratory tests of hematology, serum chemistries, liver function, coagulation studies, CRP, urinalysis, pregnancy (serum and urine), hepatitis B and C serologies, lipids, and thyroid function will be performed by each study center's local laboratory.

The following laboratory tests will be performed at the laboratories listed below:

- Cytokines, complement factor Bb, anti-PEG antibody formatio [redacted]
- Tryptase [redacted]

Additional laboratory tests may be performed centrally, depending upon the capabilities of the local laboratory.

Prior to study drug administration, samples for clinical laboratory tests are to be collected and results of serum chemistries, hematology, LFTs, and coagulation parameters (where applicable) reviewed and approved by the Investigator or qualified designee (e.g., physician's assistant, nurse practitioner).

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[a]

- Sodium
- Potassium
- Blood urea nitrogen (BUN)
- Creatinine
- C-reactive protein (CRP)
- Albumin
- Calcium
- Glucose
- Phosphate
- Lactate dehydrogenase (LDH)
- Troponin I
- Creatinine phosphokinase (CPK)
- Myocardial band enzymes of CPK (CPK-MB)

Liver Function Tests

- Aspartate transaminase (AST)
- Alanine transaminase (ALT)
- Alkaline phosphatase
- Bilirubin (total and direct)

Thyroid Function Tests

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

Coagulation Studies

- Prothrombin time (PT)
- Activated partial thromboplastin time (aPTT)
- International normalized ratio (INR)

Serology	
• Anti-PEG antibody formation (IgG and IgM)	• Hepatitis C (anti-HCV Ab)
• Hepatitis B (HBsAg and HBsAb)	
Urinalysis	
• Visual inspection for color and appearance	• Leukocytes
• pH	• Bilirubin
• Specific gravity	• Nitrite
• Ketones	• Urobilinogen
• Protein	• Microscopic inspection of sediment
• Glucose	
Complement Factors	
• Bb	• C3a[b]
Cytokines	
• Interferon-alpha (IFN-α)	• Interleukin-6 (IL-6)
• Interferon-gamma (IFN-γ)	• Interleukin-12 (IL-12)
• Tumor necrosis factor-alpha (TNF-α)	• Interferon-inducible protein-10 (IP-10)
• Interleukin-1β (IL-1β)	• Granulocyte-colony stimulating factor (G-CSF)
• IL-1 receptor antagonist (IL-1 RA)	
Other	
• β-human chorionic gonadotropin (women of child-bearing potential only)	
• Tryptase[b]	

a A non-fasting lipid panel (including total cholesterol, high density lipoprotein [HDL], low density lipoprotein [LDL], very low density lipoprotein [VLDL], and triglycerides) will be performed pre-dose on Day 0 only.

b To be performed only in the event of an infusion reaction.

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

7.2.7.1 Hematology, Serum Chemistries, and Urinalysis

Blood samples for hematology and serum chemistries and urine for urinalysis are to be collected at Screening, pre-dose on Day 0 (unless the screening evaluations were performed within the previous 72 hours) and Day 28 (or Day 21 for optional cohorts dosed once every 3 weeks), and on the Day 1, 14, 29, 42, and 56 study visits (or Days 1, 14, 22, 35, and 56 for optional cohorts dosed once every 3 weeks), or at the time of early termination, if applicable.

Prior to discharge from the hospital on Days 1 and 29 (or Days 1 and 22 for optional cohorts dosed once every 3 weeks), local serum laboratories (specifically, sodium, potassium, creatinine, albumin, calcium, glucose, and phosphate) must be reviewed by the Investigator and the results deemed not clinically significant. Any clinically significant findings must be discussed with the medical monitor to determine whether patient can be discharged and to formulate plans for patient follow-up.

Blood for CRP is to be collected pre-dose (within 10 minutes) on Days 0 and 28 (or Days 0 and 21 for optional cohorts dosed once every 3 weeks), and at 2 and 6 hours (±15 minutes), and 24 hours (± 120 minutes) post-infusion.

7.2.7.2 Liver Function Tests

Blood for LFTs is to be collected at Screening, pre-dose on Day 0 (unless the screening evaluations were performed within the previous 72 hours) and Day 28, and at the Day 1, 2, 7, 14, 29, 30, 35, 42, and 56 study visits (or Days 1, 2, 7, 14, 22, 23, 28, 35, and 56 for optional cohorts dosed once every 3 weeks), or at the time of early termination, if applicable. These samples are to be processed at the site's local laboratory.

Prior to discharge from the hospital on Days 1 and 29 (or Days 1 and 22 for optional cohorts dosed once every 3 weeks), local LFTs must be reviewed by the Investigator and the results deemed not clinically significant. Any clinically significant findings must be discussed with the medical monitor to determine whether patient can be discharged and to formulate plans for patient follow-up.

7.2.7.3 Lipid Panel

Blood for determination of the lipid panel is to be collected pre-dose on Day 0.

7.2.7.4 Coagulation Studies

Blood for coagulation studies is to be collected at Screening, pre-dose on Days 0 (unless the screening evaluations were performed within the previous 72 hours) and 28 (or Day 21 for optional cohorts dosed once every 3 weeks), at the Days 1 and 29 (or Days 1 and 22 for optional cohorts dosed once every 3 weeks) study visits.

7.2.7.5 Thyroid Function Tests

Blood for thyroid function tests is to be collected at Screening, pre-dose on Days 0 (unless the screening evaluations were performed within the previous 72 hours) and 28 (or Days 0 and 21 for optional cohorts dosed once every 3 weeks), and at Day 14, 42, and 56 (or Days 14, 35, and 56 for optional cohorts dosed once every 3 weeks) study visits, or

at the time of early termination, if applicable. These samples are to be processed at the site's local laboratory.

7.2.7.6 Complement

Blood for complement factor Bb, is to be collected pre-dose (within 10 minutes) on Days 0 and 28 (or Days 0 and 21 for optional cohorts dosed once every 3 weeks), and 30 (± 5) minutes and 2 (± 15 minutes) and 24 hours (± 120 minutes) post-infusion. If the patient experiences an infusion reaction, a blood sample for analysis of complement factors (including complement C3a) should be obtained within 1 hour of the start of the reaction.

7.2.7.7 Cytokines

Blood for cytokine assessment is to be collected pre-dose (within 10 minutes) on Days 0 and 28 (or Days 0 and 21 for optional cohorts dosed once every 3 weeks), at 2 (±15 minutes), and 6 hours (±15 minutes), and 24 hours (±120 minutes) post-infusion. If the patient experiences an infusion reaction, a blood sample for analysis of cytokines should be obtained within 1 hour of the start of the reaction.

7.2.7.8 Pregnancy Test

A serum pregnancy test will be performed for women of child-bearing potential at Screening, at the Day 56 study visit, and any time pregnancy is suspected. A urine pregnancy test will be performed pre-dose on Day 0 for women of child-bearing potential, 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).

7.2.7.9 Hepatitis B/C Status

Blood for HBV (Hepatitis B surface antibody [HbsAb], Hepatitis B surface antigen [HbsAg]), and HCV (anti-HCV) are to be collected at Screening. Patients with positive HBV or HCV serologies are not eligible for study participation.

7.3 Pharmacodynamic Assessments

7.3.1 Transthyretin Protein

Blood for serum TTR protein levels (WT and mutant) is to be collected at Screening, immediately prior (within 10 minutes) to the administration of the premedications, immediately pre-dose (within 10 minutes) on Days 0 (Baseline) and 28, and on Days 1, 2,

7, 10, 14, 21, 29, 30, 35, 38, 42, 49, 56 (end-of-study), 112, and 208 or at the time of early termination. For optional cohorts dosed once every 3 weeks, blood for serum TTR protein levels will be collected at Screening, immediately prior (within 10 minutes) to the administration of the premedications, immediately pre-dose (within 10 minutes) on Days 0 (Baseline) and 21, and on Days 1, 2, 7, 10, 14, 22, 23, 28, 31, 35, 42, 49, 56 (end-of-study), 112, and 208 or at the time of early termination. A patient will be followed every 2 weeks after Day 56 if the TTR level continues to recover but is not found to have returned to within at least 80% of the baseline value. If this occurs, the patient will be followed and discussed at each SRC meeting until the TTR level returns to within at least 80% of the baseline value. Additionally, aliquots of serum samples drawn for measurement of TTR levels will be taken and frozen, to permit testing of total, WT and mutant TTR and other types of testing related to ATTR.

Details of how samples will be collected, processed, and stored will be in the Study Laboratory Manual. The Central Laboratory for TTR analysis is:

7.3.2 Transthyretin mRNA

Blood for serum TTR mRNA is to be collected at Screening, immediately prior (within 10 minutes) to the administration of the premedications on Days 0 (Baseline) and 28, and on Days 1, 2, 29, and 30. For optional cohorts dosed once every 3 weeks, blood for serum TTR mRNA will be collected at Screening and on Days 0 (Baseline) and 21, and on Days 1, 2, 22, and 23.

The Testing Laboratory for TTR mRNA analysis is:

7.3.2.1 Vitamin A and Retinol Binding Protein

Blood for measurements of vitamin A and RBP is to be collected at Screening, immediately prior (within 10 minutes) to the administration of the premedications, immediately pre-dose (within 10 minutes) on Days 0 and 28, and on Days 1, 2, 7, 10, 14, 21, 29, 30, 35, 38, 42, 49, 56 (end-of-study), 112, and 208 or at the time of early termination. For optional cohorts dosed once every 3 weeks, blood for measurements of vitamin A and RBP will be collected at Screening, immediately prior (within 10 minutes) to the administration of the premedications, immediately pre-dose (within 10 minutes) on Days 0 (Baseline) and 21, and on Days 1, 2, 7, 10, 14, 22, 23, 28, 31, 35, 42, 49, 56 (end-of-study), 112, and 208 or at the time of early termination.

Serum samples will be evaluated by HPLC to determine vitamin A levels and by nephelometry to determine RBP levels.

The laboratory for serum vitamin A and RBP analysis is:

[redacted]

7.4 Pharmacokinetic Evaluations

7.4.1 Plasma Pharmacokinetics

The PK parameter estimates and dose proportionality profiles of ALN-TTR02 will be determined for ALN-TTR02 siRNA (ALN-18328) and liposome lipid components, DLin-MC3-DMA and PEG_{2000}-C-DMG. The plasma concentration-time profiles for ALN-18328, DLin-MC3-DMA, and PEG_{2000}-C-DMG will be determined and used in the PK analysis for all dose cohorts. In addition, free and encapsulated concentrations of siRNA will be determined in plasma at specified time points (see Table 1-1) for cohort 3 and onward, including any optional cohorts. The drug concentrations in the lower cohorts are expected to be undetectable for this analysis.

Blood sample collection times are included in the schedule of events (see Table 1-1). Plasma PK samples (siRNA and lipids) will be collected pre-dose (within 1 hour of planned dosing start), at EOI, and then 5, 10, and 30 minutes, and at 1, 2, 4, 6, 24, and 48 hours post infusion. Samples will also be collected on Days 7, 14, 21, 35, 42, 49, 56, 112, and 208 post infusion as well as at the ET visit (if applicable); for optional cohorts

dosed once every 3 weeks, samples will be collected on Days 7, 14, 21, 28, 35, 42, 56, 112, and 208 post infusion as well as at the ET visit (if applicable).

For the PK blood draws, the sampling windows are ±1 minute for the 5- and 10- minute draws; ±2 minutes for the 30-minute draws; ±5 minutes for the 1-, 2-, 4-, and 6-hour draws; and ±120 minutes for the 24- and 48-hour draws.

The EOI will usually occur at 60 minutes after the start of infusion, except in patients where the infusion has been prolonged. If the infusion is stopped, a PK blood sample will be taken when the infusion is stopped, and this sample will be treated as the EOI sample if the infusion is restarted. All remaining 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 infusion).

In the event that a patient receives only 1 dose of study drug, plasma PK samples should be collected pre-dose (within 1 hour of planned dosing start), EOI, and then 5, 10, and 30 minutes; and 1, 2, 4, 6, 24 and 48 hours post infusion; and at Day 208.

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

Pharmacokinetic analyses will consist of a non-compartmental and/or compartmental evaluation of ALN-TTR02 siRNA (ALN-18328), and of ALN-TTR02 lipids DLin-MC3-DMA and PEG_{2000}-C-DMG plasma concentration-time profiles to determine PK parameter estimates using a validated software program, [redacted]. Pharmacokinetic parameter estimates to be calculated for all patients receiving ALN-TTR02 will 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 (λ_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.

Plasma samples will be evaluated by [redacted] using a validated ATTO-Probe-HPLC assay to determine ALN-18328 concentration. The concentrations of DLin-MC3-DMA and PEG_{2000}-C-DMG in plasma samples will be evaluated by a validated liquid chromatography/mass spectrometry (LC/MS/MS) assay at [redacted]

The Central Laboratory for plasma PK analysis is:

[redacted]

7.4.2 Urine Pharmacokinetics

The PK profiles of ALN-18328 and DLin-MC3-DMA in urine will be evaluated to determine the CL_R of ALN-18328 and DLin-MC3-DMA and/or its metabolites after dosing with ALN-TTR02. Urine sample collection times are included in the schedule of assessments (see Table 1-1 or Table 12-1 for optional cohorts dosed once every 3 weeks). Urine will be collected into suitable containers and kept refrigerated. Pre-dose samples will be collected within 1 hour of the planned dosing start. For the 0 to 6 hour collection, the patient should void at time 0 hours and discard the specimen. Details of sample collection and processing for PK analyses will be included in the laboratory manual.

The Central Laboratory for urine PK analysis is:

[redacted]:

7.4.3 Banking of Serum for Future Studies

To explore the expression of hepatocyte derived proteins to further characterize the biological effects of siRNA, serum and plasma samples will be collected at selected time points (see Table 1-1).

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

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 laboratory findings, physical examination findings, ECG changes or other documents.

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 to Day 56.

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 for 28 days from the start of infusion 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 28 days following the last dose of study drug 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.

8.5 Adverse Event Reporting Period

As stated in Section 8.4, AEs will be assessed for 28 days following the last dose of study drug (Day 56 for patients receiving both doses of ALN-TTR02) 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 28 days after the last dose of study drug administration, whichever is shorter.

Serious AEs will be followed for 28 days following the last dose of study drug (Day 56 for patients receiving both doses of ALN-TTR02), 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

considerations 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.

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 in addition to the documentation on 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.
- Preliminary assignment of causality to study drug.

The report should be faxed to [redacted], as follows:

[redacted]

If follow up is required, new information should be provided to Alnylam as it becomes available using the Serious Adverse Event Reporting form. Copies of discharge summaries, consultant reports, autopsy reports, and any other relevant documents may also be requested.

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 IEC when required by national regulations.

Alnylam 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 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 has first knowledge of them.

The Investigator may be informed by Alnylam 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) which 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.

8.12 Pregnancy Reporting

A female patient with a positive pregnancy test at Screening or Day 0 is ineligible for this trial. If a female patient is determined to be pregnant within 2 months after receiving study drug, the Investigator should report the pregnancy to 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. Monitoring of the patient should continue until the outcome of the pregnancy is known.

Pregnancy occurring in the partner of a patient participating in the study should also be reported to the Investigator and Alnylam. The partner should be counseled and followed as described above.

9 STATISTICAL METHODS

9.1 Sample Size

Based on the planned dose escalation scheme (see Section 5.7.1), up to 27 patients are expected to be enrolled. Three patients are to be enrolled at each of 5 planned dose levels. An additional 3 cohorts of 3 patients may be enrolled onto 1 or more of these specified dose levels to further evaluate safety and tolerability or PD effects.

9.2 Statistical Methodology

Statistical analyses will be primarily descriptive in nature. Adverse event summaries will include tabulations of all treatment-emergent AEs, 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.

Tabulations will be presented by dose level. 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:

- Intent-to-Treat (ITT) Analysis Set: All patients who were enrolled and received study treatment. The ITT analysis set will be the primary set for the analysis of safety data.
- Per-Protocol (PP) Analysis Set: All patients in the ITT Analysis Set who had no major protocol violations.
- Pharmacokinetic (PK) Analysis Set: All patients in the ITT 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 in order to descriptively assess the comparability of dose groups. 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 duration of the infusion and dose, and the proportion of patients with modifications in the duration of infusion will be produced.

Adverse events will be summarized by MedDRA system organ class and preferred term. Separate tabulations will be produced for all treatment emergent AEs, treatment-related AEs (those considered by the Investigator as at least possibly drug related), SAEs, and discontinuations due to AEs. By-patient listings will be provided for deaths, SAEs, DLTs, and events leading to discontinuation of treatment.

Descriptive statistics will be provided for clinical laboratory data, including cytokines, as well as 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 last evaluation on study.

9.2.4 Pharmacodynamics

Serial measurements of serum TTR over a 208-day period post-dose will allow for determination of the nadir following dosing as well as the timing of the nadir post-dose and the duration of TTR suppression. A standard ELISA assay will be used to measure serum total TTR in patients. Also, TTR testing will be performed as a LC/MS assay, to measure mutant and WT TTR.

Pre- and post-dose measurements of serum TTR mRNA will assist in determining whether any observed effects of ALN-TTR02 on TTR protein are being mediated by RNAi. Transthyretin mRNA in the serum will be detected using a qRT – PCR based assay.

9.2.5 Pharmacokinetics

Pharmacokinetic analyses will consist of a non-compartmental and/or compartmental evaluation of siRNA ALN-18328 of ALN-TTR02 and DLin-MC3-DMA and PEG_{2000}-C-DMG plasma concentration-time profile to determine PK parameter estimates using a

validated software program, [redacted] Pharmacokinetic parameter estimates to be calculated for all patients 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-t}, AUC_{0-last} and $AUC_{0-\infty}$; λ_z; CL, V_{ss} and V_z. The dose proportionality of ALN-TTR02 following a single, 15-minute infusion will also be determined.

Plasma and urine samples will be evaluated by [redacted] using a validated hybridization enzyme-linked immunosorbent assay for each of the two siRNAs (both encapsulated and unencapsulated). Analysis of the concentration-time data will also be performed by [redacted]. The methods will be validated as per [redacted] Standard Operating Procedures. The PK profile of each patient will be characterized by non-compartmental analysis of each siRNA plasma concentration using validated computer software [redacted]. 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. The amount of siRNA excreted in the urine over the 2 separate and combined collection intervals will be determined and CL_R calculated for each patient, if possible.

9.2.6 Summary of Pharmacodynamic and Pharmacokinetic Analyses

Pharmacokinetic and PD data will be assessed descriptively whenever possible and by exploratory statistical comparisons. Pharmacokinetic/PD analyses will include evaluation with respect to siRNA ALN-18328 of ALN-TTR02 plasma concentrations, exposure, or the relationship of any PK/PD parameter estimate to TTR concentration after ALN-TTR02 following a single, 15-minute infusion. Attempts will be made to perform PK/PD analysis to determine PK/PD parameters (e.g., ED_{50}, EC_{50}[50% effective dose, 50% effective concentration, respectively]).

9.2.7 Interim Analysis

There is no formal interim analysis planned for this study.

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 (Appendix 6), 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 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.

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 which 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 into 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 which 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 Alnylam within 24 hours of knowledge of the event.

Any communications from regulatory agencies in regard to inspections, other studies which 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 Study Committees

An SRC will be formed that will evaluate safety and tolerability data and determine if it remains acceptable to continue dosing (administration of second dose of study drug within cohorts) or to dose escalate (between cohorts). The SRC will also be convened in the event of any potential DLT to determine whether stopping rules for accrual to a particular dose level have been met (see Section 5.7.4 for study stopping rules). The SRC is also responsible for making recommendations regarding replacement of subjects that have withdrawn before Day 56 without experiencing a DLT. In addition, the SRC may be convened at the discretion of Alnylam if important safety issues arise requiring the attention of the committee (e.g., new safety information attained in other ongoing studies with ALN-TTR02).

To ensure timely safety information exchange across the participating study centres, the SRC will be comprised of all Principal Investigators participating in the study, or their designee, Alnylam Medical Monitor, and the CRO Medical Monitor. The SRC will evaluate the available safety data post infusion including but not limited to: AEs, vital signs, pulse oximetry, 12-lead ECGs, and available laboratory parameters. The SRC will communicate frequently to ensure that patients are dosed according to the time intervals specified in Section 5.6, even as patients are being accrued across multiple centers.

The options based on SRC discussion (if stopping criteria have not been met) are to:

- Escalate the dose as planned;
- Escalate/decrease the dose to an intermediate level;

- Repeat the dose level only in the event of a safety signal (not considered severe) that indicates that any further dose escalation may affect the safety of the study patients (or to re-evaluate the PK data or to further characterize PD markers);
- Terminate the study.

The membership of the SRC and reporting structure are defined in the SRC Charter.

10.4 Ancillary Research

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

10.5 Study Record Retention

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

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

10.6 Discontinuation of the Study by Alnylam

Alnylam reserves the right to discontinue the study for clinical or administrative reasons at any time. If the site does not recruit at a reasonable rate, the study may be discontinued. 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.

10.7 Study Documentation

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

- An original signed Investigator agreement page of the protocol and any amendments;
- An IEC 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.

10.8 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 Research Practices 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 the ALN-TTR02 in the countries where this study was conducted, or until clinical development of ALN-TTR02 is halted. Throughout sample collection, storage (limited, staff only access area containing locked sample storage, and limited access sample tracking) and processing, the samples will only be handled by appropriate personnel per the laboratory's standard operating procedures.

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

10.9 Publications/Reports

Following completion of the study, the data may be considered for publication in a scientific journal or for reporting at a scientific meeting. A copy of the manuscript must be provided 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 centers 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.

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12 APPENDICES

Appendix 1: Guidelines for Acute Infusion-Related Reactions

Mild or Moderate Reaction

In the event of mild or moderate symptom(s) and/or sign(s) discontinue infusion and treat as below:

- Medication as clinically indicated (e.g., paracetamol, additional H1/H2 blocker, non-steroidal anti-inflammatory [NSAID], small volumes of physiological saline, and/or ≤5 L/min oxygen or the reaction is classified as severe);
- Re-challenge with slower infusion rate (no greater than 3 hour duration) after resolution of the reaction;
- If reaction recurs with re-challenge this patient, this will be a DLT and the patient will receive no further study drug.

Severe Reaction

In the event of severe sign(s) and symptom(s) discontinue infusion and treat as below:

- Medication as clinically indicated (e.g., fluids [>1 L saline], oxygen [>5 L/min], adrenaline, glucocorticoids, H1/H2 blocker);
- If a severe acute reaction occurs in any patient, this is a DLT and no more patients will be dosed;

Life-Threatening Reaction

- If a life-threatening reaction occurs in any patient, this is considered a DLT and no further patients will be dosed.

Appendix 2: Guidelines for Delayed Infusion-Related Reactions

Mild or Moderate Reaction

In the event of mild or moderate symptom(s) and/or sign(s) discontinue infusion and treat as below:

- Sign(s) and/or symptom(s) must be self-limiting or respond to treatment (e.g., paracetamol, narcotics, loperamide, NSAIDs, small volumes of physiological saline, and/or ≤5 L/min oxygen or the reaction is classified as severe.

Severe Reaction

Severe symptom(s) and/or sign(s):

- Requiring more intensive intervention than for moderate reaction with fluids (>1 L saline), oxygen (> 5L/min), and/or glucocorticoids;
- If severe reaction occurs in any patient, this is a DLT and no more patients will be dosed;

Life-Threatening Reaction

- If a life-threatening reaction occurs in any patient, this is considered a DLT and no further patients will be dosed.

Appendix 3: 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 4: Schedule of Assessments for Optional Cohorts Administered ALN-TTR02 Once Every Three Weeks

Up to 4 optional cohorts may be administered ALN-TTR02 once every 3 weeks. The schedule of assessments for these cohorts is provided in Table 12-1.

Table 12-1: Schedule of Assessment for Optional Cohorts Administered ALN-TTR02 Once Every Three Weeks

	Screening	Pre-Dosing		Dosing Cycles								Follow-Up		
Procedures	**D -45 to D -3**	**D -1 D 20**	**D 0 D 21 (+2 D)**	**D 0 D 21 (+2 D)**	**D 1 D 22**	**D 2 D 23**	**D 7 (±1 D) D 28 (±1 D)**	**D 10 (±2 D) D 31 (±2 D)**	**D 14 (±3 D) D 35 (±3 D)**	**D 42 (±3 D)**	**D 49 (±3 D)**	**D 56[a] (±3 D)**	**D 112 (±10 D)**	**D 208[a] (±2 W)**
Informed Consent	X													
Demographics	X													
Medical History	X		X[b]											
Inclusion/Exclusion Criteria	X		X											
Physical Examination, excluding weight	X		X[c]		X[c]	X[c]			X[c]			X		
Weight	X		X[d]											
Height	X													
Body Mass Index (BMI)	X		X											
Vital Signs[e]	X					X			X			X		
Vital Signs (Serial)[f]			X	X	X									
Echocardiogram[g]	X													
12-Lead ECG	X											X		
ECG (Serial)[h]			X	X	X									
Inpatient at Study Site			X	X	X[i]									
Cardiac Monitoring (Telemetry)[j]			X	X	X									
Pulse Oximetry (Serial)[f]			X	X	X									
Serum Pregnancy Test (females only)	X											X		
Urine Pregnancy Test (females only)[k]			X											
Hepatitis B/C Status[l]	X													
Serum Chemistry, Hematology, Urinalysis	X		X[m]		X				X			X		
Liver Function Tests[n]	X		X[m]		X	X	X		X			X		
Coagulation Studies[o]	X		X[m]		X									
Lipid Panel[p]			X											

Procedures	Screening	Pre-Dosing		Dosing Cycles								Follow-Up		
	D -45 to D -3	D -1 / D 20	D 0 / D 21 (+2 D)	D 0 / D 21 (+2 D)	D 1 / D 22	D 2 / D 23	D 7 (±1 D) / D 28 (±1 D)	D 10 (±2 D) / D 31 (±2 D)	D 14 (±3 D) / D 35 (±3 D)	D 42 (±3 D)	D 49 (±3 D)	D 56[a] (±3 D)	D 112 ±10 D)	D 208[a] (±2 W)
TTR protein, Vitamin A, and RBP in serum	X		X[q]		X	X	X	X	X	X	X	X[r]	X	X
TTR mRNA in serum	X		X		X	X								
Thyroid Function Tests[s]	X		X[m]						X			X		
Complement Bb[t]			X	X	X									
If infusion reaction: Tryptase and C3a[u]				X	X									
Premedication[v]		X	X											
Premedication reminder[w]		X												
Study Drug Administration				X[x]										
Anti-PEG Antibody Testing (IgG, IgM)	X		X				X					X		X
Cytokines and CRP[y]			X	X	X									
Plasma PK Sampling[z]			X	X	X	X	X		X	X	X	X	X	X
Urine PK Sampling[aa]			X	X	X	X	X		X	X	X	X	X	X
Exploratory Biomarkers	X		X[bb]		X	X	X	X	X	X	X	X		
Concomitant Medications	X				Continuous Monitoring									
Review/Record AEs														
Study Completion												X		

Footnotes on following pages.

a Early termination procedures: if a patient withdraws prior to Day 56, then the Days 56 and 208 visits should be performed. If a patient is withdrawn/withdraws after Day 56 and prior to Day 208, then the Day 208 visit should be performed.

b Interval medical history.

c Focused physical examination (includes head/ears/eyes/nose/throat [HEENT], cardiovascular, respiratory, abdominal, and hepatic assessments). If the screening physical examination was performed within 72 hours of Day 0, then the pre-dose (Day 0) physical examination does not need to be repeated; however, the patient's weight will be obtained.

d Weight measured on Day 0 and Day 21 will be used for calculating first and second dose, respectively.

