Official Title:	**A Phase 2, Open-Label, Multi-Dose, Dose Escalation Trial to Evaluate the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of Intravenous Infusions of ALN-TTR02 in Patients with TTR Amyloidosis**
NCT Number:	**NCT01617967**
Document Date:	**Statistical Analysis Plan, Version 1.0, 23 July 2013**

STATISTICAL ANALYSIS PLAN

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

Protocol Number:	ALN-TTR02-002
Protocol Version and Date:	Amendment 2.1: 16 January 2013 Amendment 2: 16 January 2013 Amendment 1.1: 24 September 2012 Amendment 1: 19 June 2012 Original: 03 February 2012
Name of Test Drug:	ALN-TTR02
Phase:	Phase 2
Methodology:	Multi-center, multi-national, open-label, multi-dose, dose escalation study
Sponsor:	Alnylam Pharmaceuticals, Inc. 300 Third Street Cambridge, MA 02142 USA Tel: (617) 551-8200 Fax: (617) 551-8101
Sponsor Representative:	[redacted]
Analysis Plan Date:	23 July 2013
Analysis Plan Version:	Final Version 1.0

APPROVAL SIGNATURE PAGE

Protocol Title:	A Phase 2, Open-Label, Multi-Dose, Dose Escalation Trial to Evaluate the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of Intravenous Infusions of ALN-TTR02 in Patients with TTR Amyloidosis
Sponsor:	Alnylam Pharmaceuticals, Inc. 300 Third Street Cambridge, MA 02142 USA
Protocol Number:	ALN-TTR02-002
Document Date / Version:	23 July 2013 / Final Version 1.0

Veristat, Inc. Author:

[redacted]

Signatur[redacted]

Date: 23 July 2013

Sponsor Approval

By signing this document, I acknowledge that I have read the document and approve of the planned statistical analyses described herein. I agree that the planned statistical analyses are appropriate for this study, are in accordance with the study objectives, and are consistent with the statistical methodology described in the protocol, clinical development plan, and all applicable regulatory guidances and guidelines.

I have discussed any questions I have regarding the contents of this document with the biostatistical author.

I also understand that any subsequent changes to the planned statistical analyses, as described herein, may have a regulatory impact and/or result in timeline adjustments. All changes to the planned analyses will be described in the clinical study report.

Sponsor Signatory:

[redacted]

Signatur[redacted]

Date: 23 July 2013

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation	Definition
λ_z	Elimination rate constant
AE	Adverse event
ALT	Alanine transaminase
anti-HCV Ab	Anti–hepatitis C virus antibody
aPTT	Activated partial thromboplastin time
AST	Aspartate transaminase
ATC	Anatomic therapeutic class
ATTR	Transthyretin-mediated amyloidosis
AUC	Area under the plasma concentration-time curve
$AUC_{0-\infty}$	Area under the plasma concentration-time curve extrapolated to infinity
AUC_{0-last}	Area under the plasma concentration-time curve from zero to the last measurable time point
AUC_{0-t}	Area under the plasma concentration-time curve to the last measurable concentration
AUC_p	Partial area under the plasma concentration-time curve
Bb	Activation fragment of complement fragment B
BMI	Body mass index
BUN	Blood urea nitrogen
C3a	Complement component 3a
CI	Confidence interval
CL	Systemic clearance
CL_R	Renal clearance
C_{max}	Observed maximum plasma concentration
CPK	Creatine phosphokinase
CPK-MB	Myocardial band of enzymes of creatine phosphokinase
CRF/eCRF	Case report form/Electronic case report form
CRO	Contract Research Organization

Abbreviation	**Definition**
CRP	C-reactive protein
CSR	Clinical study report
DLin-MC3-DMA	1,2-Dilinoleyloxy-N,N-dimethylpropylamine
DLT	Dose-limiting toxicity
ECG	Electrocardiogram
EOI	End of infusion
ET	Early termination
EU	European Union
G-CSF	Granulocyte-colony stimulating factor
H1/H2 blocker	Histamine H1/H2 receptor antagonist
HbsAb	Hepatitis B surface antibody
HbsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HDL	High density lipoprotein
HEENT	Head/ears/eyes/nose/throat
ICH	International Conference on Harmonization
IFN-α	Interferon-alpha
IFN-γ	Interferon-gamma
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IL-1β	Interleukin-1 beta
IL-1RA	IL-1 receptor antagonist
IL-6	Interleukin-6
IL-12	Interleukin-12
INR	International normalized ratio
IP-10	Interferon-inducible protein-10
IRR	Infusion-related reaction
ITT	Intent-to-treat

