CLINICAL STUDY PROTOCOL
ALN-TTRSC-003

A Phase 2, Open-label Extension Study to Evaluate the Long-Term Safety, Clinical Activity, and Pharmacokinetics of ALN-TTRSC in Patients with Transthyretin (TTR) Cardiac Amyloidosis Who Have Previously Received ALN-TTRSC

Original Protocol:	20 May 2014
Amendment 1:	10 July 2014
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EudraCT Number	2014-001229-34
Sponsor:	Alnylam Pharmaceuticals, Inc. 300 Third Street Cambridge, MA 02142 USA
Sponsor Contact:	[redacted] Senior Vice President Clinical Development

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 study patients are protected, consistent with the principles that have their origin in the Declaration of Helsinki.

AUTHORIZED SIGNATORIES

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

Principal Investigator

Signature _____ Date _____

Name (print) _____

Sponsor

Signature _____ Date _____

[redacted]
Senior Vice President
Clinical Development

Name (print) _____

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PROTOCOL SYNOPSIS

Protocol Title	A Phase 2, Open-label Extension Study to Evaluate the Long-Term Safety, Clinical Activity, and Pharmacokinetics of ALN-TTRSC in Patients with Transthyretin (TTR) Cardiac Amyloidosis Who Have Previously Received ALN-TTRSC
Indication	Treatment of patients with mutant or wild-type (WT) TTR cardiac amyloidosis.
Protocol Number	ALN-TTRSC-003
Phase of Development	2
Design	This is an open-label, extension study designed to evaluate the long-term safety, clinical activity, and pharmacokinetic (PK) activity of subcutaneously administered ALN-TTRSC (International Nonproprietary Name: revusiran) in patients with TTR cardiac amyloidosis who previously completed the ALN-TTRSC-002 study. Patients must be screened prior to the administration of study medication. Screening/Baseline assessments may be performed during several clinic visits. Patients must have completed the ALN-TTRSC-002 study to be eligible for this study. Eligible, consenting patients will receive 5 daily doses of ALN-TTRSC (Day 0, 1, 2, 3, and 4) and a dose at Day 7. They will then receive once weekly doses for the duration of the study through Day 1337 (or Early Termination [ET]). Doses will be administered at the clinic during Days 0 through 7. Dosing after Day 7 may be administered at home by a healthcare professional trained in the protocol or at the clinic if dosing coincides with the clinic visit for other assessments. At the discretion of the Investigator, after the Day 84 dosing visit and if the patient has not previously experienced any severe or serious adverse events (SAEs) considered related to the study drug within the previous 12 weeks, patients/caregivers may be trained by the Investigator or qualified site staff in the administration of the study drug, according to the protocol dosing requirements. Clinic visits will be performed for Screening/Baseline, dose initiation during Days 0 through 7, and then approximately every 12 weeks through the End of Study visit or ET, and approximately 28 days following the End of Study visit or ET for a Follow-up visit.

	Safety will be assessed by collection of adverse events (AEs); clinical laboratory tests, including hematology, serum chemistry, kidney and thyroid function, coagulation, and urinalysis; 12-lead electrocardiograms (ECGs); vital signs, eye examinations, and physical examinations. Safety evaluations will be performed as part of Screening and approximately every 12 weeks during the treatment period with the exception of eye examinations, which will be performed once a year. Plasma PK samples will be collected at specified time points up through Day 336. During Years 2, 3, and 4 a single PK sample will be collected each year. Pharmacodynamic (PD) evaluation will include serial measurement of serum levels of TTR at specified time points. Serum levels of vitamin A will also be evaluated as a secondary PD biomarker. Clinical efficacy will be explored through mortality, hospitalization, and Six-minute Walk Test (6-MWT). Additional clinical assessments will be performed including echocardiogram, cardiac magnetic resonance imaging (CMR), ^{99m}-Technetium (^{99m}Tc) imaging, circulating cardiac biomarkers (including N-terminal prohormone of B-type natriuretic peptide [NT-proBNP], troponins I and T), amyloid quantitation in fat pad aspirates, estimated glomerular filtration rate (eGFR), modified body mass index (mBMI), the Kansas City Cardiomyopathy Questionnaire (KCCQ), occurrence of new onset or recurrence of atrial fibrillation, pacemaker placement, change in diuretic regimen, blood pressure, New York Heart Association (NYHA) classification, and patient-reported quality of life (QoL) assessed by EQ-5D. Evaluations of clinical efficacy will be performed as part of Screening and approximately every 24 weeks during the treatment period. Disease burden and healthcare utilization will be assessed approximately every 48 weeks using a patient-reported pharmacoeconomics questionnaire. Blood samples will also be collected to evaluate exploratory biomarkers (mutant: WT TTR levels and additional cardiac and hepatic-derived proteins that may include galectin-3 and ST2) and serology and anti-drug antibodies (ADAs).
Study Sites	This study is to be conducted at approximately 6 study sites.

Investigational Drug	ALN-TTRSC is comprised of a small interfering RNA targeting mutant and WT TTR messenger ribonucleic acid with a covalently-attached triantennary N-acetylgalactosamine (GalNAc) ligand formulated in water for injection.
Dosage, Route of Administration and Duration of Treatment of Investigational Drug	Beginning on Day 0, patients will receive 5 daily subcutaneous doses of 500 mg of ALN-TTRSC. The same dose will be administered at Day 7 and then weekly from Day 14 through approximately Day 1337 (or ET).
Time on Study	The duration of patient participation in this study is up to approximately 4 years (Screening/Baseline through the Follow-up visit).
Primary Objective	To evaluate the safety and tolerability of long-term dosing with ALN-TTRSC.
Secondary Objectives	Secondary objectives of this study are: • To assess the PD effect of long-term dosing of ALN-TTRSC on serum levels of TTR. • Clinical effects of long-term dosing of ALN-TTRSC, including effect on mortality, hospitalization, and 6-MWT.
Tertiary Objectives	The tertiary objectives of this study are: • To further characterize the PK of ALN-TTRSC. • To assess the clinical effects of long-term dosing of ALN-TTRSC including: echocardiogram, cardiac biomarkers (NT-proBNP and troponin T and I), quantitation of amyloid in fat pad aspirates, CMR, KCCQ, ^{99m}Tc imaging, eGFR, NYHA class, blood pressure, mBMI, new onset or recurrence of atrial fibrillation, pacemaker placement, change in diuretic regimen, and EQ-5D QoL. • To understand disease burden and healthcare utilization.
Sample Size	Approximately 25 patients.
Inclusion and Exclusion Criteria	Each patient must meet all of the following inclusion criteria during the Screening/Baseline period to be eligible for enrollment in the study: 1. Previously received and tolerated ALN-TTRSC in Study ALN-TTRSC-002; and completed Study ALN-TTRSC-002 through the Day 90 visit;

	2. Adequate liver function, demonstrated by an aspartate transaminase and alanine transaminase ≤ 2.5 × the upper limit of normal, total bilirubin< 2 g/dL (34.2 μmol/L), and albumin > 3 g/dL (> 4.35 μmol/L); 3. Women of child-bearing potential (WOCBP) must have a negative pregnancy test, cannot be breast feeding, and must be willing to use a highly effective method of contraception prior to Screening/Baseline, throughout study participation, and for 1 month after last dose administration; 4. Males who agree to use appropriate means of contraception throughout study participation until 1 month after last dose administration; 5. Patient, or patient's legal representative, is able and willing to provide written informed consent and the patient is willing to comply with the study requirements. Each patient must not meet any of the following exclusion criteria to be eligible for enrollment in the study: 1. Estimated Glomerular Filtration Rate < 20 mL/min/1.73m^2 (using the Modification of Diet in Renal Disease [MDRD] formula); 2. Uncontrolled hypertension; 3. Uncontrolled ischemic heart disease; 4. Uncontrolled clinically significant cardiac arrhythmia; 5. Untreated hypo- or hyperthyroidism; 6. Prior major organ transplant; 7. Known or suspected systemic bacterial, viral, parasitic, or fungal infection; 8. Seropositive for hepatitis B virus, hepatitis C virus (HCV) or known to be human immunodeficiency virus positive; 9. Received an investigational agent other than tafamidis, diflunisal, doxycycline, or tauroursodeoxycholic acid, or an investigational device within 30 days prior to first dose of study drug; 10. Discontinued ALN-TTRSC-002 study due to a treatment-related AE; 11. Metastatic cancer within the past 5 years; 12. Any conditions which, in the opinion of the Investigator, would make the patient unsuitable for enrollment or could interfere with the patient's participation in, or completion of, the study.

	13. History of allergic reaction to an oligonucleotide or GalNAc.
Safety Assessments	The safety of ALN-TTRSC will be evaluated by: • Assessment of AEs • Clinical laboratory safety tests (hematology and serum chemistry, including liver function tests, thyroid function, coagulation, and urinalysis) • Vital sign measurements (blood pressure, pulse rate, oral body temperature, and respiratory rate) • 12-Lead ECG • Physical examinations • Eye examinations
Pharmacodynamic Assessments	The PD effect of ALN-TTRSC will include assessment of serum TTR and vitamin A levels.
Pharmacokinetic Assessments	Blood samples for the assessment of ALN-TTRSC concentration and possible metabolite analysis will be collected predose and at various time points postdose.
Clinical Efficacy Assessments	Clinical effects of ALN-TTRSC will be explored by: • Mortality (cardiovascular-related and all-cause) • Hospitalization (cardiovascular-related and heart-failure-related) • 6-MWT Additional exploratory clinical assessments include: • Echocardiography • Cardiac biomarkers (NT-proBNP, troponin T, and troponin I) • KCCQ • mBMI • NYHA classification • ^{99m}Tc imaging • CMR imaging with gadolinium • Fat pad aspirates for amyloid quantitation • EQ-5D QoL • Blood pressure • eGFR • New onset or recurrent atrial fibrillation • Pacemaker placement • Changes in diuretic regimen

Other Assessments	Other effects of ALN-TTRSC will be evaluated by: • Measurement of exploratory biomarkers and serology (ADAs) • Determination of pharmacoeconomic outcomes through disease burden and healthcare utilization questionnaire.
Statistical Methods	Statistical analyses will be primarily descriptive in nature. Adverse event summaries will include tabulations of all treatment-emergent AEs, treatment-related AEs, serious AEs, discontinuations due to AEs, and AEs of various grading severity. 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. 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.

Table 1: Schedule of Assessments for Clinic Visits – Year 1 and Year 2

Phase:	Screening[a]/ Baseline	Pre-dose[b]	Dose Initiation Period					Treatment Period (Doses Administered Weekly between Visits[c,d])								
Year:	Year 1												Year 2			
Study Week:			Wk 1					Wk 12	Wk 24	Wk 36	Wk 48	Wk 60	Wk 72	Wk 84	Wk 96	
Study Day:	-28 to -1	0	0	1	2	3	4	7	84	168	252	336	420	504[d]	588	672
Window (days):								±1	±14	±14	±14	±14	±14	±14	±14	±14
Procedure:																
Informed Consent[e]	X															
Study Drug Administration[f,g]			X	X	X	X	X	X	X	X	X	X	X	X	X	X
Demographics	X															
Medical History[h]	X	X														
Inclusion/Exclusion Criteria[g]	X	X														
Serology[i]	X															
Pregnancy Test[j,k]	X									X		X		X		X
Karnofsky Performance Status[j]	X									X		X		X		X
eGFR[j,l]	X	X							X	X	X	X	X	X	X	X
Safety Assessments:																
Physical Examination[j]	X	X						X	X	X	X	X	X	X	X	X
Body Weight[j]	X	X								X		X		X		X
Height	X															
Vital Signs[j,m]	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X
12-Lead ECG[j,n]	X									X		X		X		X
Serum Chemistry (including LFTs), Hematology, Urinalysis[j]	X	X		X			X	X	X	X	X	X	X	X	X	X
Coagulation[j,o]	X									X		X		X		X

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Phase:	**Screening[a]/ Baseline**	**Pre-dose[b]**	**Dose Initiation Period**					**Treatment Period (Doses Administered Weekly between Visits[c,d])**								
Year:	**Year 1**												**Year 2**			
Study Week:			**Wk 1**					**Wk 12**	**Wk 24**	**Wk 36**	**Wk 48**	**Wk 60**	**Wk 72**	**Wk 84**	**Wk 96**	
Study Day:	**-28 to -1**	**0**	**0**	**1**	**2**	**3**	**4**	**7**	**84**	**168**	**252**	**336**	**420**	**504[d]**	**588**	**672**
Window (days):								**±1**	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**
TSH[j]	X											X				X
Eye Examination	X											X				X
Review Concomitant Medications	X	X	Continuous Monitoring													
Review/Record AEs			Continuous Monitoring													
PD Assessments:																
TTR[j]	X	X				X		X	X	X	X	X	X	X	X	X
Vitamin A[j,p]	X							X		X		X		X		X
PK Assessments:																
Plasma PK Sampling[q]		X	X				X	X	X	X	X	X			X	
Clinical Efficacy Assessments:																
Review Cardiac Clinical Medical History Treatment Changes[r]									X	X	X	X	X	X	X	X
NT-proBNP, Troponin T, and Troponin I[j]	X	X							X	X	X	X	X	X	X	X
Echocardiography by Doppler	X									X		X		X		X
6-MWT[j,s]	X									X		X		X		X
NYHA Classification	X									X		X		X		X
CMR[t]	X									X		X		X		X