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 Serial measures (vital signs and pulse oximetry) are to be measured within 30 minutes pre-dose; at the end of infusion (EOI); and 30 (±5) minutes; 1, 2, and 3 (±15 minutes) hours; 6, 12, and 18 (±30 minutes) hours; and 24 (+30 minutes) hours post-infusion.

g Not needed if a normal echocardiogram has been obtained within the past 90 days.

h Serial electrocardiograms (ECGs) will be collected in 3 replicates within 30 minutes pre-dose, EOI, and 30 (±5) minutes, 2 and 4 (±15 minutes) hours, and 24 (+30 minutes) hours post-infusion.

i Patients will be hospitalized at the study site for at least 24 hours after the end of infusion of study drug. Patients may be discharged upon completion of review by the Investigator of ECG, sodium, potassium, creatinine, albumin, calcium, glucose, phosphate, and LFTs results obtained at 24-hours post-infusion, if results are deemed not clinically significant. Any clinically significant findings must be discussed with the medical monitor to determine whether patient can be discharged and to formulate plans for patient follow-up.

j Continuous cardiac monitoring will be performed via telemetry starting no later than 30 minutes prior to dosing and continuing through 24 hours (+1 hour) post-dose.

k Day 0 pre-dose only, not performed on Day 21.

l Serologies include hepatitis B surface antibody (HbsAb), hepatitis B surface antigen (HbsAg), and anti–hepatitis C virus antibody (anti-HCV Ab).

m If the parameter was assessed and meets eligibility requirements within 72 hours of Days 0 and 21, then it does not need to be repeated pre-dose (Days 0 and 21, respectively).

n Liver function tests include aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase, and bilirubin (total and direct).

o Coagulation studies include prothrombin time (PT), activated partial thromboplastin time (aPTT), and international normalized ratio (INR).

p Lipid panel (non-fasting) includes total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL), and triglycerides will be collected on Day 0 only.

q Pre-dose samples for TTR protein, vitamin A, and RBP measurements will be drawn immediately (within 10 minutes) prior to the premedications and immediately prior to dosing.

r A patient will be followed approximately every 2 weeks after Day 56 if their TTR level continues to recover but is not found to have returned to within at least 80% of the baseline value. If this occurs, the patient will be followed and discussed at each SRC meeting until the TTR level returns to within at least 80% of the baseline value.

s Thyroid function tests include thyroid stimulating hormone (TSH), thyroxine (T4), and triiodothyronine (T3).

t A blood sample will be collected for the assessment of complement Bb immediately (within 10 minutes) pre-dose, and 30 (±5) minutes, and 2 and 24 hours (±120 minutes) post infusion. If the patient experiences an infusion reaction, a blood sample for analysis of complement factors should be obtained within 1 hour of the start of the reaction.

u A blood sample for the assessment of tryptase and C3a is to be collected only in the event of an acute infusion reaction: at time of event or as soon as possible after onset, 1 hour, and 24 hours after the event.

v Premedications include 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) will be self-administered per os (PO) the evening before study drug administration. Thirty to 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. Patients enrolled in an optional cohort evaluating the use of an alternative premedication regimen, as agreed upon by the SRC, will receive the following medications at least 60 minutes prior to the start of 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). These patients will not receive any premedications the evening prior to ALN-TTR02 dosing.

w Site personnel are to call patients the day before dosing to remind them to take premedications that evening (the day before dosing). This reminder will not be needed for patients enrolled in optional cohorts evaluating the alternative premedication regimen.

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

y A blood sample for the assessment of cytokines and C-reactive protein (CRP) will be collected immediately (within 10 minutes) pre-dose, and 2 (±15 minutes), 6 (±15 minutes), and 24 hours (±120 minutes) post infusion. If the patient experiences an infusion reaction, a blood sample for analysis of cytokines should be obtained within 1 hour of the start of the reaction.

z For each dose, plasma PK samples (siRNA and lipids) will be collected pre-dose (within 1 hour of planned dosing start), EOI, and then 5, 10, and 30 minutes, and 1, 2, 4, 6, 24 and 48 hours post infusion. Samples will also be collected on Days 7, 14, 28, 35, 42, 49, 56, 112, and 208. Plasma PK on Day 0 at EOI and then 2 hours post-infusion will be analyzed for both free and encapsulated siRNA for cohort 3 and onward, including any optional cohorts. For each post dose PK blood draw, the following sampling windows are allowed: ±1 minute for the 5- and 10-minute draws; ±2 minutes for the 30-minute draws; ±5 minutes for the 1-, 2-, 4-, and 6-hour draws; and ±120 minutes for the 24- and 48-hour draws.

aa For each dose, urine PK samples will be collected pre-dose (within 1 hour of planned dosing start), and from 0-6 hours post-infusion (pooled). Samples will also be collected on Days 7, 14, 28, 35, 42, 49, 56, 112, and 208.

bb Pre-dose samples should be collected prior to infusion, but after premedications have been administered.

Appendix 5: New York Heart Association Classification of Heart Failure

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

Appendix 6: World Medical Association Declaration of Helsinki

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI

Ethical Principles for Medical Research Involving Human Patients

Recommendations guiding physicians in biomedical research involving human patients

Adopted by the 18th WMA General Assembly Helsinki, Finland, June 1964 and amended by the:

29th WMA General Assembly, Tokyo, Japan (October 1975)
35th WMA General Assembly, Venice, Italy (October 1983)
41st WMA General Assembly, Hong Kong (September 1989)
48th WMA General Assembly, Somerset West, Republic of South Africa (October 1996)
52nd WMA General Assembly, Edinburgh, Scotland (October 2000)
53rd WMA General Assembly, Washington 2002 (Note of Clarification on Paragraph 29)
55th WMA General Assembly, Tokyo 2004 (Note of Clarification on Paragraph 30)
59th WMA General Assembly, Seoul, October 2008

INTRODUCTION

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human patients, including research on identifiable human material and data. The Declaration is intended to be read as a whole and each of its constituent paragraphs should not be applied without consideration of all other relevant paragraphs.
2. Although the Declaration is addressed primarily to physicians, the WMA encourages other participants in medical research involving human patients to adopt these principles.
3. It is the duty of the physician to promote and safeguard the health of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfillment of this duty.
4. The Declaration of Geneva of the WMA binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act in the patient's best interest when providing medical care."
5. Medical progress is based on research that ultimately must include studies involving human patients. Populations that are underrepresented in medical research should be provided appropriate access to participation in research.
6. In medical research involving human patients, the well-being of the individual research patient must take precedence over all other interests.
7. The primary purpose of medical research involving human patients is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best current interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.
8. In medical practice and in medical research, most interventions involve risks and burdens.

9. Medical research is patient to ethical standards that promote respect for all human patients and protect their health and rights. Some research populations are particularly vulnerable and need special protection. These include those who cannot give or refuse consent for themselves and those who may be vulnerable to coercion or undue influence.
10. Physicians should consider the ethical, legal and regulatory norms and standards for research involving human patients in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research patients set forth in this Declaration.

BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH

11. It is the duty of physicians who participate in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research patients.
12. Medical research involving human patients must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.
13. Appropriate caution must be exercised in the conduct of medical research that may harm the environment.
14. The design and performance of each research study involving human patients must be clearly described in a research protocol. The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest, incentives for patients and provisions for treating and/or compensating patients who are harmed as a consequence of participation in the research study. The protocol should describe arrangements for post-study access by study patients to interventions identified as beneficial in the study or access to other appropriate care or benefits.
15. The research protocol must be submitted for consideration, comment, guidance and approval to a research ethics committee before the study begins. This committee must be independent of the researcher, the sponsor and any other undue influence. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research patients set forth in this Declaration. The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No change to the protocol may be made without consideration and approval by the committee.
16. Medical research involving human patients must be conducted only by individuals with the appropriate scientific training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately

qualified physician or other health care professional. The responsibility for the protection of research patients must always rest with the physician or other health care professional and never the research patients, even though they have given consent.

17. Medical research involving a disadvantaged or vulnerable population or community is only justified if the research is responsive to the health needs and priorities of this population or community and if there is a reasonable likelihood that this population or community stands to benefit from the results of the research.
18. Every medical research study involving human patients must be preceded by careful assessment of predictable risks and burdens to the individuals and communities involved in the research in comparison with foreseeable benefits to them and to other individuals or communities affected by the condition under investigation.
19. Every clinical trial must be registered in a publicly accessible database before recruitment of the first patient.
20. Physicians may not participate in a research study involving human patients unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians must immediately stop a study when the risks are found to outweigh the potential benefits or when there is conclusive proof of positive and beneficial results.
21. Medical research involving human patients may only be conducted if the importance of the objective outweighs the inherent risks and burdens to the research patients.
22. Participation by competent individuals as patients in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no competent individual may be enrolled in a research study unless he or she freely agrees.
23. Every precaution must be taken to protect the privacy of research patients and the confidentiality of their personal information and to minimize the impact of the study on their physical, mental and social integrity.
24. In medical research involving competent human patients, each potential patient must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, and any other relevant aspects of the study. The potential patient must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal. Special attention should be given to the specific information needs of individual potential patients as well as to the methods used to deliver the information. After ensuring that the potential patient has understood the information, the physician or another appropriately qualified individual must then seek the potential patient's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.

25. For medical research using identifiable human material or data, physicians must normally seek consent for the collection, analysis, storage and/or reuse. There may be situations where consent would be impossible or impractical to obtain for such research or would pose a threat to the validity of the research. In such situations the research may be done only after consideration and approval of a research ethics committee.
26. When seeking informed consent for participation in a research study the physician should be particularly cautious if the potential patient is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent should be sought by an appropriately qualified individual who is completely independent of this relationship.
27. For a potential research patient who is incompetent, the physician must seek informed consent from the legally authorized representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the population represented by the potential patient, the research cannot instead be performed with competent persons, and the research entails only minimal risk and minimal burden.
28. When a potential research patient who is deemed incompetent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorized representative. The potential patient's dissent should be respected.
29. Research involving patients who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research population. In such circumstances the physician should seek informed consent from the legally authorized representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving patients with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research should be obtained as soon as possible from the patient or a legally authorized representative.
30. Authors, editors and publishers all have ethical obligations with regard to the publication of the results of research. Authors have a duty to make publicly available the results of their research on human patients and are accountable for the completeness and accuracy of their reports. They should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results should be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest should be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

31. The physician may combine medical research with medical care only to the extent that the research is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research patients.
32. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best current proven intervention, except in the following circumstances:

- The use of placebo, or no treatment, is acceptable in studies where no current proven intervention exists; or
- Where for compelling and scientifically sound methodological reasons the use of placebo is necessary to determine the efficacy or safety of an intervention and the patients who receive placebo or no treatment will not be patient to any risk of serious or irreversible harm. Extreme care must be taken to avoid abuse of this option.

33. At the conclusion of the study, patients entered into the study are entitled to be informed about the outcome of the study and to share any benefits that result from it, for example, access to interventions identified as beneficial in the study or to other appropriate care or benefits.
34. The physician must fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never interfere with the patient-physician relationship.
35. In the treatment of a patient, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgment it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available.

Protocol ALN-TTR02-002

Amendment 2.1 Change Summary

A Phase 2, Open-Label, Multi-Dose, Dose Escalation Trial to Evaluate the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of Intravenous Infusions of ALN-TTR02 in Patients with TTR Amyloidosis

Changes included in Amendment 2.1, from Amendment 1.1 of Protocol ALN-TTR02-002 (dated 24 September 2012; original protocol dated 03 February 2012, Amendment 1 dated 19 June 2012), are itemized below and detailed in the pages that follow.

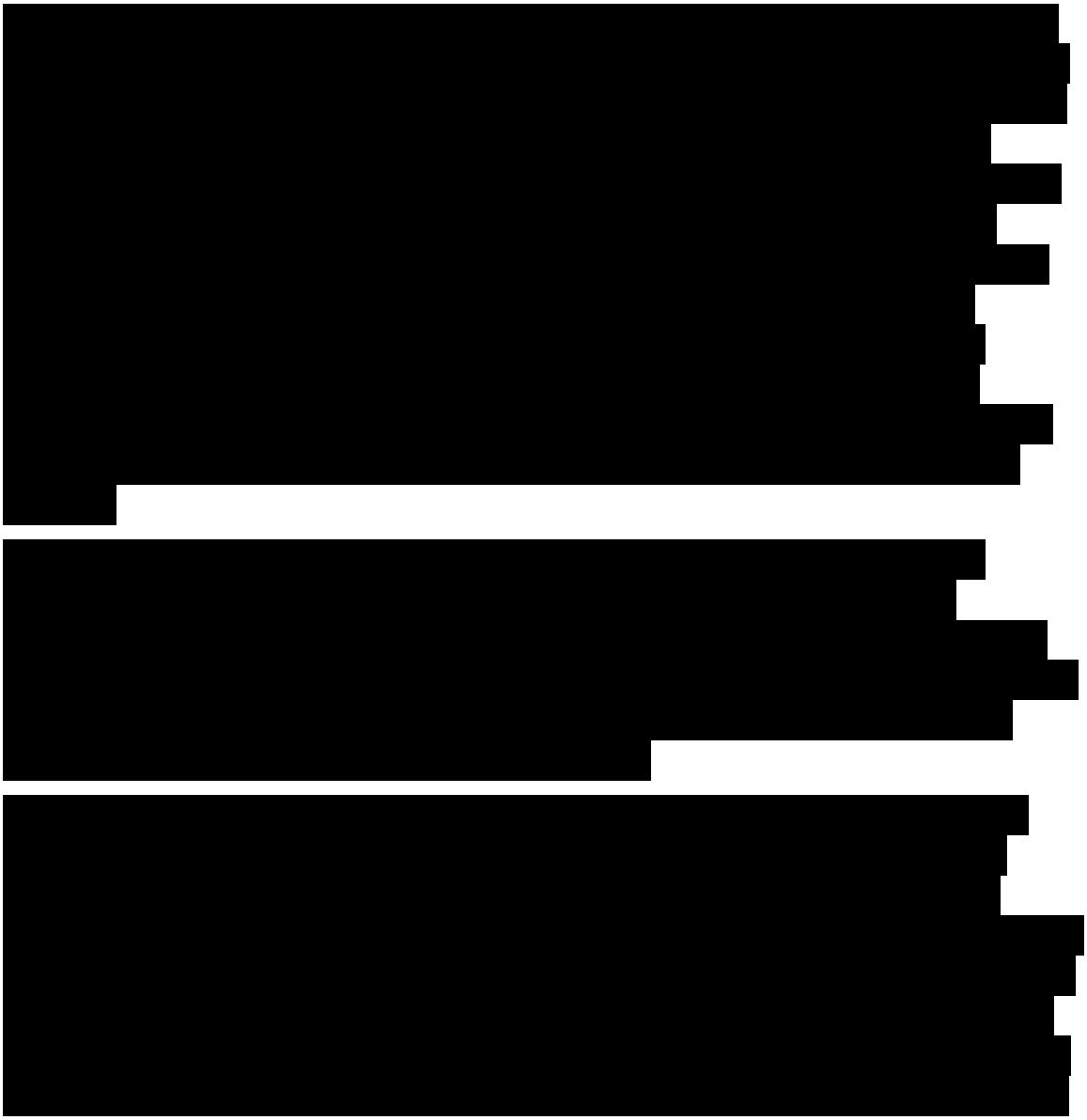

Table 1: Premedication and Infusion Rate Changes Proposed in Amendment #2.1

Medication	Current Protocol	Proposed Amendment #2.1
Dexamethasone (or equivalent)	The evening before study drug administration, 8 mg PO; self-administered. Thirty to 60 minutes prior to the start of study drug infusion, 20 mg PO; administered by study site personnel.	No dexamethasone the evening prior to ALN-TTR02 dosing. To be administered by study site personnel at least 60 minutes prior to the start of study drug infusion, 10 mg IV.
Paracetamol (or equivalent)	The evening before study drug administration, 500 mg PO; self-administered. Thirty to 60 minutes prior to the start of study drug infusion, 500 mg PO; administered by study site personnel.	No paracetamol the evening prior to ALN-TTR02 dosing. To be administered by study site personnel at least 60 minutes prior to the start of study drug infusion, 500 mg PO.
H1 blocker	Oral cetirizine 10 mg (hydroxyzine 25 mg, fexofenadine, or equivalent may be substituted if patient does not tolerate cetirizine). To be administered: The evening before study drug administration; self-administered. Thirty to 60 minutes prior to the start of study drug infusion; administered by study site personnel.	Diphenhydramine 50 mg IV (or equivalent other IV H1 blocker available at the study site). Hydroxyzine or fexofenadine 25 mg PO or cetirizine 10 mg PO may be substituted for any patient who does not tolerate IV H1 blockers. To be administered by study site personnel at least 60 minutes prior to the start of study drug infusion. (No H1 blocker the evening prior to ALN-TTR02 dosing.)
H2 blocker	Oral ranitidine 150 mg or famotidine 20 mg or equivalent other H2 blocker dose. To be administered: The evening before study drug administration; self-administered. Thirty to 60 minutes prior to the start of study drug infusion; administered by study site personnel.	Intravenous ranitidine 50 mg, famotidine 20 mg or another equivalent IV H2 blocker dose. To be administered at least 60 minutes prior to the start of study drug infusion. (No H2 blocker the evening prior to ALN-TTR02 dosing.)
Infusion rate	Over 60 minutes (~3.3 mL/min)	Approximately 1.1 mL/min over the first 15 minutes, with the remainder of the infusion administered at 3.3 mL/min (total infusion time: ~70 min).

Other changes from Amendment 1.1 to Amendment 2.1 include:

- Increased the number of optional cohorts from 3 to 5 to allow for the evaluation of once every 3 weeks dosing interval as well as an alternative premedication regimen and slower infusion rate.
- Added an additional schedule of assessments for optional cohorts administered ALN-TTR02 once every 3 weeks (refer to Appendix 4).
- The dosing regimen in the first optional cohort will be once every 4 weeks; in the remaining 4 optional cohorts the dosing regimen will be once every 3 or 4 weeks. The decision on dosing every 3 or 4 weeks will be made by the SRC based on safety.
- The alternative premedication regimen and infusion rate (detailed in Table 1) will be evaluated in up to 3 optional cohorts once the safety of the original premedication regimen and infusion rate is further established in the first 2 optional cohorts.
- Section 1.3 (Summary of Clinical Data with siRNA-LNPs) of the protocol has been simplified and primarily cross-references the IB, Edition 3, which contains the updated clinical information.
- Removed the Principal Investigators' contact information from the protocol itself. This information is included in the Study Procedures Manual.
- Made minor editorial changes (e.g., corrections of typographical, grammatical, and spelling errors as well as formatting changes and changes for consistency); these changes are not listed individually.

Text deleted is indicated by strikeout while text added is indicated by **bold** font.

Section	Prior Text	Changed To
Contact Information	**Principal Investigator** [redacted] **Principal Investigator** [redacted] **Principal Investigator** [redacted]	**~~Principal Investigator~~** [redacted] **~~Principal Investigator~~** [redacted] **~~Principal Investigator~~** [redacted]

Section	Prior Text	Changed To
	[redacted] **Principal Investigator** [redacted] **Principal Investigator** [redacted]	[redacted] ~~**Principal Investigator**~~ [redacted] ~~**Principal Investigator**~~ ~~Marcia Waddington Cruz, MD, PhD~~ [redacted]
Synopsis: Design	The dose level of ALN-TTR02 administered to any cohort in study ALN-TTR02-002 may not be higher than that deemed safe and tolerable in the Phase 1	~~The dose level of ALN-TTR02 administered to any cohort in study ALN-TTR02-002 may not be higher than that deemed safe and tolerable in the Phase 1~~

Section	Prior Text	Changed To
	single-dose study of ALN-TTR02 (Study ALN-TTR02-001).	~~single-dose study of ALN-TTR02 (Study ALN-TTR02-001).~~
Synopsis: Study Sites	This study is planned to be conducted at up to 8 study centers in Europe and South America.	This study is planned to be conducted at up to ~~8~~ **10** study centers ~~in Europe and South America~~**worldwide**.
Synopsis: Dosage, Route of Administration and Duration of Treatment of Investigational Drug	The optional cohort would be included to further confirm the safety and/or pharmacodynamic (PD) effect. If this occurs, the Independent Ethics Committees (IECs) will be informed of the expansion. Up to 3 optional cohorts are permitted in this study. All patients will receive the following premedications prior to each dose of ALN-TTR02: • Oral (PO) dexamethasone (8 mg) or equivalent administered the evening before dosing and 20 mg 30 to 60 minutes prior to start of infusion of ALN-TTR02; • Oral paracetamol (500 mg) or equivalent the evening before dosing and 30 to 60 minutes prior to the start of infusion of ALN-TTR02; • Oral H2 blocker (i.e., ranitidine 150 mg or famotidine 20 mg or equivalent other H2 blocker dose) the evening before dosing and 30 to 60 minutes prior to start of infusion of ALN-TTR02; • Oral H1 blocker, 10 mg cetirizine or equivalent (hydroxyzine 25 mg or fexofenadine may be substituted if patient does not tolerate cetirizine) the evening before dosing and 30 to 60 minutes	~~The o~~Optional cohort**(s)** would be included to further confirm the safety and/or pharmacodynamic (PD) effect **of previously evaluated dose levels, which may include evaluation of the original dosing regimen (once every 4 weeks), an alternative dosing regimen (once every 3 weeks), and/or an alternative premedication regimen and infusion rate. The dosing regimen for the first optional cohort will be once every 4 weeks, and the dosing regimen for the remaining 4 optional cohorts will be once every 3 or 4 weeks. It is anticipated that the first 2 optional cohorts will use the original premedication regimen, and the remaining 3 optional cohorts may be used to explore the alternative premedication regimen and infusion rate.** **The SRC will review all available safety data and make recommendations on dosing and premedication regimens prior to dosing of any of the optional cohorts.** ~~If this occurs, t~~The Independent Ethics Committees (IECs) will be informed of the ~~expansion~~**implementation of optional cohorts.** Up to ~~3~~ **5** optional cohorts are permitted in this study. ~~All patients will receive the following~~**Patients**

Section	Prior Text	Changed To
	prior to start of infusion of ALN-TTR02; Doses of ALN-TTR02 will be administered IV over 60 minutes by a controlled infusion device.	**receiving the original** premedication **regimen will be administered the followings** prior to each dose of ALN-TTR02: • Oral (PO) dexamethasone (8 mg) or equivalent administered the evening before dosing and 20 mg 30 to 60 minutes prior to start of infusion of ALN-TTR02; • Oral paracetamol (500 mg) or equivalent the evening before dosing and 30 to 60 minutes prior to the start of infusion of ALN-TTR02; • Oral H2 blocker (**i.e.g.**, ranitidine 150 mg or famotidine 20 mg or equivalent other H2 blocker dose) the evening before dosing and 30 to 60 minutes prior to start of infusion of ALN-TTR02; **and** • Oral H1 blocker, 10 mg cetirizine or equivalent (hydroxyzine 25 mg or fexofenadine may be substituted if patient does not tolerate cetirizine) the evening before dosing and 30 to 60 minutes prior to start of infusion of ALN-TTR02. **The alternative premedication regimen that can be used in select optional cohorts, as agreed upon by the SRC, will be administered prior to each dose of ALN-TTR02 and includes the following:** • **Intravenous (IV) dexamethasone (10 mg) or equivalent, administered at least**

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		60 minutes prior to the start of infusion of ALN-TTR02; • **Oral paracetamol (500 mg) or equivalent at least 60 minutes prior to the start of 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 start of 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 start of 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.** **For patients receiving the original premedication regimen and infusion rate,** doses of ALN-TTR02 will be administered IV over 60 minutes by a controlled infusion device **at a flow rate of approximately 3.3 mL/min.** **For patients enrolled in the optional cohorts evaluating the alternative premedication regimen and infusion rate, the flow rate of ALN-TTR02 will**

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		be approximately 1.1 mL/min during the first 15 minutes (1/3rd the original flow rate) with the remainder of the infusion administered at a flow rate of 3.3 mL/min for a total infusion time of approximately 70 minutes
Time on Study	Added language to allow an open-label extension study.	**An open-label extension study at the recommended Phase 3 dose and regimen (as derived from Study ALN-TTR02-002) is planned, which will enable the patients who enrolled in Study ALN-TTR02-002 to receive additional, long-term dosing and safety follow-up. For some of the patients, this may preclude the completion of all of the follow-up period assessments of Study ALN-TTR02-002 if they are deemed eligible to participate in the extension study prior to completion of the full follow up through Day 208. Such patients will be followed up for a minimum of 28 days after their last dose in the current ALN-TTR02-002 study prior to being enrolled in the extension study. The extension study will be implemented only after dose escalation is completed in Study ALN-TTR02-002, with post second dose follow-up through Day 208 in at least 1 cohort at the highest dose.**
Synopsis: Sample Size	Based on the entry criteria and the proposed dose escalation scheme, up to 21 patients are expected to be enrolled. Three patients are to be enrolled at each dose level and up to 3 optional cohorts of 3 patients each will be permitted.	Based on the entry criteria and the proposed dose escalation scheme, up to ~~21~~ **27** patients are expected to be enrolled. Three patients are to be enrolled at each dose level and up to ~~3~~ **5** optional cohorts of 3 patients each will be permitted.
Synopsis: Dose-Limiting	3. An infusion reaction that requires hospitalization,	3. An infusion reaction that requires hospitalization,

Section	Prior Text	Changed To
Toxicities and Stopping Criteria	despite proper premedication.	despite ~~proper~~ premedication.
Table 1.1	Schedule of Assessments Added note v. Premedications include dexamethasone (8 mg, or equivalent), paracetamol (500 mg, or equivalent), H2 blocker (i.e. 150 mg ranitidine or 20 mg famotidine), and H1 blocker (10 mg cetirizine, 25 mg hydroxyzine, fexofenadine or equivalent may be substituted) will be self-administered per os (PO) the evening before study drug administration. Thirty to 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.	Schedule of Assessments **for Cohorts Administered ALN-TTR02 Once Every Four Weeks** **Note: The schedule of assessments for optional cohorts administered ALN-TTR02 once every 3 weeks is provided in Appendix 4.** v. Premedications include dexamethasone (8 mg, or equivalent), paracetamol (500 mg, or equivalent), H2 blocker (~~i.~~ **ee.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) will be self-administered per os (PO) the evening before study drug administration. Thirty to 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**. Patients enrolled in an optional cohort evaluating the use of an alternative premedication regimen, as agreed upon by the SRC, will receive the following medications at least 60 minutes prior to the start of 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**

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		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). These patients will not receive any premedications the evening prior to ALN-TTR02 dosing.
	w. Site personnel are to call patients the day before dosing to remind them to take premedications that evening (the day before dosing).	w. Site personnel are to call patients the day before dosing to remind them to take premedications that evening (the day before dosing). **This reminder will not be needed for patients enrolled in optional cohorts evaluating the alternative premedication regimen.**
1.3: Summary of Clinical Data with siRNA-LNPs	A Phase 1 multi-centre, randomized, placebo-controlled, single-blind, single-ascending dose clinical study to evaluate the safety, tolerability, PK, and pharmacodynamic (PD) of ALN-TTR02 in healthy volunteers was approved by MHRA (EUDRACT # 2011-005291-42) and has completed dosing. ALN-TTR02 was administered as a single 60-minute IV infusion to healthy volunteers at the following doses: 10, 50, 150, 300, and 500 µg/kg (4 patients per dose level; 3 receiving ALN-TTR02 and 1 receiving placebo). The dose level of ALN-TTR02 administered to any cohort in study ALN-TTR02-002 may not be higher than that deemed safe and tolerable in the Phase 1 single-dose study of ALN-TTR02 (Study ALN-TTR02-001). Patients will be pre-medicated with	~~A Phase 1 multi-centre, randomized, placebo-controlled, single-blind, single-ascending dose clinical study to evaluate the safety, tolerability, PK, and pharmacodynamic (PD) of ALN-TTR02 in healthy volunteers was approved by MHRA (EUDRACT # 2011-005291-42) and has completed dosing. ALN-TTR02 was administered as a single 60-minute IV infusion to healthy volunteers at the following doses: 10, 50, 150, 300, and 500 µg/kg (4 patients per dose level; 3 receiving ALN-TTR02 and 1 receiving placebo). The dose level of ALN-TTR02 administered to any cohort in study ALN-TTR02-002 may not be higher than that deemed safe and tolerable in the Phase 1 single-dose study of ALN-TTR02 (Study ALN-TTR02-001). Patients will be pre-medicated with~~