Abbreviation	**Definition**
IV	Intravenous(ly)
LDH	Lactate dehydrogenase
LDL	Low density lipoprotein
LFT	Liver function test
LNP	Lipid nanoparticles
MedDRA®	Medical Dictionary for Regulatory Activities
mRNA	Messenger ribonucleic acid
PD	Pharmacodynamic
PEG_{2000}-C-DMG	3-N-[(ω-methoxy poly(ethylene glycol)2000) carbamoyl]-1,2-dimyristyloxy-propylamine
PI	Principal Investigator
PK	Pharmacokinetic
PO	Per os (orally)
PP	Per-protocol
PT	Prothrombin time
QTc	QT interval corrected for heart rate
RBC	Red blood cell
RBP	Retinol binding protein
RNAi	Ribonucleic interference
SAE	Serious adverse event
SaO_2	Arterial oxygen saturation
SAP	Statistical analysis plan
SD	Standard deviation
SI	International system of units
siRNA	Small interfering ribonucleic acid
SNALP	Stable nucleic acid lipid particles
SOC	System organ class
SRC	Safety Review Committee

Abbreviation	**Definition**
$t_{1/2}$	Terminal elimination half-life
$t_{1/2\alpha}$	Alpha half-life
$t_{1/2\beta}$	Beta half-life
T3	Triiodothyronine
T4	Thyroxine
TEAE	Treatment-emergent adverse event
t_{max}	Time of observed maximum plasma concentration
TNF-α	Tumor necrosis factor-alpha
TSH	Thyroid stimulating hormone
TTR	Transthyretin
ULN	Upper limit of normal
US/USA	United States
VLDL	Very low density lipoprotein
V_{ss}	Volume of distribution at steady state
V_z	Volume of distribution based on the terminal phase
WBC	White blood cell
WHO	World Health Organization
WT	Wild type

1. INFORMATION FROM THE STUDY PROTOCOL

1.1. Introduction and Objectives

1.1.1. Introduction

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

Mutations in the TTR gene can lead to destabilization of the tetrameric protein and disassociation of the TTR subunits into dimers and individual monomers. These misfolded TTR monomers (both mutant and wild type [WT]) can then self-assemble into amyloid fibrils.[2] The amyloid fibrils are deposited into the extracellular space of various tissues where they form amyloid plaques, with the peripheral nervous system, gastrointestinal tract, and heart being among the major sites of deposition.

There are over 100 reported TTR genetic mutations, and phenotypically these result in a spectrum of disease which is collectively referred to as TTR-mediated amyloidosis (ATTR).[3] There is a range of clinical manifestations of ATTR; the most common manifestations include some form of cardiac and/or neurologic involvement (e.g., cardiomyopathy, autonomic neuropathy, and sensory and motor neuropathy) that depends, in part, upon the particular TTR mutation and the site of amyloid deposition. Transthyretin amyloidosis is associated with severe morbidity and mortality, with a life expectancy limited to approximately 5 to 15 years from symptom onset.[4]

Because the liver is the primary source of mutant TTR, liver transplantation has been used over the past 20 years in an attempt to treat ATTR. However, the procedure is only effective in halting or slowing the progression of disease in patients with an early age of onset[5], especially for those with the V30M mutation and short disease duration prior to transplant; consequently, almost two-thirds of ATTR patients are not transplant-eligible. Tafamidis, a TTR tetramer stabilizer, was approved (November 2011) in the European Union (EU) for the treatment of ATTR in adult patients with stage 1 symptomatic polyneuropathy to delay peripheral neurologic impairment.[6] The large majority of ATTR patients do not qualify for either liver transplantation or tafamidis. In these patients, the disease is primarily managed with palliative care.

Ribonucleic interference (RNAi) is a naturally occurring cellular mechanism for regulating gene expression that is mediated by "small interfering ribonucleic acids" (siRNAs).[7] Typically, synthetic siRNAs are 19 to 23 base pair double-stranded oligonucleotides in a staggered duplex with a 2-nucleotide overhang at 1 or both of the 3' ends. Such siRNAs can be designed to target an endogenous or virally-expressed gene. When introduced into cells, the net effect of an RNAi-based pharmacological approach is the binding of the siRNA to its complementary messenger ribonucleic acid (mRNA) sequence, cleavage of this target mRNA, and suppression of the target protein.[8] 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.

Alnylam Pharmaceuticals is developing ALN-TTR02 Solution for Injection (hereafter referred to as ALN-TTR02), a synthetic investigational RNAi therapeutic comprising a siRNA targeting the TTR mRNA formulated in a lipid nanoparticle (LNP) termed AF-011. The LNP enables delivery of the siRNA primarily to the liver upon systemic administration, resulting in the down-regulation of hepatic TTR expression and, in turn, reducing serum mutant and WT TTR levels. The proposed indication for ALN-TTR02 is for the treatment of ATTR. ALN-TTR02 is intended for administration as an intravenous (IV) infusion over 1 hour.