Table 1: Schedule of Assessments for Clinic Visits – Year 1 and Year 2

Phase:	Screening[a]/ Baseline	Pre-dose[b]	Dose Initiation Period					Treatment Period (Doses Administered Weekly between Visits[c,d])								
Year:	Year 1												Year 2			
Study Week:			Wk 1					Wk 12	Wk 24	Wk 36	Wk 48	Wk 60	Wk 72	Wk 84	Wk 96
Study Day:	-28 to -1	0	0	1	2	3	4	7	84	168	252	336	420	504[d]	588	672
Window (days):								±1	±14	±14	±14	±14	±14	±14	±14	±14
^{99m}Tc Scintigraphy by 3D SPECT[u]	X											X				X
Fat Pad Aspirate Samples for Amyloid Quantification[v]	X											X				X
mBMI	X									X		X		X		X
KCCQ[j]	X									X		X		X		X
EQ-5D QoL[j]	X									X		X		X		X
Other Assessments:																
Exploratory Biomarkers/ Serology (ADA)[j,w]	X	X							X	X	X	X	X	X	X	X
Pharmacoeconomics Questionnaire[j]	X											X				X

Abbreviations: 6-MWT = Six-minute Walk Test, ^{99m}Tc = ^{99m}-Technetium, ADA = anti-drug antibody, AE = adverse event, aPTT = activated partial thromboplastin time, CMR = Cardiac magnetic resonance imaging, ECG = electrocardiogram, eGFR = estimated glomerular filtration rate, ET = early termination, HBsAb – hepatitis B surface antibody, HBsAg = hepatitis B surface antigen, HCV Ab = hepatitis C virus antibody, ICF = informed consent form, INR = international normalized ratio, KCCQ = Kansas City Cardiomyopathy Questionnaire, LFT = liver function test, LGE = late gadolinium enhancement, mBMI = modified body mass index, MDRD = Modification of Diet in Renal Disease, NT-proBNP = N-terminal prohormone of B-type natriuretic peptide, NYHA = New York Heart Association, PD = pharmacodynamic, PK = pharmacokinetic, PT = prothrombin time, QoL = quality of life; QTcB = Bazett-corrected QT interval, SPECT = single-photon emission computed tomography, TEAE = treatment-emergent adverse event, TSH = thyroid stimulating hormone, TTR = transthyretin, WOCBP = women of child-bearing potential, WT = wild-type

a Screening/Baseline assessments may be performed during several clinic visits. If the final Day 90 visit in the ALN-TTRSC-002 study is within 28 days of the first dose administered in this study, then visit procedures performed as part of the Day 90 visit do not need to be repeated at the Screening/Baseline visit. If the Day 90 visit in the ALN-TTRSC-002 study occurred within 90 days of the first dose administered in this study, the echocardiogram and CMR do not need to be repeated at the Screening/Baseline visit.

b The results from samples collected predose are not needed prior to dosing.

c The 14-day window applies to study assessments. Dosing is to maintain the weekly schedule (± 2 days).

d Refer to Table 2 for the continued Schedule of Assessments in Years 3 and 4 and for the End-of-study or Early Termination (ET) visit. For patients who discontinue early and provide consent, overall health status will be documented by either telephone contact or non-patient contact follow-up (eg, medical record check) at time points corresponding to study Day 504 (Week 72), if applicable, and study Day 1344 (Week 192).

e Prior to Screening/Baseline activities, the patient will sign and date an ICF. No study procedures should be performed prior to informed consent being obtained.

f During the treatment period, doses will be administered weekly (± 2 days). Doses may be administered and some assessments may be performed at the clinical site or at home according to Section 5.5.

g Subcutaneous injection of ALN-TTRSC into the abdomen on Day 0 (after completion of all pre-dose evaluations and procedures) and then on subsequent days into other quadrants of the abdomen. The upper arm or thigh may also be used (see the ALN-TTRSC Pharmacy Manual). Dosing on Days 1, 2, 3, and 4 must be performed approximately 24 ±4 hours after the preceding dose of study drug.

h On Day 0 pre-dose, only interval medical history will be collected. Changes in medical history during the treatment period will be captured as TEAEs or concomitant medication(s) as appropriate.

i Serology tests include HBsAb, HBsAg, and anti-HCV Ab.

j Assessment should be performed before dosing.

k The pregnancy test will be performed for WOCBP only. A serum pregnancy test should be performed at Screening/Baseline. Urine pregnancy tests at other visits.

l The serum creatinine values should be in mg/dL to 2 decimal places (e.g., 0.95 mg/dL) OR values in µmol/L to the nearest whole number (e.g., 84 µmol/L) when calculating eGFR using the MDRD study equation; see http://nkdep.nih.gov/lab-evaluation/gfr-calculators.shtml for an eGFR calculator.

m Vital sign measurements include blood pressure, pulse rate, oral body temperature, and respiratory rate. Vital signs will be measured manually in the supine position after the patient has rested comfortably for 10 minutes. Each patient's blood pressure should be taken using the same arm. Vital signs should be collected during any unscheduled clinic visit.

n For the 12-Lead ECG, each lead shall be recorded for at least 3 beats at a speed of 25 mm/s. Triplicate recordings will be obtained. The electrophysiologic parameters assessed will be rhythm, ventricular rate, PR interval, QRS duration, QT interval, ST and T-waves and QTcB.

o Coagulation studies include PT, aPTT, and INR and will be performed on all patients.

p Blood samples for vitamin A will be analyzed by a central laboratory.

q Refer to Table 4 for PK Sampling Schedule.

r Any changes to the patient's cardiac medical history and treatment regimen changes should be noted, including but not limited to: onset or recurrence of atrial fibrillation, pacemaker placement, and change in diuretic regimen.

s The 6-MWT will include 2 walks at Screening/Baseline, pre- and post-walk assessment of O_2 saturation, blood pressure, heart rate, and the Borg scale ratings for fatigue and dyspnea.

t Cardiac magnetic resonance imaging with LGE is only to be obtained in patients without contraindications (i.e. pacemakers, severe renal failure with eGFR< 30 mL/min/1.73 m^2, defibrillators, allergy to gadolinium).

u Each site can use either ^{99m}Tc-pyrophosphate as a tracer or ^{99m}Tc-3,3-diphosphono-1,2-propanodicarboxylic acid. The tracer should not be changed for the patient during the study.

v Additional timepoints may not be collected depending on the status of the screening aspirate samples.

w Aliquots of serum samples will be taken and frozen, to permit testing of the effect of ALN-TTRSC on the expression of exploratory biomarkers (WT and mutant TTR levels, additional cardiac and hepatic-derived proteins that may include galectin-3 and ST2), and serology (ADAs).

Table 2: Schedule of Assessments for Clinic Visits – Year 3 and Year 4

Phase:	**Treatment Period (Doses Administered Weekly between Visits[a])**							End of Study [b]	Follow-up [b]
Study Year	**Year 3**				**Year 4**				
Study Week	**Wk108**	**Wk120**	**Wk132**	**Wk 144**	**Wk156**	**Wk168**	**Wk180**	**Wk192**	**-**
Study Day:	756	840	924	1008	1092	1176	1260	1344/ET[b]	1372
Window (days):	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**	**±7**	**±14**
Procedure:									
Study Drug Administration[c,d]	X	X	X	X	X	X	X		
Pregnancy Test[e,f]		X		X		X		X	X
Karnofsky Performance Status[e]		X		X		X		X	X
eGFR[e,g]	X	X	X	X	X	X	X	X	X
Safety Assessments:									
Physical Examination[e]	X	X	X	X	X	X	X	X	X
Body Weight[e]		X		X		X		X	
Vital Signs[e,h]	X	X	X	X	X	X	X	X	
12-Lead ECG[e,i]				X				X	
Serum Chemistry (including LFTs), Hematology, Urinalysis[e]	X	X	X	X	X	X	X	X	X
Coagulation[e,j]				X				X	X
TSH[e]				X				X	
Eye Examination				X				X	
Review Concomitant Medications	Continuous Monitoring								
Review/Record AEs	Continuous Monitoring								
PD Assessments:									
TTR[e]	X	X	X	X	X	X	X	X	X
Vitamin A[e,k]		X		X		X		X	X
PK Assessments									
Plasma PK Sampling[l]		X				X			

Table 2: Schedule of Assessments for Clinic Visits – Year 3 and Year 4

Phase:	**Treatment Period (Doses Administered Weekly between Visits[a])**							End of Study [b]	Follow-up [b]
Study Year	**Year 3**				**Year 4**				
Study Week	**Wk108**	**Wk120**	**Wk132**	**Wk 144**	**Wk156**	**Wk168**	**Wk180**	**Wk192**	**-**
Study Day:	756	840	924	1008	1092	1176	1260	1344/ET[b]	1372
Window (days):	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**	**±7**	**±14**
Clinical Efficacy Assessments:									
Review Cardiac Clinical Medical History Treatment Changes[m]	X	X	X	X	X	X	X	X	
NT-proBNP, Troponin T, and Troponin I[e]		X		X		X		X	
Echocardiography by Doppler		X		X		X		X	
6-MWT[e,n]		X		X		X		X	
NYHA Classification		X		X		X		X	
mBMI		X		X		X		X	
KCCQ[e]		X		X		X		X	
EQ-5D QoL[e]		X		X		X		X	
Other Assessments:									
Exploratory Biomarkers/ Serology (ADA)[e,o]		X		X		X		X	
Pharmacoeconomics Questionnaire[e]				X				X	

Abbreviations: 6-MWT = Six-minute Walk Test, ^{99m}Tc = ^{99m}-Technetium, ADA = anti-drug antibody, AE = adverse event, aPTT = activated partial thromboplastin time, ECG = electrocardiogram, eGFR = estimated glomerular filtration rate, ET = early termination, HBsAb – hepatitis B surface antibody, HBsAg = hepatitis B surface antigen, HCV Ab = hepatitis C virus antibody, ICF = informed consent form, INR = international normalized ratio, KCCQ = Kansas City Cardiomyopathy Questionnaire, LFT = liver function test, LGE = late gadolinium enhancement, mBMI = modified body mass index, MDRD = Modification of Diet in Renal Disease, NT-proBNP = N-terminal prohormone of B-type natriuretic peptide, NYHA = New York Heart Association, PD = pharmacodynamic, PK = pharmacokinetic, PT = prothrombin time, QoL = quality of life; QTcB = Bazett-corrected QT interval, SPECT = single-photon emission computed tomography, TEAE = treatment-emergent adverse event, TSH = thyroid stimulating hormone, TTR = transthyretin, WOCBP = women of child-bearing potential, WT = wild-type

a The 14-day window applies to study assessments. Dosing is to maintain the weekly schedule (± 2 days).

b The End-of-study or ET visit is scheduled approximately 7 days following the last dose. The Follow-up visit is scheduled approximately 4 weeks following the End-of-study or ET visit, if applicable. Patients who discontinue early will be required to report to the clinic for an End of Study visit and a Follow-up visit. For patients who discontinue

early and have provided consent, overall health status will be documented by either telephone contact or non-patient contact follow-up (eg, medical record check) at time points corresponding to study Day 504 (Week 72), if applicable, and study Day 1344 (Week 192).

c During the treatment period, doses will be administered weekly (± 2 days). Doses may be administered at the clinical site or at home according to Section 5.5.

d Subcutaneous injection of ALN-TTRSC into the abdomen. The upper arm or thigh may also be used (see the ALN-TTRSC Pharmacy Manual).

e Assessment should be performed before dosing.

f The pregnancy test will be performed for WOCBP only. A serum pregnancy test should be performed at Screening/Baseline. Urine pregnancy tests at other visits.

g The serum creatinine values should be in mg/dL to 2 decimal places (e.g., 0.95 mg/dL) OR values in µmol/L to the nearest whole number (e.g., 84 µmol/L) when calculating eGFR using the MDRD study equation; see http://nkdep.nih.gov/lab-evaluation/gfr-calculators.shtml for an eGFR calculator.

h Vital sign measurements include blood pressure, pulse rate, oral body temperature, and respiratory rate. Vital signs will be measured manually in the supine position after the patient has rested comfortably for 10 minutes. Each patient's blood pressure should be taken using the same arm. Vital signs should be collected during any unscheduled clinic visit.