Section	Prior Text	Changed To
	dexamethasone, H1 and H2 blockers, and paracetamol prior to dosing to minimize the risk of infusion reaction. Preliminary data show that ALN-TTR02 was safe and well-tolerated and exhibited robust effects on serum TTR levels at the top doses. To date, no stopping rules have been met and there have been no early discontinuations due to adverse events. Further information on the ALN-TTR02-001 Phase 1 study can be found in the ALN-TTR02 Investigators Brochure and its Expedited Safety Report addendum dated 7 June 2012. Clinical trials with multiple different RNAi medicinal products using local or systemic administration in indications including age-related macular degeneration, respiratory syncytial virus (RSV) infection, oncology, and renal failure have been conducted.[28] Alnylam currently has 3 ongoing systemic delivery programs in the clinic evaluating the safety and efficacy of different siRNAs in 2 different LNP formulations. Specifically, the active Phase 1 clinical trials most relevant to ALN-TTR02 include ALN-PCS02, which uses the same LNP formulation, and ALN-TTR01, which uses the same siRNA as ALN-TTR02 but in a different LNP formulation. The clinical safety data from the ALN-PCS02 study in particular provides some reassurance regarding the safety of this formulation (common with ALN-TTR02) after a single dose**.**	~~dexamethasone, H1 and H2 blockers, and paracetamol prior to dosing to minimize the risk of infusion reaction. Preliminary data show that ALN-TTR02 was safe and well-tolerated and exhibited robust effects on serum TTR levels at the top doses. To date, no stopping rules have been met and there have been no early discontinuations due to adverse events. Further information on the ALN-TTR02-001 Phase 1 study can be found in the ALN-TTR02 Investigators Brochure and its Expedited Safety Report addendum dated 7 June 2012.~~ ~~Further, c~~Clinical trials with multiple different RNAi medicinal products using local or systemic administration in indications including age-related macular degeneration, respiratory syncytial virus (RSV) infection, oncology, and renal failure have been conducted.[28] Alnylam currently has **experience with** ~~34 ongoing~~ systemic delivery programs in the clinic evaluating the safety and efficacy of different siRNAs in 2 different LNP formulations**, including 1 program which employs the same AF-011 formulation as ALN-TTR02**. ~~Specifically, the active Phase 1 clinical trials most relevant to ALN-TTR02 include ALN-PCS02, which uses the same LNP formulation, and ALN-TTR01, which uses the same siRNA as ALN-TTR02 but in a different LNP formulation. The clinical safety data from the ALN-PCS02 study in particular provides some reassurance regarding the~~

Section	Prior Text	Changed To
		~~safety of this formulation (common with ALN-TTR02) after a single dose.~~ **An overview of the safety and pharmacological clinical data with these siRNA-LNPs is included in the ALN-TTR02 Investigator's Brochure (IB), Edition 3, dated 19 October 2012.** [redacted] **Furthermore, the Phase 1 data with all 4 of the siRNA-LNPs have shown that** [redacted]**.**
	ALN-PCS02-001 is a Phase 1 multi-centre, randomized, placebo-controlled, single-blind, single-ascending dose clinical study to evaluate the safety, tolerability, PK, and PD of ALN-PCS02. The study is ongoing in the United Kingdom (EudraCT #2011-000581-36). ALN-PCS02 was administered as a single 60-minute IV infusion to healthy volunteers with elevated low density lipoprotein-C (LDL-C; ≥3 mmol/L) at the following doses: 15, 45, 90, 150, and 250 μg/kg (4 patients per dose level; 3 receiving ALN-PCS02 and 1 receiving placebo). Patients were pre-medicated with dexamethasone, H1 and H2 blockers, and paracetamol prior to dosing to minimize the risk of infusion reaction. As of December 2011, 24 patients (23 males and 1 female) have been enrolled in the ALN-PCS02-001 study and 18 patients (17 males and 1 female) have received ALN-PCS02 in 6 cohorts at	**Importantly, a Phase 1 study with ALN-TTR02 was recently completed. Study ALN-TTR02-001 was a multicenter, randomized, placebo-controlled, single-blind, single-ascending dose clinical study conducted in the UK to evaluate the safety, tolerability, PK, and pharmacodynamic (PD) in healthy volunteers (EudraCT # 2011 005291-42). ALN-TTR02 was administered as a single 60-minute IV infusion to healthy volunteers at the following doses: 10, 50, 150, 300, and 500 μg/kg (4 patients per dose level; 3 receiving ALN-TTR02 and 1 receiving placebo). Patients were premedicated with dexamethasone, H1 and H2 blockers, and paracetamol prior to dosing to minimize the risk of infusion reaction. The data show that ALN-TTR02 was safe and well-tolerated and exhibited robust effects on serum TTR levels at doses ≥0.15 mg/kg.**

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	doses ranging from 15 to 250 µg/kg (including 2 cohorts dosed at the 250 µg/kg dose). No dose-limiting toxicities (DLTs) have been reported to date, and no patient has prematurely withdrawn from the study due to an adverse event (AE). There was one serious adverse event (SAE; bilateral pulmonary emboli in a patient with chronic deep vein thrombosis) considered unrelated to study drug that occurred in 1 patient dosed at 45 µg/kg. There have been no laboratory AEs related to study drug; specifically, there have been no clinically significant changes in liver function tests (LFTs), electrolytes, hematology parameters, or renal function post-dose. Mild self-limiting erythematous skin rashes not requiring treatment were seen in 6 patients dosed with ALN-PCS02 (1 each at 15 and 45 µg/kg, and in 4 patients at 0.25 mg/kg). Transient elevations in the complement pathway Bb (1.25- to 4.6-fold) have occurred in 14 patients 30 minutes post-infusion and have been observed across all dose levels and were not associated with signs or symptoms. There have been no cytokine or CRP elevations in any of the patients. Stopping rules for dose escalation have not been met, and the study is ongoing. Preliminary PD data with ALN-PCS02 showed an approximately 60% reduction in PCSK9 serum protein at the 150 and 250 µg/kg doses compared with baseline	**Lowering of TTR levels was reversible following administration of a single dose, and there were no AEs associated with lowering TTR by >90%. Further details on this study can be found in the ALN-TTR02 IB, Edition 3.** ~~ALN-PCS02-001 is a Phase 1 multi-centre, randomized, placebo-controlled, single-blind, single-ascending dose clinical study to evaluate the safety, tolerability, PK, and PD of ALN-PCS02. The study is ongoing in the United Kingdom (EudraCT #2011-000581-36). ALN-PCS02 was administered as a single 60-minute IV infusion to healthy volunteers with elevated low density lipoprotein-C (LDL-C; ≥3 mmol/L) at the following doses: 15, 45, 90, 150, and 250 µg/kg (4 patients per dose level; 3 receiving ALN-PCS02 and 1 receiving placebo). Patients were pre-medicated with dexamethasone, H1 and H2 blockers, and paracetamol prior to dosing to minimize the risk of infusion reaction. As of December 2011, 24 patients (23 males and 1 female) have been enrolled in the ALN-PCS02-001 study and 18 patients (17 males and 1 female) have received ALN-PCS02 in 6 cohorts at doses ranging from 15 to 250 µg/kg (including 2 cohorts dosed at the 250 µg/kg dose).~~ ~~No dose-limiting toxicities (DLTs) have been reported to date, and no patient has prematurely withdrawn from the study due to an adverse event (AE). There was one serious adverse event (SAE; bilateral pulmonary~~

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	levels, with a return toward baseline after ~ 28 days. The ALN-TTR01 Phase 1 clinical trial (ALN-TTR01-001) is a multi-centre, randomized, placebo-controlled, single-blind, single-ascending dose study to evaluate the safety, tolerability, PK and PD of ALN-TTR01. The study is ongoing in France, Portugal, Sweden, and the United Kingdom (EudraCT # 2009-017383-16). The data from this study are relevant to provide the effect of TTR lowering using the same siRNA as ALN-TTR02 albeit in a different formulation. ALN-TTR01 was administered as a single 15-minute IV infusion to ATTR Stage 1 patients at the following doses: 10, 30, 100, 200, 400, 700, and 1000 µg/kg (4 patients per dose level; 3 receiving ALN-TTR01 and 1 receiving placebo). Patients were pre-medicated with a similar regimen used for ALN-PCS02. A total of 32 patients were enrolled in this study (including 8 at 1000 µg/kg and 4 each at 10-700 µg/kg), of whom 24 (14 males and 10 females) received ALN-TTR01. There were no laboratory AEs and no significant changes in LFTs, electrolytes, hematology parameters, or renal function post-dose. No clinically significant changes in testosterone were observed in males. No drug-related SAEs or DLTs occurred, and no patient withdrew prematurely from the study due to an AE. There were 5 acute infusion-related reactions (IRRs): 1 each at 400 and 700 µg/kg and 3 at 1000 µg/kg, for an overall rate of 21%, all of which were mild to moderate	~~emboli in a patient with chronic deep vein thrombosis) considered unrelated to study drug that occurred in 1 patient dosed at 45 µg/kg. There have been no laboratory AEs related to study drug; specifically, there have been no clinically significant changes in liver function tests (LFTs), electrolytes, hematology parameters, or renal function post-dose.~~ ~~Mild self-limiting erythematous skin rashes not requiring treatment were seen in 6 patients dosed with ALN-PCS02 (1 each at 15 and 45 µg/kg, and in 4 patients at 0.25 mg/kg). Transient elevations in the complement pathway Bb (1.25- to 4.6-fold) have occurred in 14 patients 30 minutes post-infusion and have been observed across all dose levels and were not associated with signs or symptoms. There have been no cytokine or CRP elevations in any of the patients. Stopping rules for dose escalation have not been met, and the study is ongoing.~~ ~~Preliminary PD data with ALN-PCS02 showed an approximately 60% reduction in PCSK9 serum protein at the 150 and 250 µg/kg doses compared with baseline levels, with a return toward baseline after ~ 28 days.~~ ~~The ALN-TTR01 Phase 1 clinical trial (ALN-TTR01-001) is a multi-centre, randomized, placebo-controlled, single-blind, single-ascending dose study to evaluate the safety, tolerability, PK and PD of ALN-TTR01. The study is ongoing in France, Portugal, Sweden, and the United Kingdom (EudraCT # 2009-017383-16).~~

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	in severity, and where necessary, responded to temporary halting of the infusion followed by resumption of the infusion at a slower rate. Regardless of whether a patient experienced an IRR, all study drug infusions were successfully administered to completion. Transient elevations of Bb were observed across all dose levels, with higher induction seen in patients with acute IRRs. There have been no changes in C3, C4, or CH50. Modest elevations in both IL-6 (2 to 7-fold over baseline) and CRP (4 to 9-fold over baseline) were observed in 3 patients treated at 1000 µg/kg and were not associated with fever or chills. Preliminary plasma PK results indicate a generally dose-proportional increase in the area under the plasma concentration-time curve (AUC) and the observed maximum concentration (C_{max}) and the long term exposure in humans is higher than predicted by the preclinical animal studies. The PD effects of ALN-TTR01 were assessed through serial measurements of serum TTR protein.i There were no significant changes in TTR relative to placebo at doses below 1000 µg/kg. At 1000 µg/kg (n=5), a mean decline in TTR protein of 41% relative to baseline and placebo was observed by Day 7 post-dose (geometric mean; p=0.02), with recovery to 80% of pre-treatment level for the group by Day 28. In 1 patient, the serum TTR declined by 81%; this was accompanied by a similar decrease in RBP and vitamin A, as predicted by preclinical data with ALN-	~~The data from this study are relevant to provide the effect of TTR lowering using the same siRNA as ALN-TTR02 albeit in a different formulation. ALN-TTR01 was administered as a single 15-minute IV infusion to ATTR Stage 1 patients at the following doses: 10, 30, 100, 200, 400, 700, and 1000 µg/kg (4 patients per dose level; 3 receiving ALN-TTR01 and 1 receiving placebo). Patients were pre-medicated with a similar regimen used for ALN-PCS02. A total of 32 patients were enrolled in this study (including 8 at 1000 µg/kg and 4 each at 10-700 µg/kg), of whom 24 (14 males and 10 females) received ALN-TTR01.~~ ~~There were no laboratory AEs and no significant changes in LFTs, electrolytes, hematology parameters, or renal function post-dose. No clinically significant changes in testosterone were observed in males. No drug-related SAEs or DLTs occurred, and no patient withdrew prematurely from the study due to an AE. There were 5 acute infusion-related reactions (IRRs): 1 each at 400 and 700 µg/kg and 3 at 1000 µg/kg, for an overall rate of 21%, all of which were mild to moderate in severity, and where necessary, responded to temporary halting of the infusion followed by resumption of the infusion at a slower rate. Regardless of whether a patient experienced an IRR, all study drug infusions were successfully administered to completion. Transient elevations of Bb were observed across all dose levels, with higher induction seen in patients with acute IRRs. There have been no changes~~

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	TTR01 in NHPs and the known role of TTR in binding to and stabilizing circulating RBP. By Day 70, TTR and vitamin A had recovered to approximate pre-treatment levels without any reported signs or symptoms and did not require the need for supplementation. There were no significant changes in thyroid hormone levels or thyroid stimulating hormone (TSH) in this patient, and no adverse effects associated with TTR lowering. A similar pattern of TTR/RBP/vitamin A decrease and recovery without any impact on safety was observed in all patients on the study treated at 1.0 mg/kg who showed a response to ALN-TTR01. As with the PD data with ALN-PCS02, these data show the translatability of the PD effect from the monkey to human on a mg/kg dose basis, and show predictable reversibility. The safety of giving multiple doses of an siRNA-LNP has been established with ALN-VSP02, which uses the same first generation LNP formulation as ALN-TTR01 (see Investigator's Brochure). In a Phase 1 study performed in advanced cancer patients with liver involvement, chronic bi-weekly dosing with doses as high as 1.0 mg/kg was safe and well-tolerated using the same premedication regimen as ALN-TTR01, with no significant dose-dependent effect on liver function. Three patients with tumor shrinkage or prolonged disease stabilization who have received biweekly doses at 0.7-1.0 mg/kg for as long as 12-18 months are	~~in C3, C4, or CH50. Modest elevations in both IL-6 (2 to 7-fold over baseline) and CRP (4 to 9-fold over baseline) were observed in 3 patients treated at 1000 µg/kg and were not associated with fever or chills. Preliminary plasma PK results indicate a generally dose-proportional increase in the area under the plasma concentration-time curve (AUC) and the observed maximum concentration (C_{max}) and the long-term exposure in humans is higher than predicted by the preclinical animal studies.~~ ~~The PD effects of ALN-TTR01 were assessed through serial measurements of serum TTR protein.ii There were no significant changes in TTR relative to placebo at doses below 1000 µg/kg. At 1000 µg/kg (n=5), a mean decline in TTR protein of 41% relative to baseline and placebo was observed by Day 7 post-dose (geometric mean; p=0.02), with recovery to 80% of pre-treatment level for the group by Day 28. In 1 patient, the serum TTR declined by 81%; this was accompanied by a similar decrease in RBP and vitamin A, as predicted by preclinical data with ALN-TTR01 in NHPs and the known role of TTR in binding to and stabilizing circulating RBP. By Day 70, TTR and vitamin A had recovered to approximate pre-treatment levels without any reported signs or symptoms and did not require the need for supplementation. There were no significant changes in thyroid hormone levels or thyroid stimulating hormone (TSH) in this patient, and no adverse effects associated~~

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	continuing their treatment on an extension study. Thus, the nonclinical data with ALN-TTR02 and the preliminary clinical data with ALN-PCS02, ALN-TTR01, and ALN-VSP02 support the proposed early phase clinical trials evaluating ascending doses (single or multiple) of ALN-TTR02 (studies ALN-TTR02-001 and ALN-TTR02-002). Further information, including preliminary safety and efficacy updates from the recently completed Phase 1 trials of ALN-TTR02 in healthy volunteers and ALN-PCS02 in healthy volunteers with elevated cholesterol, can be found in the Investigator's Brochure and its Expedited Safety Report addendum dated 7 June 2012. Alnylam will immediately notify the Principal Investigators if any relevant new safety or toxicology information becomes available during the study.	~~with TTR lowering. A similar pattern of TTR/RBP/vitamin A decrease and recovery without any impact on safety was observed in all patients on the study treated at 1.0 mg/kg who showed a response to ALN-TTR01. As with the PD data with ALN-PCS02, these [redacted], and show predictable reversibility.~~ ~~The safety of giving multiple doses of an siRNA-LNP has been established with ALN-VSP02, which uses the same first generation LNP formulation as ALN-TTR01 (see Investigator's Brochure). In a Phase 1 study performed in advanced cancer patients with liver involvement, chronic bi-weekly dosing with doses as high as 1.0 mg/kg was safe and well-tolerated using the same premedication regimen as ALN-TTR01, with no significant dose-dependent effect on liver function. Three patients with tumor shrinkage or prolonged disease stabilization who have received biweekly doses at 0.7-1.0 mg/kg for as long as 12-18 months are continuing their treatment on an extension study.~~ ~~Thus, the nonclinical data with ALN-TTR02 and the preliminary clinical data with ALN-PCS02, ALN-TTR01, and ALN-VSP02 support the proposed early phase clinical trials evaluating ascending doses (single or multiple) of ALN-TTR02 (studies ALN-TTR02-001 and ALN-TTR02-002).~~ ~~Further information, including preliminary safety and~~

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		~~efficacy updates from the recently completed Phase 1 trials of ALN-TTR02 in healthy volunteers and ALN-PCS02 in healthy volunteers with elevated cholesterol, can be found in the Investigator's Brochure and its Expedited Safety Report addendum dated 7 June 2012. Alnylam will immediately notify the Principal Investigators if any relevant new safety or toxicology information becomes available during the study.~~
1.5: Dose Selection and Rationale	Two consecutive ALN-TTR02 doses, separated by a 4-week period, will be administered to patients. Study drug will be administered as a 60-minute IV infusion with the following proposed doses for each cohort: 10, 50, 150, and 300 µg/kg ALN-TTR02. In addition, cumulative safety and tolerability data observed in at least 2 patients through at least 96 hours post-second dose of the previous cohort will be reviewed by the SRC prior to administration of the second dose of study drug. The dose level of ALN-TTR02 administered to any cohort in study ALN-TTR02-002 may not be higher than that deemed safe and tolerable in the Phase 1 single-dose study of ALN-TTR02 (Study ALN-TTR02-001).	Two consecutive ALN-TTR02 doses, separated by a 4-week period **(or a 3-week period in select optional cohorts)**, will be administered to patients. Study drug will be administered as a 60-minute IV infusion with the following proposed doses for each **of the original** cohort**s**: 10, 50, 150, and 300 µg/kg ALN-TTR02. **For those patients enrolled in an optional cohort evaluating the alternative premedication regimen and infusion rate, the study drug will be administered IV over approximately 70 minutes.** In addition, cumulative safety and tolerability data observed in at least 2 patients through at least 96 hours post-second dose of the previous cohort will be reviewed by the SRC prior to administration of the second dose of study drug. ~~The dose level of ALN-TTR02 administered to any cohort in study ALN-TTR02-002 may not be higher than that deemed safe and tolerable in the Phase 1 single-dose study of ALN-TTR02 (Study ALN-TTR02-001).~~

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	The optional cohort would be included to further confirm the safety and/or PD effect. If this occurs, the Independent Ethics Committees (IECs) will be informed of the expansion. Up to 3 optional cohorts of 3 patients each are permitted in this study.	The optional cohort**(s)** would be included to further confirm the safety and/or PD effect **of previously evaluated dose levels, which may include evaluation of the original dosing regimen (once every 4 weeks), an alternative dosing regimen (once every 3 weeks), and/or an alternative premedication regimen and infusion rate. The dosing regimen for the first optional cohort will be once every 4 weeks, and the dosing regimen for the remaining 4 optional cohorts will be once every 3 or 4 weeks. It is anticipated that the first 2 optional cohorts will use the original premedication regimen, and the remaining 3 optional cohorts may be used to explore the alternative premedication regimen and infusion rate. The SRC will review all available safety data and make recommendations on dosing and premedication regimens prior to dosing of any of the optional cohorts.** If this occurs, the Independent Ethics Committees (IECs) will be informed of the ~~expansion~~**implementation of optional cohorts**. Up to ~~3~~ **5** optional cohorts of 3 patients each are permitted in this study.
1.6.1: Infusion-Related Reactions	The premedication regimen will include orally (PO) administered dexamethasone (or equivalent), paracetamol (or equivalent), and H1 and H2 blockers (as described in Section 5.5). The rate of infusion (over the course of 1 hour) will also help to reduce the potential for acute IRRs.	The premedication regimen will include ~~orally (PO) administered~~ dexamethasone (or equivalent), paracetamol (or equivalent), and H1 and H2 blockers (as described in Section 5.5). The **infusion** rate of **1 hour or longer**~~infusion (over the course of 1 hour)~~ will also help to reduce the potential for acute IRRs.
3.1: Overall Design	No patients will be a member of more than 1 treatment	No patients will be a member of more than 1 treatment

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	group.	group. **An alternative dosing regimen of 2 doses of ALN-TTR02 (at a dose previously determined by the SRC to be safe and tolerable) separated by 3 weeks may be evaluated in the optional cohort(s).**
	For patients on all dose levels other than the starting dose level of 10 μg/kg, prior to receiving the second dose the SRC will review the cumulative safety and tolerability data of at least 2 patients from the previous dose level(s) with at least 96 hours of follow-up after receiving a second dose of study drug. The dose level of ALN-TTR02 administered to any cohort in study ALN-TTR02-002 may not be higher than that deemed safe and tolerable in the Phase 1 single-dose study of ALN-TTR02 (Study ALN-TTR02-001).	For patients on all dose levels other than the starting dose level of 10 μg/kg, prior to receiving the second dose the SRC will review the cumulative safety and tolerability data of at least 2 patients from the previous dose level(s) with at least 96 hours of follow-up after receiving a second dose of study drug. ~~The dose level of ALN-TTR02 administered to any cohort in study ALN-TTR02-002 may not be higher than that deemed safe and tolerable in the Phase 1 single-dose study of ALN-TTR02 (Study ALN-TTR02-001).~~
	Eligible patients will undergo further pre-treatment assessments (performed on Day 0). All patients will receive oral premedication with dexamethasone (or equivalent), paracetamol (or equivalent), and H1 and H2 blockers the night before and 30 to 60 minutes prior to each dose of ALN-TTR02 to reduce the potential of an IRR (see Section 5.5). On Days 0 and 28, patients will receive a single dose of ALN-TTR02 administered as a 60-minute IV infusion	Eligible patients will undergo further pre-treatment assessments (performed on Day 0). ~~All~~ **Patients receiving the original premedication regimen**~~patients~~ will receive oral premedication with dexamethasone (or equivalent), paracetamol (or equivalent), and H1 and H2 blockers the night before and 30 to 60 minutes prior to each dose of ALN-TTR02 to reduce the potential of an IRR (see Section 5.5). **Those patients in an optional cohort evaluating the alternative premedication regimen, as agreed upon by the SRC, will receive IV dexamethasone (or equivalent), oral paracetamol (or equivalent), and IV H1 and H2 blockers at least 60 minutes prior to ALN-TTR02**

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		dosing; no premedication will be administered the evening prior to dosing. On Days 0 and 28, patients will receive a single dose of ALN-TTR02 administered as a 60-minute IV infusion **(for cohorts with the original premedication regimen and infusion rate), or as an approximate 70-minute IV infusion, for those patients in an optional cohort evaluating the alternative premedication regimen and infusion rate.**
4.1: Eligibility of Patients	Based on the planned dose escalation scheme (see Section 5.7.1), up to 21 patients are expected to be enrolled.	Based on the planned dose escalation scheme (see Section 5.7.1), up to ~~21~~ **27** patients are expected to be enrolled.
5.5: Premedication Plan	Premedication will be administered as follows: Added language for an alternative premedication regimen and infusion rate.	**Patients receiving the original premedication regimen will be administered the following** ~~Premedication will be administered as follows~~: **An alternative premedication regimen and infusion rate can be used in select optional cohorts, as agreed upon by the SRC. The dosing regimen for the first optional cohort will be once every 4 weeks, and the dosing regimen for the remaining 4 optional cohorts will be once every 3 or 4 weeks. It is anticipated that the first 2 optional cohorts will use the original premedication regimen, and the remaining 3 optional cohorts may be used to explore the alternative premedication regimen and infusion rate. Patients in these cohorts will not receive any premedications the evening prior to ALN-TTR02 dosing. Prior to each dose of ALN-TTR02, these**

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		patients will receive the following premedications: • **Intravenous (IV) dexamethasone (10 mg) or equivalent, administered at least 60 minutes prior to the start of infusion of ALN-TTR02;** • **Oral paracetamol (500 mg) or equivalent at least 60 minutes prior to the start of 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 start of 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 start of 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 blockers.**
5.6: Dose, Route, and Schedule of Study Drug Administration	Study drug doses will be administered as a 60-minute IV infusion 4 weeks apart. Study drug will be administered via a controlled infusion device with an extension set containing a 1.2 micron filter supplied by Alnylam or designee. Infusion products containing polyvinyl chloride, di(2-ethylhexyl)phthalate (PVC, DEHP) must NOT be used.	Study drug doses will be administered as a 60-minute IV infusion **(flow rate of approximately 3.3 mL/min)** 4 weeks apart. **The dosing of ALN-TTR02 in the optional cohorts will be once every 4 weeks for the first optional cohort and once every 3 or 4 weeks for the remaining 4 optional cohorts, at a dose previously determined by the SRC to be safe and tolerable.** Study drug will be administered via a

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		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. **For patients enrolled in an optional cohort evaluating the alternative premedication regimen, the flow rate will be approximately 1.1 mL/min during the first 15 minutes (1/3rd the original flow rate) with the remainder of the infusion taking place over 55 minutes (at a flow rate of approximately 3.3 mL/min) for a total infusion time of approximately 70 minutes.**
	The dose level of ALN-TTR02 administered to any cohort in study ALN-TTR02-002 may not be higher than that deemed safe and tolerable in the Phase 1 single-dose study of ALN-TTR02 (Study ALN-TTR02-001).	~~The dose level of ALN-TTR02 administered to any cohort in study ALN-TTR02-002 may not be higher than that deemed safe and tolerable in the Phase 1 single-dose study of ALN-TTR02 (Study ALN-TTR02-001).~~
Table 5-1	Total number of patients to be enrolled = 21 a Up to 3 optional cohorts may be included to confirm the safety, tolerability, and/or PD effect at a specific dose.	Changed the total number of patients to be enrolled from 21 patients to 27 patients a Up to ~~3~~**5** optional cohorts may be included to confirm the safety, tolerability, and/or PD effect **of previously evaluated dose levels, which may include evaluation of the original dosing regimen (once every 4 weeks), an alternative dosing regimen (once every 3 weeks), and/or an alternative premedication regimen and infusion**