ALN-TTR02, a second generation siRNA LNP formulation termed AF-011, employs the same siRNA as ALN-TTR01, which utilizes the first generation LNP (termed stable nucleic acid lipid particles [SNALP]). Both ALN-TTR01 and ALN-TTR02 are intended for the treatment of patients with ATTR; the AF-011 formulation, however, has been optimized to be more potent such that mRNA and protein reduction effects are observed at significantly lower doses with AF-011 than with the SNALP formulation.

1.1.2. Study Objectives

The primary objective of this study is to evaluate the safety and tolerability of multiple doses of ALN-TTR02.

The secondary objectives of this study are:

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

This statistical analysis plan (SAP) is designed to outline the methods to be used in the analysis of study data in order to answer the study objective(s). Populations for analysis, data handling rules, statistical methods, and formats for data presentation are provided. The statistical

analyses and summary tabulations described in this SAP will provide the basis for the results sections of the clinical study report (CSR) for this trial. Pharmacokinetic (PK) and pharmacokinetic/pharmacodynamics (PK/PD) analyses will be described in a separate analytical plan, will be performed by Alnylam Pharmaceuticals or its designated Contract Research Organization (CRO), and presented in a separate report (to be included in the CSR).

This SAP will also outline any differences in the currently planned analytical objectives relative to those planned in the study protocol.

1.2. Study Design

1.2.1. Synopsis of Study Design

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

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

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

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

Dose escalation to the next cohort will proceed after the collective safety and tolerability data through at least 96 hours post-first dose from the 3 patients in the previous cohort has been

reviewed by the SRC. If the administered dose is found to be safe and well-tolerated, dosing for the next cohort will begin no sooner than 96 hours after Patient 3 has safely received dose 1 from the previous cohort. Patients in the cohort would be dosed and reviewed for safety and tolerability following the same procedures as used for other cohorts (as stated above).

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

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

The duration of patient participation in this study is approximately 36 weeks. Patients will be screened from -45 to -3 days prior to dose administration. Eligible patients will undergo further pre-treatment assessments (performed on Day 0). Patients receiving the original premedication regimen will receive oral premedication with dexamethasone (or equivalent), paracetamol (or equivalent), and H1 and H2 blockers the night before and 30 to 60 minutes prior to each dose of ALN-TTR02 to reduce the potential of an infusion-related reaction (IRR) (see protocol Section 5.5). Those patients in an optional cohort evaluating an alternative premedication regimen, as agreed upon by the SRC, will receive IV dexamethasone (or equivalent), oral paracetamol (or equivalent), and IV H1 and H2 blockers at least 60 minutes prior to ALN-TTR02 dosing; no premedication will be administered the evening prior to dosing. On Days 0 and 28, patients on the 4-week dosing regimen will receive a single dose of ALN-TTR02 administered as a 60-minute IV infusion (for cohorts with the original premedication regimen and infusion rate), or as an approximate 70-minute IV infusion for those patients in an optional cohort evaluating the alternative premedication regimen and infusion rate. The infusion time may be extended up to 3 hours in the event of a mild or moderate infusion reaction (study drug administration will not be resumed for any patient following a severe infusion reaction). Details on the study drug administration are provided in protocol Section 5.6. Patients will be hospitalized at the study site for at least 24 hours after the end of the study drug infusion. Patients may be discharged upon Investigator review of the 24-hour electrocardiogram (ECG), liver function tests (LFTs), and a subset of serum chemistries (sodium, potassium, creatinine,

albumin, calcium, glucose, and phosphate), if results are deemed not clinically significant. Patients will return to the site for outpatient visits for safety, PK, and PD monitoring up to 208 days post-dose (see protocol Section 6 for details).

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

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

1.2.2. Randomization Methodology

Not applicable.

1.2.3. Stopping Rules and Unblinding

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

Unblinding is not applicable to this open-label study.

1.2.4. Study Procedures

The schedule of assessments, as outlined in the study protocol, is provided in Table 1-1 (for cohorts administered ALN-TTR02 once every 4 weeks) and Table 1-2 (for optional cohort(s) dosed once every 3 weeks).

Table 1-1 Schedule of Assessments for Cohorts Administered ALN-TTR02 Once Every 4 Weeks (from Protocol version 2.1 dated 16 January 2013)

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

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

Footnotes on following pages.

Note: The schedule of assessments for optional cohorts administered ALN-TTR02 once every 3 weeks is provided in Table 1-2.

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

b Interval medical history.