i For the 12-Lead ECG, each lead shall be recorded for at least 3 beats at a speed of 25 mm/s. Triplicate recordings will be obtained. The electrophysiologic parameters assessed will be rhythm, ventricular rate, PR interval, QRS duration, QT interval, ST and T-waves and QTcB.

j Coagulation studies include PT, aPTT, and INR and will be performed on all patients.

k Blood samples for vitamin A will be analyzed by a central laboratory.

l Refer to Table 4 for PK Sampling Schedule.

m Any changes to the patient's cardiac medical history and treatment regimen changes should be noted, including but not limited to: onset or recurrence of atrial fibrillation, pacemaker placement, and change in diuretic regimen.

n The 6-MWT will include 2 walks at Screening/Baseline, pre- and post-walk assessment of O_2 saturation, blood pressure, heart rate, and the Borg scale ratings for fatigue and dyspnea.

o Aliquots of serum samples will be taken and frozen, to permit testing of the effect of ALN-TTRSC on the expression of exploratory biomarkers (WT and mutant TTR levels, additional cardiac and hepatic-derived proteins that may include galectin-3 and ST2), and serology (ADAs)

Table 3: Schedule of Assessments for Weekly Dosing Days between Clinic Visits

Study Day (±14 Days):	Study Drug Administration [a,b]	Concomitant Medication, AEs, and Vital Signs [c,d]	TTR Sample [e]	Serum Chemistry and Hematology
Year 1				
Clinic Visit (Wk 1, Day 7) - See Table 1				
14	X	X	X	X
21,35,42,49,63,70,77	X	X		
28,56	X	X	X	X
Clinic Visit (Wk 12, Day 84) - See Table 1				
91,98,105,119,126,133,147,154,161	X			
112,140	X	X		
Clinic Visit (Wk 24, Day 168) - See Table 1				
175,182,189,203,210,217,231,238,245	X			
196,224	X	X		
Clinic Visit (Wk 36, Day 252) - See Table 1				
259,266,273,287,294,301,315,322,329	X			
280,308	X	X		
Clinic Visit (Wk 48, Day 336) - See Table 1				
343,350,357,371,378,385,399,406,413	X			
364,392	X	X		
Year 2				
Clinic Visit (Wk 60, Day 420) - See Table 1				
427,434,441,455,462,469,483,490,497	X			
448,476	X	X		
Clinic Visit (Wk 72, Day 504) - See Table 1				
511,518,525,539,546,553,567,574,581	X			
532,560	X	X		
Clinic Visit (Wk 84, Day 588) - See Table 1				
595,602,609,623,630,637,651,658,665	X			
616,644	X	X		
Clinic Visit (Wk 96, Day 672) - See Table 2				
679, 686, 693, 707, 714, 721, 735, 742, 749	X			
700,728	X	X		
Year 3				

Table 3: Schedule of Assessments for Weekly Dosing Days between Clinic Visits

Study Day (±14 Days):	Study Drug Administration [a,b]	Concomitant Medication, AEs, and Vital Signs [c,d]	TTR Sample [e]	Serum Chemistry and Hematology
Clinic Visit (Wk 108, Day 756) - See Table 2				
763,770,777,791,798,805,819,826,833	X			
784,812	X	X		
Clinic Visit (Wk 120, Day 840) - See Table 2				
847,854,861,875,882,889,903,910,917	X			
868,896	X	X		
Clinic Visit (Wk 132, Day 924) - See Table 2				
931,938,945,959,966,973,987,994,1001	X			
952,980	X	X		
Clinic Visit (Wk 144, Day 1008) - See Table 2				
1015,1022,1029,1043,1050,1057,1071,1078,1085	X			
1036,1064	X	X		
Year 4				
Clinic Visit (Wk 156, Day 1092) - See Table 2				
1099,1106,1113,1127,1134,1141,1155,1162, 1169	X			
1120,1148	X	X		
Clinic Visit (Wk 168, Day 1176) - See Table 2				
1183,1190,1197,1211,1218,1225,1239,1246, 1253	X			
1204,1232	X	X		
Clinic Visit (Wk 180, Day 1260) - See Table 2				
1267,1274,1281,1295,1302,1309,1323, 1330,1337	X			
1288,1316	X	X		

Abbreviations: AE = adverse event, TTR = transthyretin

a During the treatment period, doses will be administered weekly (± 2 days). Doses may be administered at the clinical site or at home according to Section 5.5.

b Subcutaneous injection of ALN-TTRSC into the abdomen on Day 0 and then on subsequent days into other quadrants of the abdomen. The upper arm or thigh may also be used (see the ALN-TTRSC Pharmacy Manual).

c Adverse events and concomitant medications information may be collected by the site via phone on a monthly basis or by a home healthcare professional.

d Vital sign measurements include blood pressure, pulse rate, oral body temperature, and respiratory rate. Vital signs will be measured manually in the supine position after the patient has rested comfortably for 10 minutes. Each patient's blood pressure should be taken using the same arm. Vital signs should be collected prior to each weekly dose, up to Day 84.

e TTR samples should be taken pre-dose.

Table 4: Pharmacokinetic Sampling Schedule

Year	Year 1							Year 2	Year 3	Year 4
Week	Wk1			Wk12	Wk24	Wk36	Wk48	Wk84	Wk120	Wk168
Study Day	0	4	7	84	168	252	336	588	840	1176
PK Collection Time										
Pre-dose (up to 1 hr)	X	X	X	X	X	X	X	X	X	X
10 min (± 5 min)	X	X								
30 min (±5 min)	X	X								
1 hr (± 10 min)	X	X								
2 hr (± 10 min)	X	X								
4 hr (± 1 hr)	X	X	X	X	X	X	X	X	X	X
8 hr (±2 hr)	X	X								

Abbreviations: hr = hour, min = minute, PK = pharmacokinetic

ABBREVIATIONS

Abbreviation	Definition
6-MWT	6-minute Walk Test
^{99m}Tc	^{99m}-Technetium
ADA	Anti-drug antibody
AE	Adverse event
AL	Amyloid light-chain
ALP	Alkaline phosphatase
ALT	Alanine transaminase
aPTT	Activated partial thromboplastin time
AST	Aspartate transaminase
ATTR	Transthyretin-mediated amyloidosis
BMI	Body mass index
BUN	Blood urea nitrogen
CMR	Cardiac magnetic resonance imaging (Cardiac MRI)
CRF	Case report form
CRO	Clinical research organization
DPD	^{99m}Tc -3,3-diphosphono-1,2-propanodicarboxylic acid
ECG	Electrocardiogram
EDC	Electronic data capture
eGFR	Estimated glomerular filtration rate
ET	Early termination
EQ-5D-QoL	EQ-5D Quality of Life questionnaire
FAC	Familial amyloidotic cardiomyopathy
FAP	Familial amyloidotic polyneuropathy
FNAFP	Fine-needle aspirate samples of the abdominal fat pad
GalNAc	N-acetylgalactosamine
GCP	Good Clinical Practice
HBsAb	Hepatitis B surface antibody
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HCV Ab	Hepatitis C virus antibody
hr	Hour
IB	Investigator's Brochure
ICF	Informed consent form
ICH	International Conference on Harmonization
IEC	Independent Ethics Committee

Abbreviation	Definition
INR	International normalized ratio
IRB	Institutional Review Board
KCCQ	Kansas City Cardiomyopathy Questionnaire
LDH	Lactate dehydrogenase
LFT	Liver function test
LGE	Late gadolinium enhancement
LVEF	Left ventricular ejection fraction
MAD	Multiple ascending dose
mBMI	Modified body mass index
MDRD	Modification of Diet in Renal Disease
MedDRA®	Medical Dictionary for Regulatory Activities
min	Minute
mRNA	Messenger ribonucleic acid
NHP	Nonhuman primate
NOAEL	No-observed-adverse-effect-level
NT-proBNP	N-terminal prohormone of B-type natriuretic peptide
NYHA	New York Heart Association
OTC	Over-the-counter
PD	Pharmacodynamic(s)
PK	Pharmacokinetic(s)
PT	Prothrombin time
QoL	Quality of life
QTcB	Bazett-corrected QT interval
RBC	Red blood cell
RBP	Retinol binding protein
RNAi	RNA interference
SAE	Serious adverse event
SC	Subcutaneous(ly)
SPECT	Single-photon emission computed tomography
siRNA	Small interfering RNA
SSA	Senile systemic amyloidosis
SUSARs	Suspected Unexpected Serious Adverse Reactions
T4	Thyroxine
TEAE	Treatment-emergent adverse event
TSH	Thyroid stimulating hormone
TTR	Transthyretin
TRACS	TTR Amyloidosis Cardiac Study

Abbreviation	Definition
ULN	Upper limit of normal
UK	United Kingdom
WBC	White blood cell
WOCBP	Women of child-bearing potential
WT	Wild-type

1. INTRODUCTION

1.1. Background and Rationale

1.1.1. Transthyretin Biology

Transthyretin (TTR), also known as prealbumin, is a tetrameric protein produced predominantly by hepatocytes (> 95% of TTR is liver-derived), with small fractions 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.[2] In the mouse, TTR plays a greater role as a carrier for T4. Whilst the absence of TTR in the TTR-knockout mouse reduces circulating levels of T4, it does not however adversely affect the concentration of the hormone in peripheral tissues.[3,4]

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 it being bound to RBP. 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.[5] The safety of lowering vitamin A through TTR suppression has been further confirmed by the absence of any vitamin A deficiency-related adverse events (AEs) in nonhuman primates (NHPs), healthy volunteers, and patients with familial amyloidotic polyneuropathy (FAP) treated with ALN-TTR02 who experienced > 80% lowering of both TTR and vitamin A.

1.1.2. Disease Overview

Transthyretin-mediated amyloidosis (ATTR) is caused by deposition of TTR amyloid fibrils in various tissues. The hereditary form of ATTR is caused by an autosomal dominant mutation in the TTR gene that leads to destabilization of the TTR tetramer and aggregation of misfolded monomers; this, in turn, results in cardiac and neuronal extracellular deposition of TTR amyloid fibrils culminating in life-threatening cardiomyopathy and/or debilitating neuropathy. There are over 100 reported TTR genetic mutations[6] that phenotypically result in at least 2 distinct clinical syndromes of ATTR: familial amyloidotic cardiomyopathy (FAC) and FAP, both of which are characterized by amyloid deposits of mutant and wild-type (WT) TTR.[7] Following the onset of symptoms in these various amyloidosis syndromes, quality of life (QoL) is severely impacted and the disease proceeds inexorably to death.

Familial amyloidotic polyneuropathy, commonly associated with the V30M mutation, occurs primarily in families from Portugal, Sweden and Japan, has an early onset (age 40-50 years), and is characterized initially by peripheral neuropathy leading to sensory and motor deficits as well as to profound autonomic dysfunction that produces disabling

gastrointestinal pathology, orthostatic hypotension, and bladder dysfunction.[8,9] As FAP progresses, it is not uncommon for patients with either V30M or non-V30M mutations to develop cardiac amyloid deposition leading to conduction defects and symptomatic cardiomyopathy. Median survival after diagnosis of FAP is approximately 10 years.[10]

Familial amyloidotic cardiomyopathy is caused predominantly by the V122I mutation that is found in up to 4% of African Americans and in over 5% of West African populations.[11] Occurring predominantly in males, V122I FAC is a late onset (age > 60 years) syndrome in which amyloid deposition is largely restricted to the heart and manifests as heart wall thickening with diastolic and systolic dysfunction, conduction defects, and arrhythmias, leading to congestive heart failure and death; neuropathy is uncommon.[2] While the rate of disease penetrance in carriers of the mutation remains undefined, it is estimated that up to 150,000 older male African Americans in the United States carry the V122I gene[12], and there could be approximately 40,000 V122I cases worldwide. Another cardiac predominant TTR mutation, T60A, manifests as a disease of the heart and the autonomic nerves with less than one quarter of patients suffering from sensorimotor neuropathy.[13] The T60A mutation affects approximately 1% of the population in northwest Ireland, but can also be found in other countries including Japan.[14,15] Median survival after diagnosis of FAC is approximately 2.5 years for V122I and 3.5 years for T60A.[13,16]

In addition to FAC, another cause of TTR cardiac amyloidosis where neuropathy is uncommon is senile systemic amyloidosis (SSA), a non-hereditary form of ATTR occurring when WT TTR deposits as amyloid primarily in heart tissue resulting in cardiomyopathy. This disease is predominantly seen in men older than 70 years[17], and the clinical presentation is similar to that described for FAC. The prevalence of SSA is unknown, but autopsy studies suggest that up to 22%-25% of individuals > 80 years old have TTR amyloid deposits in the heart, although the degree of deposition is mild in the majority of cases.[18] Median survival after diagnosis of SSA has been reported to be approximately 3.5 years.[10,19]