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		rate at a specific dose **of ALN-TTR02**.
5.7.1: Dose Escalation Procedures	The initial dose of ALN-TTR02 is 10 µg/kg. Dose escalation to 50, 150, and 300 µg/kg is planned. The dose level of ALN-TTR02 administered to any cohort in study ALN-TTR02-002 may not be higher than that deemed safe and tolerable in the Phase 1 single-dose study of ALN-TTR02 (Study ALN-TTR02-001).	The initial dose of ALN-TTR02 is 10 µg/kg. Dose escalation to 50, 150, and 300 µg/kg is planned. ~~The dose level of ALN-TTR02 administered to any cohort in study ALN-TTR02-002 may not be higher than that deemed safe and tolerable in the Phase 1 single-dose study of ALN-TTR02 (Study ALN-TTR02-001).~~
Figure 5-1	Footnote a added	**a Some optional cohorts may have the second dose of ALN-TTR02 administered 3 weeks after the first dose.**
5.7.2: Optional Cohorts	The optional cohort would be included to further confirm the safety and/or PD effect. If this occurs, the IECs will be informed of the expansion. Up to 3 optional cohorts of 3 patients each are permitted in this study.	The optional cohort would be included to further confirm the safety and/or PD effect **of previously evaluated dose levels, which may include evaluation of the original dosing regimen (once every 4 weeks), an alternative dosing regimen (once every 3 weeks), and/or to evaluate an alternative premedication regimen and infusion rate. The dosing regimen for the first optional cohort will be once every 4 weeks, and the dosing regimen for the remaining 4 optional cohorts will be once every 3 or 4 weeks. It is anticipated that the first 2 optional cohorts will use the original premedication regimen, and the remaining 3 optional cohorts may be used to explore the alternative premedication regimen and infusion rate. The SRC will review all available safety data and make recommendations on dosing and premedication regimens prior to dosing of any of the optional cohorts.** If this occurs, the IECs will be

Section	Prior Text	Changed To
		informed of the ~~expansion~~**implementation of optional cohorts**. Up to ~~3~~ **5** optional cohorts of 3 patients each are permitted in this study.
5.7.3: Dose-limiting Toxicity	A DLT is defined as: • Any life-threatening toxicity; • ALT and AST levels ≥5 × ULN or total bilirubin >2.0 mg/dL; • An infusion reaction that requires hospitalization, despite proper premedication; • Any other toxicity which in the opinion of the SRC would have precluded further dosing.	A DLT is defined as: • Any life-threatening toxicity; • ALT and AST levels ≥5 × ULN or total bilirubin >2.0 mg/dL; • An infusion reaction that requires hospitalization, despite ~~proper~~ premedication; • Any other toxicity which in the opinion of the SRC would have precluded further dosing.
6: Study Visits	Added language regarding an open-label extension study	**An open-label extension study at the recommended Phase 3 dose and regimen (as derived from Study ALN-TTR02-002) is planned, which will enable the patients who enrolled in Study ALN-TTR02-002 to receive additional, long-term dosing and safety follow-up. For some of the patients, this may preclude the completion of all of the follow-up period assessments of Study ALN-TTR02-002 if they are deemed eligible to participate in the extension study prior to completion of the full follow up through Day 208. Such patients will be followed up for a minimum of 28 days after their last dose in the current ALN-TTR02-002 study prior to being enrolled in the extension study. The extension study**

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		will be implemented only after dose escalation is completed in Study ALN-TTR02-002, with post second dose follow-up through Day 208 in at least 1 cohort at the highest dose.
	Screening evaluations are to be performed within 45 days before receiving the first dose of study drug, as indicated in Table 1-1. Patients determined to be eligible based on screening assessments will receive treatment (IV infusion of study drug) on Days 0 (Baseline) and 28 (or date of second dose administration in optional cohorts with dosing intervals greater than 4 weeks). Patients will remain hospitalized at the study site for at least 24 hours following completion of dosing to be observed for safety assessments and PK sampling. Patients will return to the study site on Days 2, 7, 10, 14, and 21 for follow-up assessments. In addition, follow-up visits after the second dose of study drug (Day 28) will occur on Days 30, 35, 38, 42, 49, and 56 (end-of-study). Patients will also return to the site for a follow-up visit on Days 112 and 208.	Screening evaluations are to be performed within 45 days before receiving the first dose of study drug, as indicated in Table 1-1 **(Appendix 4 provides the schedule of assessments for the optional cohort(s) administered ALN-TTR02 once every 3 weeks)**. Patients determined to be eligible based on screening assessments will receive treatment (IV infusion of study drug) on Days 0 (Baseline) and 28 (or date of second dose administration in optional cohorts with dosing intervals greater than 4 weeks)**; for patients in optional cohorts evaluating the alternative dosing regimen (once every 3 weeks), ALN-TTR02 will be administered on Days 0 (Baseline) and 21**. Patients will remain hospitalized at the study site for at least 24 hours following completion of dosing to be observed for safety assessments and PK sampling. Patients will return to the study site on Days 2, 7, 10, 14, and 21 for follow-up assessments. In addition, follow-up visits after the second dose of study drug (Day 28) will occur on Days 30, 35, 38, 42, 49, and 56 (end-of-study)**; patients in optional cohorts evaluating the alternative dosing regimen (once every 3 weeks) will have follow-up visits on Days 21, 22, 23, 28, 31, 35,**

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		42, 49, and 56. Patients will also return to the site for a follow-up visit on Days 112 and 208.
6.2: Pre-Dosing (Day -1 or Day 27)	(Title) Pre-Dosing (Day -1 or Day 27) On the day prior to administration of study drug (Days -1 and 27), study site personnel will contact the patient by phone to discuss the pretreatment medication they are to take that evening. On the evening before each dosing, patients are to self-administer the following medications PO: 8 mg dexamethasone or equivalent, 500 mg paracetamol or equivalent, an H1 blocker (10 mg cetirizine hydroxyzine [25 mg, fexofenadine, or equivalent may be substituted if patient does not tolerate cetirizine]), and an oral H2 blocker (i.e., ranitidine 150 mg or famotidine 20 mg or equivalent).	(Title) Pre-Dosing (Day -1 or Day 27**; Day -1 or Day 20 for optional cohorts dosed once every 3 weeks**) **For those patients receiving the original premedication regimen,** on the day prior to administration of study drug (Days -1 and 27), study site personnel will contact the patient by phone to discuss the pretreatment medication they are to take that evening. On the evening before each dosing, patients are to self-administer the following medications PO: 8 mg dexamethasone or equivalent, 500 mg paracetamol or equivalent, an H1 blocker (10 mg cetirizine hydroxyzine [25 mg, fexofenadine, or equivalent may be substituted if patient does not tolerate cetirizine]), and an oral H2 blocker (~~i.e.~~**e.g**., ranitidine 150 mg**,** ~~or~~ famotidine 20 mg**,** or equivalent **other H2 blocker).** **Patients in an optional cohort evaluating the alternative premedication regimen and infusion rate will not receive any premedication the evening prior to ALN-TTR02 dosing.**
6.3.1: Day 0 or Day 28 (+2 days)	(Title) Day 0 or Day 28 (+2 days)	(Title) Day 0 or Day 28 (+2 days)**; Day 0 or Day 21 for optional cohorts dosed once every 3 weeks**
6.3.1.1: Pre-dose	Patients will undergo the following procedures before	Patients will undergo the following procedures before

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	study drug administration on Day 0 or Day 28:	study drug administration on Day 0 or Day 28 **(Day 0 or Day 21 for optional cohorts dosed once every 3 weeks)**:
	Note: those parameters marked with an asterisk do not have to be reassessed on Days 0 or 28 if measures are obtained within 72 hours prior to dosing and meets eligibility criteria.	Note: those parameters marked with an asterisk do not have to be reassessed on Days 0 or 28 **(Days 0 or 21 for optional cohorts dosed once every 3 weeks)** if measures are obtained within 72 hours prior to dosing and meets eligibility criteria.
	• Premedicate patient 30 to 60 minutes prior to the start of study drug infusion with the following oral medications (or equivalent[s]): 20 mg dexamethasone, 500 mg paracetamol, 10 mg cetirizine, and an H2 blocker (e.g. 150 mg ranitidine, 20 mg famotidine).	• **For patients receiving the original premedication regimen, ~~P~~p**remedicate **the** patient 30 to 60 minutes prior to the start of study drug infusion with the following oral medications (or equivalent[s]): 20 mg dexamethasone, 500 mg paracetamol, 10 mg cetirizine, and an H2 blocker (e.g. 150 mg ranitidine, 20 mg famotidine). **Patients in select optional cohorts evaluating the alternative premedication regimen and infusion rate will receive the following medications (or equivalent[s]) at least 60 minutes prior to the start of ALN-TTR02 infusion:** **IV dexamethasone 10 mg, PO paracetamol 500 mg, 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 other IV**

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		H1 blocker available at the study site; or hydroxyzine or fexofenadine 25 mg PO or cetirizine 10 mg PO for any patient who does not tolerate IV diphenhydramine or other IV H1 blockers).
6.3.1.2: Administration of Study Drug	After completion of all pre-dose evaluations and procedures, administer study drug as a 60-minute IV infusion via a controlled infusion device.	After completion of all pre-dose evaluations and procedures, administer study drug as a 60-minute IV infusion **(or an approximate 70-minute IV infusion for patients in an optional cohort evaluating the alternative premedication regimen and infusion rate)** via a controlled infusion device.
6.3.1.3: Post-dose	Patients will undergo the following procedures after study drug administration on Day 0 and Day 28 (+2 days).	Patients will undergo the following procedures after study drug administration on Day 0 and Day 28 (+2 days)**; or Day 0 and Day 21 for optional cohorts dosed once every 3 weeks.**
6.3.2: Day 1 or Day 29	(Title) Day 1 or Day 29 Patients will undergo the following procedures on the day following dosing with study drug (Days 1 or 29):	(Title) Day 1 or Day 29**; Day 1 or Day 22 for optional cohorts dosed once every 3 weeks** Patients will undergo the following procedures on the day following dosing with study drug (Days 1 or 29**; or Days 1 or 22 for optional cohorts dosed once every 3 weeks**):
6.3.3: Day 2 or Day 30	(Title) Day 2 or Day 30 Patients will undergo the following procedures on Days 2 and 30:	(Title) Day 2 or Day 30**; Day 2 or Day 23 for optional cohorts dosed once every 3 weeks** Patients will undergo the following procedures on Days 2 and 30**; or Days 2 or 23 for optional cohorts dosed once every 3 weeks**:
6.3.4: Day 7 or Day 35 (±1 day)	(Title) Day 7 or Day 35 ±1 day Patients will undergo the following procedures on the	(Title) Day 7 or Day 35 ±1 day; **or Day 7 or Day 28 for optional cohorts dosed once every 3 weeks** Patients will undergo the following procedures on the

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	Day 7 and 35 study visits:	Day 7 and 35 study visits **(or Day 7 and 28 for optional cohorts dosed once every 3 weeks)**:
6.3.5: Day 10 or Day 38 (±2 day)	(Title) Day 10 or Day 38 (±2 day) Patients will undergo the following procedures on the Day 10 and 38 study visits:	(Title) Day 10 or Day 38 ±2 day**; or Day 10 or Day 31 for optional cohorts dosed once every 3 weeks** Patients will undergo the following procedures on the Day 10 and 38 study visits **(or Day 10 and 28 for optional cohorts dosed once every 3 weeks)**:
6.3.6: Day 14 or Day 42 (±3 days)	(Title) Day 14 or Day 42 (±3 days) Patients will undergo the following procedures on the Day 14 and 42 study visits:	(Title) Day 14 or Day 42 ±3 days**; or Day 14 or Day 35 for optional cohorts dosed once every 3 weeks** Patients will undergo the following procedures on the Day 14 and 42 study visits **(or Day 14 and 35 for optional cohorts dosed once every 3 weeks)**:
6.3.7: Day 21 or Day 49 (±3 days)	(Title) Day 21 or Day 49 (±3 days) Patients will undergo the following procedures on the Day 21 and 49 study visits:	(Title) Day 21 or Day 49 ±3 days**; or Day 21 or Day 42 for optional cohorts dosed once every 3 weeks** Patients will undergo the following procedures on the Day 21 and 49 study visits **(or Day 21 and 42 for optional cohorts dosed once every 3 weeks)**:
6.6: Participation in an Open-label Extension Study	Added new section	**An open-label extension study at the recommended Phase 3 dose and regimen (as derived from Study ALN-TTR02-002) is planned, which will enable the patients who enrolled in Study ALN-TTR02-002 to receive additional, long-term dosing and safety follow-up. For some of the patients, this may preclude the completion of all of the follow-up period assessments of Study ALN-TTR02-002 if they are deemed eligible to participate in the extension study prior to completion of the full follow**

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		up through Day 208. Such patients will be followed up for a minimum of 28 days after their last dose in the current ALN-TTR02-002 study prior to being enrolled in the extension study. The extension study will be implemented only after dose escalation is completed in Study ALN-TTR02-002, with post second dose follow-up through Day 208 in at least 1 cohort at the highest dose deemed safe.
7.1: Demographic Data and Medical History	Patient demographic data will be obtained during screening, and a complete medical history will be obtained during screening and updated on Days 0 and 28 as needed.	Patient demographic data will be obtained during screening, and a complete medical history will be obtained during screening and updated on Days 0 and 28 **(or Days 0 and 21 for optional cohorts dosed once every 3 weeks)** as needed.
7.2.1: Physical Examination	Body weight will be measured at Screening for assessment of eligibility, and on the Day 0 and 28 study visits.	Body weight will be measured at Screening for assessment of eligibility, and on the Day 0 and 28 **(or Day 0 and 21 for optional cohorts dosed once every 3 weeks)** study visits.
7.2.2: Vital Signs	Vital signs are to be measured at Screening and the Day 2, 14, 30, 42, and 56 (or time of early termination, if applicable) study visits and include systolic/diastolic blood pressure, pulse rate, respiration rate, and oral body temperature. On Days 0 and 28, serial vital signs are to be measured within 30 minutes pre-dose, at EOI; and at 30 (±5) minutes;1, 2, and 3 (±15 minutes) hours; 6, 12, and 18 hours (±30 minutes) post-infusion. On Days 1 and 29, serial vital signs are to be measured at 24 hours	Vital signs are to be measured at Screening and the Day 2, 14, 30, 42, and 56 (**or Days 2, 14, 23, 35, and 56 for optional cohorts dosed once every 3 weeks;** or time of early termination, if applicable) study visits and include systolic/diastolic blood pressure, pulse rate, respiration rate, and oral body temperature. On Days 0 and 28 **(or Day 21 for optional cohorts dosed once every 3 weeks)**, serial vital signs are to be measured within 30 minutes pre-dose, at EOI; and at 30 (±5) minutes;1, 2, and 3 (±15 minutes) hours; 6, 12, and 18 hours (±30 minutes) post-infusion. On Days 1

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	(+30 minutes) post-infusion.	and 29 **(or Day 22 for optional cohorts dosed once every 3 weeks)**, serial vital signs are to be measured at 24 hours (+30 minutes) post-infusion.
7.2.4: Electrocardiogram	Serial ECGs will be collected in triplicate 30 minutes prior to dosing (Days 0 and 28), EOI, and 30 (±5) minutes, 2 and 4 (±15 minutes) hours, and on Days 1 and 29 at 24 (+30 minutes) hours post-infusion. Prior to discharge from the hospital on Days 1 and 29, the ECG must be reviewed by the Investigator and the results deemed not clinically significant.	Serial ECGs will be collected in triplicate 30 minutes prior to dosing (Days 0 and 28 **[or Days 0 and 21 for optional cohorts dosed once every 3 weeks]**), EOI, and 30 (±5) minutes, 2 and 4 (±15 minutes) hours, and on Days 1 and 29 **(or Days 1 and 22 for optional cohorts dosed once every 3 weeks)** at 24 (+30 minutes) hours post-infusion. Prior to discharge from the hospital on Days 1 and 29 **(or Days 1 and 22 for optional cohorts dosed once every 3 weeks)**, the ECG must be reviewed by the Investigator and the results deemed not clinically significant.
7.2.5: Pulse Oximetry	Arterial oxygen saturation will be assessed by serial pulse oximetry on Days 0 and 28 within 30 (±5) minutes pre-dose; at EOI; at 30 (±5) minutes; at 1, 2, 3 hours (±15 minutes); and at 6, 12, and 18 hours (±15 minutes) post-infusion on Days 0 and 28; and at 24 hours (+30 minutes) post-infusion on Days 1 and 29.	Arterial oxygen saturation will be assessed by serial pulse oximetry on Days 0 and 28 **(or Days 0 and 21 for optional cohorts dosed once every 3 weeks)** within 30 (±5) minutes pre-dose; at EOI; at 30 (±5) minutes; at 1, 2, 3 hours (±15 minutes); and at 6, 12, and 18 hours (±15 minutes) post-infusion on Days 0 and 28 **(or Days 0 and 21 for optional cohorts dosed once every 3 weeks)**; and at 24 hours (+30 minutes) post-infusion on Days 1 and 29 **(or Days 1 and 22 for optional cohorts dosed once every 3 weeks)**.
7.2.7.1: Hematology, Serum Chemistries, and Urinalysis	Blood samples for hematology and serum chemistries and urine for urinalysis are to be collected at Screening,	Blood samples for hematology and serum chemistries and urine for urinalysis are to be collected at Screening,

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	pre-dose on Day 0 (unless the screening evaluations were performed within the previous 72 hours) and Day 28, and on the Day 1, 14, 29, 42, and 56 study visits, or at the time of early termination, if applicable.	pre-dose on Day 0 (unless the screening evaluations were performed within the previous 72 hours) and Day 28 **(or Day 21 for optional cohorts dosed once every 3 weeks)**, and on the Day 1, 14, 29, 42, and 56 study visits **(or Days 1, 14, 22, 35, and 56 for optional cohorts dosed once every 3 weeks)**, or at the time of early termination, if applicable.
	Prior to discharge from the hospital on Days 1 and 29, local serum laboratories (specifically, sodium, potassium, creatinine, albumin, calcium, glucose, and phosphate) must be reviewed by the Investigator and the results deemed not clinically significant. Blood for CRP is to be collected pre-dose (within 10 minutes) on Days 0 and 28, and at 2 and 6 hours (± 15 minutes), and 24 hours (± 120 minutes) post-infusion.	Prior to discharge from the hospital on Days 1 and 29 **(or Days 1 and 22 for optional cohorts dosed once every 3 weeks)**, local serum laboratories (specifically, sodium, potassium, creatinine, albumin, calcium, glucose, and phosphate) must be reviewed by the Investigator and the results deemed not clinically significant. Blood for CRP is to be collected pre-dose (within 10 minutes) on Days 0 and 28 **(or Days 0 and 21 for optional cohorts dosed once every 3 weeks)**, and at 2 and 6 hours (± 15 minutes), and 24 hours (± 120 minutes) post-infusion.
7.2.7.2: Liver Function Tests	Blood for LFTs is to be collected at Screening, pre-dose on Day 0 (unless the screening evaluations were performed within the previous 72 hours) and Day 28, and at the Day 1, 2, 7, 14, 29, 30, 35, 42, and 56 study visits, or at the time of early termination, if applicable. Prior to discharge from the hospital on Days 1 and 29,	Blood for LFTs is to be collected at Screening, pre-dose on Day 0 (unless the screening evaluations were performed within the previous 72 hours) and Day 28, and at the Day 1, 2, 7, 14, 29, 30, 35, 42, and 56 study visits **(or Days 1, 2, 7, 14, 22, 23, 28, 35, and 56 for optional cohorts dosed once every 3 weeks)**, or at the time of early termination, if applicable. Prior to discharge from the hospital on Days 1 and 29

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	local LFTs must be reviewed by the Investigator and the results deemed not clinically significant.	**(or Days 1 and 22 for optional cohorts dosed once every 3 weeks)**, local LFTs must be reviewed by the Investigator and the results deemed not clinically significant.
7.2.7.4: Coagulation Studies	Blood for coagulation studies is to be collected at Screening, pre-dose on Days 0 (unless the screening evaluations were performed within the previous 72 hours) and 28, at the Day 1 and 29 study visits.	Blood for coagulation studies is to be collected at Screening, pre-dose on Days 0 (unless the screening evaluations were performed within the previous 72 hours) and 28 **(or Day 21 for optional cohorts dosed once every 3 weeks)**, at the Day 1 and 29 **(or Day 1 and 22 for optional cohorts dosed once every 3 weeks)** study visits.
7.2.7.5: Thyroid Function Tests	Blood for thyroid function tests is to be collected at Screening, pre-dose on Days 0 (unless the screening evaluations were performed within the previous 72 hours) and 28, and at Day 14, 42, and 56 study visits, or at the time of early termination, if applicable.	Blood for thyroid function tests is to be collected at Screening, pre-dose on Days 0 (unless the screening evaluations were performed within the previous 72 hours) and 28 **(or Days 0 and 21 for optional cohorts dosed once every 3 weeks)**, and at Day 14, 42, and 56 study visits **(or Days 14, 35, and 56 for optional cohorts dosed once every 3 weeks)**, or at the time of early termination, if applicable.
7.2.7.6: Complement	Blood for complement factor Bb, is to be collected pre-dose (within 10 minutes) on Days 0 and 28, and 30 (± 5) minutes and 2 (± 15 minutes) and 24 hours (± 120 minutes) post-infusion.	Blood for complement factor Bb, is to be collected pre-dose (within 10 minutes) on Days 0 and 28 **(or Days 0 and 21 for optional cohorts dosed once every 3 weeks)**, and 30 (± 5) minutes and 2 (± 15 minutes) and 24 hours (± 120 minutes) post-infusion.

Section	Prior Text	Changed To
7.2.7.7: Cytokines	Blood for cytokine assessment is to be collected on Days 0 and 28 pre-dose (within 10 minutes), at 2 (±15 minutes), and 6 hours (±15 minutes), and 24 hours (±120 minutes) post-infusion.	Blood for cytokine assessment is to be collected **pre-dose (within 10 minutes)** on Days 0 and 28 **(or Days 0 and 21 for optional cohorts dosed once every 3 weeks)** ~~pre-dose (within 10 minutes)~~, at 2 (±15 minutes), and 6 hours (±15 minutes), and 24 hours (±120 minutes) post-infusion.
7.3.1: Transthyretin Protein	Blood for serum TTR protein levels (WT and mutant) is to be collected at Screening, immediately prior (within 10 minutes) to the administration of the premedications, immediately pre-dose (within 10 minutes) on Days 0 (Baseline) and 28, and on Days 1, 2, 7, 10, 14, 21, 29, 30, 35, 38, 42, 49, 56 (end-of-study), 112, and 208 or at the time of early termination.	Blood for serum TTR protein levels (WT and mutant) is to be collected at Screening, immediately prior (within 10 minutes) to the administration of the premedications, immediately pre-dose (within 10 minutes) on Days 0 (Baseline) and 28, and on Days 1, 2, 7, 10, 14, 21, 29, 30, 35, 38, 42, 49, 56 (end-of-study), 112, and 208 or at the time of early termination. **For optional cohorts dosed once every 3 weeks, blood for serum TTR protein levels will be collected at Screening, immediately prior (within 10 minutes) to the administration of the premedications, immediately pre-dose (within 10 minutes) on Days 0 (Baseline) and 21, and on Days 1, 2, 7, 10, 14, 22, 23, 28, 31, 35, 42, 49, 56 (end-of-study), 112, and 208 or at the time of early termination.**
7.3.2: Transthyretin mRNA	Blood for serum TTR mRNA is to be collected at Screening, immediately prior (within 10 minutes) to the administration of the premedications on Days 0 (Baseline) and 28, and on Days 1, 2, 29, and 30.	Blood for serum TTR mRNA is to be collected at Screening, immediately prior (within 10 minutes) to the administration of the premedications on Days 0 (Baseline) and 28, and on Days 1, 2, 29, and 30. **For optional cohorts dosed once every 3 weeks, blood for serum TTR mRNA will be collected at Screening and on Days 0 (Baseline) and 21, and on Days 1, 2, 22, and 23.**

Section	Prior Text	Changed To
7.3.2.1: Vitamin A and Retinol Binding Protein	Blood for measurements of vitamin A and RBP is to be collected at Screening, immediately prior (within 10 minutes) to the administration of the premedications, immediately pre-dose (within 10 minutes) on Days 0 and 28, and on Days 1, 2, 7, 10, 14, 21, 29, 30, 35, 38, 42, 49, 56 (end-of-study), 112, and 208 or at the time of early termination.	Blood for measurements of vitamin A and RBP is to be collected at Screening, immediately prior (within 10 minutes) to the administration of the premedications, immediately pre-dose (within 10 minutes) on Days 0 and 28, and on Days 1, 2, 7, 10, 14, 21, 29, 30, 35, 38, 42, 49, 56 (end-of-study), 112, and 208 or at the time of early termination. **For optional cohorts dosed once every 3 weeks, blood for measurements of vitamin A and RBP will be collected at Screening, immediately prior (within 10 minutes) to the administration of the premedications, immediately pre-dose (within 10 minutes) on Days 0 (Baseline) and 21, and on Days 1, 2, 7, 10, 14, 22, 23, 28, 31, 35, 42, 49, 56 (end-of-study), 112, and 208 or at the time of early termination.**
7.4.1: Plasma Pharmacokinetics	Samples will also be collected on Days 7, 14, 21, 35, 42, 49, 56, 112, and 208 post infusion as well as at the ET visit (if applicable).	Samples will also be collected on Days 7, 14, 21, 35, 42, 49, 56, 112, and 208 post infusion as well as at the ET visit (if applicable)**; f****or optional cohorts dosed once every 3 weeks, samples will be collected on Days 7, 14, 21, 28, 35, 42, 56, 112, and 208 post infusion as well as at the ET visit (if applicable).**
7.4.2: Urine Pharmacokinetics	Urine sample collection times are included in the schedule of assessments (see Table 1-1).	Urine sample collection times are included in the schedule of assessments (see Table 1-1 **or Table 12-1 for optional cohorts dosed once every 3 weeks**).
9.1: Sample Size	Based on the planned dose escalation scheme (see Section 5.7.1), up to 21 patients are expected to be enrolled.	Based on the planned dose escalation scheme (see Section 5.7.1), up to ~~21~~ **27** patients are expected to be enrolled.
Appendices	Added Appendix 4	**Appendix 4: Schedule of Assessments for Optional Cohorts Administered ALN-TTR02 Once**

Section	Prior Text	Changed To
		Every Three Weeks **Added Table 12-1**

Protocol ALN-TTR02-002

Amendment 2 Change Summary

A Phase 2, Open-Label, Multi-Dose, Dose Escalation Trial to Evaluate the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of Intravenous Infusions of ALN-TTR02 in Patients with TTR Amyloidosis

Changes included in Amendment 2, from Amendment 1 of Protocol ALN-TTR02-002 (dated 19 June 2012; original protocol dated 04 January 2012), are itemized below and detailed in the pages that follow.

Table 1: Premedication and Infusion Rate Changes Proposed in Amendment #2

Medication	Current Protocol	Proposed Amendment #2
Dexamethasone (or equivalent)	The evening before study drug administration, 8 mg PO; self-administered. Thirty to 60 minutes prior to the start of study drug infusion, 20 mg PO; administered by study site personnel.	No dexamethasone the evening prior to ALN-TTR02 dosing. To be administered by study site personnel at least 60 minutes prior to the start of study drug infusion, 10 mg IV.
Paracetamol (or equivalent)	The evening before study drug administration, 500 mg PO; self-administered. Thirty to 60 minutes prior to the start of study drug infusion, 500 mg PO; administered by study site personnel.	No paracetamol the evening prior to ALN-TTR02 dosing. To be administered by study site personnel at least 60 minutes prior to the start of study drug infusion, 500 mg PO.
H1 blocker	Oral cetirizine 10 mg (hydroxyzine 25 mg, fexofenadine, or equivalent may be substituted if patient does not tolerate cetirizine). To be administered: The evening before study drug administration; self-administered. Thirty to 60 minutes prior to the start of study drug infusion; administered by study site personnel.	Diphenhydramine 50 mg IV (or equivalent other IV H1 blocker available at the study site). Hydroxyzine or fexofenadine 25 mg PO or cetirizine 10 mg PO may be substituted for any patient who does not tolerate IV H1 blockers. To be administered by study site personnel at least 60 minutes prior to the start of study drug infusion. (No H1 blocker the evening prior to ALN-TTR02 dosing.)
H2 blocker	Oral ranitidine 150 mg or famotidine 20 mg or equivalent other H2 blocker dose. To be administered: The evening before study drug administration; self-administered. Thirty to 60 minutes prior to the start of study drug infusion; administered by study site personnel.	Intravenous ranitidine 50 mg, famotidine 20 mg or another equivalent IV H2 blocker dose. To be administered at least 60 minutes prior to the start of study drug infusion. (No H2 blocker the evening prior to ALN-TTR02 dosing.)
Infusion rate	Over 60 minutes (~3.3 mL/min)	Approximately 1.1 mL/min over the first 15 minutes, with the remainder of the infusion administered at 3.3 mL/min (total infusion time: ~70 min).