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

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

e Vital signs to include: blood pressure, pulse rate, oral body temperature, and respiratory rate. Parameters are to be measured in the supine position using an automated instrument after the patient has rested comfortably for 10 minutes.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

v Premedications include dexamethasone (8 mg, or equivalent), paracetamol (500 mg, or equivalent), H2 blocker (e.g., 150 mg ranitidine or 20 mg famotidine, or equivalent other H2 blocker dose), and H1 blocker (10 mg cetirizine, 25 mg hydroxyzine, fexofenadine or equivalent may be substituted) will be self-administered per os (PO) the evening before study drug administration. Thirty to 60 minutes prior to the start of study drug infusion, dexamethasone (20 mg PO, or equivalent), paracetamol (500 mg PO, or equivalent), an H2 blocker (PO), and an H1 blocker (PO) will be administered by study site personnel. Patients enrolled in an optional cohort evaluating the use of an alternative premedication regimen, as agreed upon by the SRC, will receive the following medications at least 60 minutes prior to the start of infusion of ALN-TTR02: dexamethasone (10 mg IV, or equivalent), paracetamol (PO 500 mg; or equivalent), IV H2 blocker (e.g. ranitidine 50 mg, famotidine 20 mg, or equivalent other H2 blocker dose), and IV H1 blocker (e.g., diphenhydramine 50 mg or equivalent; hydroxyzine or fexofenadine 25 mg PO or cetirizine 10 mg PO may be substituted for any patient who does not tolerate IV diphenhydramine or other IV H1 blockers). These patients will not receive any premedications the evening prior to ALN-TTR02 dosing.

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

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

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

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

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

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

Table 1-2 Schedule of Assessments for Optional Cohorts Administered ALN-TTR02 Once Every 3 Weeks (from Protocol version 2.1 dated 16 January 2013)

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

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

Footnotes on following pages.

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

b Interval medical history.

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

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

e Vital signs to include: blood pressure, pulse rate, oral body temperature, and respiratory rate. Parameters are to be measured in the supine position using an automated instrument after the patient has rested comfortably for 10 minutes.

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

v Premedications include dexamethasone (8 mg, or equivalent), paracetamol (500 mg, or equivalent), H2 blocker (e.g. 150 mg ranitidine or 20 mg famotidine, or equivalent other H2 blocker dose), and H1 blocker (10 mg cetirizine, 25 mg hydroxyzine, fexofenadine or equivalent may be substituted) will be self-administered per os (PO) the evening before study drug administration. Thirty to 60 minutes prior to the start of study drug infusion, dexamethasone (20 mg PO, or equivalent), paracetamol (500 mg PO, or equivalent), an H2 blocker (PO), and an H1 blocker (PO) will be administered by study site personnel. Patients enrolled in an optional cohort evaluating the use of an alternative premedication regimen, as agreed upon by the SRC, will receive the following medications at least 60 minutes prior to the start of infusion of ALN-TTR02: dexamethasone (10 mg IV, or equivalent), paracetamol (PO 500 mg; or equivalent), IV H2 blocker (e.g. ranitidine 50 mg, famotidine 20 mg, or equivalent other H2 blocker dose), and IV H1 blocker (e.g., diphenhydramine 50 mg or equivalent; hydroxyzine or fexofenadine 25 mg PO or cetirizine 10 mg PO may be substituted for any patient who does not tolerate IV diphenhydramine or other IV H1 blockers). These patients will not receive any premedications the evening prior to ALN-TTR02 dosing.

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

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

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

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

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

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

1.2.5. Pharmacodynamic and Safety Parameters

1.2.5.1. Pharmacodynamic Parameters

The PD evaluation will include assessment of effects of ALN-TTR02 on serum total TTR levels. Exploratory PD effects of ALN-TTR02 will be evaluated by:

- Serial measurement of circulating mutant and wild type TTR levels.
- Serial measurement of serum TTR mRNA levels.
- Serial measurement of circulating vitamin A and retinol binding protein (RBP) levels.

1.2.5.2. Safety Parameters

Safety monitoring will include assessment of adverse events (AEs), 12-lead ECGs, cardiac monitoring (telemetry), arterial oxygen saturation (SaO_2) using pulse oximetry, vital signs (blood pressure, pulse rate, oral body temperature, and respiratory rate), clinical laboratory safety tests (hematology, serum chemistry, LFTs, thyroid function parameters, serology, coagulation parameters, urinalysis, cytokines, c-reactive protein [CRP], and complement factors), and physical examinations. Patients will be closely monitored for both acute and delayed IRRs. All IRRs will be recorded as AEs.

The safety of ALN-TTR02 will be evaluated by:

- The proportion of subjects experiencing AEs, serious adverse events (SAEs), DLTs, infusion-related AEs, and AEs leading to study drug discontinuation.
- Infusion tolerability, as assessed by serial ECGs, measurement of SaO_2 by pulse oximetry, and blood pressure.
- Change from baseline in clinical laboratory test results, including hematology, serum chemistries, LFTs, and coagulation parameters.
- Change from baseline in measures of thyroid function (thyroid stimulating hormone [TSH], T4, and triiodothyronine [T3].
- Change from baseline in CRP, cytokines, and complement.
- Vital sign measurements.
- Physical examination findings.