The diagnosis of TTR cardiac amyloidosis is made typically through the discovery of heart wall thickening and diastolic dysfunction on cardiac echo in patients presenting with heart failure symptoms. These symptoms combined with endomyocardial or abdominal fat pad biopsy demonstrating TTR amyloid together with confirmation by TTR genotyping lead to the diagnosis of TTR cardiac amyloidosis. The detection of subendocardial late gadolinium enhancement (LGE) on cardiac magnetic resonance imaging (CMR) can also assist with making the diagnosis, as can cardiac uptake on ^{99m}Technetium-3,3-diphosphono-1,2-propanodicarboxylic acid (DPD) or ^{99m}Technetium -Pyrophosphate imaging, which is able to distinguish TTR from amyloid light-chain (AL) in the heart.[10, 20,21,22]

There are limited data on the natural history of the disease progression in TTR cardiac amyloidosis. One study in 9 patients reported significant increases in myocardial mass (mean increase of 8% by CMR, 22% by echocardiography) after 1 year of observation.[23] The multi-center TTR Amyloidosis Cardiac Study (TRACS) followed disease progression in 29 patients and demonstrated declines in left ventricular ejection fraction (LVEF) and 6-minute walk distance for both SSA and FAC patients over 18 months.[24]

Both FAC and SSA patients followed on TRACS exhibited a rise in the cardiac biomarker N-terminal prohormone of type-B natriuretic peptide (NT-proBNP) over 18 months. Elevation of serum biomarkers of heart failure, such as NT-proBNP, troponin I, and troponin T, which reflect increased cardiac filling pressures and myocyte injury, respectively, have been associated with poor outcome in AL amyloidosis.[25] The lowering of serum free light chains by chemotherapy in AL amyloidosis is associated with a robust decline in NT-proBNP that accompanies clinical improvement well in advance of any detectable changes on cardiac echo or CMR. It is therefore possible that the lowering of abnormal circulating TTR monomers through either inhibition of hepatic TTR protein production or stabilization of the TTR tetramer could impact NT-proBNP and clinical outcome in TTR cardiac amyloidosis patients.

Patients with TTR cardiac amyloidosis are largely managed with supportive care aimed at alleviation of heart failure symptoms, including restriction of salt intake, diuretics, pacemakers, and arrhythmia management. There are currently no approved therapies available for TTR cardiac amyloidosis. The TTR tetramer stabilizer tafamidis (Vyndaqel®) was approved in the European Union in 2011 for the treatment of early stage neuropathy in ATTR patients. A single-arm Phase 2 trial of tafamidis in 35 V122I and SSA patients has been completed; the study reported a LVEF decline of 4%, left ventricular mass increase of 14 g and Six-minute Walk Test (6-MWT) decrease of 11 meters for the pooled group of FAC and SSA patients after 12 months of treatment.[26]

Diflunisal is a generic nonsteroidal anti-inflammatory drug that binds to and stabilizes TTR in a manner similar to tafamidis.[27] A recently conducted single-arm Phase 2 study of diflunisal in 13 patients with TTR cardiac amyloidosis with a mean follow-up of approximately one year showed no significant change in cardiac structure/function or troponin/ NT-ProBNP levels. In addition, a decline in renal function was observed.[28] The combination of doxycycline and tauroursodeoxycholic acid, which has activity as a TTR amyloid fibril disrupter, is currently undergoing Phase 2 testing in FAP and SSA patients.[29,30]

Liver transplantation has been used in FAP to eliminate the mutant protein and has been shown to slow neuropathy progression in early-stage patients. However, for FAP patients with heart involvement, acceleration of the cardiac disease has been observed post-transplantation due to the continued deposition of WT TTR. For this reason, and due to the advanced age of most FAC and SSA patients, liver transplantation is contraindicated in TTR cardiac amyloidosis. While a few heart transplants have been performed in patients with cardiac amyloidosis, the procedure is often contraindicated due to the advanced age of most FAC patients. Thus, there exists a high unmet need for novel approaches to treat these patients.

1.1.3. RNA Interference

RNA interference (RNAi) is a naturally occurring cellular mechanism for regulating gene expression that is mediated by small interfering ribonucleic acids (siRNAs).[31] 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 endogenous or exogenous (infectious organisms) disease causing

genes. 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.[32] The ability to selectively and potently degrade the mRNA encoding the TTR protein using a 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.[33] As a result, various formulations and/or chemical modifications of the siRNAs are used to increase distribution to tissues, and to facilitate their uptake into the relevant cell type.

One approach that has been used successfully in humans for delivery of siRNAs to the liver employs intravenous administration of siRNAs formulated in lipid nanoparticles.[34,35] Another approach for liver-specific gene silencing has been to use subcutaneously (SC) administered siRNA conjugated to an N-acetylgalactosamine (GalNAc) ligand. It has been shown that conjugation of a triantennary GalNAc ligand to the 3' end of the sense strand of siRNA results in high affinity (nM) binding to the hepatic expressed asialoglycoprotein receptor and subsequent receptor-mediated uptake into hepatocytes. Single and multiple doses of SC administered siRNA-GalNAc conjugates have achieved sustained suppression of multiple different hepatocyte-expressed targets in rodents and NHPs, including TTR (see the ALN-TTRSC Investigator's Brochure [IB] for further details). Importantly, this approach for delivering siRNA to hepatocytes has also shown translation in humans with ALN-TTRSC.

1.1.4. Investigational Drug ALN-TTRSC Overview

ALN-TTRSC is comprised of siRNA targeting mutant and WT TTR mRNA with a covalently-attached triantennary GalNAc ligand formulated in water for injection. The siRNA consists of a 21-mer duplex oligonucleotide targeting the 3'UTR of the TTR mRNA, thereby conferring homology to WT TTR and all reported TTR mutations. The proposed indication for ALN-TTRSC is for the treatment of patients with mutant or WT TTR cardiac amyloidosis.

1.2. Summary of ALN-TTRSC Nonclinical Data

The safety pharmacology and toxicology of ALN-TTRSC was evaluated in a series of *in vitro* and *in vivo* nonclinical studies.

- Genotoxicity studies (Ames and chromosome aberration assays) were conducted in the presence and absence of metabolic activation and demonstrated that ALN-TTRSC was neither mutagenic nor clastogenic. In addition, ALN-TTRSC did not induce micronucleus formation in bone marrow of Sprague-Dawley rats.
- A safety pharmacology study was conducted with SC administered ALN-TTRSC in cynomolgus monkeys that demonstrated that the no-observed-effect-level for the cardiovascular (electrocardiogram [ECG], QT interval changes, and hemodynamics) and respiratory systems was 100 mg/kg ALN-TTRSC (the highest dose tested).

- The toxicity profile of SC administered ALN-TTRSC was evaluated in 10-dose (Days 1 through 5, 8, 15, 22, 29, and 36) toxicology studies in rats and cynomolgus monkeys. Each study included a 4-week nondosing recovery period. ALN-TTRSC is pharmacologically active in monkeys but not in rats. Repeated SC administration of ALN-TTRSC was clinically well tolerated by rats and monkeys. [redacted] The changes recovered to normal within 4 weeks after the last dose. These findings resulted in the no-observed-adverse-effect-level (NOAEL) in rats to be conservatively called as 30 mg/kg. The NOAEL in monkeys was greater or equal to the highest dose tested (≥ 300 mg/kg).
- Results from 6-month rat and 9-month monkey studies indicate that in-life and clinical pathology is consistent with results observed in the completed 10-dose (6-week) studies in rats and monkeys.

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

1.3. Summary of Clinical Data with ALN-TTRSC

Study ALN-TTRSC-001 was a Phase 1 randomized, double -blind, placebo-controlled, study ALN-TTRSC administration in healthy subjects. Subjects received single ascending doses or multiple ascending doses of up to 10 mg/kg or fixed doses of 500 or 600 mg ALN-TTRSC or placebo. In the multiple-dose cohorts, subjects received a total of 10 doses over a 35 day period administered as 5 daily SC doses followed by 5 weekly doses.

In this study, ALN-TTRSC was generally well-tolerated. The majority of treatment-emergent adverse events (AEs) were mild or moderate in severity and considered unrelated to study treatment. There were no serious adverse events (SAEs). Injection site reactions (ISRs) were the most common AEs related to study treatment. All ISRs were mild or moderate in severity and resolved without sequelae. Overall, the incidence of alanine transaminase (ALT) and aspartate transaminase (AST) elevation >1× upper limit of normal (ULN) was similar in placebo and ALN-TTRSC-treated subjects. One subject who received 600 mg ALN-TTRSC developed an asymptomatic elevation of ALT (4.3×ULN) and AST (1.9×ULN), which was defined in the protocol as a dose-limiting toxicity and resulted in study discontinuation. There were no other AEs leading to dose interruption or dose reduction. No elevations in cytokines, C-reactive protein, or other abnormal clinical findings were noted. The pharmacodynamic (PD) effect of ALN-TTRSC was dose-dependent and resulted in > 85% reduction of TTR at ≥ 5 mg/kg of ALN-TTRSC and was accompanied by reduction of RBP and vitamin A. Fixed doses of 500 mg and 600 mg ALN-TTRSC also demonstrated similar safety and PD effect over a wide range of body weights compared to that observed in cohorts with weight-based dosing.

Patients completing Study ALN-TTRSC-002 are eligible to enroll into the Phase 2, open label, extension study (ALN-TTRSC-003) designed to evaluate the safety and tolerability of long-term ALN-TTRSC dosing; additional information will be evaluated

including PK, PD, and clinical activity. Patients will receive 500 mg ALN-TTRSC weekly for approximately 4 years.

As of 05 January 2016, 25 patients have been treated with ALN TTRSC for approximately 8 months (range 1-13 months). Multiple doses of ALN TTRSC have been generally well-tolerated. The majority of AEs were mild or moderate in severity. The most common AEs related to study drug have been ISRs, usually characterized by transient erythema, pruritus, pain, or swelling at or around the injection site. Occasionally, these reactions have been recurrent, of longer duration and increased intensity. In some cases, ISRs have led to interruption or discontinuation of study drug. Elevations of ALT and AST have been observed, the majority of which have been mild, transient, <3×ULN and not associated with any changes in total bilirubin or clinical symptoms. In several subjects, the elevations may have led to interruption and/or dose reduction of study treatment. There were no serious hepatic AEs.

ALN-TTRSC-004 is an ongoing, Phase 3 randomized, double-blind, placebo-controlled study of ALN-TTRSC in patients with FAC. Patients receive ALN-TTRSC 500 mg or placebo administered as 5 daily SC doses, followed by weekly SC doses for 18 months. As of 14 January 2016, 83 patients have been treated with blinded study drug for approximately 3 months (range 0-13 months). The majority of AEs were mild or moderate in severity. The most common AEs considered related to blinded study treatment have been ISRs. One patient developed symptoms of anorexia, weight loss, and a cholestatic liver enzyme profile. The patient was hospitalized and an evaluation was consistent with drug-induced acute hepatitis and cholestasis. The event was considered possibly related to study treatment and a contributory role could not be excluded. The subject also had a number of other potential contributory factors.

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

1.4. Study Hypothesis and Rationale

The therapeutic hypothesis for treatment with ALN-TTRSC in patients with TTR cardiac amyloidosis is that lowering of serum TTR protein through inhibition of hepatic production may result in clinical benefit. Support for this approach comes from evidence that lowering of the amyloidogenic protein by ≥ 50% improves clinical outcomes in other amyloidotic disorders, including both primary AL amyloidosis and secondary Amyloid A amyloidosis.[36] The AL amyloidosis example is particularly relevant, since cardiac involvement is the main cause of morbidity and mortality, and the lowering of serum free light chains through chemotherapy and stem cell transplantation has been shown to improve cardiac function and overall survival.[37,38] In FAP, the elimination of mutant TTR by transplantation of the liver has been shown to slow neuropathy progression and improve survival in early-stage V30M patients.[39] Similarly, studies in FAP patients with tafamidis have shown that reducing circulating TTR monomers via stabilization of the TTR tetramer results in improved clinical outcomes.[40] These precedents suggest that direct and potent lowering of circulating TTR with ALN-TTRSC could potentially confer clinical benefit in patients with TTR cardiac amyloidosis.

This is an extension to the first study of ALN-TTRSC in patients with TTR cardiac amyloidosis. The primary objective of this study is to evaluate the safety and tolerability

of long-term dosing with ALN-TTRSC administered to patients with TTR cardiac amyloidosis. Secondary objectives include the assessment of the PD effect on total serum TTR and the clinical activity of long-term dosing of ALN-TTRSC.