Other changes from Amendment 1 to Amendment 2 include:

- Increased the number of optional cohorts from 3 to 5 to allow for the evaluation of once every 3 weeks dosing interval as well as an alternative premedication regimen and slower infusion rate.
- Added an additional schedule of assessments for optional cohorts administered ALN-TTR02 once every 3 weeks (refer to Appendix 4).
- The dosing regimen in the first optional cohort will be once every 4 weeks; in the remaining 4 optional cohorts the dosing regimen will be once every 3 or 4 weeks. The decision on dosing every 3 or 4 weeks will be made by the SRC based on safety.
- The alternative premedication regimen and infusion rate (detailed in Table 1) will be evaluated in up to 3 optional cohorts once the safety of the original premedication regimen and infusion rate is further established in the first 2 optional cohorts.
- Section 1.3 (Summary of Clinical Data with siRNA-LNPs) of the protocol has been simplified and primarily cross-references the IB, Edition 3, which contains the updated clinical information.
- Removed the Principal Investigators' contact information from the protocol itself. This information is included in the Study Procedures Manual.
- Made minor editorial changes (e.g., corrections of typographical, grammatical, and spelling errors as well as formatting changes and changes for consistency); these changes are not listed individually.

Text deleted is indicated by strikeout while text added is indicated by **bold** font.

Section	Prior Text	Changed To
Contact Information	**Principal Investigator** [redacted] **Principal Investigator** [redacted] **Principal Investigator** [redacted]	**~~Principal Investigator~~** [redacted] **~~Principal Investigator~~** [redacted] **~~Principal Investigator~~** [redacted]

Section	Prior Text	Changed To
	[redacted] **Principal Investigator** [redacted] **Principal Investigator** [redacted]	[redacted] **~~Principal Investigator~~** [redacted] **~~Principal Investigator~~** [redacted]
Synopsis: Design	The dose level of ALN-TTR02 administered to any cohort in study ALN-TTR02-002 may not be higher than that deemed safe and tolerable in the Phase 1	~~The dose level of ALN-TTR02 administered to any cohort in study ALN-TTR02-002 may not be higher than that deemed safe and tolerable in the Phase 1~~

Section	Prior Text	Changed To
	single-dose study of ALN-TTR02 (Study ALN-TTR02-001).	~~single-dose study of ALN-TTR02 (Study ALN-TTR02-001).~~
Synopsis: Study Sites	This study is planned to be conducted at up to 8 study centers in Europe and South America.	This study is planned to be conducted at up to ~~8~~ **10** study centers ~~in Europe and South America~~**worldwide**.
Synopsis: Dosage, Route of Administration and Duration of Treatment of Investigational Drug	The optional cohort would be included to further confirm the safety and/or pharmacodynamic (PD) effect. If this occurs, the Independent Ethics Committees (IECs) will be informed of the expansion. Up to 3 optional cohorts are permitted in this study. All patients will receive the following premedications prior to each dose of ALN-TTR02: • Oral (PO) dexamethasone (8 mg) or equivalent administered the evening before dosing and 20 mg 30 to 60 minutes prior to start of infusion of ALN-TTR02; • Oral paracetamol (500 mg) or equivalent the evening before dosing and 30 to 60 minutes prior to the start of infusion of ALN-TTR02; • Oral H2 blocker (i.e., ranitidine 150 mg or famotidine 20 mg or equivalent other H2 blocker dose) the evening before dosing and 30 to 60 minutes prior to start of infusion of ALN-TTR02; • Oral H1 blocker, 10 mg cetirizine or equivalent (hydroxyzine 25 mg or fexofenadine may be substituted if patient does not tolerate cetirizine) the evening before dosing and 30 to 60 minutes	~~The o~~Optional cohort**(s)** would be included to further confirm the safety and/or pharmacodynamic (PD) effect **of previously evaluated dose levels, which may include evaluation of the original dosing regimen (once every 4 weeks), an alternative dosing regimen (once every 3 weeks), and/or an alternative premedication regimen and infusion rate. The dosing regimen for the first optional cohort will be once every 4 weeks, and the dosing regimen for the remaining 4 optional cohorts will be once every 3 or 4 weeks. It is anticipated that the first 2 optional cohorts will use the original premedication regimen, and the remaining 3 optional cohorts may be used to explore the alternative premedication regimen and infusion rate.** **The SRC will review all available safety data and make recommendations on dosing and premedication regimens prior to dosing of any of the optional cohorts.** ~~If this occurs, t~~The Independent Ethics Committees (IECs) will be informed of the ~~expansion~~**implementation of optional cohorts.** Up to ~~3~~ **5** optional cohorts are permitted in this study. ~~All patients will receive the following~~**Patients**

Section	Prior Text	Changed To
	prior to start of infusion of ALN-TTR02; Doses of ALN-TTR02 will be administered IV over 60 minutes by a controlled infusion device.	**receiving the original** premedication **regimen will be administered the followings** prior to each dose of ALN-TTR02: • Oral (PO) dexamethasone (8 mg) or equivalent administered the evening before dosing and 20 mg 30 to 60 minutes prior to start of infusion of ALN-TTR02; • Oral paracetamol (500 mg) or equivalent the evening before dosing and 30 to 60 minutes prior to the start of infusion of ALN-TTR02; • Oral H2 blocker (**i.e.g.**, ranitidine 150 mg or famotidine 20 mg or equivalent other H2 blocker dose) the evening before dosing and 30 to 60 minutes prior to start of infusion of ALN-TTR02; **and** • Oral H1 blocker, 10 mg cetirizine or equivalent (hydroxyzine 25 mg or fexofenadine may be substituted if patient does not tolerate cetirizine) the evening before dosing and 30 to 60 minutes prior to start of infusion of ALN-TTR02. **The alternative premedication regimen that can be used in select optional cohorts, as agreed upon by the SRC, will be administered prior to each dose of ALN-TTR02 and includes the following:** • **Intravenous (IV) dexamethasone (10 mg) or equivalent, administered at least**

Section	Prior Text	Changed To
		60 minutes prior to the start of infusion of ALN-TTR02; • **Oral paracetamol (500 mg) or equivalent at least 60 minutes prior to the start of 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 start of 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 start of 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.** **For patients receiving the original premedication regimen and infusion rate,** doses of ALN-TTR02 will be administered IV over 60 minutes by a controlled infusion device **at a flow rate of approximately 3.3 mL/min.** **For patients enrolled in the optional cohorts evaluating the alternative premedication regimen and infusion rate, the flow rate of ALN-TTR02 will**

Section	Prior Text	Changed To
		be approximately 1.1 mL/min during the first 15 minutes (1/3rd the original flow rate) with the remainder of the infusion administered at a flow rate of 3.3 mL/min for a total infusion time of approximately 70 minutes
Time on Study	Added language to allow an open-label extension study.	**An open-label extension study at the recommended Phase 3 dose and regimen (as derived from Study ALN-TTR02-002) is planned, which will enable the patients who enrolled in Study ALN-TTR02-002 to receive additional, long-term dosing and safety follow-up. For some of the patients, this may preclude the completion of all of the follow-up period assessments of Study ALN-TTR02-002 if they are deemed eligible to participate in the extension study prior to completion of the full follow up through Day 208. Such patients will be followed up for a minimum of 28 days after their last dose in the current ALN-TTR02-002 study prior to being enrolled in the extension study. The extension study will be implemented only after dose escalation is completed in Study ALN-TTR02-002, with post second dose follow-up through Day 208 in at least 1 cohort at the highest dose.**
Synopsis: Sample Size	Based on the entry criteria and the proposed dose escalation scheme, up to 21 patients are expected to be enrolled. Three patients are to be enrolled at each dose level and up to 3 optional cohorts of 3 patients each will be permitted.	Based on the entry criteria and the proposed dose escalation scheme, up to ~~21~~ **27** patients are expected to be enrolled. Three patients are to be enrolled at each dose level and up to ~~3~~ **5** optional cohorts of 3 patients each will be permitted.
Synopsis: Dose-Limiting	3. An infusion reaction that requires hospitalization,	3. An infusion reaction that requires hospitalization,

Section	Prior Text	Changed To
Toxicities and Stopping Criteria	despite proper premedication.	despite ~~proper~~ premedication.
Table 1.1	Schedule of Assessments Added note v. Premedications include dexamethasone (8 mg, or equivalent), paracetamol (500 mg, or equivalent), H2 blocker (i.e. 150 mg ranitidine or 20 mg famotidine), and H1 blocker (10 mg cetirizine, 25 mg hydroxyzine, fexofenadine or equivalent may be substituted) will be self-administered per os (PO) the evening before study drug administration. Thirty to 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.	Schedule of Assessments **for Cohorts Administered ALN-TTR02 Once Every Four Weeks** **Note: The schedule of assessments for optional cohorts administered ALN-TTR02 once every 3 weeks is provided in Appendix 4.** v. Premedications include dexamethasone (8 mg, or equivalent), paracetamol (500 mg, or equivalent), H2 blocker (~~i.~~ **ee.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) will be self-administered per os (PO) the evening before study drug administration. Thirty to 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**. Patients enrolled in an optional cohort evaluating the use of an alternative premedication regimen, as agreed upon by the SRC, will receive the following medications at least 60 minutes prior to the start of 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**

Section	Prior Text	Changed To
		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). These patients will not receive any premedications the evening prior to ALN-TTR02 dosing.
	w. Site personnel are to call patients the day before dosing to remind them to take premedications that evening (the day before dosing).	w. Site personnel are to call patients the day before dosing to remind them to take premedications that evening (the day before dosing). **This reminder will not be needed for patients enrolled in optional cohorts evaluating the alternative premedication regimen.**
1.3: Summary of Clinical Data with siRNA-LNPs	A Phase 1 multi-centre, randomized, placebo-controlled, single-blind, single-ascending dose clinical study to evaluate the safety, tolerability, PK, and pharmacodynamic (PD) of ALN-TTR02 in healthy volunteers was approved by MHRA (EUDRACT # 2011-005291-42) and has completed dosing. ALN-TTR02 was administered as a single 60-minute IV infusion to healthy volunteers at the following doses: 10, 50, 150, 300, and 500 µg/kg (4 patients per dose level; 3 receiving ALN-TTR02 and 1 receiving placebo). The dose level of ALN-TTR02 administered to any cohort in study ALN-TTR02-002 may not be higher than that deemed safe and tolerable in the Phase 1 single-dose study of ALN-TTR02 (Study ALN-TTR02-001). Patients will be pre-medicated with	~~A Phase 1 multi-centre, randomized, placebo-controlled, single-blind, single-ascending dose clinical study to evaluate the safety, tolerability, PK, and pharmacodynamic (PD) of ALN-TTR02 in healthy volunteers was approved by MHRA (EUDRACT # 2011-005291-42) and has completed dosing. ALN-TTR02 was administered as a single 60-minute IV infusion to healthy volunteers at the following doses: 10, 50, 150, 300, and 500 µg/kg (4 patients per dose level; 3 receiving ALN-TTR02 and 1 receiving placebo). The dose level of ALN-TTR02 administered to any cohort in study ALN-TTR02-002 may not be higher than that deemed safe and tolerable in the Phase 1 single-dose study of ALN-TTR02 (Study ALN-TTR02-001). Patients will be pre-medicated with~~

Section	Prior Text	Changed To
	dexamethasone, H1 and H2 blockers, and paracetamol prior to dosing to minimize the risk of infusion reaction. Preliminary data show that ALN-TTR02 was safe and well-tolerated and exhibited robust effects on serum TTR levels at the top doses. To date, no stopping rules have been met and there have been no early discontinuations due to adverse events. Further information on the ALN-TTR02-001 Phase 1 study can be found in the ALN-TTR02 Investigators Brochure and its Expedited Safety Report addendum dated 7 June 2012. Clinical trials with multiple different RNAi medicinal products using local or systemic administration in indications including age-related macular degeneration, respiratory syncytial virus (RSV) infection, oncology, and renal failure have been conducted.[28] Alnylam currently has 3 ongoing systemic delivery programs in the clinic evaluating the safety and efficacy of different siRNAs in 2 different LNP formulations. Specifically, the active Phase 1 clinical trials most relevant to ALN-TTR02 include ALN-PCS02, which uses the same LNP formulation, and ALN-TTR01, which uses the same siRNA as ALN-TTR02 but in a different LNP formulation. The clinical safety data from the ALN-PCS02 study in particular provides some reassurance regarding the safety of this formulation (common with ALN-TTR02) after a single dose**.**	~~dexamethasone, H1 and H2 blockers, and paracetamol prior to dosing to minimize the risk of infusion reaction. Preliminary data show that ALN-TTR02 was safe and well-tolerated and exhibited robust effects on serum TTR levels at the top doses. To date, no stopping rules have been met and there have been no early discontinuations due to adverse events. Further information on the ALN-TTR02-001 Phase 1 study can be found in the ALN-TTR02 Investigators Brochure and its Expedited Safety Report addendum dated 7 June 2012.~~ ~~Further, c~~Clinical trials with multiple different RNAi medicinal products using local or systemic administration in indications including age-related macular degeneration, respiratory syncytial virus (RSV) infection, oncology, and renal failure have been conducted.[28] Alnylam currently has **experience with** ~~3~~**4** ~~ongoing~~ systemic delivery programs in the clinic evaluating the safety and efficacy of different siRNAs in 2 different LNP formulations**, including 1 program which employs the same AF-011 formulation as ALN-TTR02**. ~~Specifically, the active Phase 1 clinical trials most relevant to ALN-TTR02 include ALN-PCS02, which uses the same LNP formulation, and ALN-TTR01, which uses the same siRNA as ALN-TTR02 but in a different LNP formulation. The clinical safety data from the ALN-PCS02 study in particular provides some reassurance regarding the~~

Section	Prior Text	Changed To
		~~safety of this formulation (common with ALN-TTR02) after a single dose.~~ **An overview of the safety and pharmacological clinical data with these siRNA-LNPs is included in the ALN-TTR02 Investigator's Brochure (IB), Edition 3, dated 19 October 2012.** [redacted] **s. Furthermore, the Phase 1 data with all 4 of the siRNA-LNPs have shown that the** [redacted]
	ALN-PCS02-001 is a Phase 1 multi-centre, randomized, placebo-controlled, single-blind, single-ascending dose clinical study to evaluate the safety, tolerability, PK, and PD of ALN-PCS02. The study is ongoing in the United Kingdom (EudraCT #2011-000581-36). ALN-PCS02 was administered as a single 60-minute IV infusion to healthy volunteers with elevated low density lipoprotein-C (LDL-C; ≥3 mmol/L) at the following doses: 15, 45, 90, 150, and 250 µg/kg (4 patients per dose level; 3 receiving ALN-PCS02 and 1 receiving placebo). Patients were pre-medicated with dexamethasone, H1 and H2 blockers, and paracetamol prior to dosing to minimize the risk of infusion reaction. As of December 2011, 24 patients (23 males and 1 female) have been enrolled in the ALN-PCS02-001 study and 18 patients (17 males and 1 female) have received ALN-PCS02 in 6 cohorts at	**Importantly, a Phase 1 study with ALN-TTR02 was recently completed. Study ALN-TTR02-001 was a multicenter, randomized, placebo-controlled, single-blind, single-ascending dose clinical study conducted in the UK to evaluate the safety, tolerability, PK, and pharmacodynamic (PD) in healthy volunteers (EudraCT # 2011 005291-42). ALN-TTR02 was administered as a single 60-minute IV infusion to healthy volunteers at the following doses: 10, 50, 150, 300, and 500 µg/kg (4 patients per dose level; 3 receiving ALN-TTR02 and 1 receiving placebo). Patients were premedicated with dexamethasone, H1 and H2 blockers, and paracetamol prior to dosing to minimize the risk of infusion reaction. The data show that ALN-TTR02 was safe and well-tolerated and exhibited robust effects on serum TTR levels at doses ≥0.15 mg/kg.**

Section	Prior Text	Changed To
	doses ranging from 15 to 250 µg/kg (including 2 cohorts dosed at the 250 µg/kg dose). No dose-limiting toxicities (DLTs) have been reported to date, and no patient has prematurely withdrawn from the study due to an adverse event (AE). There was one serious adverse event (SAE; bilateral pulmonary emboli in a patient with chronic deep vein thrombosis) considered unrelated to study drug that occurred in 1 patient dosed at 45 µg/kg. There have been no laboratory AEs related to study drug; specifically, there have been no clinically significant changes in liver function tests (LFTs), electrolytes, hematology parameters, or renal function post-dose. Mild self-limiting erythematous skin rashes not requiring treatment were seen in 6 patients dosed with ALN-PCS02 (1 each at 15 and 45 µg/kg, and in 4 patients at 0.25 mg/kg). Transient elevations in the complement pathway Bb (1.25- to 4.6-fold) have occurred in 14 patients 30 minutes post-infusion and have been observed across all dose levels and were not associated with signs or symptoms. There have been no cytokine or CRP elevations in any of the patients. Stopping rules for dose escalation have not been met, and the study is ongoing. Preliminary PD data with ALN-PCS02 showed an approximately 60% reduction in PCSK9 serum protein at the 150 and 250 µg/kg doses compared with baseline	**Lowering of TTR levels was reversible following administration of a single dose, and there were no AEs associated with lowering TTR by >90%. Further details on this study can be found in the ALN-TTR02 IB, Edition 3.** ~~ALN-PCS02-001 is a Phase 1 multi-centre, randomized, placebo-controlled, single-blind, single-ascending dose clinical study to evaluate the safety, tolerability, PK, and PD of ALN-PCS02. The study is ongoing in the United Kingdom (EudraCT #2011-000581-36). ALN-PCS02 was administered as a single 60-minute IV infusion to healthy volunteers with elevated low density lipoprotein-C (LDL-C; ≥3 mmol/L) at the following doses: 15, 45, 90, 150, and 250 µg/kg (4 patients per dose level; 3 receiving ALN-PCS02 and 1 receiving placebo). Patients were pre-medicated with dexamethasone, H1 and H2 blockers, and paracetamol prior to dosing to minimize the risk of infusion reaction. As of December 2011, 24 patients (23 males and 1 female) have been enrolled in the ALN-PCS02-001 study and 18 patients (17 males and 1 female) have received ALN-PCS02 in 6 cohorts at doses ranging from 15 to 250 µg/kg (including 2 cohorts dosed at the 250 µg/kg dose).~~ ~~No dose-limiting toxicities (DLTs) have been reported to date, and no patient has prematurely withdrawn from the study due to an adverse event (AE). There was one serious adverse event (SAE; bilateral pulmonary~~

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	levels, with a return toward baseline after ~ 28 days. The ALN-TTR01 Phase 1 clinical trial (ALN-TTR01-001) is a multi-centre, randomized, placebo-controlled, single-blind, single-ascending dose study to evaluate the safety, tolerability, PK and PD of ALN-TTR01. The study is ongoing in France, Portugal, Sweden, and the United Kingdom (EudraCT # 2009-017383-16). The data from this study are relevant to provide the effect of TTR lowering using the same siRNA as ALN-TTR02 albeit in a different formulation. ALN-TTR01 was administered as a single 15-minute IV infusion to ATTR Stage 1 patients at the following doses: 10, 30, 100, 200, 400, 700, and 1000 µg/kg (4 patients per dose level; 3 receiving ALN-TTR01 and 1 receiving placebo). Patients were pre-medicated with a similar regimen used for ALN-PCS02. A total of 32 patients were enrolled in this study (including 8 at 1000 µg/kg and 4 each at 10-700 µg/kg), of whom 24 (14 males and 10 females) received ALN-TTR01. There were no laboratory AEs and no significant changes in LFTs, electrolytes, hematology parameters, or renal function post-dose. No clinically significant changes in testosterone were observed in males. No drug-related SAEs or DLTs occurred, and no patient withdrew prematurely from the study due to an AE. There were 5 acute infusion-related reactions (IRRs): 1 each at 400 and 700 µg/kg and 3 at 1000 µg/kg, for an overall rate of 21%, all of which were mild to moderate	~~emboli in a patient with chronic deep vein thrombosis) considered unrelated to study drug that occurred in 1 patient dosed at 45 µg/kg. There have been no laboratory AEs related to study drug; specifically, there have been no clinically significant changes in liver function tests (LFTs), electrolytes, hematology parameters, or renal function post-dose.~~ ~~Mild self-limiting erythematous skin rashes not requiring treatment were seen in 6 patients dosed with ALN-PCS02 (1 each at 15 and 45 µg/kg, and in 4 patients at 0.25 mg/kg). Transient elevations in the complement pathway Bb (1.25- to 4.6-fold) have occurred in 14 patients 30 minutes post-infusion and have been observed across all dose levels and were not associated with signs or symptoms. There have been no cytokine or CRP elevations in any of the patients. Stopping rules for dose escalation have not been met, and the study is ongoing.~~ ~~Preliminary PD data with ALN-PCS02 showed an approximately 60% reduction in PCSK9 serum protein at the 150 and 250 µg/kg doses compared with baseline levels, with a return toward baseline after ~ 28 days.~~ ~~The ALN-TTR01 Phase 1 clinical trial (ALN-TTR01-001) is a multi-centre, randomized, placebo-controlled, single-blind, single-ascending dose study to evaluate the safety, tolerability, PK and PD of ALN-TTR01. The study is ongoing in France, Portugal, Sweden, and the United Kingdom (EudraCT # 2009-017383-16).~~

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	in severity, and where necessary, responded to temporary halting of the infusion followed by resumption of the infusion at a slower rate. Regardless of whether a patient experienced an IRR, all study drug infusions were successfully administered to completion. Transient elevations of Bb were observed across all dose levels, with higher induction seen in patients with acute IRRs. There have been no changes in C3, C4, or CH50. Modest elevations in both IL-6 (2 to 7-fold over baseline) and CRP (4 to 9-fold over baseline) were observed in 3 patients treated at 1000 µg/kg and were not associated with fever or chills. Preliminary plasma PK results indicate a generally dose-proportional increase in the area under the plasma concentration-time curve (AUC) and the observed maximum concentration (C_{max}) and the long term exposure in humans is higher than predicted by the preclinical animal studies. The PD effects of ALN-TTR01 were assessed through serial measurements of serum TTR protein.i There were no significant changes in TTR relative to placebo at doses below 1000 µg/kg. At 1000 µg/kg (n=5), a mean decline in TTR protein of 41% relative to baseline and placebo was observed by Day 7 post-dose (geometric mean; p=0.02), with recovery to 80% of pre-treatment level for the group by Day 28. In 1 patient, the serum TTR declined by 81%; this was accompanied by a similar decrease in RBP and vitamin A, as predicted by preclinical data with ALN-	~~The data from this study are relevant to provide the effect of TTR lowering using the same siRNA as ALN-TTR02 albeit in a different formulation. ALN-TTR01 was administered as a single 15-minute IV infusion to ATTR Stage 1 patients at the following doses: 10, 30, 100, 200, 400, 700, and 1000 µg/kg (4 patients per dose level; 3 receiving ALN-TTR01 and 1 receiving placebo). Patients were pre-medicated with a similar regimen used for ALN-PCS02. A total of 32 patients were enrolled in this study (including 8 at 1000 µg/kg and 4 each at 10-700 µg/kg), of whom 24 (14 males and 10 females) received ALN-TTR01.~~ ~~There were no laboratory AEs and no significant changes in LFTs, electrolytes, hematology parameters, or renal function post-dose. No clinically significant changes in testosterone were observed in males. No drug-related SAEs or DLTs occurred, and no patient withdrew prematurely from the study due to an AE. There were 5 acute infusion-related reactions (IRRs): 1 each at 400 and 700 µg/kg and 3 at 1000 µg/kg, for an overall rate of 21%, all of which were mild to moderate in severity, and where necessary, responded to temporary halting of the infusion followed by resumption of the infusion at a slower rate. Regardless of whether a patient experienced an IRR, all study drug infusions were successfully administered to completion. Transient elevations of Bb were observed across all dose levels, with higher induction seen in patients with acute IRRs. There have been no changes~~

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	TTR01 in NHPs and the known role of TTR in binding to and stabilizing circulating RBP. By Day 70, TTR and vitamin A had recovered to approximate pre-treatment levels without any reported signs or symptoms and did not require the need for supplementation. There were no significant changes in thyroid hormone levels or thyroid stimulating hormone (TSH) in this patient, and no adverse effects associated with TTR lowering. A similar pattern of TTR/RBP/vitamin A decrease and recovery without any impact on safety was observed in all patients on the study treated at 1.0 mg/kg who showed a response to ALN-TTR01. As with the PD data with ALN-PCS02, these data show [redacted] and show predictable reversibility. The safety of giving multiple doses of an siRNA-LNP has been established with ALN-VSP02, which uses the same first generation LNP formulation as ALN-TTR01 (see Investigator's Brochure). In a Phase 1 study performed in advanced cancer patients with liver involvement, chronic bi-weekly dosing with doses as high as 1.0 mg/kg was safe and well-tolerated using the same premedication regimen as ALN-TTR01, with no significant dose-dependent effect on liver function. Three patients with tumor shrinkage or prolonged disease stabilization who have received biweekly doses at 0.7-1.0 mg/kg for as long as 12-18 months are	~~in C3, C4, or CH50. Modest elevations in both IL-6 (2 to 7-fold over baseline) and CRP (4 to 9-fold over baseline) were observed in 3 patients treated at 1000 µg/kg and were not associated with fever or chills. Preliminary plasma PK results indicate a generally dose-proportional increase in the area under the plasma concentration-time curve (AUC) and the observed maximum concentration (C_{max}) and the long-term exposure in humans is higher than predicted by the preclinical animal studies.~~ ~~The PD effects of ALN-TTR01 were assessed through serial measurements of serum TTR protein.ii There were no significant changes in TTR relative to placebo at doses below 1000 µg/kg. At 1000 µg/kg (n=5), a mean decline in TTR protein of 41% relative to baseline and placebo was observed by Day 7 post-dose (geometric mean; p=0.02), with recovery to 80% of pre-treatment level for the group by Day 28. In 1 patient, the serum TTR declined by 81%; this was accompanied by a similar decrease in RBP and vitamin A, as predicted by preclinical data with ALN-TTR01 in NHPs and the known role of TTR in binding to and stabilizing circulating RBP. By Day 70, TTR and vitamin A had recovered to approximate pre-treatment levels without any reported signs or symptoms and did not require the need for supplementation. There were no significant changes in thyroid hormone levels or thyroid stimulating hormone (TSH) in this patient, and no adverse effects associated~~

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	continuing their treatment on an extension study. Thus, the nonclinical data with ALN-TTR02 and the preliminary clinical data with ALN-PCS02, ALN-TTR01, and ALN-VSP02 support the proposed early phase clinical trials evaluating ascending doses (single or multiple) of ALN-TTR02 (studies ALN-TTR02-001 and ALN-TTR02-002). Further information, including preliminary safety and efficacy updates from the recently completed Phase 1 trials of ALN-TTR02 in healthy volunteers and ALN-PCS02 in healthy volunteers with elevated cholesterol, can be found in the Investigator's Brochure and its Expedited Safety Report addendum dated 7 June 2012. Alnylam will immediately notify the Principal Investigators if any relevant new safety or toxicology information becomes available during the study.	~~with TTR lowering. A similar pattern of TTR/RBP/vitamin A decrease and recovery without any impact on safety was observed in all patients on the study treated at 1.0 mg/kg who showed a response to ALN-TTR01. As with the PD data with ALN-PCS02, these data show [redacted] and show predictable reversibility.~~ ~~The safety of giving multiple doses of an siRNA-LNP has been established with ALN-VSP02, which uses the same first generation LNP formulation as ALN-TTR01 (see Investigator's Brochure). In a Phase 1 study performed in advanced cancer patients with liver involvement, chronic bi-weekly dosing with doses as high as 1.0 mg/kg was safe and well-tolerated using the same premedication regimen as ALN-TTR01, with no significant dose-dependent effect on liver function. Three patients with tumor shrinkage or prolonged disease stabilization who have received biweekly doses at 0.7-1.0 mg/kg for as long as 12-18 months are continuing their treatment on an extension study.~~ ~~Thus, the nonclinical data with ALN-TTR02 and the preliminary clinical data with ALN-PCS02, ALN-TTR01, and ALN-VSP02 support the proposed early phase clinical trials evaluating ascending doses (single or multiple) of ALN-TTR02 (studies ALN-TTR02-001 and ALN-TTR02-002).~~ ~~Further information, including preliminary safety and~~