2. SUBJECT POPULATION

2.1. Population Definitions

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

- Intent-to-Treat (ITT) Analysis Set: All patients who were enrolled and received study treatment.
- Per-Protocol (PP) Analysis Set: All patients in the ITT analysis set who had no major protocol violations.
- Pharmacokinetic (PK) Analysis Set: All patients in the ITT analysis set who have adequate data to determine a full PK profile.

The ITT analysis set is the primary population for the analysis of PD and safety parameters. A secondary analysis of PD parameters will be performed for the PP analysis set. The PK analysis set is the primary population for the analysis of PK parameters.

2.2. Protocol Violations

At the discretion of the Sponsor, major protocol violations, as determined by a review of the data prior to unblinding of the study results and the conduct of statistical analyses, may result in the removal of a subject's data from the PP analysis set. Medpace will be responsible for producing the final protocol violation file (formatted as a Microsoft Excel file), which will be reviewed by Veristat and Alnylam; this file will include a description of the protocol violation and clearly identify whether or not this violation warrants exclusion from the PP analysis set. This file will be finalized prior to hard database lock.

All protocol violations will be presented in a data listing.

3. GENERAL STATISTICAL METHODS

3.1. Sample Size Justification

Based on the planned dose escalation scheme, up to 27 patients are expected to be enrolled. Three patients are to be enrolled at each of 4 planned dose levels. An additional 5 cohorts of 3 patients may be enrolled onto 1 or more of these specified dose levels to further evaluate safety and tolerability or PD effects. The sample size was chosen based on previous experience in Phase 2 studies and was not based on power calculations.

3.2. General Methods

All data listings that contain an evaluation date will contain a relative study day. Pre-treatment and on-treatment study days are numbered relative to the day of study medication dosing, which is designated as Day 0. For example, the day prior to study medication will be Day -1 and the day after study medication will be Day 1.

All output will be incorporated into Microsoft Word or Excel files, or Adobe Acrobat PDF files, sorted and labeled according to the International Conference on Harmonisation (ICH) recommendations, and formatted to the appropriate page size(s).

Tabulations will be produced for appropriate demographic, baseline, efficacy, and safety parameters. For categorical variables, summary tabulations of the number and percentage of subjects within each category (with a category for missing data) of the parameter will be presented. For continuous variables, the number of subjects, mean, median, standard deviation (SD), minimum, and maximum values will be presented.

All data will be presented in by-subject data listings.

As this is a Phase 2 dose-escalation trial, formal statistical hypothesis testing will not be performed. Although exploratory confidence intervals (CIs) will be presented as described below, no formal statistical conclusions will be made based on these estimates.

Subjects will be analyzed in the cohort to which they were originally assigned. Data will be tabulated for each dose group (i.e., dose level, regimen, and pre-medication regimen).

Laboratory data collected and recorded as below the limit of detection will be set equal to the lower limit of detection for the calculation of summary statistics.

3.3. Computing Environment

All descriptive statistical analyses will be performed using SAS statistical software version 9.3 or higher, unless otherwise noted. Adverse events will be coded for summarization using the Medical Dictionary for Regulatory Activities (MedDRA® version 15.0). Concomitant medications will be coded using the World Health Organization (WHO) Drug Dictionary (March 2012 version).

3.4. Baseline Definitions

Unless noted otherwise, baseline will be defined as the Day 0 pre-dose value when non-missing, otherwise the latest value from amongst any screening values will be used (i.e., the measurement closest to and prior to dosing will be considered baseline). For PD parameters (TTR, RBP, Vitamin A), baseline will be defined as the average of all pre-dose values (i.e., Screening and Day 0 pre-dose values).

3.5. Methods of Pooling Data

Data will be summarized separately for each dose group (i.e., dose level and regimen). Data from expansion cohorts that are dosed at the same level and regimen as a previous cohort will be pooled with the previous cohort for presentation by dose group. In addition, safety data will be presented overall.

Data will be tabulated separately for dose groups dosed 4 weeks apart and dose groups dosed 3 weeks apart, if applicable.

3.6. Adjustments for Covariates

No formal statistical analyses that adjust for possible covariate effects are planned.

3.7. Multiple Comparisons/Multiplicity

Multiplicity is not of concern for this Phase 2 study with a descriptive interpretation.

3.8. Subpopulations

Summaries of serial TTR will be presented by genotype (V30M versus other) and use of TTR stabilizer (tafamidis or diflunisal use versus none).

3.9. Withdrawals, Dropouts, Loss to Follow-up

Subjects who voluntarily withdraw are termed dropouts. Dropouts may be replaced following discussion with the Investigator and Sponsor.