1.5. Dose Selection and Rationale

Clinical data demonstrate that multiple doses of ALN-TTRSC up to 10 mg/kg and fixed doses of 500 mg ALN-TTRSC have been safe and well-tolerated in healthy volunteers. In addition, similar PK, PD effects, and safety of ALN-TTRSC was observed in patients with TTR cardiac amyloidosis. Based on these data, the proposed 500 mg dose for this study is expected to result in sustained TTR reduction ≥ 85%. Patients enrolled in study ALN-TTRSC-003 will receive ALN-TTRSC at a fixed dose of 500 mg administered as 5 daily doses during Week 1, followed by weekly doses for the duration of the study through Day 1337 (or Early Termination [ET]). The longer dosing on the extension study is supported by the overall favorable benefit/risk profile of ALN-TTRSC to date and the absence of new findings in the ongoing long-term toxicology studies in rats and monkeys.

1.6. Risk-Benefit Assessment

Please see the ALN-TTRSC IB, Guidance to the Investigator for expanded risk/benefit assessment.

1.6.1. Injection Site Reactions

ISRs have been observed in subjects receiving ALN-TTRSC in clinical studies. The majority have been mild or moderate in severity. Typically, the reactions consist of localized erythema, pain, swelling, pruritus, or rash at or around the injection site, and are transient in nature. Occasionally, these reactions have been of longer duration, more severe in nature and/or recurrent. In some subjects, this resulted in discontinuation of ALN-TTRSC. If such reactions occur, rotation of the injection site (e.g., to the extremities) is recommended as a potential mitigation strategy (see Section 5.5). Dose reduction may be considered in some cases after consultation with the Medical Monitor (see Section 5.6).

1.6.2. Abnormal Liver Function Tests

Elevations of AST and/or ALT have been seen in subjects receiving ALN-TTRSC in completed and ongoing clinical studies. The majority of abnormalities have been mild and transient elevations of ALT and/or AST <3×ULN, and are not associated with changes in total bilirubin or clinical symptoms. In some subjects, higher ALT and/or AST elevations have been observed and led to interruption or discontinuation of study treatment. Serious hepatic adverse events have also been reported, including a case of possible drug-induced hepatitis and cholestasis; in this case a contributory role of study drug could not be excluded.

The risk of liver toxicity is lowered by requiring patients to have adequate liver function at study entry, as well as regular monitoring of LFTs during the study. If a patient develops clinical signs or symptoms such as unexplained anorexia, fatigue, jaundice, or dark urine, the Investigator should consider liver injury as a potential etiology.

Dose reduction may be considered in some cases after consultation with the Medical Monitor (see Section 5.6).

1.6.3. Vitamin A Lowering

The suppression of serum levels of TTR and RBP is expected to result in the lowering of circulating vitamin A levels. Preclinical and clinical data have shown that the lowering of circulating vitamin A associated with suppression of RBP does not result in severe vitamin A deficiency. Furthermore, there are individuals with RBP/TTR mutations who have life-long low levels of circulating vitamin A and are essentially in good health, suggesting that there are compensatory transport mechanisms for vitamin A that are yet undescribed.[41] This has also been confirmed in TTR knockout mice, which do not exhibit any manifestations of vitamin A deficiency, with vitamin A uptake by most tissues continuing in the absence of RBP.[42] Provided there is adequate vitamin A in the diet, tissue stores of vitamin A should not be affected by the lowering of TTR and RBP. However, as the vitamin A content of the diet may vary between different countries and different individuals within a country, all patients on the study will be asked to take a supplement containing the recommended daily allowance of vitamin A of 2500-3000 IU.

1.7. Urgent Safety Measures

In accordance with UK Law (Medicines for Human Use [Clinical Trials] as amended: Statutory Instrument 1031 Part 4 Section 30) the Sponsor and Investigator may take appropriate urgent safety measures in order to protect the subjects of a clinical trial against any immediate hazard to their health or safety. If such measures are taken, the Sponsor shall immediately (no later than 3 days from the date the measures are taken) give written notice to the licensing authority and the relevant Independent Ethics Committee (IEC) of the measures taken and the circumstances giving rise to those measures.

2. STUDY OBJECTIVES

2.1. Primary Objective

The primary objective of the study is to evaluate the safety and tolerability of long-term dosing with ALN-TTRSC.

2.2. Secondary Objectives

Secondary objectives of this study are to assess the PD effect of long-term dosing of ALN-TTRSC on serum levels of TTR and the clinical effects of long-term dosing of ALN-TTRSC, including effect on mortality, hospitalization, and 6-MWT.

2.3. Tertiary Objectives

Tertiary objectives of the study are:

- To further characterize the PK of ALN-TTRSC.
- To assess the clinical effects of long-term dosing of ALN-TTRSC including: echocardiogram, cardiac biomarkers (NT-proBNP and troponin T and I), quantitation of amyloid in fat pad aspirates, CMR, Kansas City Cardiomyopathy Questionnaire (KCCQ), ^{99m}-Technetium (^{99m}Tc) imaging, estimated glomerular filtration rate (eGFR), New York Heart Association (NYHA) class, blood pressure, modified body mass index (mBMI), new onset or recurrence of atrial fibrillation, pacemaker placement, change in diuretic regimen, and EQ-5D Quality of Life (EQ-5D-QoL) questionnaire.
- To understand disease burden and healthcare utilization.

3. STUDY PLAN

3.1. Overall Design

Protocol ALN-TTRSC-003 is an open-label, extension study designed to evaluate the long-term safety, clinical activity, and PK of subcutaneously administered ALN-TTRSC in patients with TTR cardiac amyloidosis who previously completed the ALN-TTRSC-002 study.

The study will be conducted either at newly added study sites or at the same study sites where patients completed participation in Study ALN-TTRSC-002.

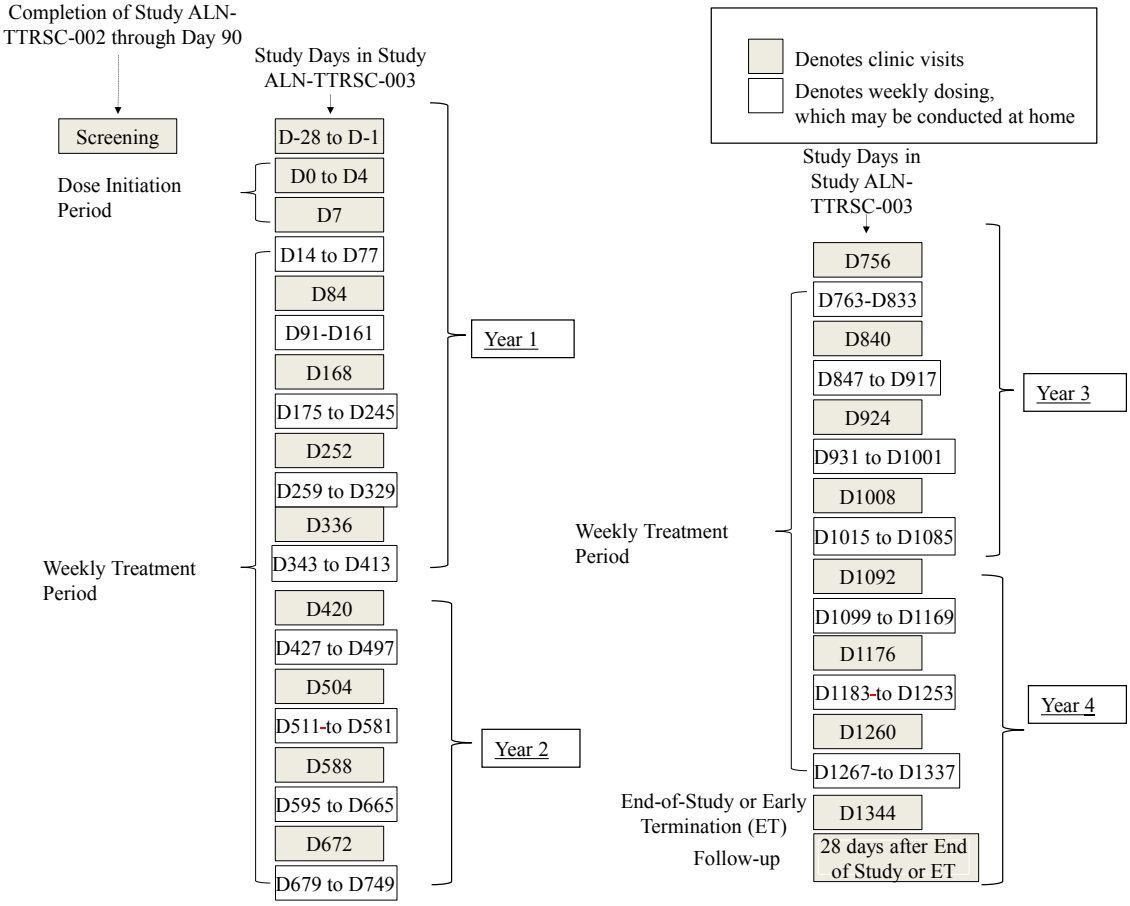
Eligible, consenting patients will receive 5 daily doses of ALN-TTRSC (Day 0, 1, 2, 3, and 4) and a dose at Day 7. They will then receive once weekly doses for the duration of the study through Day 1337 (or ET). Doses will be administered at the clinic during Days 0 through 7. Dosing after Day 7 may be administered at home by a healthcare professional trained in the protocol, or at the clinic if dosing coincides with the clinic visit for other assessments. At the discretion of the Investigator, after the Day 84 dosing visit, and if the patient has not experienced any severe adverse events or SAEs considered related to the study drug within the previous 12 weeks, patients/caregivers may be trained by the Investigator or qualified site staff in the administration of the study drug, according to the protocol dosing requirements.

Patients must be screened prior to administration of study medication under this protocol. Screening/Baseline assessments may be performed during several clinic visits. Patients must have completed the ALN-TTRSC-002 study to be eligible for this study. If the final (Day 90) visit in the ALN-TTRSC-002 study is within 28 days of the first dose administered in this study, then visit procedures performed as part of the Day 90 visit do not need to be repeated at the Screening/Baseline visit. If the final (Day 90) visit in the ALN-TTRSC-002 study occurred within 90 days of the first dose administered in this study, the echocardiogram and CMR do not need to be repeated at the Screening/Baseline visit. Assessments not conducted as part of the ALN-TTRSC-002 Day 90 visit must be completed at the Screening/Baseline visit.

Clinic visits will be performed for Screening/Baseline, dose initiation during Days 0 through 7, and then approximately every 12 weeks through the End of Study visit (or ET), and at approximately 28 days following the End of Study visit (or ET) for a Follow-up visit.

A schematic of the protocol design is presented in Figure 1.

Figure 1: Study Schematic



3.2. Safety Assessments

Safety evaluations will be performed as part of Screening/Baseline and approximately every 12 weeks during the treatment period (with the exception of eye examinations, which will be performed once a year). Some safety evaluations such as vital sign measurement and clinical laboratory tests will be performed more frequently.

Safety monitoring will include assessment of AEs, clinical laboratory tests (hematology, serum chemistry, thyroid function, coagulation, and urinalysis), 12-lead ECGs, vital signs (blood pressure, pulse rate, oral body temperature, and respiratory rate), eye examinations, and physical examinations.

3.3. Pharmacodynamic Assessments

Pharmacodynamic evaluation will include serial measurement of serum levels of TTR at specified time points. Serum levels of vitamin A will also be evaluated as a secondary PD biomarker.

3.4. Pharmacokinetic Assessments

Samples for assessment of plasma concentration of ALN-TTRSC and possible metabolite analysis will be collected at various time points.

3.5. Exploratory Clinical Efficacy Assessments

Clinical efficacy will be explored through mortality, hospitalization, and 6-MWT. Additional clinical assessments will be performed including echocardiogram, CMR, ^{99m}Tc imaging, circulating cardiac biomarkers (including NT-proBNP, troponins I and T), amyloid quantitation in fat pad aspirates, eGFR, mBMI, KCCQ, new onset or recurrence of atrial fibrillation, pacemaker placement, change in diuretic medication regimen, blood pressure, NYHA classification, and patient-reported QoL assessed by EQ-5D. Evaluations of clinical efficacy will be performed as part of Screening and approximately every 24 through 48 weeks during the treatment period.

Other Assessments

Blood samples will also be collected to evaluate exploratory biomarkers (mutant: WT TTR levels and additional cardiac and hepatic-derived proteins that may include galectin-3 and ST2) and serology (anti-drug antibody [ADAs]).

Aliquots of serum samples will be taken and frozen, to permit testing of the effect of ALN-TTRSC on the expression of these exploratory biomarkers and serology.

Disease burden and healthcare utilization will be assessed approximately every 48 weeks using a patient reported pharmacoeconomics questionnaire.

4. PATIENT POPULATION

4.1. Eligibility of Patients

Approximately 25 patients who completed participation in Study ALN-TTRSC-002 are expected to be enrolled in this extension study.