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		~~efficacy updates from the recently completed Phase 1 trials of ALN-TTR02 in healthy volunteers and ALN-PCS02 in healthy volunteers with elevated cholesterol, can be found in the Investigator's Brochure and its Expedited Safety Report addendum dated 7 June 2012. Alnylam will immediately notify the Principal Investigators if any relevant new safety or toxicology information becomes available during the study.~~
1.5: Dose Selection and Rationale	Two consecutive ALN-TTR02 doses, separated by a 4-week period, will be administered to patients. Study drug will be administered as a 60-minute IV infusion with the following proposed doses for each cohort: 10, 50, 150, and 300 µg/kg ALN-TTR02. In addition, cumulative safety and tolerability data observed in at least 2 patients through at least 96 hours post-second dose of the previous cohort will be reviewed by the SRC prior to administration of the second dose of study drug. The dose level of ALN-TTR02 administered to any cohort in study ALN-TTR02-002 may not be higher than that deemed safe and tolerable in the Phase 1 single-dose study of ALN-TTR02 (Study ALN-TTR02-001).	Two consecutive ALN-TTR02 doses, separated by a 4-week period **(or a 3-week period in select optional cohorts)**, will be administered to patients. Study drug will be administered as a 60-minute IV infusion with the following proposed doses for each **of the original** cohort**s**: 10, 50, 150, and 300 µg/kg ALN-TTR02. **For those patients enrolled in an optional cohort evaluating the alternative premedication regimen and infusion rate, the study drug will be administered IV over approximately 70 minutes.** In addition, cumulative safety and tolerability data observed in at least 2 patients through at least 96 hours post-second dose of the previous cohort will be reviewed by the SRC prior to administration of the second dose of study drug. ~~The dose level of ALN-TTR02 administered to any cohort in study ALN-TTR02-002 may not be higher than that deemed safe and tolerable in the Phase 1 single-dose study of ALN-TTR02 (Study ALN-TTR02-001).~~

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	The optional cohort would be included to further confirm the safety and/or PD effect. If this occurs, the Independent Ethics Committees (IECs) will be informed of the expansion. Up to 3 optional cohorts of 3 patients each are permitted in this study.	The optional cohort**(s)** would be included to further confirm the safety and/or PD effect **of previously evaluated dose levels, which may include evaluation of the original dosing regimen (once every 4 weeks), an alternative dosing regimen (once every 3 weeks), and/or an alternative premedication regimen and infusion rate. The dosing regimen for the first optional cohort will be once every 4 weeks, and the dosing regimen for the remaining 4 optional cohorts will be once every 3 or 4 weeks. It is anticipated that the first 2 optional cohorts will use the original premedication regimen, and the remaining 3 optional cohorts may be used to explore the alternative premedication regimen and infusion rate. The SRC will review all available safety data and make recommendations on dosing and premedication regimens prior to dosing of any of the optional cohorts.** If this occurs, the Independent Ethics Committees (IECs) will be informed of the ~~expansion~~**implementation of optional cohorts**. Up to ~~3~~ **5** optional cohorts of 3 patients each are permitted in this study.
1.6.1: Infusion-Related Reactions	The premedication regimen will include orally (PO) administered dexamethasone (or equivalent), paracetamol (or equivalent), and H1 and H2 blockers (as described in Section 5.5). The rate of infusion (over the course of 1 hour) will also help to reduce the potential for acute IRRs.	The premedication regimen will include ~~orally (PO) administered~~ dexamethasone (or equivalent), paracetamol (or equivalent), and H1 and H2 blockers (as described in Section 5.5). The **infusion** rate of **1 hour or longer**~~infusion (over the course of 1 hour)~~ will also help to reduce the potential for acute IRRs.
3.1: Overall Design	No patients will be a member of more than 1 treatment	No patients will be a member of more than 1 treatment

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	group.	group. **An alternative dosing regimen of 2 doses of ALN-TTR02 (at a dose previously determined by the SRC to be safe and tolerable) separated by 3 weeks may be evaluated in the optional cohort(s).**
	For patients on all dose levels other than the starting dose level of 10 μg/kg, prior to receiving the second dose the SRC will review the cumulative safety and tolerability data of at least 2 patients from the previous dose level(s) with at least 96 hours of follow-up after receiving a second dose of study drug. The dose level of ALN-TTR02 administered to any cohort in study ALN-TTR02-002 may not be higher than that deemed safe and tolerable in the Phase 1 single-dose study of ALN-TTR02 (Study ALN-TTR02-001).	For patients on all dose levels other than the starting dose level of 10 μg/kg, prior to receiving the second dose the SRC will review the cumulative safety and tolerability data of at least 2 patients from the previous dose level(s) with at least 96 hours of follow-up after receiving a second dose of study drug. ~~The dose level of ALN-TTR02 administered to any cohort in study ALN-TTR02-002 may not be higher than that deemed safe and tolerable in the Phase 1 single-dose study of ALN-TTR02 (Study ALN-TTR02-001).~~
	Eligible patients will undergo further pre-treatment assessments (performed on Day 0). All patients will receive oral premedication with dexamethasone (or equivalent), paracetamol (or equivalent), and H1 and H2 blockers the night before and 30 to 60 minutes prior to each dose of ALN-TTR02 to reduce the potential of an IRR (see Section 5.5). On Days 0 and 28, patients will receive a single dose of ALN-TTR02 administered as a 60-minute IV infusion	Eligible patients will undergo further pre-treatment assessments (performed on Day 0). ~~All~~ **Patients receiving the original premedication regimen**~~patients~~ will receive oral premedication with dexamethasone (or equivalent), paracetamol (or equivalent), and H1 and H2 blockers the night before and 30 to 60 minutes prior to each dose of ALN-TTR02 to reduce the potential of an IRR (see Section 5.5). **Those patients in an optional cohort evaluating the alternative premedication regimen, as agreed upon by the SRC, will receive IV dexamethasone (or equivalent), oral paracetamol (or equivalent), and IV H1 and H2 blockers at least 60 minutes prior to ALN-TTR02**

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		dosing; no premedication will be administered the evening prior to dosing. On Days 0 and 28, patients will receive a single dose of ALN-TTR02 administered as a 60-minute IV infusion **(for cohorts with the original premedication regimen and infusion rate), or as an approximate 70-minute IV infusion, for those patients in an optional cohort evaluating the alternative premedication regimen and infusion rate.**
4.1: Eligibility of Patients	Based on the planned dose escalation scheme (see Section 5.7.1), up to 21 patients are expected to be enrolled.	Based on the planned dose escalation scheme (see Section 5.7.1), up to ~~21~~ **27** patients are expected to be enrolled.
5.5: Premedication Plan	Premedication will be administered as follows: Added language for an alternative premedication regimen and infusion rate.	**Patients receiving the original premedication regimen will be administered the following** ~~Premedication will be administered as follows~~: **An alternative premedication regimen and infusion rate can be used in select optional cohorts, as agreed upon by the SRC. The dosing regimen for the first optional cohort will be once every 4 weeks, and the dosing regimen for the remaining 4 optional cohorts will be once every 3 or 4 weeks. It is anticipated that the first 2 optional cohorts will use the original premedication regimen, and the remaining 3 optional cohorts may be used to explore the alternative premedication regimen and infusion rate. Patients in these cohorts will not receive any premedications the evening prior to ALN-TTR02 dosing. Prior to each dose of ALN-TTR02, these**

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		patients will receive the following premedications: • **Intravenous (IV) dexamethasone (10 mg) or equivalent, administered at least 60 minutes prior to the start of infusion of ALN-TTR02;** • **Oral paracetamol (500 mg) or equivalent at least 60 minutes prior to the start of 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 start of 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 start of 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 blockers.**
5.6: Dose, Route, and Schedule of Study Drug Administration	Study drug doses will be administered as a 60-minute IV infusion 4 weeks apart. Study drug will be administered via a controlled infusion device with an extension set containing a 1.2 micron filter supplied by Alnylam or designee. Infusion products containing polyvinyl chloride, di(2-ethylhexyl)phthalate (PVC, DEHP) must NOT be used.	Study drug doses will be administered as a 60-minute IV infusion **(flow rate of approximately 3.3 mL/min)** 4 weeks apart. **The dosing of ALN-TTR02 in the optional cohorts will be once every 4 weeks for the first optional cohort and once every 3 or 4 weeks for the remaining 4 optional cohorts, at a dose previously determined by the SRC to be safe and tolerable.** Study drug will be administered via a

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		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. **For patients enrolled in an optional cohort evaluating the alternative premedication regimen, the flow rate will be approximately 1.1 mL/min during the first 15 minutes (1/3rd the original flow rate) with the remainder of the infusion taking place over 55 minutes (at a flow rate of approximately 3.3 mL/min) for a total infusion time of approximately 70 minutes.**
	The dose level of ALN-TTR02 administered to any cohort in study ALN-TTR02-002 may not be higher than that deemed safe and tolerable in the Phase 1 single-dose study of ALN-TTR02 (Study ALN-TTR02-001).	~~The dose level of ALN-TTR02 administered to any cohort in study ALN-TTR02-002 may not be higher than that deemed safe and tolerable in the Phase 1 single-dose study of ALN-TTR02 (Study ALN-TTR02-001).~~
Table 5-1	Total number of patients to be enrolled = 21 a Up to 3 optional cohorts may be included to confirm the safety, tolerability, and/or PD effect at a specific dose.	Changed the total number of patients to be enrolled from 21 patients to 27 patients a Up to ~~3~~**5** optional cohorts may be included to confirm the safety, tolerability, and/or PD effect **of previously evaluated dose levels, which may include evaluation of the original dosing regimen (once every 4 weeks), an alternative dosing regimen (once every 3 weeks), and/or an alternative premedication regimen and infusion**

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		rate at a specific dose **of ALN-TTR02**.
5.7.1: Dose Escalation Procedures	The initial dose of ALN-TTR02 is 10 µg/kg. Dose escalation to 50, 150, and 300 µg/kg is planned. The dose level of ALN-TTR02 administered to any cohort in study ALN-TTR02-002 may not be higher than that deemed safe and tolerable in the Phase 1 single-dose study of ALN-TTR02 (Study ALN-TTR02-001).	The initial dose of ALN-TTR02 is 10 µg/kg. Dose escalation to 50, 150, and 300 µg/kg is planned. ~~The dose level of ALN-TTR02 administered to any cohort in study ALN-TTR02-002 may not be higher than that deemed safe and tolerable in the Phase 1 single-dose study of ALN-TTR02 (Study ALN-TTR02-001).~~
Figure 5-1	Footnote a added	**a Some optional cohorts may have the second dose of ALN-TTR02 administered 3 weeks after the first dose.**
5.7.2: Optional Cohorts	The optional cohort would be included to further confirm the safety and/or PD effect. If this occurs, the IECs will be informed of the expansion. Up to 3 optional cohorts of 3 patients each are permitted in this study.	The optional cohort would be included to further confirm the safety and/or PD effect **of previously evaluated dose levels, which may include evaluation of the original dosing regimen (once every 4 weeks), an alternative dosing regimen (once every 3 weeks), and/or to evaluate an alternative premedication regimen and infusion rate. The dosing regimen for the first optional cohort will be once every 4 weeks, and the dosing regimen for the remaining 4 optional cohorts will be once every 3 or 4 weeks. It is anticipated that the first 2 optional cohorts will use the original premedication regimen, and the remaining 3 optional cohorts may be used to explore the alternative premedication regimen and infusion rate. The SRC will review all available safety data and make recommendations on dosing and premedication regimens prior to dosing of any of the optional cohorts.** If this occurs, the IECs will be

Section	Prior Text	Changed To
		informed of the ~~expansion~~**implementation of optional cohorts**. Up to ~~3~~ **5** optional cohorts of 3 patients each are permitted in this study.
5.7.3: Dose-limiting Toxicity	A DLT is defined as: • Any life-threatening toxicity; • ALT and AST levels ≥5 × ULN or total bilirubin >2.0 mg/dL; • An infusion reaction that requires hospitalization, despite proper premedication; • Any other toxicity which in the opinion of the SRC would have precluded further dosing.	A DLT is defined as: • Any life-threatening toxicity; • ALT and AST levels ≥5 × ULN or total bilirubin >2.0 mg/dL; • An infusion reaction that requires hospitalization, despite ~~proper~~ premedication; • Any other toxicity which in the opinion of the SRC would have precluded further dosing.
6: Study Visits	Added language regarding an open-label extension study	**An open-label extension study at the recommended Phase 3 dose and regimen (as derived from Study ALN-TTR02-002) is planned, which will enable the patients who enrolled in Study ALN-TTR02-002 to receive additional, long-term dosing and safety follow-up. For some of the patients, this may preclude the completion of all of the follow-up period assessments of Study ALN-TTR02-002 if they are deemed eligible to participate in the extension study prior to completion of the full follow up through Day 208. Such patients will be followed up for a minimum of 28 days after their last dose in the current ALN-TTR02-002 study prior to being enrolled in the extension study. The extension study**

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		will be implemented only after dose escalation is completed in Study ALN-TTR02-002, with post second dose follow-up through Day 208 in at least 1 cohort at the highest dose.
	Screening evaluations are to be performed within 45 days before receiving the first dose of study drug, as indicated in Table 1-1. Patients determined to be eligible based on screening assessments will receive treatment (IV infusion of study drug) on Days 0 (Baseline) and 28 (or date of second dose administration in optional cohorts with dosing intervals greater than 4 weeks). Patients will remain hospitalized at the study site for at least 24 hours following completion of dosing to be observed for safety assessments and PK sampling. Patients will return to the study site on Days 2, 7, 10, 14, and 21 for follow-up assessments. In addition, follow-up visits after the second dose of study drug (Day 28) will occur on Days 30, 35, 38, 42, 49, and 56 (end-of-study). Patients will also return to the site for a follow-up visit on Days 112 and 208.	Screening evaluations are to be performed within 45 days before receiving the first dose of study drug, as indicated in Table 1-1 **(Appendix 4 provides the schedule of assessments for the optional cohort(s) administered ALN-TTR02 once every 3 weeks)**. Patients determined to be eligible based on screening assessments will receive treatment (IV infusion of study drug) on Days 0 (Baseline) and 28 (or date of second dose administration in optional cohorts with dosing intervals greater than 4 weeks)**; for patients in optional cohorts evaluating the alternative dosing regimen (once every 3 weeks), ALN-TTR02 will be administered on Days 0 (Baseline) and 21**. Patients will remain hospitalized at the study site for at least 24 hours following completion of dosing to be observed for safety assessments and PK sampling. Patients will return to the study site on Days 2, 7, 10, 14, and 21 for follow-up assessments. In addition, follow-up visits after the second dose of study drug (Day 28) will occur on Days 30, 35, 38, 42, 49, and 56 (end-of-study)**; patients in optional cohorts evaluating the alternative dosing regimen (once every 3 weeks) will have follow-up visits on Days 21, 22, 23, 28, 31, 35,**

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		42, 49, and 56. Patients will also return to the site for a follow-up visit on Days 112 and 208.
6.2: Pre-Dosing (Day -1 or Day 27)	(Title) Pre-Dosing (Day -1 or Day 27) On the day prior to administration of study drug (Days -1 and 27), study site personnel will contact the patient by phone to discuss the pretreatment medication they are to take that evening. On the evening before each dosing, patients are to self-administer the following medications PO: 8 mg dexamethasone or equivalent, 500 mg paracetamol or equivalent, an H1 blocker (10 mg cetirizine hydroxyzine [25 mg, fexofenadine, or equivalent may be substituted if patient does not tolerate cetirizine]), and an oral H2 blocker (i.e., ranitidine 150 mg or famotidine 20 mg or equivalent).	(Title) Pre-Dosing (Day -1 or Day 27**; Day -1 or Day 20 for optional cohorts dosed once every 3 weeks**) **For those patients receiving the original premedication regimen,** on the day prior to administration of study drug (Days -1 and 27), study site personnel will contact the patient by phone to discuss the pretreatment medication they are to take that evening. On the evening before each dosing, patients are to self-administer the following medications PO: 8 mg dexamethasone or equivalent, 500 mg paracetamol or equivalent, an H1 blocker (10 mg cetirizine hydroxyzine [25 mg, fexofenadine, or equivalent may be substituted if patient does not tolerate cetirizine]), and an oral H2 blocker (~~i.e~~**e.g**., ranitidine 150 mg**,** ~~or~~ famotidine 20 mg**,** or equivalent **other H2 blocker).** **Patients in an optional cohort evaluating the alternative premedication regimen and infusion rate will not receive any premedication the evening prior to ALN-TTR02 dosing.**
6.3.1: Day 0 or Day 28 (+2 days)	(Title) Day 0 or Day 28 (+2 days)	(Title) Day 0 or Day 28 (+2 days)**; Day 0 or Day 21 for optional cohorts dosed once every 3 weeks**
6.3.1.1: Pre-dose	Patients will undergo the following procedures before	Patients will undergo the following procedures before

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	study drug administration on Day 0 or Day 28:	study drug administration on Day 0 or Day 28 **(Day 0 or Day 21 for optional cohorts dosed once every 3 weeks)**:
	Note: those parameters marked with an asterisk do not have to be reassessed on Days 0 or 28 if measures are obtained within 72 hours prior to dosing and meets eligibility criteria.	Note: those parameters marked with an asterisk do not have to be reassessed on Days 0 or 28 **(Days 0 or 21 for optional cohorts dosed once every 3 weeks)** if measures are obtained within 72 hours prior to dosing and meets eligibility criteria.
	• Premedicate patient 30 to 60 minutes prior to the start of study drug infusion with the following oral medications (or equivalent[s]): 20 mg dexamethasone, 500 mg paracetamol, 10 mg cetirizine, and an H2 blocker (e.g. 150 mg ranitidine, 20 mg famotidine).	• **For patients receiving the original premedication regimen, ~~P~~p**remedicate **the** patient 30 to 60 minutes prior to the start of study drug infusion with the following oral medications (or equivalent[s]): 20 mg dexamethasone, 500 mg paracetamol, 10 mg cetirizine, and an H2 blocker (e.g. 150 mg ranitidine, 20 mg famotidine). **Patients in select optional cohorts evaluating the alternative premedication regimen and infusion rate will receive the following medications (or equivalent[s]) at least 60 minutes prior to the start of ALN-TTR02 infusion:** **IV dexamethasone 10 mg, PO paracetamol 500 mg, 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 other IV**

Section	Prior Text	Changed To
		H1 blocker available at the study site; or hydroxyzine or fexofenadine 25 mg PO or cetirizine 10 mg PO for any patient who does not tolerate IV diphenhydramine or other IV H1 blockers).
6.3.1.2: Administration of Study Drug	After completion of all pre-dose evaluations and procedures, administer study drug as a 60-minute IV infusion via a controlled infusion device.	After completion of all pre-dose evaluations and procedures, administer study drug as a 60-minute IV infusion **(or an approximate 70-minute IV infusion for patients in an optional cohort evaluating the alternative premedication regimen and infusion rate)** via a controlled infusion device.
6.3.1.3: Post-dose	Patients will undergo the following procedures after study drug administration on Day 0 and Day 28 (+2 days).	Patients will undergo the following procedures after study drug administration on Day 0 and Day 28 (+2 days)**; or Day 0 and Day 21 for optional cohorts dosed once every 3 weeks.**
6.3.2: Day 1 or Day 29	(Title) Day 1 or Day 29 Patients will undergo the following procedures on the day following dosing with study drug (Days 1 or 29):	(Title) Day 1 or Day 29**; Day 1 or Day 22 for optional cohorts dosed once every 3 weeks** Patients will undergo the following procedures on the day following dosing with study drug (Days 1 or 29**; or Days 1 or 22 for optional cohorts dosed once every 3 weeks**):
6.3.3: Day 2 or Day 30	(Title) Day 2 or Day 30 Patients will undergo the following procedures on Days 2 and 30:	(Title) Day 2 or Day 30**; Day 2 or Day 23 for optional cohorts dosed once every 3 weeks** Patients will undergo the following procedures on Days 2 and 30**; or Days 2 or 23 for optional cohorts dosed once every 3 weeks**:
6.3.4: Day 7 or Day 35 (±1 day)	(Title) Day 7 or Day 35 ±1 day Patients will undergo the following procedures on the	(Title) Day 7 or Day 35 ±1 day; **or Day 7 or Day 28 for optional cohorts dosed once every 3 weeks** Patients will undergo the following procedures on the

Section	Prior Text	Changed To
	Day 7 and 35 study visits:	Day 7 and 35 study visits **(or Day 7 and 28 for optional cohorts dosed once every 3 weeks)**:
6.3.5: Day 10 or Day 38 (±2 day)	(Title) Day 10 or Day 38 (±2 day) Patients will undergo the following procedures on the Day 10 and 38 study visits:	(Title) Day 10 or Day 38 ±2 day**; or Day 10 or Day 31 for optional cohorts dosed once every 3 weeks** Patients will undergo the following procedures on the Day 10 and 38 study visits **(or Day 10 and 28 for optional cohorts dosed once every 3 weeks)**:
6.3.6: Day 14 or Day 42 (±3 days)	(Title) Day 14 or Day 42 (±3 days) Patients will undergo the following procedures on the Day 14 and 42 study visits:	(Title) Day 14 or Day 42 ±3 days**; or Day 14 or Day 35 for optional cohorts dosed once every 3 weeks** Patients will undergo the following procedures on the Day 14 and 42 study visits **(or Day 14 and 35 for optional cohorts dosed once every 3 weeks)**:
6.3.7: Day 21 or Day 49 (±3 days)	(Title) Day 21 or Day 49 (±3 days) Patients will undergo the following procedures on the Day 21 and 49 study visits:	(Title) Day 21 or Day 49 ±3 days**; or Day 21 or Day 42 for optional cohorts dosed once every 3 weeks** Patients will undergo the following procedures on the Day 21 and 49 study visits **(or Day 21 and 42 for optional cohorts dosed once every 3 weeks)**:
6.6: Participation in an Open-label Extension Study	Added new section	**An open-label extension study at the recommended Phase 3 dose and regimen (as derived from Study ALN-TTR02-002) is planned, which will enable the patients who enrolled in Study ALN-TTR02-002 to receive additional, long-term dosing and safety follow-up. For some of the patients, this may preclude the completion of all of the follow-up period assessments of Study ALN-TTR02-002 if they are deemed eligible to participate in the extension study prior to completion of the full follow**

Section	Prior Text	Changed To
		up through Day 208. Such patients will be followed up for a minimum of 28 days after their last dose in the current ALN-TTR02-002 study prior to being enrolled in the extension study. The extension study will be implemented only after dose escalation is completed in Study ALN-TTR02-002, with post second dose follow-up through Day 208 in at least 1 cohort at the highest dose deemed safe.
7.1: Demographic Data and Medical History	Patient demographic data will be obtained during screening, and a complete medical history will be obtained during screening and updated on Days 0 and 28 as needed.	Patient demographic data will be obtained during screening, and a complete medical history will be obtained during screening and updated on Days 0 and 28 **(or Days 0 and 21 for optional cohorts dosed once every 3 weeks)** as needed.
7.2.1: Physical Examination	Body weight will be measured at Screening for assessment of eligibility, and on the Day 0 and 28 study visits.	Body weight will be measured at Screening for assessment of eligibility, and on the Day 0 and 28 **(or Day 0 and 21 for optional cohorts dosed once every 3 weeks)** study visits.
7.2.2: Vital Signs	Vital signs are to be measured at Screening and the Day 2, 14, 30, 42, and 56 (or time of early termination, if applicable) study visits and include systolic/diastolic blood pressure, pulse rate, respiration rate, and oral body temperature. On Days 0 and 28, serial vital signs are to be measured within 30 minutes pre-dose, at EOI; and at 30 (±5) minutes;1, 2, and 3 (±15 minutes) hours; 6, 12, and 18 hours (±30 minutes) post-infusion. On Days 1 and 29, serial vital signs are to be measured at 24 hours	Vital signs are to be measured at Screening and the Day 2, 14, 30, 42, and 56 (**or Days 2, 14, 23, 35, and 56 for optional cohorts dosed once every 3 weeks;** or time of early termination, if applicable) study visits and include systolic/diastolic blood pressure, pulse rate, respiration rate, and oral body temperature. On Days 0 and 28 **(or Day 21 for optional cohorts dosed once every 3 weeks)**, serial vital signs are to be measured within 30 minutes pre-dose, at EOI; and at 30 (±5) minutes;1, 2, and 3 (±15 minutes) hours; 6, 12, and 18 hours (±30 minutes) post-infusion. On Days 1

Section	Prior Text	Changed To
	(+30 minutes) post-infusion.	and 29 **(or Day 22 for optional cohorts dosed once every 3 weeks)**, serial vital signs are to be measured at 24 hours (+30 minutes) post-infusion.
7.2.4: Electrocardiogram	Serial ECGs will be collected in triplicate 30 minutes prior to dosing (Days 0 and 28), EOI, and 30 (±5) minutes, 2 and 4 (±15 minutes) hours, and on Days 1 and 29 at 24 (+30 minutes) hours post-infusion. Prior to discharge from the hospital on Days 1 and 29, the ECG must be reviewed by the Investigator and the results deemed not clinically significant.	Serial ECGs will be collected in triplicate 30 minutes prior to dosing (Days 0 and 28 **[or Days 0 and 21 for optional cohorts dosed once every 3 weeks]**), EOI, and 30 (±5) minutes, 2 and 4 (±15 minutes) hours, and on Days 1 and 29 **(or Days 1 and 22 for optional cohorts dosed once every 3 weeks)** at 24 (+30 minutes) hours post-infusion. Prior to discharge from the hospital on Days 1 and 29 **(or Days 1 and 22 for optional cohorts dosed once every 3 weeks)**, the ECG must be reviewed by the Investigator and the results deemed not clinically significant.
7.2.5: Pulse Oximetry	Arterial oxygen saturation will be assessed by serial pulse oximetry on Days 0 and 28 within 30 (±5) minutes pre-dose; at EOI; at 30 (±5) minutes; at 1, 2, 3 hours (±15 minutes); and at 6, 12, and 18 hours (±15 minutes) post-infusion on Days 0 and 28; and at 24 hours (+30 minutes) post-infusion on Days 1 and 29.	Arterial oxygen saturation will be assessed by serial pulse oximetry on Days 0 and 28 **(or Days 0 and 21 for optional cohorts dosed once every 3 weeks)** within 30 (±5) minutes pre-dose; at EOI; at 30 (±5) minutes; at 1, 2, 3 hours (±15 minutes); and at 6, 12, and 18 hours (±15 minutes) post-infusion on Days 0 and 28 **(or Days 0 and 21 for optional cohorts dosed once every 3 weeks)**; and at 24 hours (+30 minutes) post-infusion on Days 1 and 29 **(or Days 1 and 22 for optional cohorts dosed once every 3 weeks)**.
7.2.7.1: Hematology, Serum Chemistries, and Urinalysis	Blood samples for hematology and serum chemistries and urine for urinalysis are to be collected at Screening,	Blood samples for hematology and serum chemistries and urine for urinalysis are to be collected at Screening,