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

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

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

Data collected at an ET visit will be included in Day 56 or Day 208 tabular summaries. That is, the Day 28 summary will include actual observed values from Day 56 along with data collected from any ET visits prior to Day 56. Similarly, the Day 208 summary will include data from subjects completing the study, along with any data collected at ET visits between Day 56 and Day 208.

3.10. Missing, Unused, and Spurious Data

In general, there will be no substitutions made to accommodate missing data points. All data recorded on the case report form (CRF) will be included in data listings that will accompany the CSR. Data collected at multiple time points throughout the study will be presented in chronological order in the data listings according to assessment date/time.

Handling of partial dates for determining whether an AE is treatment-emergent is discussed in Section 4.5.2. Handling of partial dates for determining concomitant medication is discussed in Section 4.5.6.

3.11. Visit Windows

It is expected that all visits will occur according to the protocol schedule of assessments. All data will be tabulated per the evaluation visit as recorded on the CRF even if the assessment is outside of the window for that study visit. In data listings, the study day relative to Day 0 (day of first dose) will be presented.

Unscheduled visits will be included in data listings, but no assignment to a study visit will be made for the purpose of summary tabulations.

3.12. Interim Analyses

No interim analyses are planned for this study.

4. STUDY ANALYSES

4.1. Subject Disposition

Subject disposition will be tabulated and include the number enrolled, the number dosed, the number in each subject population for analysis, the number who completed the study (as indicated on the Study Completion CRF), the number who withdrew prior to completing the study and reason(s) for withdrawal. Summary data will be presented by dose group and overall.

A by-subject data listing of study completion information including the reason for premature study withdrawal, if applicable, will be presented.

4.2. Demographics and Baseline Characteristics

Demographics and baseline characteristics will be summarized and presented by dose group and overall for the ITT population.

Age, height, weight, and BMI will be summarized using descriptive statistics (number of subjects, mean, SD, median, minimum, and maximum). Sex, race, ethnicity, and country will be summarized by presenting the numbers and percentages of subjects in each category.

Baseline disease characteristics will be summarized by presenting the numbers and percentages of subjects with or without the V30M mutation and with or without prior exposure to TTR02. Concurrent use of TTR stabilizer (Tafamidis or Diflunisal) will also be summarized.

All demographic and baseline data will be provided in data listings.

Medical history and prior surgeries will be presented in a data listing. Pregnancy and virology test results will be presented in data listings.

4.3. Pharmacodynamic Evaluation

4.3.1. Total Serum TTR (ELISA)

Serial measurements and changes from baseline of serum TTR through Day 208 will be summarized for each scheduled time point using descriptive statistics. Percent change from baseline will also be summarized. Ninety-five percent CIs for percent change will be presented. An overall summary of percent reduction in TTR will include the maximum percent reduction per dose group, dose group means (SD) at individual and group nadirs (defined below), and dose group means (SD) at Day 28 and Day 56 (Day 21 and Day 42 for optional cohorts dosed every 3 weeks).

The individual nadir TTR value, defined as the lowest observed TTR value from Day 1 to the Day 28 premedication assessment (Day 21 for optional cohorts dosed every 3 weeks), will be

determined for each subject. The nadir value will be plotted for each subject, grouped by dose group. Dose group means will be indicated on the plot. The value of the percent reduction associated with the nadir will be plotted similarly. The nadir value from the Day 29 to Day 56 interval (Day 22 to Day 42 for optional cohorts dosed every 3 weeks) will also be determined and plotted.

The group nadir TTR value, defined as the lowest observed mean percent TTR reduction from baseline from Day 1 to the Day 28 premedication assessment (Day 21 for optional cohorts dosed every 3 weeks), will be determined for each dose/regimen group. The group nadir value will be plotted for each group, along with standard error bars. The group nadir value from the Day 29 to Day 56 interval (Day 22 to Day 42 for optional cohorts dosed every 3 weeks) will also be determined and plotted.

The area under the concentration-time curve (AUC) will be calculated for serum TTR. The AUC will be calculated using the daily TTR concentrations starting from baseline, defined as the average of all pre-dose values, and continuing to the Day 28 premedication assessment (Day 21 for optional cohorts dosed every 3 weeks). The trapezoidal rule will be used for the calculation of AUC. For the purpose of this AUC calculation, it is assumed that the collection times are in multiples of equal 24 hour intervals. For example, the interval from Day 2 to Day 4 will be 48 hours, irrespective of actual assessment times. AUC will be summarized using descriptive statistics. A similar calculation and summary will be performed for the AUC calculated from Day 28 to Day 56 (Day 21 to Day 42 for optional cohorts dosed every 3 weeks).