4.2. Inclusion Criteria

Each patient must meet all of the following inclusion criteria during the Screening/Baseline period to be eligible for enrollment in this study:

1. Previously received and tolerated ALN-TTRSC in Study ALN-TTRSC-002; and completed Study ALN-TTRSC-002 through the Day 90 visit;
2. Adequate liver function, demonstrated by an AST and ALT ≤2.5 × ULN, total bilirubin< 2 g/dL (34.2 μmol/L), and albumin > 3 g/dL (> 4.35 μmol/L);
3. Women of child-bearing potential (WOCBP) must have a negative pregnancy test, cannot be breast feeding, and must be willing to use a highly effective method of contraception (see Section 7.9.1) prior to Screening/Baseline, throughout study participation, and for 1 month after last dose administration;
4. Males who agree to use appropriate means of contraception (see Section 7.9.2) throughout study participation until 1 month after last dose administration;
5. Patient, or patient's legal representative, is able and willing to provide written informed consent and the patient is willing to comply with the study requirements.

4.3. Exclusion Criteria

Each patient must not meet any of the following exclusion criteria to be eligible for enrollment in the study:

1. Estimated Glomerular Filtration Rate < 20 mL/min/1.73 m^2 (using the Modification of Diet in Renal Disease [MDRD] formula);
2. Uncontrolled hypertension;
3. Uncontrolled ischemic heart disease;
4. Uncontrolled clinically significant cardiac arrhythmia;
5. Untreated hypo- or hyperthyroidism;
6. Prior major organ transplant;
7. Known or suspected systemic bacterial, viral, parasitic, or fungal infection;
8. Seropositive for hepatitis B virus, hepatitis C virus (HCV) or known to be HIV positive;

9. Received an investigational agent other than tafamidis, diflunisal, doxycycline, or tauroursodeoxycholic acid, or an investigational device within 30 days prior to first dose of study drug;
10. Discontinued ALN-TTRSC-002 due to a treatment-related AE;
11. Metastatic cancer within the past 5 years;
12. Any conditions which, in the opinion of the Investigator, would make the patient unsuitable for enrollment or could interfere with the patient's participation in, or completion of, the study.
13. History of allergic reaction to an oligonucleotide or GalNAc.
14. Blinding Procedure

This is an open-label, multi-dose study; all patients will receive ALN-TTRSC. No blinding procedures are needed.

4.4. Patient Withdrawal

Patients are free to withdraw from the study at any time and for any reason, without penalty to their continuing medical care. For those patients who withdraw early, every effort should be made to complete the ET visit (see Section 4.4.2 for handling of patients who withdraw).

4.4.1. Reasons for Withdrawal

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

- Is in violation of the protocol.
- Experiences a serious or intolerable AE.
- Becomes pregnant.
- Requests to be withdrawn from the study (patient withdrawal of consent).
- Is found to be considerably non-compliant with the protocol-required ALN-TTRSC dosing visits.

A patient may also be withdrawn from the study if, in the Investigator's opinion, he or she is unable to continue. The Investigator will also withdraw the patient from the study upon the request of Alnylam, including if Alnylam terminates the study. Upon occurrence of a serious or intolerable AE, the Investigator or designee will make every possible attempt to confer with Alnylam before discontinuing the patient.

Missing an occasional dose of study medication will not necessarily result in the patient being withdrawn from the study. However, if a patient misses 2 consecutive doses of study medication, the Investigator at the site and the Medical Monitor will discuss the appropriate management measures up to and including withdrawal from the study.

4.4.2. Handling of Withdrawals

In the event a patient withdraws or is withdrawn from the study, the Investigator will inform the Medical Monitor at the clinical research organization (CRO) and Alnylam 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.

When a patient withdraws or is withdrawn from the study, every effort should be made to conduct a complete ET visit as soon as possible following the patient's withdrawal, as described in Table 2.

If a patient is withdrawn for an AE, appropriate medical care should be provided and, if possible, the AE should be followed until resolution.

A patient who fails to return for final evaluations will be contacted by the site in an attempt to have the patient 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.

For patients who discontinue early and have provided consent, overall health status will be documented by telephone contact or non-patient contact follow-up (eg, medical record check) at the scheduled time points.

4.4.3. Replacements

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

5. STUDY MEDICATION

5.1. Presentation of Study Drug

The study drug, ALN-TTRSC, is comprised of a siRNA targeting mutant and WT TTR mRNA with a covalently attached triantennary GalNAc ligand formulated in water for injection.

The ALN-TTRSC Solution for Injection will be supplied as a sterile 200 mg/mL solution and packaged in a glass vial. The container closure system consists of a United States Pharmacopeia/European Pharmacopoeia Type I borosilicate glass vial, a Teflon-faced butyl rubber stopper and an aluminum flip-off cap.

5.2. Preparation of Study Drug

The study drug will be prepared by investigational site staff, a healthcare professional, the patient, or caregiver according to the procedures detailed in the Pharmacy Manual.

5.3. Storage of Study Drug

Study drug may be dispensed only by the Investigator, by a staff member specifically authorized by the Investigator, or by a pharmacist, as appropriate. If a patient will receive weekly doses between visits at home, arrangements should be made to allow the required number of vials to be stored either at the site, a homecare health agency, or the patient's home for storage between doses.

All study drug will be stored upright, refrigerated, and protected from light in the storage area of the site pharmacy, homecare health agency, or in a patient's home. Any deviation from the recommended storage conditions should be reported.

No special procedures for the safe handling of ALN-TTRSC are required.

Alnylam, or a designee, will be permitted, upon request, to audit the supplies, storage, dispensing procedures, and records, as applicable.

No study drug may be administered to any person not enrolled in the trial.

Additional storage details are provided in the Pharmacy Manual.

5.4. Labeling and Packaging of Study Drug

All packaging and labeling as well as the preparation of ALN-TTRSC will be in compliance with Good Manufacturing Practice 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 sites will include all appropriate local labeling requirements on the vial and external packaging. Sample labels will be submitted to health authorities, per local country submission requirements.

5.5. Dose, Route, and Schedule of Study Drug Measurement of Patient Compliance

Patients meeting the eligibility criteria will initially receive a daily SC dose of 500 mg of ALN-TTRSC over 5 consecutive days (Days 0, 1, 2, 3, and 4). Doses should be administered 24 ±4 hours apart.

Patients will then receive the same dose of ALN-TTRSC on Day 7 and then weekly from Day 14 through approximately Day1337 (or ET). The Day 7 dose should be administered within ±1 day of the planned dosing date. The weekly doses starting on Day 14 should be administered within ±2 days of the planned dosing date.

Study drug injection during clinic visits will be administered by qualified site staff under the supervision of the Investigator or designee. Dosing after Day 7 may be administered at home by a healthcare professional trained in the protocol, or at the clinic if dosing coincides with the clinic visit for other assessments. The preferred site of injection is the abdomen. Optional additional sites are the upper arms and thighs. The site of injection will be rotated. If a local reaction around the injection site occurs, photographs may be taken.

At the discretion of the Investigator, after the Day 84 dosing visit, and if the patient has not experienced any severe or serious AEs considered related to the study drug within the previous 12 weeks, patients/caregivers may be trained by the Investigator or qualified site staff in the administration of the study drug, according to the protocol dosing requirements. Training of the patient/caregiver in study dosing should be recorded in the site source documentation.

Detailed instructions can be found in the Pharmacy Manual.

5.6. Criteria for Dose Interruption, Dose Modification, or Discontinuation of Study Drug

During the study, if a patient has an elevation of AST, ALT, or total bilirubin (unless due to Gilbert's Syndrome) >3×ULN, repeat assessments of AST, ALT, and total bilirubin should be performed within 72 hours. The patient should be followed closely, with LFT assessments performed weekly, and as medically indicated to guide patient management. Close monitoring may be discontinued when ALT, AST, and total bilirubin return to ≤3×ULN. Additional, and repeat assessments, may be performed at the discretion of the Investigator.

- Dosing will be held for the following liver function test abnormalities: AST or ALT elevation of ≥3 ×ULN in association with either total bilirubin elevation of ≥1.5 ×ULN or gastrointestinal symptoms including nausea, vomiting and/or abdominal pain;
- AST or ALT elevation ≥5 ×ULN

Under these circumstances, the Medical Monitor and Investigator will decide on the appropriate action regarding frequency of repeat liver function tests and resumption of dosing.

Dose-modification criteria:

If a study drug-related AE occurs in a patient that the Investigator judges as presenting a potential risk to the patient for further dosing, the Medical Monitor and Investigator will review all available safety data for that patient and for all other patients enrolled in the study. Based on this review it may be decided to discontinue further administration of ALN-TTRSC, or, if the Investigator concurs, the patient may resume ALN-TTRSC at the same dose or, at a lower dose of 250 mg.

Missed doses:

If a patient misses a dose of study drug and is able to take the dose prior to their next dose, they should receive the missed dose as soon as possible, otherwise the missed dose will not be made up. Patients should never receive more than one dose of study drug a day.

5.7. Measurement of Patient Compliance

Treatment compliance with study drug administration is dependent on the proper preparation and administration of SC injections by study site personnel, the patient's home healthcare professional, or the patient/caregiver who has been trained in study drug administration, as well as attendance by the patient to the clinic. Patients/caregivers will be provided with a diary with which to record study drug administration between clinic visits. Patients will be permitted to miss an occasional dose of study drug. However, if a patient misses 2 consecutive doses, the Investigator, in consultation with the Medical Monitor, will discuss whether the patient will be able to continue on the study. Patients/caregivers who are administering study drug at home should be instructed to contact the study site if 2 consecutive doses are missed.

5.8. Study Drug Accountability

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

Patients/caregivers who have been trained to administer study drug at home between clinic visits will be dispensed an adequate supply of ALN-TTRSC. The number of vials dispensed to the patient will be recorded in the study drug accountability log. At each subsequent clinic visit, patients will be required to return all used and unused study drug vials to the site and the number of bottles will be recorded in the study drug accountability log by the site staff.

Drug accountability records and inventory will be available for verification by the Alnylam Monitor or designee. At the completion of the study, there will be a final reconciliation of all study drugs. Remaining study drug (all used, partially used, and unused vials) will be returned to Alnylam or its agent 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 re-dispensed to a different patient.

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

5.9. Concomitant Medication / Treatment

Use of any investigational agent other than tafamidis, diflunisal, doxycycline, or tauroursodeoxycholic acid, or an investigational device within 30 days prior to the first dose of study medication in this protocol is prohibited during study participation.

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

The Investigator should review over-the-counter (OTC) and or herbal preparations to ensure that these are not potentially disease modifying, and advise patients to limit heavy alcohol consumption.

Use of all concomitant medications from Screening/Baseline through the Follow-up visit will be recorded on the patient's CRF. This will include all prescription drugs, herbal preparations, OTC medications, vitamins, and minerals. Any changes in medications during the study will also be recorded on the CRF.

Any concomitant medication 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. For coding of concomitant medication, an internationally recognized and accepted coding dictionary will be used.

6. STUDY ASSESSMENTS

Details of the timing of each assessment are presented in the Schedule of Assessments for Clinic Visits for Year 1 and Year 2 (Table 1), the Schedule of Assessments for Clinic Visits for Year 3 and Year 4 (Table 2), the Schedule of Assessments for Weekly Dosing Days Between Clinic Visits (Table 3), and the Pharmacokinetic Sampling Schedule (Table 4).

Screening/Baseline assessments may be performed during several clinic visits. If the final Day 90 visit in the ALN-TTRSC-002 study is within 28 days of the first dose administered in this study, then visit procedures performed as part of the Day 90 visit do not need to be repeated at the Screening/Baseline visit. If the Day 90 visit in the ALN-TTRSC-002 study occurred within 90 days of the first dose administered in this study, the echocardiogram and CMR do not need to be repeated at the Screening/Baseline visit. The results from samples collected predose are not needed prior to dosing.

The 14-day window for clinic visits applies to study assessments. Dosing is to maintain the weekly schedule (± 2 days).

The End-of-study or ET visit is scheduled approximately 7 days following the last dose. The Follow-up visit is scheduled approximately 4 weeks following the End-of-study or ET visit, if applicable. Patients who discontinue early will be required to report to the clinic for an End-of-study visit and a Follow-up visit.

6.1. Demographic Data and Medical History

Patient demographic data will be obtained at Screening/Baseline. A complete medical history will be obtained at Screening/Baseline and will include past or present conditions of the following: general, head and neck, eyes, ears, nose and throat, chest/respiratory, heart/cardiovascular, gastrointestinal/liver, gynecological/urogenital, musculoskeletal/extremities, dermatological/skin, neurological/psychiatric, endocrine/metabolic, hematologic/lymphatic, allergies/drug sensitivities, past surgeries, substance abuse, or any other disease or disorder. The patient's HIV status will also be obtained.

Any AEs from the previous study (ALN-TTRSC-002) that are ongoing on Day 0 will be considered as part of the medical history and recorded as such on the CRF.

On Day 0 pre-dose, the medical history for each patient will be updated with any changes during the interval since the last recording. Any changes in medical history will be evaluated against the inclusion and exclusion criteria to determine the patient's continued eligibility for the study.