Section	Prior Text	Changed To
	pre-dose on Day 0 (unless the screening evaluations were performed within the previous 72 hours) and Day 28, and on the Day 1, 14, 29, 42, and 56 study visits, or at the time of early termination, if applicable.	pre-dose on Day 0 (unless the screening evaluations were performed within the previous 72 hours) and Day 28 **(or Day 21 for optional cohorts dosed once every 3 weeks)**, and on the Day 1, 14, 29, 42, and 56 study visits **(or Days 1, 14, 22, 35, and 56 for optional cohorts dosed once every 3 weeks)**, or at the time of early termination, if applicable.
	Prior to discharge from the hospital on Days 1 and 29, local serum laboratories (specifically, sodium, potassium, creatinine, albumin, calcium, glucose, and phosphate) must be reviewed by the Investigator and the results deemed not clinically significant. Blood for CRP is to be collected pre-dose (within 10 minutes) on Days 0 and 28, and at 2 and 6 hours (± 15 minutes), and 24 hours (± 120 minutes) post-infusion.	Prior to discharge from the hospital on Days 1 and 29 **(or Days 1 and 22 for optional cohorts dosed once every 3 weeks)**, local serum laboratories (specifically, sodium, potassium, creatinine, albumin, calcium, glucose, and phosphate) must be reviewed by the Investigator and the results deemed not clinically significant. Blood for CRP is to be collected pre-dose (within 10 minutes) on Days 0 and 28 **(or Days 0 and 21 for optional cohorts dosed once every 3 weeks)**, and at 2 and 6 hours (± 15 minutes), and 24 hours (± 120 minutes) post-infusion.
7.2.7.2: Liver Function Tests	Blood for LFTs is to be collected at Screening, pre-dose on Day 0 (unless the screening evaluations were performed within the previous 72 hours) and Day 28, and at the Day 1, 2, 7, 14, 29, 30, 35, 42, and 56 study visits, or at the time of early termination, if applicable. Prior to discharge from the hospital on Days 1 and 29,	Blood for LFTs is to be collected at Screening, pre-dose on Day 0 (unless the screening evaluations were performed within the previous 72 hours) and Day 28, and at the Day 1, 2, 7, 14, 29, 30, 35, 42, and 56 study visits **(or Days 1, 2, 7, 14, 22, 23, 28, 35, and 56 for optional cohorts dosed once every 3 weeks)**, or at the time of early termination, if applicable. Prior to discharge from the hospital on Days 1 and 29

Section	Prior Text	Changed To
	local LFTs must be reviewed by the Investigator and the results deemed not clinically significant.	**(or Days 1 and 22 for optional cohorts dosed once every 3 weeks)**, local LFTs must be reviewed by the Investigator and the results deemed not clinically significant.
7.2.7.4: Coagulation Studies	Blood for coagulation studies is to be collected at Screening, pre-dose on Days 0 (unless the screening evaluations were performed within the previous 72 hours) and 28, at the Day 1 and 29 study visits.	Blood for coagulation studies is to be collected at Screening, pre-dose on Days 0 (unless the screening evaluations were performed within the previous 72 hours) and 28 **(or Day 21 for optional cohorts dosed once every 3 weeks)**, at the Day 1 and 29 **(or Day 1 and 22 for optional cohorts dosed once every 3 weeks)** study visits.
7.2.7.5: Thyroid Function Tests	Blood for thyroid function tests is to be collected at Screening, pre-dose on Days 0 (unless the screening evaluations were performed within the previous 72 hours) and 28, and at Day 14, 42, and 56 study visits, or at the time of early termination, if applicable.	Blood for thyroid function tests is to be collected at Screening, pre-dose on Days 0 (unless the screening evaluations were performed within the previous 72 hours) and 28 **(or Days 0 and 21 for optional cohorts dosed once every 3 weeks)**, and at Day 14, 42, and 56 study visits **(or Days 14, 35, and 56 for optional cohorts dosed once every 3 weeks)**, or at the time of early termination, if applicable.
7.2.7.6: Complement	Blood for complement factor Bb, is to be collected pre-dose (within 10 minutes) on Days 0 and 28, and 30 (± 5) minutes and 2 (± 15 minutes) and 24 hours (± 120 minutes) post-infusion.	Blood for complement factor Bb, is to be collected pre-dose (within 10 minutes) on Days 0 and 28 **(or Days 0 and 21 for optional cohorts dosed once every 3 weeks)**, and 30 (± 5) minutes and 2 (± 15 minutes) and 24 hours (± 120 minutes) post-infusion.

Section	Prior Text	Changed To
7.2.7.7: Cytokines	Blood for cytokine assessment is to be collected on Days 0 and 28 pre-dose (within 10 minutes), at 2 (±15 minutes), and 6 hours (±15 minutes), and 24 hours (±120 minutes) post-infusion.	Blood for cytokine assessment is to be collected **pre-dose (within 10 minutes)** on Days 0 and 28 **(or Days 0 and 21 for optional cohorts dosed once every 3 weeks)** ~~pre-dose (within 10 minutes)~~, at 2 (±15 minutes), and 6 hours (±15 minutes), and 24 hours (±120 minutes) post-infusion.
7.3.1: Transthyretin Protein	Blood for serum TTR protein levels (WT and mutant) is to be collected at Screening, immediately prior (within 10 minutes) to the administration of the premedications, immediately pre-dose (within 10 minutes) on Days 0 (Baseline) and 28, and on Days 1, 2, 7, 10, 14, 21, 29, 30, 35, 38, 42, 49, 56 (end-of-study), 112, and 208 or at the time of early termination.	Blood for serum TTR protein levels (WT and mutant) is to be collected at Screening, immediately prior (within 10 minutes) to the administration of the premedications, immediately pre-dose (within 10 minutes) on Days 0 (Baseline) and 28, and on Days 1, 2, 7, 10, 14, 21, 29, 30, 35, 38, 42, 49, 56 (end-of-study), 112, and 208 or at the time of early termination. **For optional cohorts dosed once every 3 weeks, blood for serum TTR protein levels will be collected at Screening, immediately prior (within 10 minutes) to the administration of the premedications, immediately pre-dose (within 10 minutes) on Days 0 (Baseline) and 21, and on Days 1, 2, 7, 10, 14, 22, 23, 28, 31, 35, 42, 49, 56 (end-of-study), 112, and 208 or at the time of early termination.**
7.3.2: Transthyretin mRNA	Blood for serum TTR mRNA is to be collected at Screening, immediately prior (within 10 minutes) to the administration of the premedications on Days 0 (Baseline) and 28, and on Days 1, 2, 29, and 30.	Blood for serum TTR mRNA is to be collected at Screening, immediately prior (within 10 minutes) to the administration of the premedications on Days 0 (Baseline) and 28, and on Days 1, 2, 29, and 30. **For optional cohorts dosed once every 3 weeks, blood for serum TTR mRNA will be collected at Screening and on Days 0 (Baseline) and 21, and on Days 1, 2, 22, and 23.**

Section	Prior Text	Changed To
7.3.2.1: Vitamin A and Retinol Binding Protein	Blood for measurements of vitamin A and RBP is to be collected at Screening, immediately prior (within 10 minutes) to the administration of the premedications, immediately pre-dose (within 10 minutes) on Days 0 and 28, and on Days 1, 2, 7, 10, 14, 21, 29, 30, 35, 38, 42, 49, 56 (end-of-study), 112, and 208 or at the time of early termination.	Blood for measurements of vitamin A and RBP is to be collected at Screening, immediately prior (within 10 minutes) to the administration of the premedications, immediately pre-dose (within 10 minutes) on Days 0 and 28, and on Days 1, 2, 7, 10, 14, 21, 29, 30, 35, 38, 42, 49, 56 (end-of-study), 112, and 208 or at the time of early termination. **For optional cohorts dosed once every 3 weeks, blood for measurements of vitamin A and RBP will be collected at Screening, immediately prior (within 10 minutes) to the administration of the premedications, immediately pre-dose (within 10 minutes) on Days 0 (Baseline) and 21, and on Days 1, 2, 7, 10, 14, 22, 23, 28, 31, 35, 42, 49, 56 (end-of-study), 112, and 208 or at the time of early termination.**
7.4.1: Plasma Pharmacokinetics	Samples will also be collected on Days 7, 14, 21, 35, 42, 49, 56, 112, and 208 post infusion as well as at the ET visit (if applicable).	Samples will also be collected on Days 7, 14, 21, 35, 42, 49, 56, 112, and 208 post infusion as well as at the ET visit (if applicable)**; f****or optional cohorts dosed once every 3 weeks, samples will be collected on Days 7, 14, 21, 28, 35, 42, 56, 112, and 208 post infusion as well as at the ET visit (if applicable).**
7.4.2: Urine Pharmacokinetics	Urine sample collection times are included in the schedule of assessments (see Table 1-1).	Urine sample collection times are included in the schedule of assessments (see Table 1-1 **or Table 12-1 for optional cohorts dosed once every 3 weeks**).
9.1: Sample Size	Based on the planned dose escalation scheme (see Section 5.7.1), up to 21 patients are expected to be enrolled.	Based on the planned dose escalation scheme (see Section 5.7.1), up to ~~21~~ **27** patients are expected to be enrolled.
Appendices	Added Appendix 4	**Appendix 4: Schedule of Assessments for Optional Cohorts Administered ALN-TTR02 Once**

Section	Prior Text	Changed To
		Every Three Weeks **Added Table 12-1**

Protocol ALN-TTR02-002

Amendment 1.1 Change Summary

A Phase 2, Open-Label, Multi-Dose, Dose Escalation Trial to Evaluate the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of Intravenous Infusions of ALN-TTR02 in Patients with TTR Amyloidosis

Changes included in Amendment 1.1 to Protocol ALN-TTR02-002, dated 24 September 2012, are itemized below and detailed on the following page.

- Pages 39 and 50 of the Protocol were updated to add requested language from the French CEC (*insert name of EC)* regarding AST and ALT levels and receipt of second dose. Statements were added to the pertinent sections of the protocol so that if after receipt of the first dose of study drug the patient's ALT and AST levels are reported to be greater than 2.5 times the upper limit of normal, the patient will not receive the second dose of study drug.
- Made minor editorial changes (e.g., corrections of typographical, grammatical, and spelling errors as well as formatting changes and changes for consistency); these changes are not listed individually.

Text deleted is indicated by strikeout while text added is indicated by **bold** font.

Section	Prior Text	Changed To
3.1 Overall Design	Within each of the cohorts, the first patient will receive their first dose and if the dose is well tolerated (per Section 5.7.1~~5.7.3~~, Dose-limiting Toxicity), Patients 2 and 3 will receive their first dose at that same dose level no sooner than 48 hours apart, with Patient 2 receiving a dose of study drug no sooner than 48 hours after the dosing of Patient 1. Similar to the first dose, the second dose will be administered to patients 4 weeks after the first dose in a sequential manner with at least 48 hours separating dosing of each patient.	Within each of the cohorts, the first patient will receive their first dose and if the dose is well tolerated (per Section 5.7.1~~5.7.3~~, Dose-limiting Toxicity), Patients 2 and 3 will receive their first dose at that same dose level no sooner than 48 hours apart, with Patient 2 receiving a dose of study drug no sooner than 48 hours after the dosing of Patient 1. Similar to the first dose, the second dose will be administered to patients 4 weeks after the first dose in a sequential manner with at least 48 hours separating dosing of each patient. **If after the first dose, ALT and AST are > 2.5 x ULN, that patient will not receive their second dose.**

Section	Prior Text	Changed To
5.7.1 Dose-escalation Procedures	Within each of the cohorts, the first patient will receive their first dose and if the dose is well tolerated, per Section 5.7.1~~5.7.3~~ (Dose-limiting Toxicity), Patients 2 and 3 will receive their first dose at that same dose level no sooner than 48 hours apart, with Patient 2 receiving a dose of study drug no sooner than 48 hours after the dosing of Patient 1. Similar to the first dose, the second dose will be administered to patients 4 weeks after the first dose in a sequential manner with at least 48 hours separating dosing of each patient.	Within each of the cohorts, the first patient will receive their first dose and if the dose is well tolerated, per Section 5.7.1~~5.7.3~~ (Dose-limiting Toxicity), Patients 2 and 3 will receive their first dose at that same dose level no sooner than 48 hours apart, with Patient 2 receiving a dose of study drug no sooner than 48 hours after the dosing of Patient 1. Similar to the first dose, the second dose will be administered to patients 4 weeks after the first dose in a sequential manner with at least 48 hours separating dosing of each patient. **If after the first dose, ALT and AST are > 2.5 x ULN, that patient will not receive their second dose.**

Protocol ALN-TTR02-002

Amendment 1 Change Summary

A Phase 2, Open-Label, Multi-Dose, Dose Escalation Trial to Evaluate the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of Intravenous Infusions of ALN-TTR02 in Patients with TTR Amyloidosis

Changes included in Amendment 1 to Protocol ALN-TTR02-002, dated 19 June 2012, are itemized below and detailed in the pages that follow.

- Added pertinent information from the ongoing chronic GLP toxicology study in non-human primates (Study 504265); also, reference is made to the Expedited Safety Report addendum dated 7 June 2012 to the IB, which contains the details of the nonclinical finding from this study.
- Made additional references, where relevant, to the Expedited Safety Report addendum dated 7 June 2012, which in addition to providing pertinent data from the ongoing chronic GLP toxicology study in non-human primates, also includes preliminary safety and efficacy updates from the Phase 1 trials of ALN-TTR02 in healthy volunteers (ALN-TTR02-001) and ALN-PCS02 in healthy volunteers with elevated cholesterol (ALN-PCS02-001).
- In light of the favorable single-dose safety data that have emerged from the recently completed Phase 1 trials of ALN-TTR02 and ALN-PCS02 at doses greater than or equal to the top dose on this study (detailed in Expedited Safety Report addendum dated 7 June 2012 to the IB), we have removed the requirement that the SRC review safety data on all patients within a dose level following first dose prior to any patient at that dose level receiving their second dose.
- Removed the 500 μg/kg dose cohort (highest dose) since the ALN-TTR02-001 study has completed enrollment and demonstrated adequate pharmacology at the 0.3 mg/kg dose.
- Having removed the top dose, thus reducing the total number of patients in the study from 24 to 21. Instead, the number of optional cohorts has been increased from 3 to 4 to allow for further evaluation of safety and/or pharmacodynamic effect.
- Extended the post-dose on-site observation period from 6 hours to 24 hours post-infusion [redacted]
- Added the following additional safety evaluations to further ensure patient safety:
  - Continuous cardiac monitoring (telemetry) to be performed during dosing and for 24 hours post-dose (from Days 0 to 1 and again from Days 28 to 29).

  - Serial vital signs and pulse oximetry assessments at 12 and 18 hours (±30 minutes), and 24 (+30 minutes) hours post-infusion.
  - Serial electrocardiogram at 24 (+30 minutes) hours post-infusion.
- Added specific instructions, consistent with that contained in the ALN-TTR02-01 protocol, for dosing of patients who weigh 105 kg or less.
- Language regarding Early Termination visits was made consistent with the Informed Consent Form.
- Made minor editorial changes (e.g., corrections of typographical, grammatical, and spelling errors as well as formatting changes and changes for consistency); these changes are not listed individually.

Text deleted is indicated by strikeout while text added is indicated by **bold** font.

Section	Prior Text	Changed To
Contact Information	[redacted]	[redacted]
Synopsis: Design	Dosing within a cohort: The first patient will receive their first dose and if the dose is well tolerated, Patients 2 and 3 will receive their first dose at that same dose level no sooner than 48 hours apart, with Patient 2 receiving a dose of study drug no sooner than 48 hours after the dosing of Patient 1. Cumulative safety and tolerability data on all patients will be reviewed by the SRC after the last patient has at least 96 hours of follow-up and prior to the administration of each patient within the cohort's second dose. If deemed safe and tolerated by the SRC, patients will receive their 2nd dose of study drug approximately 4 weeks after receiving their first dose. Similar to the first dose, the second dose will be administered to patients in a sequential manner with at least 48 hours separating dosing of each patient.	Dosing within a cohort: The first patient will receive their first dose and if the dose is well tolerated, **per Section 5.7.3 (Dose-limiting Toxicity),** Patients 2 and 3 will receive their first dose at that same dose level no sooner than 48 hours apart, with Patient 2 receiving a dose of study drug no sooner than 48 hours after the dosing of Patient 1. ~~Cumulative safety and tolerability data on all patients will be reviewed by the SRC after the last patient has at least 96 hours of follow-up and prior to the administration of each patient within the cohort's second dose. If deemed safe and tolerated by the SRC, patients will receive their 2nd dose of study drug approximately 4 weeks after receiving their first dose.~~ Similar to the first dose, the second dose will be administered to patients **4 weeks after the first dose** in a sequential manner with at least 48 hours separating dosing of each patient.

Section	Prior Text	Changed To
	Dosing of the next cohort: Collective cohort safety and tolerability data on Patients 1 to 3 through at least 96 hours post-first dose will be reviewed by the SRC; if the administered dose is found to be safe and well tolerated, dosing for the next protocol-specified dose level will begin no sooner than 96 hours after Patient 3 had safely received dose 1 from the previous cohort. Patients in the cohort would be dosed and reviewed for safety and tolerability following the same procedures as used for other cohorts (as stated above). Prior to receiving the second dose, the SRC will review the cumulative safety and tolerability data of at least 2 patients from the previous cohort with at least 96 hours of follow-up after receiving a second dose of study drug.	Dosing of the next cohort: Collective cohort safety and tolerability data on Patients 1 to 3 through at least 96 hours post-first dose will be reviewed by the SRC; if the administered dose is found to be safe and well tolerated, dosing for the next ~~protocol-specified dose level~~**cohort** will begin no sooner than 96 hours after Patient 3 had safely received dose 1 from the previous cohort. Patients in the cohort would be dosed and reviewed for safety and tolerability following the same procedures as used for other cohorts (as stated above). **For patients on all dose levels other than the starting dose level of 10 µg/kg**, prior to receiving the second dose, the SRC will review the cumulative safety and tolerability data of at least 2 patients from the previous ~~cohort~~ **dose level(s)** with at least 96 hours of follow-up after receiving a second dose of study drug.
Synopsis Dosage, Route of Administration and Duration of Treatment of Investigational Drug	Five cohorts are planned to be enrolled to evaluate 2 consecutive doses, 4 weeks apart of the following dose levels: 10, 50, 150, 300, and 500 µg/kg ALN-TTR02. Based on the interim evaluation of safety data, it may also be decided by the SRC that an optional cohort may be utilized which will include 3 additional patients. These optional cohorts may occur at a previously tested dose that was found to be safe and where stopping rules were not met or at an intermediate dose level.	~~Five~~ **Four** cohorts are planned to be enrolled to evaluate 2 consecutive doses, 4 weeks apart of the following dose levels: 10, 50, 150, **and** 300~~, and 500~~ µg/kg ALN-TTR02. Based on the interim evaluation of safety data, it may also be decided by the SRC that an optional cohort may be utilized which will include 3 additional patients. **Dosing in** these optional cohorts may occur at a previously tested dose that was found to be safe and where stopping rules were not met or at an intermediate dose level.
	Up to 3 optional cohorts are permitted in this study.	Up to **4**~~3~~ optional cohorts are permitted in this study.
	Patients will be observed at the clinic for up to 6 hours	Patients will **remain hospitalized at the study site for at**

Section	Prior Text	Changed To
	following completion of dosing on Days 0 and 28, for safety assessments and pharmacokinetic (PK) sampling.	**least 24 hours** ~~be observed at the clinic for up to 6 hours~~ following completion of dosing on Days 0 and 28, for safety assessments and pharmacokinetic (PK) sampling.
Synopsis Sample Size	Based on the entry criteria and the proposed dose escalation scheme, up to 24 patients are expected to be enrolled.	Based on the entry criteria and the proposed dose escalation scheme, up to ~~24~~ **21** patients are expected to be enrolled.
Synopsis Safety Assessments	Safety evaluations will include assessment of adverse events (AEs), electrocardiograms (ECGs), arterial oxygen saturation (SaO_2) using pulse oximetry, vital signs (blood pressure, pulse rate, oral body temperature, and respiration rate), clinical laboratory safety tests (hematology, serum chemistry, liver function tests, thyroid function parameters, serology, coagulation parameters, urinalysis, cytokines, C-reactive protein, complement factors), and physical examinations.	Safety evaluations will include assessment of adverse events (AEs), electrocardiograms (ECGs), **cardiac monitoring (telemetry),** arterial oxygen saturation (SaO_2) using pulse oximetry, vital signs (blood pressure, pulse rate, oral body temperature, and respiration rate), clinical laboratory safety tests (hematology, serum chemistry, liver function tests, thyroid function parameters, serology, coagulation parameters, urinalysis, cytokines, C-reactive protein, complement factors), and physical examinations.
Table 1-1: Schedule of Assessments	*Changes to timing of assessments*: Vital signs Vital signs (serial) ECG (serial) Pulse oximetry (serial) *Assessments added*: Inpatient at study site Cardiac monitoring (telemetry)	*Changes to timing of assessments:* Vital signs: removed D1/D29 time point Vital signs (serial): added D1/D29 time point ECG (serial): added D1/D29 time point Pulse oximetry: added D1/D29 time point *Assessments added:* Inpatient at study site: scheduled for the D0/D28 (predose); D0/D28 (postdose) and D1/D29 time points Cardiac monitoring (telemetry): scheduled for the D0/D28 (predose); D0/D28 (postdose) and D1/D29 time points
	Footnote f: Serial measures (vital signs and pulse	*Footnote f:* Serial measures (vital signs and pulse oximetry) are to be measured within 30 minutes pre-dose;

Section	Prior Text	Changed To
	oximetry) are to be measured within 30 minutes pre-dose; at the end of infusion (EOI); and 30 (±5) minutes; and 1, 2, 3, and 6 (±15 minutes) hours post-infusion.	at the end of infusion (EOI); and 30 (±5) minutes; 1, 2, **and** 3 **(±15 minutes) hours;** 6**, 12, and 18 (±30 minutes) hours; and 24** (+**30** minutes) hours post-infusion.
	Footnote h: Serial electrocardiograms (ECGs) will be collected in 3 replicates within 30 minutes pre-dose, EOI, and 30 (±5) minutes, and 2 and 4 (±15 minutes) hours post-infusion	*Footnote h:* Serial electrocardiograms (ECGs) will be collected in 3 replicates within 30 minutes pre-dose, EOI, and 30 (±5) minutes~~, and~~ 2 and 4 (±15 minutes) hours**, and 24 (+30 minutes) hours** post-infusion
	Added footnote i.	**Patients will be hospitalized at the study site for at least 24 hours after the end of infusion of study drug. Patients may be discharged upon completion of review by the Investigator of ECG, sodium, potassium, creatinine, albumin, calcium, glucose, phosphate, and LFTs results obtained at 24-hours post-infusion, if results are deemed not clinically significant. Any clinically significant findings must be discussed with the medical monitor to determine whether patient can be discharged and to formulate plans for patient follow-up.**
	Added footnote j.	**Continuous cardiac monitoring will be performed via telemetry starting no later than 30 minutes prior to dosing and continuing through 24 hours (+1 hour) post-dose.**

Section	Prior Text	Changed To
Section 1.2: Summary of ALN-TTR02 Non-clinical Data	[redacted]	[redacted]
	Further information can be found in the ALN-TTR02 Investigator's Brochure.	Further information can be found in the ALN-TTR02 Investigator's Brochure **and its Expedited Safety Report addendum dated 7 June 2012**.

Section	Prior Text	Changed To
Section 1.3: Summary of Clinical Data with siRNA-LNPs	Although no clinical data are yet available with ALN-TTR02, a Phase 1 multi-centre, randomized, placebo-controlled, single-blind, single-ascending dose clinical study to evaluate the safety, tolerability, PK, and pharmacodynamic (PD) of ALN-TTR02 in healthy volunteers is currently approved by MHRA (EUDRACT # 2011-005291-42) and will begin dosing shortly. ALN-TTR02 will be administered as a single 60-minute IV infusion to healthy volunteers at the same doses proposed for this study: 10, 50, 150, 300, and 500 µg/kg (4 patients per dose level; 3 receiving ALN-TTR02 and 1 receiving placebo). The dose level of ALN-TTR02 administered to any cohort in study ALN-TTR02-002 may not be higher than that deemed safe and tolerable in the Phase 1 single-dose study of ALN-TTR02 (Study ALN-TTR02-001). Patients will be pre-medicated with dexamethasone, H1 and H2 blockers, and paracetamol prior to dosing to minimize the risk of infusion reaction.	~~Although no clinical data are yet available with ALN-TTR02,~~ A Phase 1 multi-centre, randomized, placebo-controlled, single-blind, single-ascending dose clinical study to evaluate the safety, tolerability, PK, and pharmacodynamic (PD) of ALN-TTR02 in healthy volunteers ~~is currently~~ **was** approved by MHRA (EUDRACT # 2011-005291-42) and ~~will begin dosing shortly~~ **has completed dosing**. ALN-TTR02 ~~will be~~ **was** administered as a single 60-minute IV infusion to healthy volunteers at the ~~same~~ **following** doses ~~proposed for this study~~: 10, 50, 150, 300, and 500 µg/kg (4 patients per dose level; 3 receiving ALN-TTR02 and 1 receiving placebo). The dose level of ALN-TTR02 administered to any cohort in study ALN-TTR02-002 may not be higher than that deemed safe and tolerable in the Phase 1 single-dose study of ALN-TTR02 (Study ALN-TTR02-001). Patients will be pre-medicated with dexamethasone, H1 and H2 blockers, and paracetamol prior to dosing to minimize the risk of infusion reaction. **Preliminary data show that ALN-TTR02 was safe and well-tolerated and exhibited robust effects on serum TTR levels at the top doses. To date, no stopping rules have been met and there have been no early discontinuations due to adverse events. Further information on the ALN-TTR02-001 Phase 1 study can be found in the ALN-TTR02 Investigators Brochure and its Expedited Safety Report addendum dated 7 June 2012.**

Section	Prior Text	Changed To
	Further information can be found in the Investigator's Brochure.	Further information**, including preliminary safety and efficacy updates from the recently completed Phase 1 trials of ALN-TTR02 in healthy volunteers and ALN-PCS02 in healthy volunteers with elevated cholesterol,** can be found in the Investigator's Brochure **and its Expedited Safety Report addendum dated 7 June 2012**.
Section 1.5: Dose Selection and Rationale	Study drug will be administered as a 60-minute IV infusion with the following proposed doses for each cohort: 10, 50, 150, 300, and 500 µg/kg ALN-TTR02.	Study drug will be administered as a 60-minute IV infusion with the following proposed doses for each cohort: 10, 50, 150, **and** 300~~, and 500~~ µg/kg ALN-TTR02.
	Added information from the ongoing chronic GLP toxicology study in non-human primates (Study 504265). The top dose proposed in the study (500 µg/kg) [redacted]	The top dose proposed in the study (~~500~~ **300** µg/kg) is [redacted]. **Preliminary safety data are available from ongoing Phase 1 trials with ALN-TTR02 and ALN-PCS02. In the ALN-TTR02-001 Phase 1 trial in healthy volunteers, these same dose levels were found to be safe and well-tolerated. Furthermore, ALN-PCS02, which uses the same second generation LNP formulation as ALN-TTR02, was safe and well-tolerated at doses up to 400 µg/kg in healthy volunteers with elevated cholesterol. See the ALN-TTR02 Investigators Brochure and its Expedited Safety Report addendum dated 7 June 2012 for updated information on the ALN-TTR02 and ALN-PCS02 Phase 1 trials.**
	Additionally, a conservative dosing approach is being taken. In each cohort, patients will be dosed in a sequential fashion with a minimum of 48 hours elapsing between patients. Collective cohort safety and tolerability data on the 3 patients from the prior cohort through at least	Additionally, a conservative dosing approach is being taken. In each cohort, patients will be dosed in a sequential fashion with a minimum of 48 hours elapsing between patients. Collective cohort safety and tolerability data on the 3 patients from the prior cohort through at least