TTR levels over time will be plotted. Each subject's TTR levels will be joined by a line, and each subject within a dose group will have a different symbol. A separate plot will be produced for each dose group. In addition, the average of each dose group (with standard error bars) will be plotted versus time. Dose group means (with standard error bars) for percent change from baseline will be plotted over time.

The pharmacodynamics assessment for use in the PK/PD analysis will be similar to that described above, but may include additional PD analyses which will be used for the purpose of PK/PD analysis. The methods for the PK/PD analysis will be described in the PK and PK/PD analysis plans and the analysis result will be reported in the separate PK and PK/PD report which will be appended to the CSR.

4.3.2. Vitamin A and Retinol Binding Protein

Observed values and changes from baseline in vitamin A through Day 208 will be summarized for each scheduled time point using descriptive statistics. Percent change from baseline will also be summarized. Ninety-five percent CIs for percent change will be presented.

Vitamin A levels over time will be plotted. Each subject's vitamin A levels will be joined by a line, and each subject within a dose group will have a different symbol. A separate plot will be produced for each dose group. In addition, the average of each dose group (with standard error bars) will be plotted versus time. Dose group means (with standard error bars) for percent change from baseline will be plotted over time. Percent reduction from baseline at individual and group nadirs will be plotted as described above for serum TTR.

The above summaries will also be performed for RBP.

A scatter plot of total serum TTR protein versus vitamin A and RBP, all normalized to their respective baseline values, will be produced. The plot will include Spearman's correlation coefficients (ρ) and the p-values from the tests of ρ = zero, to assess the strength of the correlations between serum TTR and vitamin A and between serum TTR and RBP.

4.4. Pharmacokinetic Evaluations

All tabulations of PK data will be presented in a separate report appended to the CSR.

4.5. Safety Analyses

Safety analyses will be conducted using the ITT analysis set.

4.5.1. Study Drug Exposure

Duration of infusion will be summarized using descriptive statistics. A summary of the numbers and percentages of subjects with a dose interruption will be provided. Duration of interruption will be summarized using descriptive statistics. Summary statistics will be provided for the total drug amount received by the subject and the total volume infused.

All data related to study medication administration, including details on inpatient hospitalization for dosing, will be presented in a data listing.

4.5.2. Adverse Events

Adverse events will be displayed in tables and data listings using MedDRA system organ class (SOC) and preferred term.

Analyses of AEs will be performed for those events that are considered treatment-emergent, where treatment-emergent is defined per protocol as any AE with onset during or after the administration of study medication through 28 days after dosing, or any event that was present pre-infusion but worsened in intensity or was subsequently considered drug-related by the Investigator.

When determining whether an AE is treatment-emergent, partial dates will be handled as follows. If the day of the month is missing, the onset day will be set to the first day of the month unless it is the same month and year as study treatment. In this case, in order to conservatively report the event as treatment-emergent, the onset date will be assumed to be the date of treatment. If the onset day and month are both missing, the day and month will be assumed to be January 1, unless the event occurred in the same year as the study treatment. In this case, the event onset will be imputed as the day of treatment, in order to report the event conservatively as treatment-emergent. A missing onset date will be imputed as the day of treatment. If the resulting imputed onset date is later than a non-missing date of resolution, then the imputed onset date will be set to the resolution date. The imputed data is used solely for the purpose of determining whether an AE is treatment-emergent; actual dates as recorded on the eCRF will be presented in subject listings.

Adverse events are summarized by subject incidence rates; therefore, in any tabulation, a subject contributes only once to the count for a given SOC or preferred term.

The numbers and percentages of subjects with any treatment-emergent adverse event (TEAE), with any TEAE assessed by the Investigator as related to treatment (definitely or possibly related), with any TEAE that is severe in intensity, with any serious TEAE, with any TEAE leading to discontinuation of study medication, or with any TEAE considered a DLT will be summarized by dose group and overall.

Tabulations by SOC and preferred term will be produced for all TEAEs, for all related TEAEs, all TEAEs of severe intensity, all treatment-emergent IRRs, and for all serious TEAEs. In these tabulations, each subject will contribute only once (i.e., the most related occurrence or the most intense occurrence) to each of the incidence rates in the descriptive analysis, regardless of the number of episodes of a given event (preferred term). The most commonly occurring TEAEs, defined as those events experienced by at least 10% of all subjects, will be tabulated by preferred term in decreasing order in frequency.

Separate tables will present AE incidence rates by maximum relationship to study drug and by maximum severity. Subjects who report multiple occurrences of the same AE (preferred term) will be classified according to the most related or most severe occurrence, respectively.

No formal hypothesis-testing of AE incidence rates will be performed.

All reported AEs will be provided in data listings, including AEs that are not treatment-emergent.

By-subject listings also will be provided for the following: subject deaths, SAEs, AEs leading to drug discontinuation, and DLTs.