6.2. Karnofsky Performance Status

At the scheduled time points, each patient's Karnofsky Performance Status will be assessed before study drug dosing; see Appendix 1 for a sample of the Karnofsky Scale.[43]

6.3. Estimated Glomerular Filtration Rate

Estimated glomerular filtration rate will be calculated using the MDRD formula. If this parameter met eligibility criteria at Screening/Baseline, then dosing can proceed prior to receiving pre-dose Day 0 results.

The formula is as follows:

$$\text{GFR (mL/min/1.73 m}^2\text{)} = 175 \times (S_{cr})^{-1.154} \times (\text{Age})^{-0.203} \times (0.742 \text{ if female}) \times (1.212 \text{ if African American}) \text{ (conventional units)}$$

This formula estimates GFR using 4 variables (serum creatinine, age, ethnicity, and gender). For precision, the serum creatinine values should be in mg/dL to 2 decimal places (eg, 0.95 mg/dL) OR values in µmol/L to the nearest whole number (e.g., 84 µmol/L).[44] The following eGFR calculator can be used to determine eGFR: http://nkdep.nih.gov/lab-evaluation/gfr-calculators.shtml.

6.4. Safety Assessments

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

6.4.1. Physical Examination

At the scheduled time points, physical examinations should be performed before study drug dosing and will include the examination of the following: general appearance, head, eyes, ears, nose and throat, chest/respiratory, heart/cardiovascular, gastrointestinal/liver, musculoskeletal/extremities, dermatological/skin, thyroid/neck, lymph nodes, and neurological/psychiatric.

6.4.2. Body Weight and Height

At the scheduled time points, body weight (measured in kilograms [kg]) will be obtained before study drug dosing; height will also be measured.

6.4.3. Vital Signs

At the scheduled time points, vital signs will be measured before study drug dosing. Vital sign measurements include blood pressure, pulse rate, oral body temperature, and respiratory rate. Vital signs will be measured manually in the supine position 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 obtained in degrees Celsius, heart rate will be counted for a full minute and recorded in beats per minute, and respirations will be counted for a full minute and recorded in breaths per minute.

Vital signs should also be collected during any unscheduled clinic visit, and before dosing at home, up to Day 84. For the safety of the patient, additional vital signs may be added at the discretion of the Investigator.

6.4.4. Electrocardiogram

At the scheduled time points, standard computerized 12-lead ECG recordings will be obtained in triplicate before study drug dosing. Each lead shall be recorded for at least 3

beats at a speed of 25 mm/sec. The electrocardiographic parameters assessed will be rhythm, ventricular rate, PR interval, QRS duration, QT interval, ST and T waves, and Bazett-corrected QT interval (QTcB).

The Investigator or designee is responsible for reviewing the ECGs to assess whether the results are within normal limits and to determine the clinical significance of the results. These assessments will be recorded on the CRF. For any clinically significant abnormal results (eg, ischemic ECG changes, wave/interval changes, or arrhythmia), the Investigator must contact the Medical Monitor to discuss continued participation of the patient in the study.

6.4.5. Clinical Laboratory Tests

At the scheduled time points, samples for clinical laboratory tests will be collected before study drug dosing. The following clinical laboratory tests will be performed by each study site's local laboratory or a central laboratory:

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 Chemistry

- Sodium
- Potassium
- Blood urea nitrogen (BUN)
- Creatinine
- Uric acid
- Lactate dehydrogenase (LDH)
- Glucose
- Phosphate
- Albumin
- Calcium
- Carbon dioxide
- Chloride

Liver Function Tests

- AST
- ALT
- Alkaline phosphatase (ALP)
- Bilirubin (total and direct)

Coagulation Studies

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

Thyroid Function Test

- Thyroid stimulating hormone (TSH)

Serology Parameters

- Hepatitis B surface antigens (HBsAg)
- Hepatitis B surface antibody (HBsAb)
- Anti-hepatitis C virus antibody (HCV Ab)

Urinalysis

- Visual inspection for appearance and color
- pH (dipstick)
- Specific gravity
- Ketones
- Protein
- Glucose
- Bilirubin
- Nitrite
- RBCs
- Urobilinogen
- Leukocytes
- Microscopy (if clinically indicated)

Urine Pregnancy Testing (WOCBP only)

- β-human chorionic gonadotropin

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

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.

6.4.6. Pregnancy Test

A serum pregnancy test should be performed at Screening/Baseline for WOCBP and a urine pregnancy test may be used at all other visits; urine pregnancy tests will be performed at the study site. Samples for the pregnancy test should be collected before study drug dosing. The results of the pregnancy test at Screening/Baseline 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 the pregnancy outcome is known (see Section 7.10).

6.4.7. Eye Examination

At the scheduled time points, an eye examination will be performed. These examinations will include assessment of visual acuity and slit-lamp evaluation and visual field, where possible. Visual acuity should be evaluated at the beginning of each specified visit in the study (ie, prior to slit-lamp examination). Visual acuity testing should be done with best (most recent) correction. Further details regarding the eye examinations will be provided in the Study Reference Manual.

6.4.8. Concomitant Medications

All medications the patient receives during the Screening/Baseline period through the Follow-up visit will be recorded. The indication for the medication must be assessed to determine if a new AE is present. After Day 84, the site should contact the patient by phone at least monthly to collect information on concomitant medications between clinic visits. See Section 5.9 for more details on prohibited and acceptable concomitant medications.

6.4.9. Adverse Events

Adverse events will be collected beginning with the first dose of study drug and through the Follow-up visit. After Day 84, the site should contact the patient by phone at least monthly to collect information on adverse events between clinic visits. See Section 7 for more details on the collection and reporting of AEs.

6.5. Pharmacodynamic Assessments

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

6.5.1. Transthyretin

At the scheduled time points, blood for serum total TTR levels will be collected before study drug dosing and assessed using an enzyme linked immunosorbent assay method.

6.5.2. Vitamin A

At the scheduled time points, blood for serum vitamin A levels will be collected before study drug dosing and analyzed at a central laboratory.

6.6. Pharmacokinetic Evaluations

Blood samples for assessment of ALN-TTRSC concentration and possible metabolite analysis will be collected as noted in the Pharmacokinetic Sampling Schedule (Table 4). Please refer to the Study Laboratory Manual for details on the sample collection and processing for plasma PK samples.

6.7. Clinical Efficacy Assessments

The clinical efficacy assessments are briefly described below. Further details on performing clinical efficacy assessments will be provided in the Study Reference Manual.

6.7.1. Review of Cardiac Medical History and Treatment Changes

Any changes to the patient's cardiac medical history and treatment regimen changes should be recorded. Changes to be documented may include, but are not limited to, onset or recurrence of atrial fibrillation, pacemaker placement, and change in diuretic regimen.

6.7.2. NT-proBNP, Troponin T, and Troponin I

At the scheduled time points, blood samples for the quantification of troponin T, troponin I, and NT-proBNP (biomarkers of cardiac status) will be collected before study drug dosing. Quantification of these biomarkers will be performed at a central laboratory.

6.7.3. Echocardiography

An echocardiogram with Doppler will be used for assessment of cardiac structure and function. Echocardiography will be performed. Details for image acquisition and upload for central review can be found in the Echocardiogram Site Instruction Manual.

6.7.4. Six-Minute Walk Test

The 6-MWT will be performed to assess functional exercise capacity and will include pre- and post-walk assessment of O_2 saturation, blood pressure, heart rate, and the Borg scale rating for dyspnea and fatigue. The Screening/Baseline assessment will include a practice and an actual 6-MWT test 1 hour apart; at subsequent visits the 6-MWT should be performed before study drug dosing. Attempts should be made to perform the test approximately the same time of the day during the visits.

The 6-MWT will be administered by staff trained in the protocol, as per the American Thoracic Society guidelines.[45]

6.7.5. NYHA Classification

NYHA classification will be recorded; the NYHA classification scale is provided in Appendix 2.

6.7.6. Cardiac Magnetic Resonance Imaging

Cardiac MRI with LGE will be performed on patients without contraindications (ie, pacemakers, severe renal failure with eGFR < 30 mL/min/1.73 m^2, defibrillators, or allergy to gadolinium).

Details for image acquisition and upload for central review can be found in the MRI Instruction Manual.

6.7.7. Modified Body Mass Index

Assessment of nutritional status will be measured by the mBMI, which has been linked to autonomic dysfunction. Since the mBMI takes into account the serum albumin values along with body mass index (BMI; function of height and weight), it corrects for hypoalbuminemia and is considered more reflective of nutritional status than BMI alone.

6.7.8. Kansas City Cardiomyopathy Questionnaire

The KCCQ is a self-administered, 23-item questionnaire developed to provide a better description of health-related QoL in patients with chronic heart failure.[46] The KCCQ assesses physical function, symptoms, social function, self-efficacy, and knowledge, and QoL. At the scheduled time points, this questionnaire should be completed before study drug dosing.

6.7.9. EQ-5D Quality of Life

Quality of life will be assessed through the use of the EQ-5D-5L, a standardized 5 question instrument for use as a measure of health outcomes. At the scheduled time points, This questionnaire should be completed before study drug dosing.

6.7.10. Technetium Imaging

Technetium (^{99m}Tc) imaging by 3D single-photon emission computed tomography (SPECT) will be performed, where possible, depending upon availability of the ^{99m}Tc imaging tracer. ^{99m}-Tc-Pyrophosphate or DPD can be used as a tracer. The tracer should not be changed for the patient during the study.

Details for the procedure can be found in the Technetium Site Imaging Manual.

6.7.11. Fat Pad Aspirates

At the scheduled time points, two fine-needle aspirate samples of the abdominal fat pad (FNAFP) for amyloid quantification will be collected. Additional timepoints may not be collected depending on the status of the screening aspirate samples. Details on FNAFP collection, processing, and shipment will be in the Study Reference Manual.

6.8. Other Assessments

6.8.1. Exploratory Biomarkers and Serology

At the scheduled time points, aliquots of serum samples will be taken before study drug dosing and frozen to permit testing of the effect of ALN-TTRSC on the expression of exploratory biomarkers (mutant: WT TTR levels, additional cardiac and hepatic-derived proteins that may include galectin-3 and ST2) and serology (ADA).

Mutant and WT TTR will be assessed using a mass spectrometry (MS) method.

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

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.

6.8.2. Pharmacoeconomics Questionnaire

The burden of disease and healthcare utilization will be assessed using a patient-reported pharmacoeconomics questionnaire. At the scheduled time points, this questionnaire should be completed before study drug dosing.

7. REPORTING ADVERSE EVENTS

7.1. 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.

Because reductions in TTR and vitamin A are expected responses to ALN-TTRSC, reductions in these parameters will not be considered AEs. While circulating vitamin A levels will be lowered, this is not expected to result in signs or symptoms of severe vitamin A deficiency.

7.1.1. Adverse Events of Clinical Interest

The following events are considered to be AEs of clinical interest:

- ISRs
- Hepatic AEs, including LFT abnormalities considered clinically significant by the Investigator

7.2. 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 and may require intervention to prevent one of the other outcomes listed above. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias, convulsions, or the development of drug dependency or abuse.

7.3. 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). Between clinic visits, the Investigator or designated study site staff should contact the patient by telephone on at least a monthly basis to collect additional information about any medically relevant health changes that may have occurred since the study visit. The patient should also be instructed to contact the study site to report any changes in health status.

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

7.4. Adverse Event Reporting

The Investigator is responsible for reporting all AEs that are observed or reported after the first dose of study drug through the Follow-up visit regardless of their relationship to study drug or clinical significance.

Any medical condition that is present when a patient is screened and does not deteriorate is captured as medical history and 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 that occur after the first dose of study drug must be reported in detail on the appropriate CRF page and recorded in the site's source records. The description of the AE will include the onset time and date, duration, severity, seriousness (see Section 7.8) relationship to study drug, action taken, and outcome (including time and date of resolution, if applicable). Adverse events resulting from concurrent illnesses, concomitant medications, or progression of disease states must also be reported. The Medical Dictionary for Regulatory Activities (MedDRA®) will be used to code AEs.

For AEs that are considered AEs of clinical interest, additional clinical information may be collected based upon the severity or nature of the event.

If a patient has ISRs meeting any of the following criteria, the Investigator, or designee, should contact the Medical Monitor and submit a supplemental ISR eCRF:

- ISRs that are recurrent and/or demonstrate a pattern of increasing severity
- Any ISR that is determined to be severe and/or a cause for study drug discontinuation
- Any ISR which, in the opinion of the Investigator, requires further medical evaluation or treatment

In some cases, where it is medically appropriate, further evaluation may include photographs, referral to a dermatologist, skin biopsy, or other laboratory testing. If a biopsy was obtained, the Sponsor may request that the biopsy also be reviewed by a central dermatopathologist. To better understand the safety profile of the study drug, additional analysis of biopsy tissue may be performed as allowed by local regulations.