Section	Prior Text	Changed To
	96 hours post-dose will be reviewed by the Safety Review Committee (SRC) prior to approving dosing for the next protocol-specified dose level. In addition, cumulative safety and tolerability data observed through at least 96 hours post-second dose of the previous cohort will be reviewed by the SRC prior to administration of the second dose of study drug.	96 hours post-dose will be reviewed by the Safety Review Committee (SRC) prior to approving dosing for the next ~~protocol-specified~~ dose level. In addition, cumulative safety and tolerability data observed **in at least 2 patients** through at least 96 hours post-second dose of the previous cohort will be reviewed by the SRC prior to administration of the second dose of study drug.
	The top dose proposed in the study (500 µg/kg) is [redacted].	The top dose proposed in the study (~~500~~ **300** µg/kg) is [redacted]
	Up to 3 optional cohorts may be enrolled, each comprised of 3 patients, to further evaluate a dose level or dosing intervals greater than 4 weeks. The optional cohorts will follow the same treatment schema as cohorts 1 to 5, as outlined in Section 5.6.	**Based on the interim evaluation of safety data, it may also be decided by the SRC that an optional cohort may be utilized which will include 3 additional patients. Dosing in these optional cohorts may occur at a previously tested dose that was found to be safe and where stopping rules were not met or at an intermediate dose level. Patients in the optional cohort would be dosed and reviewed for safety and tolerability following the same procedures as used for other cohorts. The optional cohort would be included to further confirm the safety and/or PD effect. If this occurs, the Independent Ethics Committees (IECs) will be informed of the expansion. Up to 3 optional cohorts of 3 patients each are permitted in this study**. ~~Up to 3 optional cohorts may be enrolled, each comprised of 3 patients, to further evaluate a dose level or dosing intervals greater than 4 weeks.~~ The optional cohorts will follow the same treatment schema as cohorts 1 to ~~5~~**4**, as

Section	Prior Text	Changed To
		outlined in Section 5.6.
Section 3.1: Overall Design	Two doses, separated by 4 weeks, of ALN-TTR02 (10, 50, 150, 300, and 500 µg/kg) will be investigated in 5 sequential cohorts comprised of 3 patients each. No patients will be a member of more than 1 treatment group. Three optional cohorts may also be included to further explore the safety and PD effect at any dose or the PD effect of extending the dosing intervals of ALN-TTR02 beyond 4 weeks (see Section 5.7.2).	Two doses, separated by 4 weeks, of ALN-TTR02 (10, 50, 150, **and** 300~~, and 500~~ µg/kg) will be investigated in ~~5~~ **4** sequential cohorts comprised of 3 patients each. No patients will be a member of more than 1 treatment group. ~~Three optional cohorts may also be included to further explore the safety and PD effect at any dose or the PD effect of extending the dosing intervals of ALN-TTR02 beyond 4 weeks (see Section 5.7.2).~~
	Within each of the cohorts, patients will be dosed in a sequential fashion with a minimum of 48-hours of observation between each patient. Continuation of dosing (administration of second dose) of the current cohort and dose escalation to the next cohort will proceed after the collective safety and tolerability data for Patients 1 to 3 of the current cohort through at least 96 hours post-first dose are reviewed by the SRC. The continuation dosing (administration of second dose) of subsequent cohorts is dependent upon the SRC determining administration of second doses of study drug were safe and tolerable in previous cohorts.	Within each of the cohorts, patients will be dosed in a sequential fashion with a minimum of 48-hours of observation between each patient. Continuation of dosing (administration of second dose) of the current cohort and dose escalation to the next cohort will proceed after the collective safety and tolerability data for Patients 1 to 3 of the current cohort through at least 96 hours post-first dose are reviewed by the SRC. The continuation dosing (administration of second dose) of subsequent cohorts is dependent upon the SRC determining administration of second doses of study drug were safe and tolerable in previous cohorts. **Within each of the cohorts, the first patient will receive their first dose and if the dose is well tolerated (per Section 5.7.3, Dose-limiting Toxicity), Patients 2 and 3 will receive their first dose at that same dose level no sooner than 48 hours apart, with Patient 2 receiving a dose of study drug no sooner than 48 hours after the dosing of Patient 1. Similar to the first dose, the second**

Section	Prior Text	Changed To
		dose will be administered to patients 4 weeks after in a sequential manner with at least 48 hours separating dosing of each patient. **Dose escalation to the next cohort will proceed after the collective safety and tolerability data through at least 96 hours post-first dose from the 3 patients in the previous cohort has been reviewed by the SRC. If the administered dose is found to be safe and well tolerated, dosing for the next cohort will begin no sooner than 96 hours after Patient 3 has safely received dose 1 from the previous cohort. Patients in the cohort would be dosed and reviewed for safety and tolerability following the same procedures as used for other cohorts (as stated above).** **For patients on all dose levels other than the starting dose level of 10 μg/kg, prior to receiving the second dose the SRC will review the cumulative safety and tolerability data of at least 2 patients from the previous dose level(s) with at least 96 hours of follow-up after receiving a second dose of study drug.** **The dose level of ALN-TTR02 administered to any cohort in study ALN-TTR02-002 may not be higher than that deemed safe and tolerable in the Phase 1 single-dose study of ALN-TTR02 (Study ALN-TTR02-001).**
	Patients will be observed for 6 hours after ALN-TTR02 dose administration and will return to the site for out-	**Patients will be hospitalized at the study site for at least 24 hours after the end of the study drug infusion.**

Section	Prior Text	Changed To
	patient visits for safety, PK, and PD monitoring up to 208 days post-dose (see Section 6 for details).	**Patients may be discharged upon Investigator review of the 24 hour ECG, LFTs, and a subset of serum chemistries (sodium, potassium, creatinine, albumin, calcium, glucose, and phosphate), if results are deemed not clinically significant.** ~~Patients will be observed for 6 hours after ALN-TTR02 dose administration and~~ will return to the site for out-patient visits for safety, PK, and PD monitoring up to 208 days post-dose (see Section 6 for details).
	The SRC will evaluate safety in the study and determine if it remains acceptable to proceed with dosing (administration of second dose) or dose escalate per their safety review charter.	The SRC will evaluate safety in the study and determine if it remains acceptable to ~~proceed with dosing (administration of second dose) or~~ dose escalate **or administer the second dose to the next dose level** per their safety review charter.
Section 3.2: Safety Assessments	Safety monitoring will include assessment of AEs, 12-lead ECGs, arterial oxygen saturation (SaO_2) using pulse oximetry, vital signs (blood pressure, pulse rate, oral body temperature, and respiratory rate), clinical laboratory safety tests (hematology, serum chemistry, liver function tests, thyroid function parameters, serology, coagulation parameters, urinalysis, cytokines, CRP, and complement factors), and physical examinations.	Safety monitoring will include assessment of AEs, 12-lead ECGs, **cardiac monitoring (telemetry),** arterial oxygen saturation (SaO_2) using pulse oximetry, vital signs (blood pressure, pulse rate, oral body temperature, and respiratory rate), clinical laboratory safety tests (hematology, serum chemistry, LFTs, thyroid function parameters, serology, coagulation parameters, urinalysis, cytokines, CRP, and complement factors), and physical examinations.
Section 4.1: Eligibility of Patients	Based on the planned dose escalation scheme (see Section 5.7.1), up to 24 patients are expected to be enrolled.	Based on the planned dose escalation scheme (see Section 5.7.1), up to ~~24~~ **21** patients are expected to be enrolled.

Section	Prior Text	Changed To
Section 5.2: Preparation of Study Drug	Added instructions for calculating study drug dose to be consistent with ALN-TTR02-001.	**Subjects who weigh 105 kg or more will receive ALN-TTR02 dosing based on an assumption of a body weight of 104 kg.**
Section 5.6: Dose, Route, and Schedule of Study Drug Administration	Study drug doses will be administered as a 60-minute IV infusion.	Study drug doses will be administered as a 60-minute IV infusion **4 weeks apart**.
	Patients will remain at the study site for 6 hours following completion of dosing on Day 0 and Day 28 (or date of second dose administration in optional cohorts with dosing intervals greater than 4 weeks), to be observed for safety assessments and PK sampling.	Patients will remain **hospitalized** at the study site for **at** least ~~6~~ **24** hours following completion of dosing on Day 0 and Day 28 ~~(or date of second dose administration in optional cohorts with dosing intervals greater than 4 weeks)~~, to be observed for safety assessments and PK sampling.
	The planned study drug doses are 10, 50, 150, 300, and 500 µg/kg, with safety monitoring between patients and interim review by the SRC after each cohort's dose administration.	The planned study drug doses are 10, 50, 150, **and** 300~~, and 500~~ µg/kg, ~~with safety monitoring between patients and interim review by the SRC after each cohort's dose administration~~.
	Added instructions for calculating study drug dose to be consistent with ALN-TTR02-001.	**Subjects who weigh 105 kg or more will receive ALN-TTR02 dosing based on an assumption of a body weight of 104 kg.**
	Within each cohort, the first patient will receive their first dose and if the dose is well tolerated, Patient 2 will receive their first dose of study drug no sooner than 48 hours after the dosing of Patient 1. Similarly, if no safety or tolerability issues are observed with the first 2 patients, Patient 3 will receive their first dose no sooner than 48 hours after Patient 2. After all patients in a cohort have at least 96 hours of follow-up, the cumulative safety and	~~Within each cohort, the first patient will receive their first dose and if the dose is well tolerated, Patient 2 will receive their first dose of study drug no sooner than 48 hours after the dosing of Patient 1. Similarly, if no safety or tolerability issues are observed with the first 2 patients, Patient 3 will receive their first dose no sooner than 48 hours after Patient 2. After all patients in a cohort have at least 96 hours of follow-up, the cumulative safety and~~

Section	Prior Text	Changed To
	tolerability data on all patients will be reviewed by the SRC prior to the administration of each cohort's second dose. If deemed safe and tolerated by the SRC, Patients 1-3 will receive their second dose of study drug approximately 4 weeks after receiving their first dose. Patients will continue to be dosed in a sequential fashion with a minimum of a 48-hour observation period prior to the dosing of the subsequent patient. Prior to escalating to the next cohort, each of the 3 patients in the current cohort must have received study drug with at least 96 hours of follow-up, for that cohort to be eligible for SRC review. If the administered dose is found to be safe and well tolerated, dosing for the next protocol-specified dose level will begin no sooner than 7 days after Patient 3 in the previous cohort had safely received the first dose of study drug. Prior to administration of the second dose of study drug, the SRC will review the cumulative safety and tolerability data of at least 2 patients from the previous cohort with at least 96 hours of follow-up after receiving the second dose of study drug. If deemed safe and tolerable by the SRC, patients in the current cohort will receive their second dose of study drug.	~~tolerability data on all patients will be reviewed by the SRC prior to the administration of each cohort's second dose. If deemed safe and tolerated by the SRC, Patients 1-3 will receive their second dose of study drug approximately 4 weeks after receiving their first dose. Patients will continue to be dosed in a sequential fashion with a minimum of a 48-hour observation period prior to the dosing of the subsequent patient.~~ ~~Prior to escalating to the next cohort, each of the 3 patients in the current cohort must have received study drug with at least 96 hours of follow-up, for that cohort to be eligible for SRC review. If the administered dose is found to be safe and well tolerated, dosing for the next protocol-specified dose level will begin no sooner than 7 days after Patient 3 in the previous cohort had safely received the first dose of study drug. Prior to administration of the second dose of study drug, the SRC will review the cumulative safety and tolerability data of at least 2 patients from the previous cohort with at least 96 hours of follow-up after receiving the second dose of study drug. If deemed safe and tolerable by the SRC, patients in the current cohort will receive their second dose of study drug.~~ **Within each of the cohorts, the first patient will receive their first dose and if the dose is well tolerated, per Section 5.7.3 (Dose-limiting Toxicity), Patients 2 and 3 will receive their first dose at that same dose level no sooner than 48 hours apart, with Patient 2 receiving a dose of study drug no sooner than 48 hours after the**

Section	Prior Text	Changed To
		dosing of Patient 1. Similar to the first dose, the second dose will be administered to patients in a sequential manner with at least 48 hours separating dosing of each patient. **Dose escalation to the next cohort will proceed after the collective safety and tolerability data through at least 96 hours post-first dose from the 3 patients in the previous cohort has been reviewed by the SRC. If the administered dose is found to be safe and well tolerated, dosing for the next cohort will begin no sooner than 96 hours after Patient 3 has safely received dose 1 from the previous cohort. Patients in the cohort would be dosed and reviewed for safety and tolerability following the same procedures as used for other cohorts (as stated above).** **For patients on all dose levels other than the starting dose level of 10 μg/kg, prior to receiving the second dose the SRC will review the cumulative safety and tolerability data of at least 2 patients from the previous dose level(s) with at least 96 hours of follow-up after receiving a second dose of study drug.**
Figure 5-1	Removed previous figure	Replaced figure with one that accurately depicts the dosing as described in this amendment. This figure has been moved from Section 5.6 to Section 5.7.1.
Table 5-1	Last row containing Cohort 5 (500 μg/kg dose)	This last row has been removed.

Section	Prior Text	Changed To
	Total enrollment: Up to 24 patients	Total enrollment: Up to ~~24~~ **21** patients
	Footnote a: Up to 3 optional cohorts may be included to confirm the safety, tolerability, and/or PD effect at a specific dose. Each optional cohort will be comprised of 3 patients	Up to 3 optional cohorts may be included to confirm the safety, tolerability, and/or PD effect at a specific dose. **Dosing in these optional cohorts may occur at a previously tested dose that was found to be safe and where stopping rules were not met or at an intermediate dose level.** Each optional cohort will be comprised of 3 patients
Section 5.7.1 Dose Escalation Procedures	Dose escalation to 50, 150, 300, and 500 µg/kg is planned.	Dose escalation to 50, 150, **and** 300~~, and 500~~ µg/kg is planned.
	Study drug doses will be escalated sequentially after the SRC reviews safety data, as detailed in the SRC Charter, collected from all 3 patients at the current dose level. The SRC will evaluate safety in the study and determine if it remains acceptable to dose escalate per their safety review charter after each cohort based on pre-established stopping rules. Collective post-first dose safety and tolerability data on the 3 patients in a cohort through at least 96 hours post-dose will be reviewed by the SRC, and if the administered dose is found to be safe and well tolerated, dosing of the next protocol-specified dose level will occur. For any DLT, accrual to that dose level will stop, all dosing will be discontinued, and dose escalation will end, pending evaluation of all available safety data by the SRC.	~~Study drug doses will be escalated sequentially after the SRC reviews safety data, as detailed in the SRC Charter, collected from all 3 patients at the current dose level. The SRC will evaluate safety in the study and determine if it remains acceptable to dose escalate per their safety review charter after each cohort based on pre-established stopping rules. Collective post-first dose safety and tolerability data on the 3 patients in a cohort through at least 96 hours post-dose will be reviewed by the SRC, and if the administered dose is found to be safe and well tolerated, dosing of the next protocol-specified dose level will occur. For any DLT, accrual to that dose level will stop, all dosing will be discontinued, and dose escalation will end, pending evaluation of all available safety data by the SRC.~~ **Within each of the cohorts, the first patient will receive their first dose and if the dose is well tolerated, per**

Section	Prior Text	Changed To
		Section 5.7.3 (Dose-limiting Toxicity), Patients 2 and 3 will receive their first dose at that same dose level no sooner than 48 hours apart, with Patient 2 receiving a dose of study drug no sooner than 48 hours after the dosing of Patient 1. Similar to the first dose, the second dose will be administered to patients 4 weeks after the first dose in a sequential manner with at least 48 hours separating dosing of each patient. **Dose escalation to the next cohort will proceed after the collective safety and tolerability data through at least 96 hours post-first dose from the 3 patients in the previous cohort has been reviewed by the SRC. If the administered dose is found to be safe and well tolerated, dosing for the next cohort will begin no sooner than 96 hours after Patient 3 has safely received dose 1 from the previous cohort. Patients in the cohort would be dosed and reviewed for safety and tolerability following the same procedures as used for other cohorts (as stated above).** **For patients on all dose levels other than the starting dose level of 10 μg/kg, prior to receiving the second dose the SRC will review the cumulative safety and tolerability data of at least 2 patients from the previous dose level(s) with at least 96 hours of follow-up after receiving a second dose of study drug.**

Section	Prior Text	Changed To
Section 5.7.2 Optional Cohorts	Based on the interim evaluation of safety data, it may also be decided by the SRC that an optional cohort may be utilized to include 3 additional patients. These optional cohorts will be included to further confirm the safety and/or PD effect. Up to 3 optional cohorts are permitted in this study. The dose of study drug administered to patients in these cohorts can be a previously tested dose that was found to be safe and where stopping rules were not met or at an intermediate dose level. Patients in any optional cohort would be dosed and reviewed for safety and tolerability following the same procedures as used for other cohorts. The Independent Ethics Committee (IEC) will be informed of any optional cohorts utilized.	~~Based on the interim evaluation of safety data, it may also be decided by the SRC that an optional cohort may be utilized to include 3 additional patients. These optional cohorts will be included to further confirm the safety and/or PD effect. Up to 3 optional cohorts are permitted in this study. The dose of study drug administered to patients in these cohorts can be a previously tested dose that was found to be safe and where stopping rules were not met or at an intermediate dose level. Patients in any optional cohort would be dosed and reviewed for safety and tolerability following the same procedures as used for other cohorts.~~ ~~The Independent Ethics Committee (IEC) will be informed of any optional cohorts utilized.~~ **Based on the interim evaluation of safety data, it may also be decided by the SRC that an optional cohort may be utilized which will include 3 additional patients. Dosing in these optional cohorts may occur at a previously tested dose that was found to be safe and where stopping rules were not met or at an intermediate dose level. Patients in the optional cohort would be dosed and reviewed for safety and tolerability following the same procedures as used for other cohorts. The optional cohort would be included to further confirm the safety and/or PD effect. If this occurs, the IECs will be informed of the expansion. Up to 3 optional cohorts of 3 patients each are permitted in this study.**

Section	Prior Text	Changed To
Section 5.7.3 Dose-limiting Toxicity	• Any other toxicity which in the opinion of the SRC would have precluded administration of a second dose.	• Any other toxicity which in the opinion of the SRC would have precluded ~~administration of a second dose~~**further dosing**.
Section 5.11 Suggested Guidelines for Management of Infusion Reactions	After dosing, patients should be discharged from the clinic no sooner than 6 hours post-dose with directions on self-administration of medications as needed to ameliorate potential delayed reactions to study drug infusion, such as fever, chills, and myalgia.	After dosing, patients ~~should~~ **will** be discharged from the ~~clinic~~ **hospital** no sooner than ~~6~~ **24** hours post-dose**. Prior to discharge, the 24-hour ECG and local serum chemistries and LFTs must be reviewed by the Investigator as described in Section 3.1. Patients will be discharged** with directions on self-administration of medications as needed to ameliorate potential delayed reactions to study drug infusion, such as fever, chills, and myalgia.
Section 6 Study Visits	Patients will remain at the study site for 6 hours following completion of dosing to be observed for safety assessments and PK sampling. All study center visits will be conducted on an out-patient basis. After receiving the first dose of study drug, patients will have follow-up visits on Days 1, 2, 7, 10, 14, and 21. In addition, follow-up visits after the second dose of study drug (Day 28) will occur on Days 29, 30, 35, 38, 42, 49, and 56 (end-of-study).	Patients will remain **hospitalized** at the study site for **at least ~~6~~ 24** hours following completion of dosing to be observed for safety assessments and PK sampling. ~~All study center visits will be conducted on an out-patient basis. After receiving the first dose of study drug,~~ patients will ~~have follow-up visits~~**return to the study site** on Days ~~1,~~ 2, 7, 10, 14, and 21 **for follow-up assessments**. In addition, follow-up visits after the second dose of study drug (Day 28) will occur on Days ~~29,~~ 30, 35, 38, 42, 49, and 56 (end-of-study).

Section	Prior Text	Changed To
	All patients who discontinue the study before Day 56 will be encouraged to return to the study site for the Days 56 and 208 study visits. All patients who discontinue the study after Day 56 and prior to Day 208 will be encouraged to complete the Day 208 visit.	All patients who discontinue the study before Day 56 will ~~be encouraged to~~ return to the study site for the**ir Early Termination visit for** Day~~s~~ 56 **assessments.**~~and 208 study visits.~~ All patients who discontinue the study after Day 56 and prior to Day 208 will ~~be encouraged to complete~~**return to the study site for their Early Termination visit for** ~~the~~ Day 208 ~~visit~~**assessments**.
Section 6.3.1.1 Pre-dose	Obtain serial measurements of:	Obtain ~~serial~~ measurements of:
	Added cardiac monitoring.	**Initiate cardiac monitoring (telemetry) 30 minute pre-dose.**
Section 6.3.1.3 Post-dose	Patients will remain at the study site for 6 hours following completion of dosing, to be observed for safety assessments and PK sampling. For non-PK assessments performed 30 minutes post-infusion, a window of ±5 minutes is allowed, a window of ±15 minutes is allowed for assessments performed through 6 hours post-infusion, and a window of ±120 minutes is allowed for assessments performed 24 hours post-infusion.	Patients will remain **hospitalized** at the study site for **at least** ~~6~~ **24** hours following completion of dosing, to be observed for safety assessments and PK sampling. For non-PK assessments performed 30 minutes post-infusion, a window of ±5 minutes is allowed, a window of ±15 minutes is allowed for assessments performed **between 1 and 3 hours, and a window of ±30 minutes for** ~~through~~ 6 **through 18** hours post-infusion~~, and a window of ±120 minutes is allowed for assessments performed 24 hours post-infusion~~.
	• Obtain serial measurement of vital signs at the end of infusion (EOI) and at 30 minutes and 1, 2, 3, and 6 hours post-infusion.	• Obtain serial measurement of vital signs at the end of infusion (EOI) and at 30 minutes and 1, 2, 3, ~~and 6~~**, 12, and 18** hours post-infusion.
	Added cardiac monitoring.	**Continue cardiac monitoring (telemetry) through 24 (+1) hour post-dose.**

Section	Prior Text	Changed To
	Perform serial pulse oximetry at EOI and at 30 minutes and 1, 2, 3, and 6 hours post-infusion.	Perform serial pulse oximetry at EOI and at 30 minutes and 1, 2, 3, ~~and~~ 6**, 12, and 18** hours post-infusion.
	Patients will remain in the hospital for a total of 6 hours before they are discharged.	Patients will remain in the hospital for ~~a total of 6~~**at least 24** hours. ~~before they are discharged.~~
Section 6.3.2 Day 1 or Day 29	Added serial pulse oximetry, ECG, and cardiac monitoring time points. Clarified when vital signs were to be measured. • Measure vital signs.	• Measure vital signs **24 (+30 minutes) hours post-infusion**. • **Perform serial pulse oximetry at 24 (+30 minutes) hours post-infusion.** • **Perform serial 12-lead ECGs in triplicate at 24 (+30 minutes) hours post-infusion.** • **Conclude cardiac monitoring (telemetry) at 24 (+1) hours post-infusion.**
	Added discharge instructions.	**Patients will be discharged from the study site after the ECG and local serum chemistries and LFTs have been reviewed by the Investigator and deemed not clinically significant.**
Section 6.4 Early Termination	Patients coming off study before Day 56 will be encouraged to complete the Days 56 and 208 study visits. Patients coming off study after Day 56 and before Day 208 will be encouraged to complete the Day 208 study visit.	Patients coming off study before Day 56 will ~~be encouraged~~**return to the study site** to complete **their Early Termination visit which will include** ~~the~~ Day~~s~~ 56 ~~and 208 study visits~~**assessments**. Patients coming off study after Day 56 and before Day 208 will ~~be encouraged~~**return to the study site to complete their Early Termination visit which will include** ~~to complete the~~ Day 208 ~~study visit~~**assessments**.

Section	Prior Text	Changed To
Section 7.2.2 Vital Signs	Vital signs are to be measured at Screening and the Day 1, 2, 14, 29, 30, 42, and 56 (or time of early termination, if applicable) study visits and include systolic/diastolic blood pressure, pulse rate, respiration rate, and oral body temperature.	Vital signs are to be measured at Screening and the Day ~~1~~, 2, 14, ~~29,~~ 30, 42, and 56 (or time of early termination, if applicable) study visits and include systolic/diastolic blood pressure, pulse rate, respiration rate, and oral body temperature.
	On Days 0 and 28, serial vital signs are to be measured within 30 minutes pre-dose, at EOI, and at 30 (±5) minutes and 1, 2, 3, and 6 hours (±15 minutes) post-infusion.	On Days 0 and 28, serial vital signs are to be measured within 30 minutes pre-dose, at EOI; and at 30 (±5) minutes; ~~and~~ 1, 2, **and** 3 **(±15 minutes) hours;** ~~and~~ 6**, 12, and 18** hours (~~±15~~ **30** minutes) post-infusion. **On Days 1 and 29, serial vital signs are to be measured at 24 hours (+30 minutes) post-infusion.**
Section 7.2.4 Electrocardiogram	Serial ECGs will be collected in triplicate 30 minutes prior to dosing (Days 0 and 28), EOI, and 30 (±5) minutes, and 2 and 4 (±15 minutes) hours post-infusion.	Serial ECGs will be collected in triplicate 30 minutes prior to dosing (Days 0 and 28), EOI, and 30 (±5) minutes, ~~and~~ 2 and 4 (±15 minutes) hours**, and on Days 1 and 29 at 24 (+30 minutes) hours** post-infusion.
	Added discharge instructions.	**Prior to discharge from the hospital on Days 1 and 29, the ECG must be reviewed by the Investigator and the results deemed not clinically significant. Any clinically significant findings must be discussed with the medical monitor to determine whether patient can be discharged and to formulate plans for patient follow-up.**

Section	Prior Text	Changed To
Section 7.2.5 Pulse Oximetry	Arterial oxygen saturation will be assessed by serial pulse oximetry on Days 0 and 28 within 30 minutes pre-dose, at EOI, and at 30 (±5) minutes and 1, 2, 3, and 6 hours (±15 minutes) post-infusion on Days 0 and 28.	Arterial oxygen saturation will be assessed by serial pulse oximetry on Days 0 and 28 within 30 **(±5)** minutes pre-dose; at EOI;~~, and~~ at 30 (±5) minutes**;** ~~**and**~~ **at** 1, 2, 3 **hours (±15 minutes);** and **at** 6**, 12, and 18** hours (±15 minutes) post-infusion on Days 0 and 28**; and at 24 hours (+30 minutes) post-infusion on Days 1 and 29**.
Section 7.2.6 Cardiac Monitoring	Added cardiac monitoring instructions.	**Continuous cardiac monitoring will be performed via telemetry starting no later than 30 minutes prior to dosing and continuing through 24 hours (+1 hour) post-dose.**
Section 7.2.7.1 Hematology, Serum Chemistries, and Urinalysis	Added discharge instructions.	**Prior to discharge from the hospital on Days 1 and 29, local serum laboratories (specifically, sodium, potassium, creatinine, albumin, calcium, glucose, and phosphate) must be reviewed by the Investigator and the results deemed not clinically significant. Any clinically significant findings must be discussed with the medical monitor to determine whether patient can be discharged and to formulate plans for patient follow-up.**
Section 7.2.7.2 Liver Function Tests	Added discharge instructions.	**Prior to discharge from the hospital on Days 1 and 29, local LFTs must be reviewed by the Investigator and the results deemed not clinically significant. Any clinically significant findings must be discussed with the medical monitor to determine whether patient can be discharged and to formulate plans for patient follow-up.**

Section	Prior Text	Changed To
Section 9.1: Sample Size	Based on the planned dose escalation scheme (see Section 5.7.1), up to 24 patients are expected to be enrolled.	Based on the planned dose escalation scheme (see Section 5.7.1), up to ~~24~~ **21** patients are expected to be enrolled.