4.5.3. Laboratory Data

Clinical laboratory values will be expressed in SI units.

Descriptive statistics will be provided for the actual values and changes from baseline to each on-study evaluation for each clinical laboratory parameter, including hematology, clinical chemistry, LFTs, thyroid function tests, and coagulation studies. Percent change from baseline will also be summarized.

Descriptive statistics will be provided for CRP and cytokines at pre-dose and 2, 6, and 24 hours post-dose. Change from pre-dose will also be summarized. Fold change, as defined below, will be summarized for CRP. Complement factor (Bb) will be summarized at pre-dose, and 30 minutes, 2 hours and 24 hours post-dose. Change and fold change from pre-dose will also be summarized. Separate summaries will be presented for Dose 1 and Dose 2.

Fold change from pre-dose is defined as the ratio of the post-dose value to the pre-dose value. Maximum fold change is defined as the ratio of the maximum post-dose value to the pre-dose value. Maximum fold change from pre-dose for cytokines, complement factor (Bb), and CRP will be represented graphically in the form of a semi-$\log_{10}$ plot. Maximum fold change will be plotted for each subject, grouped by dose level. Data recorded as below the limit of detection will be set equal the lower limit of detection for the calculation of fold change. Separate plots will be presented for Dose 1 and Dose 2.

For each continuous laboratory parameter, results will be categorized as low, normal, or high based on the laboratory normal ranges. Shift tables will be employed to summarize the pre-dose category versus the post-dose category, where the post-dose category will be based on the maximum observed value (in absolute value) subsequent to each dose. For dose 1, the maximum value shall be selected from assessments performed through the Day 28 pre-dose

value (Day 21 for the 3-week regimen). All out-of-range and clinically significant laboratory results will be identified in subject data listings.

Figures will be produced for each dose group that display each subject's AST, ALT, alkaline phosphatase, and bilirubin (total and direct) results over time; 1 figure will be produced for each dose group and liver function test parameter, with lines presenting subject results over time. In addition, treatment group means, with standard error bars, will be plotted over time. Similar plots will be produced for complement factor (Bb) and cytokines. A vertical reference line will identify the nominal Dose 2 dosing day (i.e., Day 28 for the 4-week regimen or Day 21 for the 3-week regimen).

Urinalysis results will be included in subject listings but will not be included in summary tables.

Tryptase and C3a will be included in subject listings for any subjects experiencing an IRR.

Results of anti-PEG antibody testing will be listed if any positive results are reported.

All laboratory data, including the Day 0 fasting lipid panel, will be provided in data listings. Laboratory values outside of the normal ranges will be listed separately, together with comments as to their clinical significance.

4.5.4. Vital Signs and Physical Examination

Descriptive statistics will be provided for serial vital signs collected on each dosing day, including blood pressure, pulse rate, oral body temperature and respiration rate. Pulse oximetry (SaO_2) will be included with serial vital sign summaries. Change from pre-dose to each post-dose assessment will also be summarized. Treatment group means with standard error bars will be plotted over time for each serial vital sign assessment. Separate plots will be presented for Dose 1 and Dose 2. Routine vital signs will be included in subject listings but will not be summarized.

Vital sign measurements will be presented for each subject in a data listing. All physical examination findings will be presented in a data listing.

4.5.5. Electrocardiogram

Descriptive statistics will be provided for serial ECGs collected on each dosing day, including ventricular rate, PR interval, QRS duration, and QT interval. Bazett's formula will be used to calculate the heart rate corrected QT interval (QTcB). The mean of the triplicate values will be used for calculating summary statistics. Change from pre-dose to each post-dose assessment

will also be summarized. Routine 12-lead ECGs will be included in subject listings but will not be summarized.

Electrocardiogram data for each subject will be provided in a data listing.

4.5.6. Concomitant Medications

Concomitant medications will be defined as those medications that were initiated after first study drug administration or those that were ongoing at the time of the first study drug administration. If a start date of a medication is missing, the drug will be assumed to be concomitant, unless an end date indicates otherwise. If an end date is missing or the medication is ongoing, the medication will be considered concomitant.

Concomitant medications will be coded using the WHO Drug Dictionary. Subject incidence will be tabulated by Anatomic Therapeutic Class (ATC) and preferred term for each dose group and overall; subjects will only count once for each ATC or preferred term in the event that they have multiple records of the same ATC or preferred term in the database. Medications taken as protocol specified premedications will be summarized separately.

The use of concomitant medications, including protocol-specified premedications, will be included in a by-subject data listing. Premedications will also be listed separately.

5. CHANGES TO PLANNED ANALYSES

As of this date, there have been no changes between the protocol-defined statistical analyses and those presented in this SAP.

6. REFERENCES

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7. CLINICAL STUDY REPORT APPENDICES

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