For patients with hepatic AEs, additional information, including, clinical history, course of event, and local laboratory results to monitor LFT levels or other laboratory parameters, may be collected.

7.5. Adverse Event Reporting Period

All AEs that occur after the first dose of study drug must be followed to satisfactory resolution or for a period of 28 days from the last dose of study drug, whichever is the shorter, or the AE is deemed by the Investigator to be chronic or the patient to be stable.

Serious AEs will be followed until resolved or until the SAE is considered by the Investigator to be chronic or the patient to be stable.

7.6. Assessment of Causality

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

Definitely Related:	A clinical event, including laboratory test abnormality, occurring in a plausible time relationship to the medication administration, and which cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug should be clinically plausible.
Possibly Related:	A clinical event, including laboratory test abnormality, with a reasonable time sequence to the medication administration, but which could also be explained by concurrent disease or other drugs or chemicals. Information on the drug withdrawal may be lacking or unclear.
Unlikely Related:	A clinical event, including laboratory test abnormality, with little or no temporal relationship to medication administration, and which other drugs, chemicals, or underlying disease provide plausible explanations.
Not Related:	A clinical event, including laboratory test abnormality, that has no temporal relationship to the medication or has more likely alternative etiology.

7.7. Assessment of Severity

The severity of each AE is to be classified as mild, moderate, severe, or life threatening according to the following criteria:

Mild:	Mild events are those which require minimal or no treatment and do not interfere with the patient's daily activities.
Moderate:	Moderate events are those which cause sufficient discomfort to interfere with normal everyday activities and may require minor analgesics or antiemetics.

Severe:	Severe events are those which interrupt a patient's usual daily activity and may require systemic drug therapy or other therapy.
Life Threatening:	Life threatening events put the patient's life at risk.

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).

7.8. 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 seriousness criteria above (Section 7.2) must be reported to Alnylam, or designee, within 24 hours from the time that site personnel first learn of the event, using the Serious Adverse Event Report provided. All SAEs must be reported regardless of the relationship to study drug. The SAE must also be recorded on the standard CRF pages.

The initial report should include at least the following information:

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

In the first instance, AE reporting will be via electronic data capture (EDC). For names, addresses, telephone, and fax numbers for SAE reporting (eg, in case the EDC system is down), see information included in the SAE Reporting Procedure.

If follow up is required, new information should be provided to Alnylam, or designee, as it becomes available using the Serious Adverse Event Report. 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 or Sub-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 or Sub-investigator until satisfactory resolution or the Investigator or Sub-investigator deems the SAE to be chronic or stable. Clinical, laboratory, and diagnostic measures should be employed by the Investigator or Sub-investigator as needed to adequately determine the etiology of the event.

The Investigator will be responsible for reporting all SAEs to the IEC/Institutional Review Board (IRB). Alnylam, or designee, will be responsible for reporting of all relevant events to the regulatory authorities according to all applicable regulations.

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

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

7.9. Contraceptive Requirements

7.9.1. Requirements for Women of Child-bearing Potential

Women of child-bearing potential may be included in this study and include any female who has experienced menarche and who is not postmenopausal or permanently sterilized (eg, tubal occlusion, hysterectomy, or bilateral salpingectomy).

Women of child-bearing potential must have a negative pregnancy test and must be using 1 highly effective method of contraception in combination with a barrier method from the signing of the informed consent form (ICF), throughout study participation, and for 1 month after the last dose of ALN-TTRSC. Highly effective methods of birth control result in a low failure rate (ie, less than 1% per year).[47]

For the purpose of this study, one of the following combinations of contraceptive methods must be used by WOCBP:

- Hormonal: established use of oral (except low-dose gestagens [eg, lynestrenol and norethisterone]), implantable, injectable, or transdermal methods of conception in conjunction with spermicide, condom, or diaphragm;
- Placement of an intrauterine device in conjunction with spermicide or condom;
- Placement of an intrauterine system in conjunction with spermicide or condom;
- Bilateral tubal occlusion in conjunction with spermicide or condom;
- Surgical sterilization of male partner (with the appropriate post-vasectomy documentation of the absence of sperm in the ejaculate; for female patients on the study, the vasectomized male partner should be the sole partner for that patient) in conjunction with spermicide, condom, or diaphragm;

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

Interaction between ALN-TTRSC and hormonal contraceptives is not anticipated; there were no effects of ALN-TTRSC on the reproductive organs (including histopathology) in any of the animal (rat and NHP) studies conducted to date. Moreover, ALN-TTRSC showed no inhibition in vitro of cytochrome P_{450} (CYP)1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4/5 (both testosterone and midazolam were used as substrates in human liver microsomes).

7.9.2. Requirements for Male Patients

Males who agree to use appropriate means of contraception throughout study participation until 1 month after the last dose of ALN-TTRSC may be included in this study (see Section 7.9.1).

It is unknown if ALN-TTRSC poses any potential risk of drug exposure through the ejaculate. Male patients (including men who have had vasectomies), particularly those with partners who are pregnant, should use condoms (accompanied with spermicidal foam, gel, film, cream, or suppository) as the appropriate means of contraception for the duration of the study and until 1 month after study drug administration.

Males should also abstain from sperm donation after the first dose of study drug through study participation and for 1 month after the last dose of ALN-TTRSC.

7.10. Pregnancy Reporting

A female patient with a positive pregnancy test at Screening/Baseline is ineligible for this study. If a female patient becomes pregnant during this study, the patient must be instructed to stop all study drug administration. The Investigator or Sub-investigator must report a patient pregnancy (or a pregnancy of the partner of a patient participating in the study if consent has been obtained from the partner) to Alnylam or its agency within 24 hours of being notified of the pregnancy. Details of the pregnancy will be reported on a pregnancy report form. The patient/partner shall receive any necessary counseling regarding the risks of continuing the pregnancy and the possible effects on the fetus.

Monitoring of the patient/partner by the Investigator will continue until the conclusion of the pregnancy, and the Investigator will document the outcome of the pregnancy, which will be reported to Alnylam. If the outcome of the pregnancy meets the criteria for an SAE (ie, postpartum complication, spontaneous abortion, stillbirth, neonatal death, or congenital anomaly), then the Investigator should follow the procedures for reporting an SAE outlined above.

8. STATISTICAL METHODS

8.1. Sample Size

Approximately 25 patients who completed Study ALN-TTRSC-002 will be enrolled in this extension study.

8.2. Statistical Methodology

Statistical analyses will be primarily descriptive in nature. Adverse event summaries will include tabulations of all TEAEs, treatment-related AEs, SAEs, discontinuations due to AEs, and AEs of various grading severity.

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.

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.

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

8.2.1. Populations to be Analyzed

The following populations (ie, analysis sets) will be identified:

- Safety Analysis Set: All patients who receive at least one dose of ALN-TTRSC will be included in the safety analysis.
- PD Analysis Set: All patients who received at least one dose of ALN-TTRSC and have at least one blood sample post-dose assessment.
- PK Analysis Set: All patients who receive at least one dose of ALN-TTRSC and have at least one blood draw postdose to determine plasma concentrations of ALN-TTRSC will be included in PK analyses.

8.2.2. Baseline Evaluations

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

8.2.3. Safety Analyses

Adverse events will be summarized by MedDRA® system organ class and preferred term. Separate tabulations will be produced for all TEAEs, treatment-related AEs (those considered by the Investigator as at least possibly drug related), SAEs, discontinuations due to AEs, and AEs of various grading severity.

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

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

Details of any abnormalities will be included in patient listings.

8.2.4. Clinical Activity Assessments

Change from baseline by time point for 6-MWT will be summarized, where change from baseline is predose value (or final assessment from ALN-TTRSC-002, if a pre-dose assessment in this study is not required). Change from baseline will also be summarized where baseline is derived from predose assessments in ALN-TTRSC-002.

Frequency of hospitalization, cardiovascular hospitalization, and heart failure hospitalization will be summarized. All-cause mortality and cardiovascular mortality will be summarized using Kaplan-Meier curves.

Descriptive statistics will be presented for all clinical activity variables (NT-proBNP, troponin I, troponin T concentrations; selected echocardiography parameters; mBMI; NYHA classification; CMR imaging parameters). Patient reported QoL and disease burden will be assessed by summary statistics for the EQ-5D and KCCQ. Summary statistics will be provided for observed values and changes from baseline.

8.2.5. Healthcare Utilization Assessments

The observed values and changes from baseline in a health care utilization questionnaire will be summarized.

8.2.6. Pharmacodynamics

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

8.2.7. Pharmacokinetics

Pharmacokinetic parameters will be calculated, when feasible, by noncompartmental methods using standard modeling software. Pharmacokinetic parameters will be summarized and presented, and mean (± standard error of the mean) concentration-time profiles will be generated by dose.

8.2.8. Interim Analysis

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

8.2.9. Missing Data

For continuous and categorical variables, data will be analyzed as reported, unless otherwise specified. No imputation will be performed unless clearly identified.

For time-to-event variables, such as overall survival, subjects missing death will be right-censored.

9. 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.

9.1. Data Handling and Quality Assurance

9.1.1. Case Report Forms

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

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

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

9.1.2. 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 written contact. The monitor will maintain current personal knowledge of the study through observation, review of study records and source documentation and discussion of the conduct of the study with the Investigator and staff.

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

9.1.3. Inspections

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

9.2. Regulatory Guidelines

This study will be performed in accordance with the clinical trial agreement, the protocol, all applicable government laws, regulations, and guidances where the study is being conducted including policies with foundations in the World Health Organization Declaration of Helsinki, the International Conference on Harmonization (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use, the Health Insurance Portability and Accountability Act of 1996, and all other applicable medical privacy laws and regulations.

9.2.1. Independent Ethics Committee Institutional Review Board

National regulations and ICH require that approval be obtained from an IEC or an IRB prior to participation of patients in research studies. Prior to the study onset, the protocol, any protocol amendments, informed consent forms (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 or IRB.

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

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

The Investigator is responsible for obtaining continuing review of the clinical research at least annually or more often if specified by the IEC or IRB. 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 or IRB is constituted and operates in accordance with Federal and ICH GCP and any local regulations.

9.2.2. Regulatory Authorities

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

9.2.3. 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 or IRB that approved the study. Amendments to the protocol must be submitted in writing to the Investigator's IEC or IRB and the Regulatory Authority for approval prior to patients being enrolled under the amended protocol.

9.2.4. Informed Consent Form

Written informed consent in compliance with ICH and local regulations will be obtained from each patient prior to undergoing any protocol-specific tests or procedures that are not part of routine care.

Alnylam will provide an ICF template to the Investigator for use in developing a site-specific ICF. Prior to submission of the site-specific ICF to the IEC/IRB, the site-specific ICF must be reviewed and approved by Alnylam. Any changes requested by the IEC/IRB must also be approved by Alnylam. The final IEC/IRB 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 will be given a full explanation of the study and be allowed to read the ICF. Once the Investigator is assured that the patient understands the commitments of participating in the study, the patient 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.

For patients who discontinue early and have provided consent, overall health status will be documented by telephone contact or non-patient contact follow-up (eg, medical record check) at the scheduled time points in an attempt to minimize the extent of missing data.

9.2.5. 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/IRB per their local reporting requirements, or at least annually and at the conclusion of the study. The reports will be made available to Alnylam or designee.

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

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

9.2.6. Financial Disclosure Reporting Obligations

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

9.3. Ancillary Research

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

9.4. Study Record Retention

Essential documents should be retained for the period of time required by applicable local law. The essential documents include the signed and dated final protocol, signed and dated amendments(s), if applicable, signed and dated Curriculum Vitae of the

Investigators, copies of the completed CRFs, signed ICFs, IEC/IRB approval and all related correspondence, financial agreements, regulatory approval, drug accountability, study correspondence, and patient identification codes. Records will not be destroyed without informing Alnylam in writing and giving Alnylam the opportunity to store the records for a longer period of time at Alnylam's expense.

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

9.5. Discontinuation of the Study by Alnylam

Alnylam reserves the right to discontinue the study for clinical or administrative reasons at any time. Should the study be terminated and/or the site closed for whatever reason, all documentation and study drug pertaining to the study must be returned to Alnylam or its representative, and the Investigators, IEC/IRB, and Regulatory Authorities will be promptly informed of the termination and the reason for the decision. The Investigator should promptly inform the patients and assure appropriate therapy and follow-up.

9.6. Confidentiality

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

The Investigator must treat all of the information related to the study and the compiled data as confidential, the use of which is for the purpose of conducting the study. Alnylam must approve any transfer of information to any person(s) or entities not directly involved in the study.

9.7. Publications/Reports

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

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

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11. APPENDICES

Appendix 1: Karnofsky Scale

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

Appendix 2: New York Heart Association Classification of Heart Failure

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